QATAR UNIVERSITY

COLLEGE OF ARTS AND SCIENCES

# IMPACT ON LOCAL PEARL OYSTER PINCTADA RADIATA EXPOSED TO CHRONIC LEVELS OF CHLORINE

BY

ARWA SHAIF ALFAQIH

A Project Submitted to

the Faculty of the College of Arts and Sciences

in Partial Fulfillment of the Requirements for the Degree of

Masters of Science in Environmental Sciences

June 2019

# COMMITTEE PAGE

The members of the Committee approve the Project of Arwa Shaif Alfaqih

defended on 16/05/2019.

_____

Dr. Radhouan Ben-Hamadou
Project/Dissertation Supervisor

_____

Dr. Allal Ouhtit
Committee Member

_____

Dr. Mohamed AlGhouti
Committee Member

# ABSTRACT

ALFAQIH, ARWA, SHAIF., Masters : May : 2019, Environmental Sciences

Title: Impact on local pearl oyster Pinctada radiata exposed to chronic levels of chlorine and chlorinated by-products.

Supervisor of Project: Dr. Radhouan Ben-Hamadou.

The marine environment is facing major challenges due to several natural and anthropological stressors, including chemical and thermal pollutions released by coastal industries using seawater for cooling purposes. The Arabian Gulf is not an exception and during the last decades, the increased coastal industrial and urban development had just added more pressures on the already extreme environment. Chlorine is widely used in industrial processes for mainly i. Sterilization of sewage and pollutants and ii. Preventing the accumulation of organisms on hard substrates (bio-fouling) such as in power or desalination plants.

We carried out a laboratory experiment to identify the effects of exposure to chlorine on biological and physiological performance of the local pearl oyster *Pinctada radiata*. The experiment was run during 75 days at ExxonMobil Research Qatar with adult oysters (originally collected from AlWakra coastal waters) being exposed to two different concentration of chlorine (0.025 and 0.1 mg/l) and a control treatment.

Our results suggested that exposure to chlorine had non-significant effects on the mortality, growth and physiology (respiration rate) of healthy oysters. Analysis of the mortality registered throughout the experiments in all treatments (Kaplan Meier curves) suggested a higher final mortality rate (31.48%) for the oysters exposed to Cl concentration

at 0.025 mg/l. This final rate was significantly different when compared with the other two treatments. In contract with this result, final mortality rates of oysters from the control treatment (11.76%) and exposed to 0.1 mg/l of chlorine (14.81%) were not significantly different.

Such non-effect of chlorine on mortality was also confirmed in oysters endpoints related to growth (i.e. length, width and height increments) and physiological (i.e. respiration rate).

Nevertheless, the experiment suggested that the size class of the considered oysters had a significant impact on mortality and growth, with larger specimens showing relatively lower performance than the smaller oysters.

These double evidences may be related to chemoreceptors located at the margins of the mantle of the oysters that may act as detectors of hazardous pollutants in the water and cause the closing of the valves. In addition, when transfer the oysters from its natural ecosystem to artificial one, outdoors affect in the laboratory as temperature, light; all lead to stress and decreasing in the physiological performance of oysters.

This will lead to both, decrease in feeding and prevention from toxicity, resulting in a reduced growth and lower susceptibility, respectively.

# DEDICATION

*The project, all the practical section and also the final result, belong to my own effort. I mentioned all the references of any data, information, graphs or tables of previous researchers done to help me in my project.*

*Arwa Shaif Alfaqih*

# ACKNOWLEDGMENTS

I ought to renowned my unlimited grateful thanks to Allah who always stand with me and gave me the power to do and finish this project,

I like to thank my supervisor Dr. Radhouan Ben Hamadou and also Dr. Pedro, Ms. Zenaba and Mr. Mohammed Sherif. I will send my wholehearted thanks to Dr. Suhur, Dr.Nantid and Ms. Bernice, all staff from EMRQ (ExxonMobil Research Center, Qatar) for helping me in the laboratory section.

I like to thanks my family especially my mother for pushing me to continue my study and my sister Mshael. I can't say words to appreciate my lovely friend Dr. Aisha for advising me and being patient with me to receive the last step of my project.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

## 1.1 Marine environment and challenges

Several environmental challenges are facing the aquatic marine environments. Oil spills have been identified as a major stressor due to their harmful effect on the marine environment and associated human economic activities. Deep water Horizon was reported as a main petroleum spill in 2010 in South Korea and because of this episode, it draw international attention to its effect on water and associated biota. Recently, substantial improvement have been made in various sectors consisting of precaution and readiness measures taken to enhance oil release recovery and improvement strategies to characterize the oil released in to the marine environment, and determination of mitigation responses. Nevertheless, several challenges remain associated with both low temperature and severe ecological conditions such as in the North Frigid Zone (Chin et al., 2019).

Polychlorinated biphenyls (PCBs) are a group of industrial chemical components that their production have been considerable declined through the 1960s, due to the associated environmental issues about their adverse effects on the natural environment and human beings. Contamination by Polychlorinated biphenyls (PCBs) at the end 1980s in the ocean were mainly from atmospheric source. Several studies conducted in the following years showed highest concentrations of the PCB s in the Northern Hemisphere oceans. In the ocean's ground water, concentrations of PCBs are generally at or less than 1 pg/l.

Recent researches studied subsurface maxima for PCBs in the North Atlantic Ocean (Sun et al., 2016). Significant development has been made to identify the existence and establish the profiles of PCBs in the seawater by determining the dynamics of their biogeochemical

forms inside the marine waters inside the marine waters. Specifically, the coupling of PCB elements to the biological pump has encouraged the investigation of why PCBs found in the oligotrophic zone (Lohmann & Dachs, 2019).

Debris in the marine environment are recognized as a global environmental issue (Sheppard, 2019). Several international researches are investigating debris distribution and their impact on habitats and associated biota in the world ocean at depths between (50–7000 m), however only a limited information is gathered so far, due to the difficulty in conducting large scale studies (Angiolillo, 2019). Anthropogenic and environmental consideration affect particles spatial arrangement that are released from land in to coastal waters (Barbier, 2011). Wind and currents can be used as drivers to infer the distribution of persistent particles that disperse at large distances affecting their behavior into the water column before sinking and potential accumulation areas (Eriksen et al., 2016). 50% to 80% of debris or wastes in the marine environment are resulting in the large use of plastics in land, this high percentage of persistence is caused by their large resistance against disintegration (Potera, 2013). Plastic particles found in the sea because of human activity like fishing, become trapped on 70% of marine rocks. High occurrence of plastic debris may affect marine organisms and their niches. Consistent preservation strategies and concrete mitigation measures are needed to preserve ocean communities that are under increasing stress (Angiolillo, 2019). As a result of the industrial and social activities in the coastline, marine life can be threatened (Lynette et al., 2019).

Above 320 million items of plastics debris are found all around the world every year. Alongside, the introduction of plastic waste into the marine environment is of global concern, resulting on the immediate and recurrent impact on organic systems,

marine fauna, and indirectly harming economic activities. Due to the large distribution and dynamics of plastic debris, these are acting as vectors of diseases and contaminations, spreading adsorbed microorganisms and pollutants among and between basins of the world ocean (Luís et al., 2019).

### 1.2 Major sources in marine pollution

There are many major sources of pollutions that affect marine ecosystem and they can be natural as climatic change (Weis, 2015), or introducing of invasive species (Hayes & Sliwa, 2003). Anthropogenic affect in the sea water include heavy metals as Zn, Hg (Buccolieri et al., 2006), petroleum or oil drops (García et al., 2006) and desalination (Miller, 2015). Desalination of saline water can be achieved either by thermal desalination technologies or by membrane technologies. Reverse osmosis (RO) represents the membrane separation technology while multi stage flushing (MSF) and multi effect distillation (MED) represent the thermal technologies (Peñate & García-Rodríguez, 2012). Currently, the global market of thermal desalination is about 35% while the RO market share is up to 61% (Altaee et al., 2013). However, in Gulf Cooperation Council (GCC) countries the market share of RO technology represents 30% and 70% for the thermal desalination (Hoda et al., 2017). The high demand of MSF and MED technologies in the GCC countries is due to their capability in treating harsh seawater with high salinity, temperature, and impurities to produce high quality product (Altaee et al., 2013). Thermal desalination processes are known to have a high quality freshwater, however, these processes experience major drawbacks characterized by the disposal of the concentrated byproduct known as the Rejected Brine (RB) into the water bodies and environment (Afrasiabi & Shahbazali, 2011). Discharging the rejected brine leads to eutrophication, pH and temperature, increasing the level of heavy

metals in the marine environment, and also increase disinfectants properties that lead to several problems to marine biota (Afrasiabi & Shahbazali, 2011). The environmental impacts resulted from Rejected Brine disposal can be minimized by reducing the amount of disposed RB through following Zero-liquid discharge (ZLD) practice.

Bio-fouling is the growth and accumulation of marine organisms on the surfaces of petroleum pipeline that are submerged in the seawater (Munk et al., 2009). These lead to severe problems and risks from the time when used to build large building structures that are enough for constant immersion , because of that they use chlorine and chlorination co-product to stop the growth of marine organism . This chlorinated by-products is a major contaminant found in the seawater because it release and caused oxidizing of chemicals as Br, which has negative affect on the marine environment (Rostron & Rehana, 2015).

There are 23 power stations shoreline in USA mainly in California city, these situations use of seawater in reducing water temperature by condenser processes (Resources Agency, 1973), influences of thermal releases on the marine environment have received increasing interests (Adams et al., 1969).

The Arabian Gulf countries have been subjected to growing development of their manufacturing assets couple to an increasing of human population needs, which result in the generation and then release of pollutants in to the pelagic ecosystem. These contaminants contain considerable amount may lead to genetic disorder of the marine organisms in the receiving environment. Accordingly, the effects of chemicals contaminants such as TPHs “Total Petroleum Hydrocarbons “and PAHs “Polycyclic Aromatic Hydrocarbons “ were investigated in marine organisms in Qatar. Samples of the pearl oyster *Pinctada radiata* used as bio-indicator of marine pollution were collected from

2 different sites in Qatar, (South of Al Khor and Doha Harbour. Results showed higher aneuploidy levels (abnormality of chromosomal number) in highly impacted sites (Leitão et al., 2017).

The special spreading of different bio-chlorinated complexes was explored in the Gulf region. Through 2000–2001 industrialized PCB was measured from Qatar and Oman by collected samples from precipitation in the coast region and living organism as numerous mollusks and fish in the water (Mora et al., 2005).

Coastal region is mostly affected by anthropogenic activities. Indeed, seawater is commonly used in industrial intermediate cooling system (Abuzinada et al., 2008).

In the sea, when chlorine is introduced to water, associated to high salinity, bromide (Br) is oxidized and when reacting with hypochlorite forms hypobromous acid (HOBr). This response is fast, with 99% change inside 10 seconds at greatest salinity and not exceed15 seconds indeed at half of salinity.

When chlorination (which is the process of adding chlorine to water) is performed in salty water, these oxidants Br become more toxic to living organisms (e.g. mollusks) marine bivalves (Jenner et al., 2003).

### 1.3 Chlorine and chlorine by products in the marine environment

Chlorine (Cl), from the group of halogens, is widely used in industrial processes in the coastal zone for mainly i. Sterilization of sewage and other wastes and ii. Preventing the accumulation of organisms on hard substrates (bio-fouling) such as in power or desalination plants (Beauchamp, 1969; Jolley et al., 1978).

Indeed, coastal electrical power stations are using massive amount of noxious chlorine (Cl) as an anti-fouling, mainly within their cooling system. Chlorination of seawater may result

additionally in the formation of toxic nonmetallic elements like “fluorine (F), chlorine (Cl) and bromine (Br) beside the free monochloramine ($NH_2Cl$) (Lewis, 1966), chlorinated hydrocarbon mixtures (Jolley, 1975) and complexes of Br (Dove, 1970).

Both; time of exposure and temperature are the primary ecological factors that determine the toxicological impact of halogens (Cl, Br, I and F)(Davis & Middaugh, 1976).

Cl and Chlorine by-products are lethal to marine biota and relatively harmful to the receiving ecosystems (Brungs, 1976; Morgan & Carpenter, 1978; Hall et al., 1981, 1982)

Studied conducted by (Blogoslawski et al., 1976) concluded that there are variation between salty and inland water, chlorination and its ability to be oxidized Bromine to hybobromide happened in the seawater.

In April 2008, a research conducted by (Macdonald et al., 2011) notifications showed that Pulse-Chlorination® (P-C®) which is Cooling System of seawater by adding Cl and improving the quality of the polluted water. This European methods had an efficient result at large depositing reduction. Result showed that, all marine organisms were died, and if there were breakable remaining shatters of oysters with dark color shells, they had been possibly existing before starting of Pulse- Chlorination ® Technique.

### 1.4 Effects of chlorine on marine life

In previous study conducted by Capuzzo (1977) on young fishes and zooplanktons suggested that the type of chlorine reaction seems to lower metabolic functions and the respiratory performance of fishes by 50%. Chlorination also reduce the energy that oysters need it to close their valve with limited duration (Van Wijk et al., 1989), or the accumulation of metabolic molecules would force the animals to open their valves and be in contact with the surroundings (Akberali & Black, 1980). This susceptibility is

nevertheless variable among marine organisms. For instance, the Atlantic oyster *Crassostrea virginica* showed consistent higher resistance than another bivalve *Donax serra* with <10% of death rate when exposed to Cl in concentrations ranging 0.35 to 0.85 ppm for 2 weeks (Scott & Middaugh, 1978).

Importance of Cl as a toxicant is the result of its substantial usage as biofouling agent at coastal power plants. Formation of Cl or Cl co-product in the seawater may also lead to the production of Br or F toxicants in addition to the release of Cl , including dichloramine ($NHCl_2$) (Lewis, 1966), organochloride complexes (Jolley, 1975) and Br complexes (Dove, 1970). After chlorination happened in the marine ecosystem, complexes are formed such as Cl or F, and they can interact with Br, natural materials, $NH_3$ and any compounds that contain nitrogen. Thus the overall toxicity resulting from cooling systems using Cl can also differ from one location to another, due to variations in relative concentrations in Cl, F, Br or I (Capuzzo, 1977).

The highest accumulation of oceanic autotrophic plankton on coastal power plants cooling system in California, result in reducibility of the technique from 41.7 % in to 33.7% and the highest number of phytoplankton was mainly between June to August (Briand, 1975).

Previous study done by (Capuzzo, 1977) on larval lobsters showed the reduction on metabolic activity including growth and standard respiratory rate $P< 0.01$ when *Homarus americanus* tested with 1.0/ mg of chloramine or free chlorine for just 1 hour (Capuzzo, 1977). Research conducted by (Capuzzo et al., 1976) showed the variance affecting of released Cl and $NH_2Cl$ on juvenile phase of crustacean (lobsyer) *H. americanus*, resulted on more toxicity effect under $NH_2Cl$, lowering respiration rates.

### 1.5 Oysters as bio-indicator of marine pollution

Oysters, a filter feeder bivalve associated to the seabed, are commonly used as bio-indicator of marine aquatic pollution. The Qatar local pearl oyster *Pinctada radiata* was used in an eco-toxicological investigation to study the biological effects of pollutants is Qatari waters (Leitão et al., 2017). Authors quantified toxicants' bioaccumulation and aneuploidy (presence of abnormal number of chromosomes in a cell) in the pearl oyster in two sampling sites (Al Khor and Doha harbor). Results suggested that local chronical contamination by organic pollutants such as TPHs and PAHs and trace metals resulted in different scores of aneuploidy. Contamination of seawater and sediments were associated to neighboring adjacent industrial activities (Ross, 2003).

Marine bivalves are using to evaluate the health of marine ecosystem and quality of environmental improvement (Viarengo et al., 2007).

Adding of Cl lower than 0.3 ppm to *Donax serra*, lead to immediate closing of valves within 6 hours, while half percentage death of *D.serra*, when expose to 0.6 ppm of Cl concentration .Chemical Receptors And Mantle Edge Of *Donax serra* allow the fast discovery of Cl and direct closing of the valve, and give this type of marine bivalves the ability to defend against chlorine and chlorine co-product (Stenton & Brown, 1994). Effects of chlorine acute exposure (short-term exposure at high concentration) on survival and growth of marine invertebrates was investigated by Waugh (1964). The author tested crustacean juvenile of the *Elminius modestus* to Cl treatments between 0.5 to 5.0 mg/l for less than 15 min and observed improvement and obvious progress to survival. Nevertheless, mortality of *E. modestus* showed after exposed to treatment with chlorine concentration more than 0.5 mg/l (Waugh, 1964). Pesticide used in agriculture field to

destroyed undesirable organism. It can be migrates from terrestrial to seawater and result in marine pollution (Solan & Whiteley, 2016). The accumulations of pesticide in various types of oysters done by (Mora et al., 2005), and (Table1) showed pearl oysters from Abu Dhabi are the best bio-indicators of marine pesticide pollution.

Figure 1:Organochloride Conc. Of oysters in different places of GCC country (Mora et al., 2005).

Table 6
Chlorinated hydrocarbon concentrations (ng $g^{-1}$ dry weight) in bivalve molluscs

| Compound | UAE | | | | | | Qatar | Bahrain | | Oman | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Jebel Ali | | Abu Dhabi | | Akkah Head | | Ras Al Nouf | BAPCO | Meridien Hotel | Al Sawadi | Ras Al Hamra | Ras Al Yei | Hilf | Mirbat |
| | Pearl oysters | Pen Shells | Rock scallops | Pearl oysters | Pearl oysters | Rock oysters | Clams | Pearl oysters | Pearl oysters | Rock oysters | Rock oysters | Rock oysters | Rock oysters | Rock oysters |
| Dry/wet weight ratio | 0.162 | 0.263 | 0.169 | 0.155 | 0.203 | 0.273 | 0.129 | 0.13 | 0.18 | 0.23 | 0.22 | 0.23 | 0.24 | 0.22 |
| HEOM (mg $g^{-1}$)[a] | 35 | 21 | 39 | 54 | 39 | 97 | 27 | 32 | 37 | 92 | 52 | 87 | 78 | 93 |
| HCB | 0.025 | 0.016 | 0.073 | 0.29 | 0.013 | 0.024 | 0.057 | 0.019 | 0.024 | 0.028 | 0.018 | 0.043 | 0.017 | 0.034 |
| α-HCH | 0.014 | 0.027 | <0.005 | 0.083 | <0.005 | 0.028 | 0.17 | 0.012 | 0.010 | 0.027 | 0.006 | 0.004 | 0.004 | 0.008 |
| β-HCH | 0.050 | 0.18 | 0.027 | 0.085 | 0.021 | 0.24 | 0.23 | 0.035 | 0.029 | 0.11 | 0.12 | 0.14 | 0.038 | 0.16 |
| Lindane | 0.021 | 0.026 | 0.006 | 0.026 | <0.004 | <0.004 | 0.15 | 0.083 | 0.054 | 0.013 | 0.014 | 0.011 | 0.023 | 0.016 |
| ∑HCHs | 0.085 | 0.233 | 0.033 | 0.194 | 0.021 | 0.268 | 0.55 | 0.13 | 0.093 | 0.15 | 0.14 | 0.155 | 0.065 | 0.184 |
| *p,p′*-DDT | 0.11 | 0.026 | 0.22 | 0.54 | 0.028 | 0.18 | 0.14 | 0.350 | 0.260 | 2.00 | 0.25 | 0.34 | 1.90 | 0.69 |
| ∑DDTs | 0.137 | 0.106 | 1.077 | 5.881 | 0.133 | 1.815 | 0.154 | 1.495 | 0.708 | 3.383 | 0.897 | 1.74 | 4.644 | 1.566 |
| *cis*-Chlordane | <0.006 | 0.27 | 0.17 | 0.19 | 0.025 | 0.47 | n.a. | 0.092 | 0.150 | <0.006 | 0.016 | <0.006 | <0.006 | <0.006 |
| *trans*-Chlordane | <0.006 | 0.011 | 0.011 | 0.078 | 0.021 | 0.059 | <0.006 | <0.009 | 0.049 | 0.095 | 0.012 | 0.16 | 0.076 | 0.54 |
| *trans*-Nonachlor | <0.004 | <0.004 | <0.004 | <0.004 | <0.004 | 0.015 | <0.004 | 0.009 | 0.044 | 0.020 | 0.15 | 0.13 | 0.010 | 0.21 |
| Heptachlor | <0.003 | <0.003 | 0.004 | 0.060 | <0.003 | <0.003 | <0.003 | <0.004 | <0.004 | <0.003 | <0.003 | <0.003 | <0.003 | <0.003 |
| Aldrin | <0.003 | 0.014 | 0.022 | 0.11 | 0.012 | 0.020 | <0.003 | <0.003 | <0.003 | 0.031 | 0.063 | 0.071 | 0.12 | 0.072 |
| Dieldrin | 0.016 | 0.60 | 0.22 | 1.2 | 0.055 | 0.23 | 0.006 | 0.100 | 0.310 | 0.53 | 0.79 | 0.32 | 0.15 | 1.00 |
| Endrin | <0.013 | <0.013 | 0.043 | 0.080 | 0.18 | 0.38 | <0.013 | <0.014 | <0.014 | <0.009 | 0.12 | 0.30 | 0.27 | 0.48 |
| α-Endosulfan | <0.004 | <0.004 | <0.004 | <0.004 | <0.004 | <0.004 | <0.004 | <0.002 | <0.002 | 0.075 | 0.081 | <0.001 | n.a. | 0.076 |
| β-Endosulfan | <0.005 | 0.039 | <0.005 | 0.094 | 0.027 | <0.005 | <0.005 | 0.025 | 0.014 | 0.058 | 0.040 | 0.009 | 0.030 | 0.040 |
| Endosulfan sulfate | <0.006 | 0.016 | <0.006 | <0.006 | <0.006 | <0.006 | <0.006 | 0.041 | 0.029 | <0.002 | 0.032 | 0.034 | n.a. | 0.012 |
| Aroclor 1254 | 0.21 | 0.25 | 1.2 | 3.7 | <0.078 | 1.1 | 1.3 | 3.000 | 1.600 | 1.90 | 1.60 | 1.20 | 2.70 | 1.30 |
| Aroclor 1260 | 0.13 | <0.062 | 0.34 | 3.0 | <0.062 | 0.30 | 0.11 | 2.400 | 0.590 | 0.77 | 1.30 | 0.34 | 4.40 | 1.00 |
| ∑PCBs | 0.203 | 0.303 | 1.14 | 3.59 | 0.131 | 0.515 | 0.245 | 5.79 | 2.80 | 2.36 | 3.22 | 1.19 | 4.77 | 1.98 |

n.a. = Not analysed.
[a] Hexane extractable organic matter.

S. de Mora et al. / Marine Pollution Bulletin 50 (2005) 835–849

## 1.6 Previous studies on effects of chlorine on oyster

Diverse researches have been conducted to investigate effects of acute or chronic exposure to Cl on mollusks.

When seawater continues receive the liberations from chlorine and chlorine co-product with residual levels between 0.01 - 0.05 mg/liter, the growth and physiological performance of adult *Crassostrea virginica* were inhibited (Richardson, Burton & Stavola, 1982).

Bongers et al. (1977) studied survival rate among bivalves exposed for 2 weeks to various

chlorine concentrations ranging between 0.035 to 0.085 mg/liter and there were no mortality of *C. virginica*.

Scott & Middaugh (1978) studied the outcome of chronic exposure to Cl on bivalve *C. virginica* and demonstrated substantial mortality for concentrations between 3.20 to 5.60 mg/liter. Results showed strong association of Cl toxicity with variations in temperature, suggesting a seasonal variation of the effects of Cl exposure on bivalves. Such findings were also corroborated by Scott &Vernberg (1979).

The results obtained by Leonard et al. (1981) who studied the effect of chlorine toxicity on *Crassostrea virginica*, were consistent with results from Waugh (1964) who concluded that *Ostrea edulis* exposed to high concentrations of chlorine for periods from 3 to 20min, had non-significant result in mortality on the bivalve larvae at concentration equal to 10 mg/liter.

Previous studies done by Rhoderick et al. (1977) and Liden et al. (1980) concluded that adult bivalves (more than 1 year old) are able to live between 7 to 14 days under high concentration of chlorine-produced oxidant. The observed high mortality rate at the beginning of the experiment was related to the stress associated with the transfer of the bivalve *Pinctada radiata* from the natural environment to the experimental aquarium. Scott et al. (1980) conclude that, in summer the chlorination products can create high amount of poisonous outcomes. This cause biological shocked for the mature bivalve *Crassostrea virginica*, then inhibit valve grow up and close.

### 1.7 The Arabian Gulf and anthropogenic stressors

Contrasting with its cultural, social, identity and commercial values in the neighboring countries, the Arabian Gulf is considered as a naturally extreme environment, exposed to

high variations in temperature, salinity, and UV light (Al-Saleh et al., 1999; Price et al., 1993). Seawater in the Arabian Gulf (also referred to as Persian Gulf) is regard as tropical and warm, where sea surface temperature approaches 35$^{o}$C in summer. Naturally, tropical water has a great number of organisms including microorganisms, plants or animals (e.g. encrusting or sessile macroinvertebrates) that has the ability to attach to hard substrate immersed in the water, known as biofouling. This process is of major concern for marine vessels or coastal industries using seawater for engine cooling, condensers or valves. Indeed, the attachment of these organisms leads to a severe problem in the process of seawater intake in desalination process; in addition to the oil industry that uses great amount of seawater. Biofouling is the phenomenon of growth and attachment of marine organisms on the surfaces of engineered structures that are submerged in the marine environment. This phenomenon occurs naturally and leads to severe problems and risks for coastal structures constantly immersed (Rostron & Rehana, 2015). As a result of the local and regional increase in the industrial development and the pressure of human activities on the marine environment, Arabian Gulf countries are being substantially affected. To address such biological fouling of industrial structures, anti-biofouling solutions have been used, including addition of chemical biocide into used seawater to prevent the formation of biological growth.

Therefore, a large percentage of contaminants is introduced into the marine environment composed of potentially genotoxic elements (Abdel-Wahab et al., 2009). Industrial cooling is one of the technologies considered as a critical process due to the outcomes that resulted in severe environmental impacts on the quality of water and indirectly the population health in the Arabian Gulf, including in Qatar (Adenekan et al ., 2009). The environmental

impacts on the marine environment are a consequence of using chemical materials. These materials are used for the purpose of controlling the presence of attached organisms on the surfaces; however, this leads to produce large amount of pollutants categorized into toxic and/or carcinogenic (Abdel-Wahab et al., 2009).

Furthermore, the presence of the chemical pollutants leads to subsequent impacts on the receiving marine ecosystem and usually the associated food web. Moreover, a serious risk could be observed to human health as seawater is heavily used locally as the source of drinking water in the Arabian Gulf, through desalination (Abdel-Wahab et al., 2009). The Arabian Gulf region is indeed strongly reliant on seawater characteristics as a source of freshwater throughout desalination (Price et al., 1993). The Arabian Gulf is a semi-enclosed sea with an extreme evaporation rate and low freshwater discharge rate from land leading to a poor dilution and slower dispersion rate of pollutants (Sheppard et al., 2010). Beside these environmental limitations on water circulation and self-purification in the Arabian Gulf, the development of the industrial sector and the increase in the population increased the stress related to environmental risk (pollution). Consequently, the pollution risk developed regionally is exacerbated and considered more severe compared to open marine systems (Sheppard et al., 2010)

Recently, the marine environment in Qatar experienced an increased level of pressures, including inputs of urban and industrial wastewaters, and the resuspension of sediment as a result of coastal dredging (Sheppard et al., 2010). Portion of contaminants occurring in the Qatari marine environment consists of genotoxic, carcinogenic, and mutagenic materials. In which the genotoxic substances are able to alter the genetic makeup at non-lethal and non-cytotoxic concentrations. Ultimately, the impact of toxic compounds can be

noticed at the subcellular level before being noticeable at higher levels of biological organization (Zuykov et al., 2013).

### 1.8 Environmental impacts of industrial activities in Qatar

Because of the growing industrial activities in the coastal zone around the Arabian Gulf, industries are using chlorinated products into cooling seawaters to avoid biofouling in hard structures. The produced chlorinated seawater is then discharged into the marine environment, usually coupled to increased temperature, and generate other chlorinated by-products (CBPs) when chlorine interacts with organic matter, naturally occurring in the marine environment (Rostron and Rehana, 2015). New regulations have been established in Qatar to control the release of chlorine (Cl) used as anti-biofouling, namely in waters for cooling system. The maximum concentration of released chlorine in the water used for cooling system should not be higher than 0.05 mg/L. (Abdel-Wahab et al., 2009).

The main environmental concern with this operation is the detrimental impacts of the released chlorinated by-products in the receiving marine environment (Mora et al., 2005). The effect of chlorinated residual seawater on Qatari marine ecosystem has been poorly investigated, contrasting with a fair amount of evidences, conducted globally, on the negative effects of such pollution on the marine environment. A previous study conducted by Sheridan (1981) revealed a decrease in survival and growth rates of the Atlantic oyster *Crassostrea virginica* when subjected to chronic exposure of chlorinated seawater. Another recent study demonstrated that the oyster *Ostrea edulis* could survive in seawater with chlorine concentration below 20ppm, while the barnacles *Elminius modestus* showed to be more sensitive and massive mortalities were recorded at the same level of chlorine (Duncan, 2008). Because of the continuous industrial use of chlorine, as an oxidizing agent,

in Qatar, it is crucial to investigate the effects of this pollutant and its by-products on the marine environment in order to determine the magnitude of such pollution and the related environmental impacts.

### 1.9 Chlorine and Chlorinated by products in Qatar marine environment

In Qatar, massive volumes of seawater are daily used for cooling purposes then discard back into the Arabian Gulf and these outcomes should be controlled by adding chlorine for bio-fouling (Abdel-Wahab et al., 2009). Consequently, chlorine based biocide substances is used in order to prevent biofouling and it is considered as the most commonly technique in reducing biofouling. The solicitation of $O_3$ , UV radioactivity, surface active agents , automatic washing (Langford, 1977), radiant concentration (Yang et al., 2000), or monitoring potential hydrogen pH (Yukselen et al., 2003) or heat for cooling system could be used to reduce biofouling too. Biofouling is regarded as the main operational limiting factor of water marine cooling system. It is formed by the growing of microorganisms on the cooling hard structures, creating the so-called biofilm; on the top of which other living organism will attach or recruit subsequently. Biofilms are commonly found on the surfaces of industrial heat exchange, decreasing the efficiency of the process (Goodman, 1987). When Biocide added into the seawater, oxidation reaction happened between Br and other complex (Abdel-Wahab et al., 2009).

The most commonly used process in minimizing the biofouling is the use of biocides (primarily chlorine) (Bott, 2011). Despite the high performance of this solution in prevent biofouling, it is hard to control the reactions that happens when release chlorine in the seawater. Studies have been conducted in order to understand the impact of high CBPs concentration on marine life.

*P. radiata* is a strong fouling organism, able to seriously decrease the effectiveness of seawater cooling methods (Macdonald et al., 2009). QatarGas has examined the fouling susceptibility by using *P. radiata* as the target organism. Another study conducted on the coastal waters of Ras Laffan Industrial City (RLIC) in Qatar revealed that beyond 2 kilometers from the discharge, concentrations of CBPs, associated with cooling water discharges, were lower than the analytical detection limits (Adenekan et al., 2009). In addition, Liquefied Natural Gas (LNG) production at RLIC involves the use of important amounts of marine water in the cooling systems to prevent accumulation of organism on the system. The seawater used in the cooling systems is mixed with NaClO to reach up to 1.5 ppm of $Cl_2$. The study concluded that CBPs especially bromoform ($CHBr_3$) was the highest in concentration in the collected seawater samples (Adenekan et al ., 2009). After discharge of that remaining oxidizing agent from chlorination activities, they can interact with organic elements then form poisonous and cancer causing agent that affect marine environment and also people who desalinate seawater for drinking purpose, especially in Gulf region (Abdel-Wahab et al., 2009).

### 1.10 Hypotheses

We hypothesize that exposed Qatari local oysters *Pinctada radiata* to different level of chlorine (0.025-0.1 mg\liter) will show:

- Higher mortality at high concentration 0.1 mg\l
- Decrease in weight, length, width or height.
- Disturbance (reduction) in their respiration rate at exposure with 0.1 mg\l

## 1.11 Objectives

The general aim of this study is to investigate the effect of industrial chlorine on marine life. The local pearl oyster, *Pinctada radiata*, is used in an experimental approach as biological model exposed to different concentrations of chlorine and the impacts on the biology and physiology of the oyster are identified. The specific objectives of the study are:

1- To investigate the impact of chlorinated seawater on the survival and growth of the pearl oyster

2- To investigate the impact of chlorinated seawater on the physiological performance of the pearl oyster.

# CHAPTER 2: MATERIALS AND METHODS

## 2.1 Sampling

Specimens of the local pearl oyster *Pinctada radiata* were collected from Al-Wakrah (N25.14507, E51.62258) on January 2nd 2017, in order to evaluate the potential influence of chlorine exposure on the biology and physiology of this indicator species. 350 oyster individuals were then transferred to ExxonMobil Research Qatar (EMRQ) for an initial acclimation period prior to initiate the experiment. Collected oysters were measured and weighted after reception at EMRQ (Figure 1) and only adults (larger than 38mm) were retained for the experiment. The morphometric ranges of considered individuals are reported in (Table 2) and detailed individual measurements reported in (Appendix B).

Table 1: Morphometric of the collected oysters "weight- length-height and width (For individual details, see Appendix B)

| Weight (gm) | Length(mm) | Height(mm) | Width(mm) |
|---|---|---|---|
| 7.969-35.922 | 38.89-62.27 | 37.44-58.04 | 13.02-24.35 |

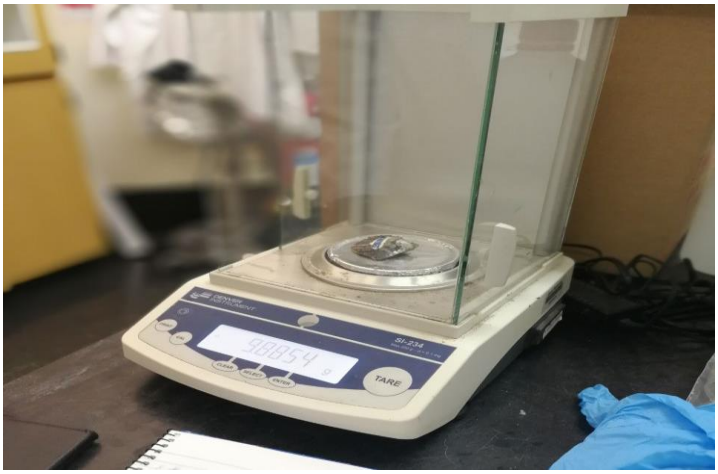


Figure 2: weighting of the oysters at laboratory after collection from the sampling site

## 2.2 Experimental design

Considered specimens of *Pinctada radiata* for this experiment were exposed to chlorine and chlorination by-products at EMRQ's laboratory. Oysters were subjected to one of the 3 experimental settings (with 2 replicate each): a control artificial seawater (with no addition of chlorine), 0.025 mg\liter and 0.1 mg\liter of chlorine for an exposure period of 10-11 weeks (Table 3). According to the by-laws issued by the "Supreme Council for the Environment and Natural Reserves No. (4) for the year 2005 depicting the executive regulations for the law of environmental protection issued by Legislative Decree No. 30 of 2002" in Qatar; the legal remaining Cl concentration in the receiving marine environment should be less than 0.05mg\liter , and in our experiment we considered two concentrations at higher (0.1 mg/l) and lower (0.025 mg/l) levels.

The artificial seawater was prepared by adding 400 gm of reef commercial salt dissolve it in 1000 liter of deionized water.

Table 2: Experimental design of the three aquarium with the different concentrations of Cl including replication n=2 of each tanks

| | | |
|---|---|---|
| Control A | 0.025 mg of chlorine A | 0.1 mg of chlorine A |
| 0.1 mg of chlorine B | Control B | 0.025 mg of chlorine A |

The chlorine were prepared for the experiment as followed:

1. 2.5 g of Sodium hypochloride was weighed and dissolved in 1 liter of ultrapure Milli-Q water. The solution was kept in flask on a magnetic stirrer and allowed to stir for 6 hours.
2. The solution was kept overnight undisturbed to allow any undissolved particles to settle down.
3. Chlorine in this solution was measured using a DR 2800 spectrophotometer.
4. Based on the above measurement, enough solution was taken and mixed thoroughly in 2.5 liters of Milli-Q water in an amber bottle to get two 2.5 liter bottles of about 110ppm and 2 bottles of 30 ppm of chlorine solution.
5. Two bottles with 110 ppm were used to give a dose of 0.1 mg/l of chlorine per spiking.
6. Two bottles with 30 ppm were used to give a dose of 0.025 mg/ l of chlorine per spiking.
7. Chlorine in the oyster tanks were measured at regular intervals by collecting water sample immediately after a spiking.

The dimensions of the water tanks were 24 (height) cm X 80 (length) cm X 40 (width) cm. 60 liters of seawater were added to each tank “aquarium”. In each tank, approximately 59 oysters were added randomly (Figure 2).

Figure 3: Random distribution of selected oysters in the aquaria (different treatments)

The collected oysters were labeled individually and their morphometrics (i.e. weight, height, length and width) measured and recorded. Oysters were then assigned randomly to one of the 6 aquaria, comprising 3 treatments and 2 replicate aquarium each) (see also Appendix C)

Oysters were fed daily, outside the tanks in a separate container (to maintain the water quality inside the tanks, limiting organic inputs), with a mixtures of algae and rice powder.

### 2.3 Monitoring of water quality

All aquaria were kept at room temperature (23 ±2°C) throughout the duration of the experiment. The seawaters of the 6 aquaria were monitored and kept in the same conditions of salinity 40 ±2 psu, using a refractometer (Figure 3) and pH 7.0 ±0.2 , using HACH pH meter ( Figure 4 ).

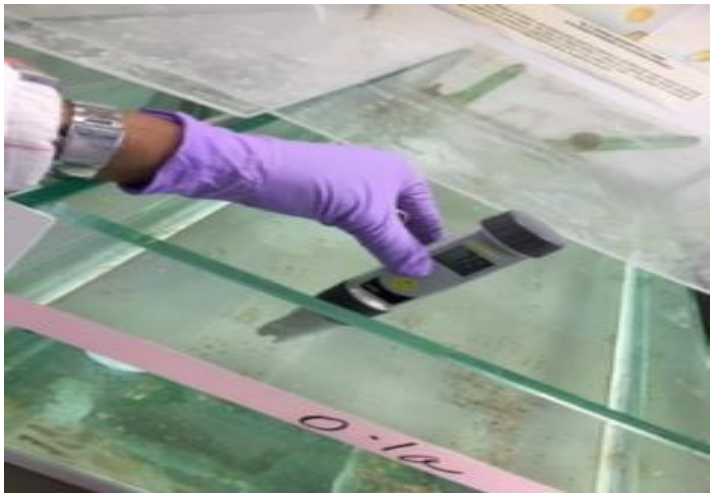

Figure 4: Refractometer for salinity measurement

Figure 5: Hydrogen parameter for measurement acidity or alkalinity of water

Separated water filters were added to each tank to keep a convenient water quality and prevent accumulation of any undesired organic particulate matter. These filters were cleaned weekly.

To keep a suitable water quality into the tanks, water flow and circulation were controlled. The water inside the aquaria was analyzed twice a week and renewed depending on the concentration of nitrates and nitrites (between 20%-50% renewal). Other water quality parameters (salinity, pH, Nitrate ($NO_3$), Nitrite ($NO_2^-$), Ammonium (NH4), Phosphate ($PO_4$), Calcium (Ca) and Magnesium (Mg)) were monitored and maintained at acceptable levels through water exchange (Table 4)

Table 3: Water quality parameters

| Parameters | Range | Frequency | Method use |
|---|---|---|---|
| Temperature | 23 +/- 2°C | Daily | digi-thermometer |
| Salinity | 40 +/- 2 | Daily | Refractometer |
| PH | 7.0+/- 0.2 | Daily | HACH pH meter |
| $NO_3^-$ | <0.5 mg\L | Weekly | JBL©waterkit |
| $NO_2^-$ | 0.1-0.2 mg\L | Weekly | JBL©waterkit |
| NH4 | 0.1-0.4 mg\L | Weekly | JBL©waterkit |
| $PO_4$ | 0.02-0.05 mg\L | Weekly | JBL©waterkit |
| Cl | 0.025 mg\L<br>0.1 mg\L | Daily | DR 2800 Spectrophotometer |

Chlorine concentrations were measured daily, using DR 2800 Spectrophotometer, in order to compensate the losses of Cl through evaporation. A chlorine concentration (0.1mg\L-0.025mg\L) was maintained in the 4 treatment tanks, by adding Cl high concentrated solutions (Figure 5).

Figure 6: Preparation of different Cl concentrations

### 2.4 Monitoring of oysters performance

Oysters were daily observed, the dead ones were removed immediately to maintain the aquaria water quality. The labels of the dead oysters were recorded and measured their morphometrics (weight- length- width- height). The survival curve (Kaplan Meier) was recorded for each treatment and compared using a log-rank test.

### 2.5 Assessment of oysters' respiration rate

18 oysters were used (3 per tanks were chosen randomly). First, 3 oysters were placed into cleaned glass jars covered with aluminum, filled with water from the original tank, sealed and then incubated in dark. A blank control with pre-filtered (0.22 µm) seawater from the same tank was also incubated in the same conditions. Oxygen concentrations were then measured at 4 different times (T0, T1, T2, T3) using a portable optical oxygen analyzer (101, OxySense[1]). Prior to the oxygen measurements the jars were gently shaken to ensure

[1] http://www.oxysense.com/

moderate mixing of water and prevent stratification. Oxygen saturation levels were measured until $O_2$ saturation reaches 70% of the initial value or after 5 consecutive measurements (Figure 6) (see also Appendix C for details).

The respiration rate ($VO_2$) was then calculated for each time interval, by applying the following equation:

$$VO_2 = [V/(W \times t)] \times [(Cie - Cfe) - (Cic - Cfc)]$$

Where:

*V* = volume of the respirometer or jar (*l*);

*W* = weight of the oyster in the respirometer (gr),

*t* = measured period (H);

*Cie* and *Cfe* = initial and final oxygen concentrations in the experimental respirometer;

*Cic* and *Cfc* = initial and final average of oxygen concentration in the corresponding control respirometers.


Figure 7: Arrangement of *Pinctada radiata* into individual jars (top pictures) for measuring oxygen concentrations using the OxySense platform (bottom pictures)

# CHAPTER 3: RESULTS

## 3.1 Water quality

Water quality parameters (Nitrate $NO_3^-$, Nitrite $NO_2^-$ , Ammonium $NH_4^+$ and Phosphate $PO_4^{3-}$**)** in all treatments/tanks were measured throughout the experiment (Table 5)**.** There were no significant differences on these water parameter between the sixth aquariums (Table 5). This was achieved by the use of the closed and controlled water system in the experiment. Nitrate concentrations become high between 30-160 mg\l in the time period between 2nd and 3rd sampling times and following the rule ("If the concentration more than >>1") we have exchanged 2.5 liters from the tanks by newly generated artificial seawater.

Table 4: Monitored concentration of nutrients in the aquariums throughout the experiment

| Date | Tank | NH4 mg\L | NO3 mg\L | PO4 mg\L | NO2 mg\L |
|---|---|---|---|---|---|
| 9.1.2017 | control A | 0.1 | 0.5-1 | 0.02-0.05 | 0.1 |
| 9.1.2017 | 0.025 A | 0.1 | 0.5-1 | 0.02-0.05 | 0.1 |
| 9.1.2017 | 0.1A | 0.2 | 0.5-1 | 0.02-0.05 | 0.1 |
| 9.1.2017 | control B | 0.2 | 0.5-1 | 0.02-0.05 | 0.1-0.2 |
| 9.1.2017 | 0.025 B | 0.2 | 0.5-1 | 0.02-0.05 | 0.2 |
| 9.1.2017 | 0.1B | 0.2-0.4 | 0.5-1 | 0.02-0.05 | 0.1 |
| 29.1.2017 | control A | <0.05 | 80 | 0.8-1.2 | >>1 |

| Date | Tank | NH4 mg\L | NO3 mg\L | PO4 mg\L | NO2 mg\L |
|---|---|---|---|---|---|
| 29.1.2017 | 0.025 A | 1 | 80 | 0.4 | >>1 |
| 29.1.2017 | control B | <0.05 | 80-160 | 0.8-1.2 | >>1 |
| 29.1.2017 | 0.025 B | <0.05 | 80-160 | 0.4 | >>1 |
| 29.1.2017 | 0.1B | 0.05-0.1 | 40-80 | 0.8 | >>1 |
| 26.2.2017 | control A | <0.05 | 30 | 1.2 | 0.05 |
| 26.2.2017 | 0.025 A | <0.05 | 30 | 1.2 | >>1 |
| 26.2.2017 | 0.1A | <0.05 | 50 | 1.2 | >>1 |
| 26.2.2017 | control B | <0.05 | 30 | 1.2 | 0.05 |
| 26.2.2017 | 0.025 B | 0.1 | 50 | 1.2 | >>1 |
| 26.2.2017 | 0.1B | <0.05 | 50 | 1.2 | >>1 |

### 3.2 Survival

The mortality was high in the following days after distributing the oysters into the different tanks, at different concentration of chlorine. We considered this period (first 4 days) as an acclimation period and we initiated the calculation of the mortality rate in the different treatments starting from Day-5 of the experiment. The survival curves, or Kaplan Meier curves, were generated (Figure 7) and a statistical Log-rank cross comparison test performed (Table 6)

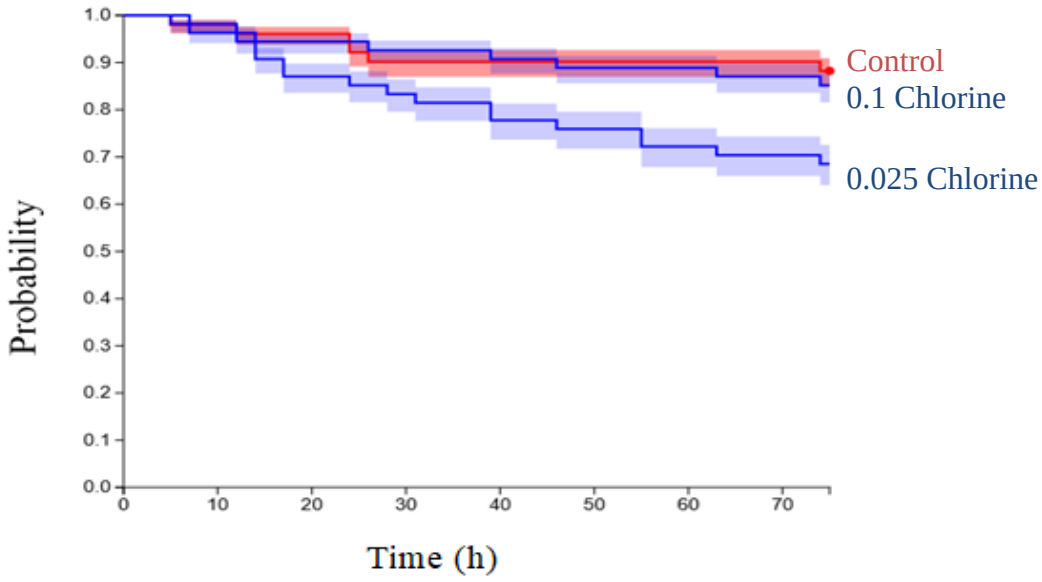


Figure 8: Survival curves (Kaplan Meier) for the three treatments and after an acclimation period of 4 days.

Log rank test showed a significant higher hazard rate of the treatment (Chlorine 0.025) compared with the Control and Chlorine 0.1 mg/l. In the other hand, there are no significant difference in the survival between Control and Cl with 0.1 mg/l concentration (Table 6) (For individual details, see Appendix A)

After completion of the experiment (75 days), final mortality rate was significantly higher for the treatment Chlorine 0.025 (31.48%) compared with Chlorine 0.1 (14.81%) and the lowest for the Control treatment (11.76%). The latter two were not significantly different as suggested by the Log-rank test.

Table 5: Results of the Log-rank cross-comparison test between Kaplan Meier Survival curves of the three treatments

| Cross Comparison between treatments | Log rank test |
|---|---|
| Control vs 0.025 | The survival rates and curves differ ($z = 2.39$, $p = 0.0169$) |
| Control vs 0.1 | No significant difference between survival ($z = 0.43$, $p = 0.67$) |
| 0.025 vs 0.1 | The survival rates and curves differ ($z = 2.06$, $p = 0.0396$) |

**The relationship between size of oysters and mortality**

Considering that the differences in the mortality due to concentrations of chlorine was not significant, we investigated the possibility that the differences are due to the random sampling variability after allowing for the effects of differences in oysters' Size Class. The difference in the average mortality between the different Size Classes is greater than would be expected by chance, after allowing for effects of differences in Treatment (For individual details, see Appendix A)

Table 6: Results of ANOVA to investigate effects on mortality by Treatment and Size Class of oysters. Significant differences are reported in bold (P)

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 2 | 0.184 | 0.0919 | 2.061 | 0.162 |
| Size Class | 4 | 1.165 | 0.291 | 6.532 | **0.003** |
| Treatment x Size Class | 8 | 0.863 | 0.108 | 2.420 | 0.067 |
| Residual | 15 | 0.669 | 0.0446 | | |
| Total | 29 | 2.880 | 0.0993 | | |

Despite, the highest mortality of oysters was in tanks with 0.025 of Cl concentration. It appears that applied increasing chlorine concentrations had no effect on the mortality of oysters (For individual details, see Appendix A), mortality was, nevertheless, significantly increased with length (Figure 8). Highest final mortality rates were observed in bigger sized oysters regardless of the treatment. So there is significant effect of size classes on mortality, while no significant differences were observed between the different treatments of Chlorine for the same response parameter (i.e. mortality).

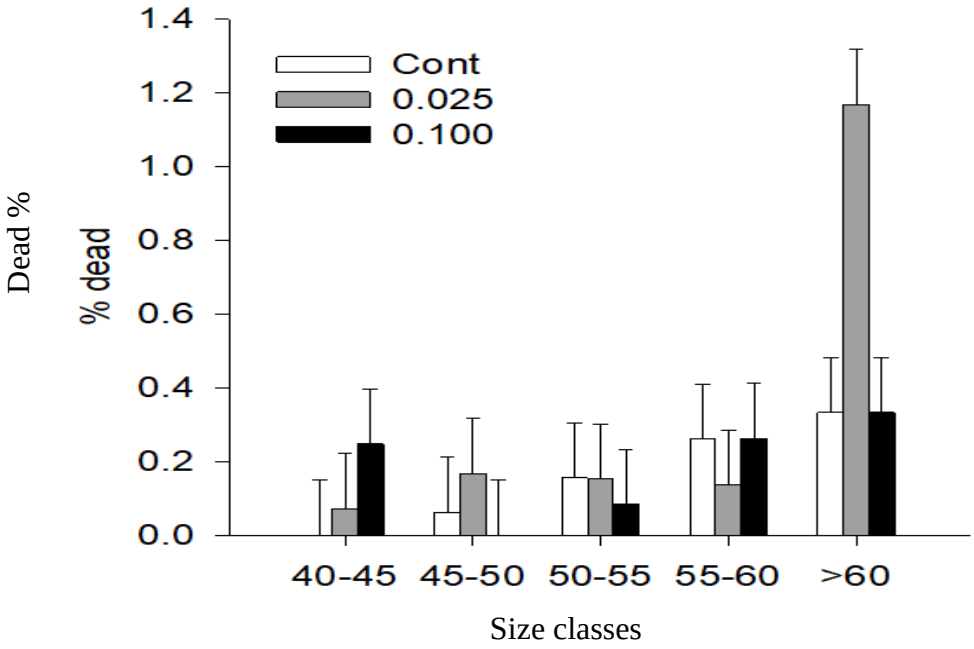


Figure 9: Final Mortality of oysters, at different size classes, after being exposed to different concentration of Cl.

### 3.3 Growth and morphometric variability

**The effect of treatment and size classes on height's increase of oysters**

Considering that the differences in the mortality due to concentrations of chlorine was not significant, we also investigated the possibility that the differences in height are due to the random sampling variability after allowing for the effects of differences in oysters' size Class. Indeed, the difference in the mean values of heights among the different levels of size Classes is greater than would be expected by chance after allowing effects of differences treatment (p=0.014) (For individual details, see Appendix B)

Table 7: Results of ANOVA to investigate effects of Treatment and Size Class variability on oysters' height. Significant differences are reported in bold (P)

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 2 | 0.0202 | 0.0101 | 2.600 | 0.107 |
| Size Class | 4 | 0.0696 | 0.0174 | 4.488 | **0.014** |
| Treatment x Size Class | 8 | 0.0415 | 0.00519 | 1.339 | 0.298 |
| Residual | 15 | 0.0582 | 0.00388 | | |
| Total | 29 | 0.189 | 0.00653 | | |

Generally oyster's increments in height significantly increased while size classes decreased, except for bigger oysters (larger than 60mm) exposed to 0.1 of Cl concentration (Figure 9). At the end, we conclude that there is no significant difference between increments of height of oysters exposed to different Cl concentrations.

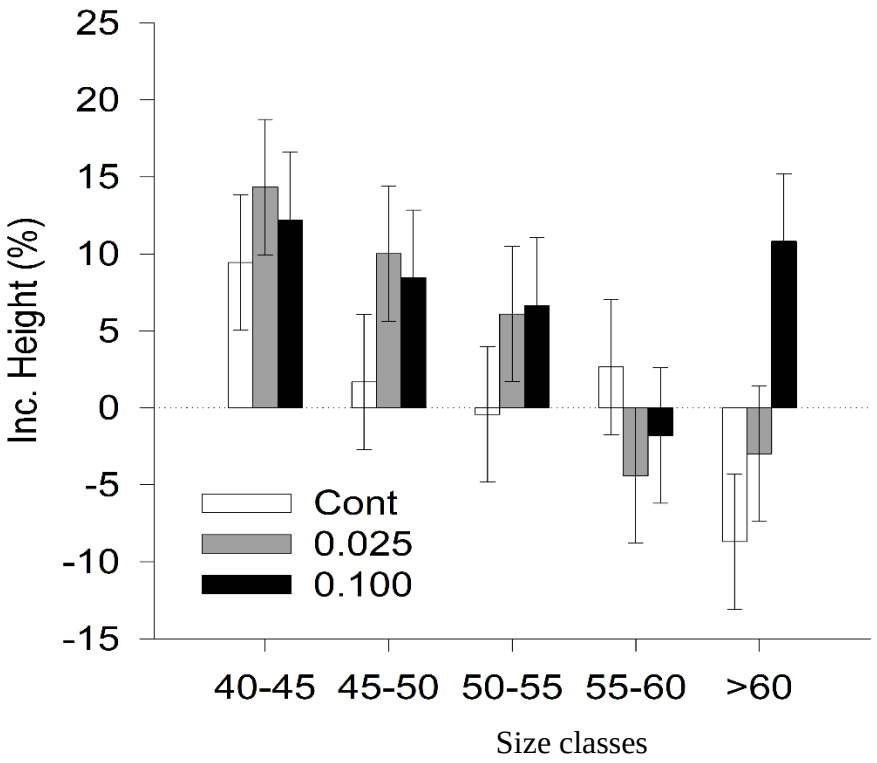


Figure 10: Increment in height of oysters, distributed among different size classes, exposed to different Cl concentrations.

**The effect of treatment and size classes on length's increments of oysters**

Considering that the differences in the length due to concentrations of chlorine was not significant, we investigated the possibility that the differences are due to the random sampling variability after allowing for the effects of differences in oysters' size classes. The difference in the mean values of increment of oyster's lengths among the different Size classes is greater than would be expected by chance after allowing for effects of differences in Treatment ($p<0.001$) (For individual details, see Appendix A).

Table 8: Results of ANOVA to investigate effects of Treatment and Size Class variability on oysters' length. Significant differences are reported in bold (P)

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 2 | 0.000292 | 0.000146 | 0.273 | 0.765 |
| Size Class | 4 | 0.0188 | 0.00469 | 8.780 | <**0.001** |
| Treatment x Size Class | 8 | 0.00604 | 0.000755 | 1.412 | 0.269 |
| Residual | 15 | 0.00802 | 0.000534 | | |
| Total | 29 | 0.0331 | 0.00114 | | |

There was a decreasing trend in oyster's length increment for all tanks when Size classes were increasing. Moreover, negative length increments were recorded in all treatments for oysters larger than 55mm (Figure 10). Lastly, we conclude that there is no significant effect of increased Cl concentrations on length increments of exposed oysters.

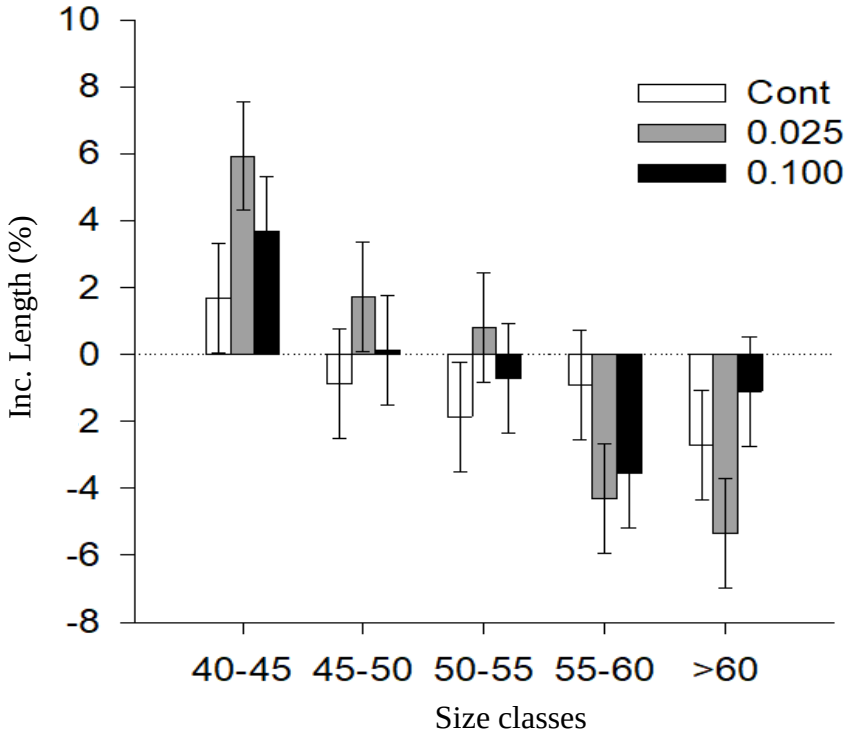


Figure 11: Increment in length of oysters, distributed among different size classes, exposed to different Cl concentrations.

**The effect of treatment and size classes on width's increments of oysters**

Considering that the differences in the width of oysters due to concentrations of chlorine was not significant, we investigated the possibility that these differences are due to the random sampling variability after allowing for the effects of differences in oysters' Size classes. The difference in the mean values of width increments among the different levels of Size classes is greater than would be expected by chance after allowing for effects of differences in Treatment (p=0.019) (For individual details, see Appendix B)

Table 9: Results of ANOVA to investigate effects of Treatment and Size Class variability on oysters' width. Significant differences are reported in bold (P)

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 2 | 0.000156 | 0.0000780 | 0.0836 | 0.920 |
| Size Class | 4 | 0.0155 | 0.00386 | 4.138 | **0.019** |
| Treatment x Size Class | 8 | 0.0152 | 0.00190 | 2.031 | 0.113 |
| Residual | 15 | 0.0140 | 0.000934 | | |
| Total | 29 | 0.0448 | 0.00154 | | |

There was a trend of decreasing width increments of oysters for all tanks when Size classes were increasing. A negative width increment was observed in all tanks for oysters larger than 50mm (Figure 11). Lastly, we conclude that there is no significant effect of increased Cl concentrations on width increments of exposed oysters.

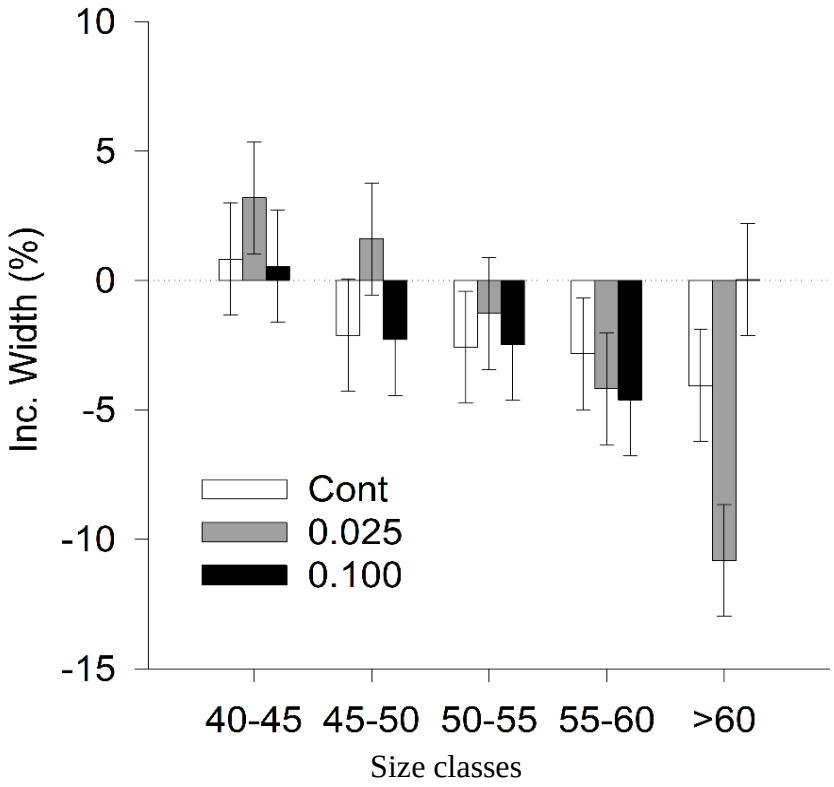


Figure 12: Increment in width of oysters, distributed among different size classes, exposed to different Cl concentrations.

## 3.4 Physiological performance

### Effect of Chlorine on respiration rate

Respiration rates were measured for oysters exposed to the different concentrations of chlorine and during two consecutive incubation periods (within individual respirometers) (Table 11). Respiration rates were consistently higher for oysters exposed to 0.1 mg/l when compared with oysters exposed to 0.025 mg/l chlorine concentration. Oysters maintained in the control tanks were showing intermediate levels of respiration rates. No significant effect of exposure to chlorine on the respiration rates of oysters was then identified. (For

individual details, see Appendix C)

Table 10: Respiration rates of oysters collected from the different Treatments and measured during three consecutive timings in sealed respirometers

| Treatment | Number of respirometers | T1 | | T2 | | T3 | |
|---|---|---|---|---|---|---|---|
| | | Mean | STD | Mean | STD | Mean | STD |
| **Control** | 6 | 0.647 | 0.361 | 0.671 | 0.242 | 0.648 | 0.290 |
| **0.025** | 6 | 0.360 | 0.364 | 0.409 | 0.159 | 0.573 | 0.119 |
| **0.1** | 6 | 0.520 | 0.361 | 0.710 | 0.285 | 0.723 | 0.208 |

The difference in the mean values of oxygen consumption among the different levels of respirometers is greater than would be expected by chance, after allowing for effects of differences in Treatment ($P < 0.001$).

Table 11: Results of ANOVA to investigate effects of different Cl concentration on oxygen consumption. Significant differences are reported in bold (P)

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 2 | 4.676 | 2.338 | 2.342 | 0.138 |
| respirometer | 3 | 124.742 | 41.581 | 41.650 | **<0.001** |
| Treatment x respirometer | 6 | 23.641 | 3.940 | 3.947 | 0.021 |
| Residual | 12 | 11.980 | 0.998 | | |
| Total | 23 | 165.038 | 7.176 | | |

The oxygen concentration showed a consistent decrease from T2 to T3, in all respirometers and for all treatments (Figure 12). This decrease was greatly due to the oysters' oxygen consumption throughout the period of incubation, since the decrease of oxygen concentration in the blank respirometers (with no oysters) was minimal. ). Lastly, we conclude that there is no significant effect of increased Cl concentrations on respiration rate of exposed oysters.

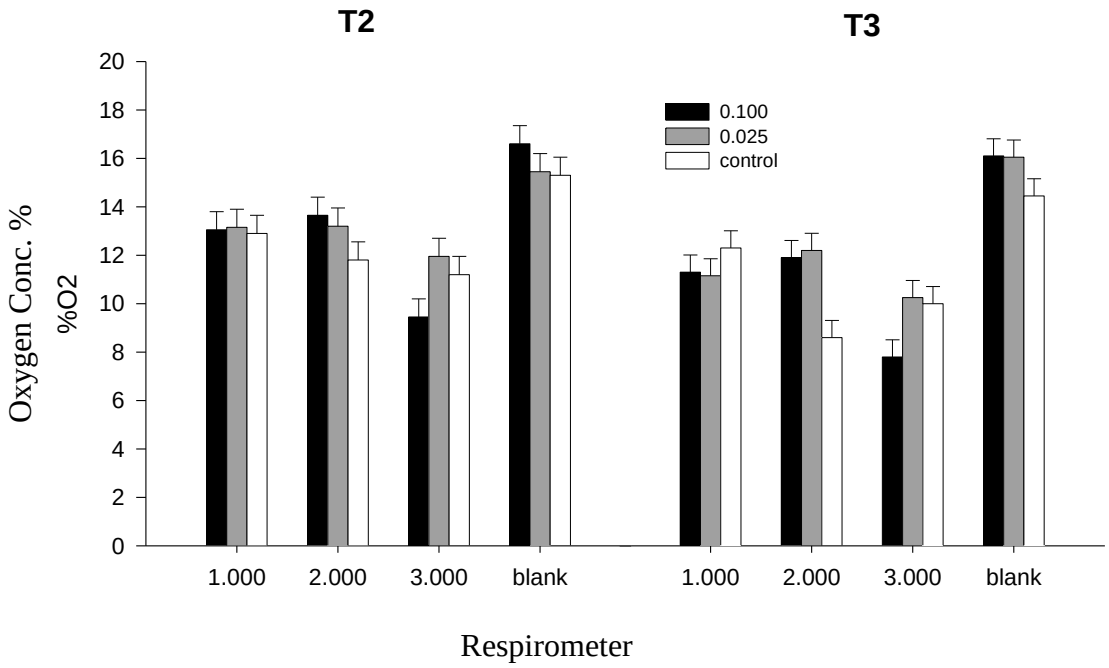


Figure 13: Remaining oxygen concentration in respirometers after consumptions by oysters during two consecutive incubation periods

# CHAPTER 4: DISCUSSION

We carried out a laboratory experiment to identify the effects of exposure to chlorine on biological and physiological performance of the local pearl oyster *Pinctada radiata*. The experiment was run during 75 days at ExxonMobil Research Qatar with adult oysters (originally collected from AlWakra coastal waters) being exposed to two different concentration of chlorine (0.025 and 0.1 mg/l) and a control treatment. The water quality within the experimental tanks (i.e. temperature, pH, salinity, $NO_3^-$, $NO_2^-$, $NH_4$ and $PO_4$) was maintained throughout the experiment at adequate levels. Nitrate value increased significantly at all tanks during the third week of the experiment and a water renew was conducted to recover adequate levels. This increase in $NO_3$ was most probably due to the preceding mortality of oysters, during the first phase of the experiment. Indeed, organic degradation of died oysters until removal from the tanks should have increased the nitrate concentrations in the closed water system. Similarly, both concentrations of ammonium and phosphate increased at the same period, but at lower alarming levels (Table 4).

Our results suggested that exposure to chlorine had non-significant effects on the mortality, growth and physiology (respiration rate) of healthy oysters. This outcome is in agreement with previous studies conducted by Leonard et al. (1981) who found similar non-effect on three life stage of the Atlantic oyster *Crassostrea virginica* exposed to different concentration of chlorine. Similarly, Waugh (1964) concluded that when *Ostrea edulis* were exposed to high concentrations of Chlorine produced oxidant (CPO - 10 mg/l) for 3 to 20 min, no significant effects on juvenile larvae were observed.

The mortality rate of *P. radiata* was the highest at first days of the experiment, interpreted

as acclimation period and accordingly these mortality events were censored from the final results. Analysis of the mortality registered throughout the experiments in all treatments (Kaplan Meier curves) suggested a higher final mortality rate (31.48%) for the oysters exposed to Cl concentration at 0.025 mg/l. This final rate was significantly different when compared with the other two treatments. In contract with this result, final mortality rates of oysters from the control treatment (11.76%) and exposed to 0.1 mg/l of chlorine (14.81%) were not significantly different. Similarly, the mortality along the 75 days of the experiments (Figure7) showed that only mortality of oysters from the treatment (0.025mg/l) was significantly higher than the other two treatments, which were in their turn not significantly different from each other. These findings suggest confidently that applied chlorine concentrations may not be accounted as inducing higher mortality of exposed adult oysters (*P. radiata*). Our findings comparatively contrasted with previous results conducted on the pearl oyster, exposed during 1 day to sodium hypochlorite (NaClO) at a concentration of 0.47 ppm and 1.25 ppm, and where mortality was significantly dependent on increased concentration of NaClO (Göksu et al., 2002). Nevertheless, these concentrations of sodium hypochlorite (a chlorine by-product) are equivalent to outflow concentrations of chlorine above the observed and allowed in Qatar coastal waters, which may explain the differences in our work's findings.

Additionally, we were intrigued by the mortality incidence among larger oysters and we computed an ANOVA analysis that demonstrated that variation in size classes was responsible for significant differences in final mortality rates, with larger oysters more susceptible to mortality than the smaller counterparts, regardless of the applied treatment. Such susceptibility to mortality of larger specimens used in toxicity tests was also

confirmed in previous studies (Rhoderick et al., 1977; Liden et al., 1980) who exposed adult bivalves to chlorine-produced oxidants for period of 7 and 14 days, respectively.

This variability in response parameters due to variation in size classes and not of the chlorine exposure was also found for growth endpoints (length, width and height increments) (Figure 9; Figure 10 and Figure 11).

Indeed, our results suggested that exposure to chlorine, at the used concentrations, was not responsible in the observed variability of oyster's growth (considering all three parameters: length, width and height increments). In the contrary, variation in oysters' size classes was determinant for their growth variation, with growth consistently decreasing when size increased, and this regardless of the applied treatment of chlorine. These findings may be explained by the possibility of chemical receptors located in the bivalve siphons to detect toxicants (e.g. chlorine) and induce closing of the valves, or, when transferred oysters from their natural environment to the artificial one led to a level of stress that prevented them from feeding and then reducing exposure to toxicants. This may also explain the decrease in the morphometrics of the exposed oysters, since oysters were refrained, through this behavioral protective response, from feeding conveniently. Such suggestion was also evocated to explain the non-effect of chemical toxicants on the biology of the clam *Donax serra* (Stenton & Brown, 1994).

Alternatively, we may explain this decrease in growth performance of the maintained oysters when considering the feeding process that was applied throughout this experiment. Indeed, feeding was limited in time (daily feeding in a separate container during 1h/day); probably not allowing convenient intake by oysters of their nutritional requirements to sustain normal growth. This may have affected the physiological performance and growth

of the oysters in all treatments. Such suggestion was also evocated to explain the non-effect of chemical toxicants on the biology of the clam *Donax serra* (Stenton & Brown, 1994).

Such non-conclusive results were also found in our study for the effect of chlorine exposure on the physiology of oysters. Indeed, statistical analysis of generated respiration rates among the different treatments were not significantly different, suggesting that treatments with different concentrations of chlorine were not responsible in modifications on respiration rates of the oysters. These findings contrast with previous studies on larvae of lobsters (*Homarus americanus*) that showed the reduction on metabolic activity including growth and respiratory activities when the larvae were exposed to 1 mg/l of chloramine or free chlorine for just 1 hour (Capuzzo, 1977). Capuzzo et al. (1976) found also similar effect of the same lobster (*H. americanus*) but on juvenile specimens, where lowered level of respiration rates were registered for lobsters exposed to chlorine and $NH_2Cl$ (chloramine). Once again, the different feeding behavior between bivalves and crustaceans (i.e. lobster) and capacity of the bivalves to protect themselves from the surrounding water (by closing of the valves) may explain this observed higher susceptibility of non-shelled organisms to dissolved or particulate chemical pollutants.

These findings obtained in our study, should nevertheless not discard the potential effect of chlorine on other life stages of the oyster *P. radiata* or on other marine biota, since the chemical toxicity of chlorine and chlorinated by-products was already established (Leonard et al., 1981).

# CHAPTER 5: CONCLUSION

Because of the increase in the industrial activities alongside the coasts of the Arabian Gulf region, various industries are using chlorinated products into cooling seawaters to avoid biofouling in hard structures. Coastal power and desalination plants are not an exception and are using massive amount of noxious Chlorine. Chlorination of seawater may additionally results in the formation of toxic nonmetallic elements like fluorine (F), chlorine (Cl) and bromine (Br) beside free mono-chloramine ($NH_2Cl$), chlorinated hydrocarbon mixtures and complexes of Br. The experiment run here, on the local pearl oyster *Pinctada radiata*, exposed to chlorine concentrations (0.025 and 0.1 ppm) for 75 days demonstrated that chlorine released in the natural marine environment in Qatar has no significant effect on the mortality, growth and physiology of this bivalve. Nevertheless, the experiment suggested that the size class of the considered oysters had a significant impact on mortality and growth, with larger specimens showing relatively lower performance than the smaller oysters.

These double evidences may be related to chemoreceptors located at the margins of the mantle of the oysters that may act as detectors of hazardous pollutants in the water and cause the closing of the valves. This will lead to both, decrease in feeding and prevention from toxicity, resulting in a reduced growth and lower susceptibility, respectively. In addition, when transfer the oysters from its natural ecosystem to artificial one, outdoors affect in the laboratory as temperature, light; all lead to stress and decreasing in the physiological performance of oysters.

An alternative explanation was also suggested, highlighting the feeding process applied to

all oysters. Indeed the limited duration for which the oysters were kept in a separate container in presence of feed (microalgae + rice powder) may have limited feed intake and negatively affected the growth of all oysters from the different tanks.

Nonetheless, considering the chemical toxicity of chlorine, further investigation of the eco-toxicity of Cl by using other endpoints (e.g. oxidative stress) or on other biological models (with different feeding behavior) should be conducted.

# REFERENCES


Abdel-Wahab, A., Linke, P., Alfadala, H., El-Halwagi, M., & Batchelor, B. (2009). Towards a Holistic Approach to the Sustainable Use of Seawater for Process Cooling. Proceedings of the 1st Annual Gas Processing Symposium, 332-340. doi:10.1016/b978-0-444-53292-3.50041-9.

Abuzinada, A. H., Barth, H. J., Krupp, F., Böer, B., & Al Abdessalaam, T. Z. (2008) Editors: Protecting the Gulf's Marine Ecosystems from Pollution. Birkhäuser Verlag/Switzerland, 285 pp.

Adams, J. R. (1969). Ecological Investigations around Some Thermal Power Stations in California Tidal Waters. Chesapeake Science,10(3/4), 145. doi:10.2307/1350451.

Adenekan, A. E., Kolluru, V. S., & Smith, J. P. (2009). Transport and Fate of Chlorinated By-Products Associated with Cooling Water Discharges. Proceedings of the 1st Annual Gas Processing Symposium, 341-353. doi:10.1016/b978-0-444-53292-3.50042-0.

Afrasiabi, N., & Shahbazali, E. (2011). RO brine treatment and disposal methods. Desalination and Water Treatment,35(1-3), 39-53. doi:10.5004/dwt.2011.3128.

Akberali, H. B., & Black, J. E. (1980). Behavioural responses of the bivalve Scrobicularia plana (da Costa) subjected to short-term copper (Cu II) concentrations. Marine Environmental Research,4(2), 97-107. doi:10.1016/0141-1136(80)90048-3.

Al-Saleh, I., Al-Doush, I., & Echeverria-Quevedo, A. (1999). Residues of Pesticides in Grains Locally Grown in Saudi Arabia. Bulletin of Environmental Contamination and Toxicology,63(4), 451-459. doi:10.1007/s001289901001 .

Altaee, A., Mabrouk, A., & Bourouni, K. (2013). A novel Forward osmosis membrane

pretreatment of seawater for thermal desalination processes. Desalination,326, 19-29. doi:10.1016/j.desal.2013.07.008.

Angiolillo, M. (2019).Chapter 14 - Debris in Deep Water .Volume III: Ecological Issues and Environmental Impacts, Pages 251-268.

Barbier, E. B. (2011.). The protective value of estuarine and coastal ecosystems. Handbook on the Economics of Ecosystem Services and Biodiversity,27-39. doi:10.4337/9781781951514.00008.

Beauchamp, R. S. (1969). The Use of Chlorine in the Cooling Water System of Coastal Power Station. Chesapeake Science,10(3/4), 280. doi:10.2307/1350468.

Blogoslawski,W . J., Farrel, L., Garceau,R .,& Derrig, P. (1976). Production of oxidants in ozonised sea water. In: Proceedings of the Second lnternationaI Symposium on Ozone Technology (Rice, R. G., Pichet, P. & VINCENT, M-A. (Eds.)), Ozone Press Int., Jamesville, NY, 671-81.

Bongers, L. H., Bradley, B., Burton, D. T.,Holland, A. F., &. Lidev,L. H. (1977). Biotoxicity of bromine chloride and chlorine treated power plant condenser cooling water effluents. Martin Marietta Corp., Environ. Tech. Center, Baltimore, Md. 61 p.

Bott, T. R. (2011). Industrial biofouling. Amsterdam: Elsevier.

Braithwaite, R., & Mcevoy, L. (2004). Marine Biofouling on Fish Farms and Its Remediation. Advances in Marine Biology,215-252. doi:10.1016/s0065-2881(04)47003-5.

Briand, F. J. (1975). Effects of power-plant cooling systems on marine phytoplankton. Marine Biology,33(2), 135-146. doi:10.1007/bf00390718.

Brungs, W. A. (1976). Effects of wastewater and cooling water chlorination on aquatic life, US Environ. Prot. Agency Report No. EPA-600/3-76-036, 45 pp.

Buccolieri, A., Buccolieri, G., Cardellicchio, N., Dellatti, A., Leo, A. D., & Maci, A. (2006). Heavy metals in marine sediments of Taranto Gulf (Ionian Sea, Southern Italy). Marine Chemistry,99(1-4), 227-235. doi:10.1016/j.marchem.2005.09.009.

Capuzzo, J. M. (1977). The effects of free chlorine and chloramine on growth and respiration rates of larval lobsters (Homarus Americanus). Water Research,11(12), 1021-1024. doi:10.1016/0043-1354(77)90001-x.

Capuzzo, J. M., Lawrence, S. A., & Davidson, J. A. (1976). Combined toxicity of free chlorine, chloramine and temperature to stage I larvae of the American lobster Homarus americanus. Water Research,10(12), 1093-1099. doi:10.1016/0043-1354(76)90042-7.

Chin,B.Ye. X., Zhang, B., Jing, L.,& Lee,.K. (2019). Chapter 22 - Marine Oil Spills Preparedness and Countermeasures .Volume III: Ecological Issues and Environmental Impacts, Pages 407-426.

Davis,W. P., & Middaugh, D. P., (1976). A review of the impact of chlorination processes upon marine ecosystems. In The Environmental Impact of Water Chlorina- tion, Proceedings (Edited by R. L. Jolley), pp. 299-325.

Dove, R. A. (1970). Reactions of small dosages of chlorine in sea water. Central Electricity Generating Board, Research Report 42/70, File No. 0.3070/ID, Job No. 10665. Univ. of Southampton, England.

Duncan,G.( 2008) .Observations on the effects of chlorine on the larvae of oysters (Ostrea edulis (L)and barnacles (Elminius modestus (Darwin)). Annals of Applied Biology. Volume 54, Issue 3.

Eriksen, M., Thiel, M., & Lebreton, L. (2016). Nature of Plastic Marine Pollution in the Subtropical Gyres. The Handbook of Environmental Chemistry Hazardous Chemicals

Associated with Plastics in the Marine Environment,135-162. doi:10.1007/698_2016_123.

Göksu C., Evik F., &Findik O .(2002) Lethal chlorine concentrations for the pearl oyster Pinctada radiata (Leach, 1814). Turkish Journal of Veterinary and Animal

Sciences 26(1): 157-160200.

Goodman, P. D. (1987). Effect of chlorination on materials for sea water cooling systems: A review of chemical reactions. British Corrosion Journal,22(1), 56-62. doi:10.1179/000705987798271785.

García-Borboroglu, P., Boersma, P. D., Ruoppolo, V., Reyes, L., Rebstock, G. A., Griot, K., Silva, R. P. (2006). Chronic oil pollution harms Magellanic penguins in the Southwest Atlantic. Marine Pollution Bulletin,52(2), 193-198. doi:10.1016/j.marpolbul.2005.11.004.

Hall, L. W., Burton, D. T., Liden, L. H., & Heath, A. G. (1981). An Interpretative Literature Analysis Evaluating the Effects of Power Plant Chlorination on Freshwater Organisms. CRC Critical Reviews in Toxicology,9(1), 1-20. doi:10.3109/10408448109059561.

Hall, L. W., Burton, D. T., Liden, L. H., & Garey, J. (1982). Power Plant Chlorination Effects on Estuarine and Marine Organisms. CRC Critical Reviews in Toxicology,10(1), 27-47. doi:10.3109/10408448209033630.

Hayes, K. R., & Sliwa, C. (2003). Identifying potential marine pests—a deductive approach applied to Australia. Marine Pollution Bulletin,46(1), 91-98. doi:10.1016/s0025-326x(02)00321-1.

Hoda A. Mansour Mansour Et Al. (2017). Dyes Bio-Sorption by two Marine Algae and Their Applications on Industrial Effluents from Borg El-Arab region, Egypt. Egyptian Journal of Botany, 0(0), 0-0. doi:10.21608/ejbo.2017.1199

Jenner, H .A ., Polman, H .J .G., & Wijck, R. (2003) Four years experience with a new chlorine dosing regime against macrofouling. Presented at VGB (Verband der Großkessel-Besitzer e.V.) meeting 2003 at Essen.

Jolley, R. L., Gorchev, H. & Hamilton, J. R., D. H. (1978). Water chlorination environmental impact and health effects, Vol. 2. Ann Arbor Sci. Publ. Inc., Ann Arbor, MI, 909 pp.

Jolley, R. L. (1975). Chlorine containing organic constituents in chlorinated effluents. J. Wat. Pollut. Control Fed. 47, 601-618.

Langford T. (1977) Environmental management of coastal cooling discharges in Hong kong, Chemistry & Industry: 612-616.sevier, Amsterdam, p 332–340.

Leitão, A., Al-Shaikh, I., Hassan, H., Hamadou, R. B., & Bach, S. (2017). First genotoxicity assessment of marine environment in Qatar using the local Pearl oyster Pinctada radiata. Regional Studies in Marine Science,11, 23-31. doi:10.1016/j.rsma.2017.02.001.

Leonard, B., Dennis, T., & Ann, M. (1981) .A comparison of Ozone and Chlorine toxicity of three life stages of the American oyster Crassostrea virginica .Academy of Natural Sciences of Philadelphia, Benedict Estuarine Research Laboratory, Benedict, Maryland 20612, USA.

Lewis, B. G. (1966). Chlorination and mussel control. I: The chemistry of chlorinated sea water: A review of the literature. Central Electricity Research Laboratories, Laboratory Note No, England. RD/L/N 106/66, Leatherhead.

Liden, L. H., Burton, D. T., Bongers, L. H .,& Holland, A. F. (I 980). Effects of chlorobrominated and chlorinated waters on estuarine organisms. J. Water Pollut. Control. Fed., 52, 173-82.

Lohmann, R., & Dachs, J .(2019). Chapter 15 -Polychlorinated Biphenyls in the Global Ocean. Volume III: Ecological Issues and Environmental Impacts, Pages 269-282.

Luís Gabriel A.Barboza, AndrésCózar, Barbara C.G.Gimenez ,Thayanne Lima Barros, Peter J.Kershaw,& Lúcia Guilhermino. (2019). Chapter 17 - Macroplastics Pollution in the Marine Environment. .Volume III: Ecological Issues and Environmental Impacts, Pages 305-328.

Lynette H.L , Eliza C.Heery, &Peter A.Todd.(2019). Chapter 26 – Shoreline Defenses.Volume III: Ecological Issues and Environmental Impacts, Pages 491-504.

Lyons, B., Thain, J., Stentiford, G., Hylland, K., Davies, I., & Vethaak, A. (2010). Using biological effects tools to define Good Environmental Status under the European Union Marine Strategy Framework Directive. Marine Pollution Bulletin,60(10), 1647-1651. doi:10.1016/j.marpolbul.2010.06.005.

Macdonald, I. A., Polman, H. J., Jenner, H. A., & Quyam, S. Q. (2011). Pulse-Chlorination®: Anti-Fouling Optimization in Seawater Cooling Systems. Operational and Environmental Consequences of Large Industrial Cooling Water Systems,287-301. doi:10.1007/978-1-4614-1698-2_12.

Macdonald, R. L. (2009). Response to Letter by Vergouwen et al. Stroke, 40(2). doi:10.1161/strokeaha.108.538520

Miller, S., Shemer, H., & Semiat, R. (2015). Energy and environmental issues in desalination. Desalination,366, 2-8. doi:10.1016/j.desal.2014.11.034.

Mora, S. D., Fowler, S. W., Tolosa, I., Villeneuve, J., & Cattini, C. (2005). Chlorinated hydrocarbons in marine biota and coastal sediments from the Gulf and Gulf of Oman. Marine Pollution Bulletin,50(8), 835-849. doi:10.1016/j.marpolbul.2005.02.022.

Morgan, R. P., & Carpenter, E. J. (1978). Biocides. Power Plant Entrainment,95-134. doi:10.1016/b978-0-12-631050-4.50007-8.

Munk, T., Kane, D., & Yebra, D. (2009). The effects of corrosion and fouling on the performance of ocean-going vessels: A naval architectural perspective. Advances in Marine Antifouling Coatings and Technologies,148-176. doi:10.1533/9781845696313.1.148.

Peñate, B., & García-Rodríguez, L. (2012). Current trends and future prospects in the design of seawater reverse osmosis desalination technology. Desalination,284, 1-8. doi:10.1016/j.desal.2011.09.010

Potera, C. (2013). Microbes Occupy, May Be Degrading Plastic Debris in Oceans. Microbe Magazine,8(11), 438-439. doi:10.1128/microbe.8.438.1.

Rhoderick, J. C., Block, R. M., Roosenburg, K. G. & ROOSENBURG, W. H. (1977). Effects of chlorination to the American oyster, Crassostrea virginica, at two temperatures. Assoc. Southeastern Biol. Bull., 24, 80.

Richardson, L. B., Burton, D. T., & Stavola, A. M. (1982). A comparison of ozone and chlorine toxicity to three life stages of the American oyster Crassostrea virginica. Marine Environmental Research,6(2), 99-113. doi:10.1016/0141-1136(82)90024-1.

Price, A.R.G., Sheppard, C.R.C., & Roberts, C.M.( 1993). The Gulf: its biological setting. Marine Pollution Bulletin 27, 5–15.

Ross, P. S. (Ed.). (2003). Reviews of Environmental Contamination and Toxicology. Reviews of Environmental Contamination and Toxicology. doi:10.1007/b97458.

Rostron,R ., & Rehana, P . (2015). Biofouling of seawater intake grilles - causes and mitigation Strategies. International Journal of Advanced Research, Volume 3, Issue 9:1399- 1407

Resources Agency (1973). The energy dilemma: California's 20 year power plant sighting plan. Sacrament of Department of Water Resources.

Rhodrick, J. C., Block, R. M., Drobeck, K. G.,& Roosenburg, W. H. (1977). Effects of chlorination

to the American oyster, Crassostrea virginica, at two temperatures. Assoc. Southeastern Biol. Bull., 24, 80.

Scott, G. I., Middaugh., Crane, A. M.., & N. H. McGlothlin. (1980). Physiological effects of chlorine produced oxidants and uptake of chlorination by-products in the American oyster, Crassostrea virginica (Gmelin), p. 501-516.

Scott, G. I., & Vernberg, W. B. (1979). Seasonal Effects Of Chlorine Produced Oxidants On The Growth, Survival And Physiology Of The American Oyster, Crassostrea Virginica (Gmenlin). Marine Pollution,415-435. doi:10.1016/b978-0-12-718260-5.50025-6.

Scott, G. I., & Middaugh, D. P. (1978). Seasonal chronic toxicity of chlorination to the American oyster, Crassostrea virginica (G), p. 311-328. In R. L. Jolley, H. Gorchev, and D. H. Hamilton, Jr. (eds.), Water Chlorination: Environmental Impact and Health Effects, Vol. 2. Ann Arbor Science Publish- ers, Inc., Ann Arbor, Michigan.

Sheppard, C., Al-Husiani, M., Al-Jamali, F., Al-Yamani, F.,& Baldwin, R. (2010). The Gulf: a young sea in decline. Mar. Pollut. Bull. 60, 13–38.

Sheppard, C. (2019). World Seas: An Environmental Evaluation: Volume III: Ecological Issues and Environmental Impacts. Academic Press.

Sheridan, P. F. (1981). Effects of Continuous Chlorination on Spat of the American Oyster (Crassostrea virginica). Estuaries,4(4), 376. doi:10.2307/1352164.

Solan, M., & Whiteley, N. M. (2016). Stressors in the marine environment: Physiological and ecological responses, societal implications. Oxford: Oxford University Press.

Stenton, J. M. E., & Brown, A. C.(1994). Exposure of the sandy beach bivalve Donax Serra Roding to a heated and chlorinated effluent II effects of chlorine on borrowing and survival, South Africa. Zoology Department. Journal of Shellfish Research, Vol 13, No 2,451-454.

Sun, C., Soltwedel, T., Bauerfeind, E., Adelman, D. A., & Lohmann, R. (2016). Depth Profiles of Persistent Organic Pollutants in the North and Tropical Atlantic Ocean. Environmental Science & Technology,50(12), 6172-6179. doi:10.1021/acs.est.5b05891.

Van Wijk, K., J. M. E. Stenton-Dozey & A. C. Brown. (1989). Oxycon- formation in the burrowing bivalve Donax serra Röding. J. Moll. Stud. 55:133-134.

Waugh, G. D. (1964). Observations on the effects of chlorine on the larvae of oysters (Ostrea edulis (L.)) and barnacles (Elminius modestus (Darwin)). Annals of Applied Biology,54(3), 423-440. doi:10.1111/j.1744-7348.1964.tb01206.x

Weis, J. S. (2015). Marine pollution: What everyone needs to know. New York, NY: Oxford University Press.

Yang, L. (2000). Natural disinfection of wastewater in marine outfall fields. Water Research,34(3), 743-750. doi:10.1016/s0043-1354(99)00209-2.

Yukselen, M. A., Calli, B., Gokyay, O., & Saatci, A. (2003). Inactivation of coliform bacteria in Black Sea waters due to solar radiation. Environment International,29(1), 45-50. doi:10.1016/s0160-4120(02)00144-7.

Zuykov, M., Pelletier, E., & Harper, D. A. (2013). Bivalve mollusks in metal pollution studies: From bioaccumulation to biomonitoring. Chemosphere,93(2), 201-208. doi:10.1016/j.chemosphere.2013.05.001.

# APPENDICES

## 6.1 Appendix A: Mortality of oysters

Dependent Variable: % dead

**Normality Test:** Passed (P = 0.056)

**Equal Variance Test:** Failed (P < 0.050)

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 2 | 0.184 | 0.0919 | 2.061 | 0.162 |
| Size Class | 4 | 1.165 | 0.291 | 6.532 | 0.003 |
| Treatment x Size Class | 8 | 0.863 | 0.108 | 2.420 | 0.067 |
| Residual | 15 | 0.669 | 0.0446 | | |
| Total | 29 | 2.880 | 0.0993 | | |

Least square means for Treatment:

| Group | Mean |
|---|---|
| Control | |
| Cl 0.0250 | |
| Cl 0.1000 | |

Std Err of LS Mean = 0.0668

Least square means for Size Class:

| Group | Mean |
|---|---|
| 40-45 | 0.106 |
| 45-50 | 0.0764 |
| 50-55 | 0.131 |
| 55-60 | 0.220 |
| >60 | 0.611 |

Std Err of LS Mean = 0.0862

Least square means for Treatment x Size Class:

| Group | Mean |
|---|---|
| Control x 40-45 | |
| Control x 45-50 | |
| Control x 50-55 | |
| Control x 55-60 | |
| Control x >60 | |
| 0.025 x 40-45 | |
| 0.025 x 45-50 | |
| 0.025 x 50-55 | |
| 0.025 x 55-60 | |
| 0.025 x >60 | |
| 0.100 x 40-45 | |
| 0.100 x 45-50 | |

| Group | |
|---|---|
| 0.100 x 50-55 | |
| 0.100 x 55-60 | |
| 0.100 x >60 | |

Std Err of LS Mean = 0.149

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method):

Comparisons for factor: **Size Class**

| **Comparison** | **Diff of Means** | **p** | **Q** | **P** | **P<0.050** |
|---|---|---|---|---|---|
| >60 vs. 45-50 | 0.535 | 5 | 6.204 | 0.004 | Yes |
| >60 vs. 40-45 | 0.505 | 4 | 5.857 | 0.004 | Yes |
| >60 vs. 50-55 | 0.481 | 3 | 5.575 | 0.004 | Yes |
| >60 vs. 55-60 | 0.391 | 2 | 4.537 | 0.006 | Yes |
| 55-60 vs. 45-50 | 0.144 | 4 | 1.667 | 0.649 | No |
| 55-60 vs. 40-45 | 0.114 | 3 | 1.320 | 0.628 | Do Not Test |
| 55-60 vs. 50-55 | 0.0895 | 2 | 1.038 | 0.474 | Do Not Test |
| 50-55 vs. 45-50 | 0.0542 | 3 | 0.629 | 0.898 | Do Not Test |
| 50-55 vs. 40-45 | 0.0243 | 2 | 0.282 | 0.845 | Do Not Test |
| 40-45 vs. 45-50 | 0.0299 | 2 | 0.347 | 0.810 | Do Not Test |

**6.2 Kaplan Meier**

| Day | Event | Group |
|---|---|---|
| First day of the experiments till the last day | Dead oysters=1<br>Survival oysters= 0 | Control<br>Cl concentration= 0.1<br>Cl concentration= 0.025 |

| Day | Event | Group | Day | Event | Group | Day | Event | Group |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | Cont | 1 | 1 | 0.025 | 1 | 1 | 0.1 |
| 1 | 1 | Cont | 1 | 1 | 0.025 | 1 | 1 | 0.1 |
| 1 | 1 | Cont | 1 | 1 | 0.025 | 1 | 1 | 0.1 |
| 1 | 1 | Cont | 1 | 1 | 0.025 | 1 | 1 | 0.1 |
| 1 | 1 | Cont | 1 | 1 | 0.025 | 1 | 1 | 0.1 |
| 1 | 1 | Cont | 5 | 1 | 0.025 | 7 | 1 | 0.1 |
| 1 | 1 | Cont | 7 | 1 | 0.025 | 12 | 1 | 0.1 |
| 1 | 1 | Cont | 12 | 1 | 0.025 | 14 | 1 | 0.1 |
| 5 | 1 | Cont | 14 | 1 | 0.025 | 26 | 1 | 0.1 |
| 12 | 1 | Cont | 14 | 1 | 0.025 | 39 | 1 | 0.1 |
| 24 | 1 | Cont | 17 | 1 | 0.025 | 46 | 1 | 0.1 |
| 24 | 1 | Cont | 17 | 1 | 0.025 | 63 | 1 | 0.1 |
| 26 | 1 | Cont | 24 | 1 | 0.025 | 74 | 1 | 0.1 |
| 74 | 1 | Cont | 28 | 1 | 0.025 | 75 | 0 | 0.1 |

| Day | Event | Group | Day | Event | Group | Day | Event | Group |
|---|---|---|---|---|---|---|---|---|
| 75 | 0 | Cont | 31 | 1 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 39 | 1 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 39 | 1 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 46 | 1 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 55 | 1 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 55 | 1 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 63 | 1 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 74 | 1 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |

| Day | Event | Group | Day | Event | Group | Day | Event | Group |
|---|---|---|---|---|---|---|---|---|
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |

| Day | Event | Group | Day | Event | Group | Day | Event | Group |
|---|---|---|---|---|---|---|---|---|
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |
| 75 | 0 | Cont | 75 | 0 | 0.025 | 75 | 0 | 0.1 |

## 6.3 Appendix B: Growth

***Height***

Balanced Design

Dependent Variable: Inc. Height

**Normality Test:** Passed (P = 0.549)

**Equal Variance Test:** Failed (P < 0.050)

| **Source of Variation** | **DF** | **SS** | **MS** | **F** | **P** |
|---|---|---|---|---|---|
| Treatment | 2 | 0.0202 | 0.0101 | 2.600 | 0.107 |
| Size Class | 4 | 0.0696 | 0.0174 | 4.488 | 0.014 |
| Treatment x Size Class | 8 | 0.0415 | 0.00519 | 1.339 | 0.298 |
| Residual | 15 | 0.0582 | 0.00388 | | |
| Total | 29 | 0.189 | 0.00653 | | |

Least square means for Treatment:

| Group | Mean |
|---|---|
| Control | |
| Cl 0.0250 | |
| Cl 0.1000 | |

Std Err of LS Mean = 0.0197

Least square means for Size Class:

| Group | Mean |
|---|---|
| 40-45 | |
| 45-50 | |
| 50-55 | |
| 55-60 | |
| >60 | |

Std Err of LS Mean = 0.0

Least square means for Treatment x Size Class:

| Group | Mean |
|---|---|
| Control x 40-45 | [illegible] |
| Control x 45-50 | [illegible] |
| Control x 50-55 | [illegible] |
| Control x 55-60 | [illegible] |

| | |
|---|---|
| Control x >60 | 0 |
| 0.025 x 40-45 | 4 |
| 0.025 x 45-50 | 0 |
| 0.025 x 50-55 | 0 |
| 0.025 x 55-60 | 9 |
| 0.025 x >60 | 8 |
| 0.100 x 40-45 | 2 |
| 0.100 x 45-50 | 3 |
| 0.100 x 50-55 | 6 |
| 0.100 x 55-60 | 9 |
| 0.100 x >60 | 0 |

Std Err of LS Mean = 0.0440

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method):

Comparisons for factor: **Size Class**

| **Comparison** | **Diff of Means** | **p** | **Q** | **P** | **P<0.050** |
|---|---|---|---|---|---|
| 40-45 vs. 55-60 | 0.132 | 5 | 5.179 | 0.017 | Yes |
| 40-45 vs. >60 | 0.123 | 4 | 4.830 | 0.018 | Yes |
| 40-45 vs. 50-55 | 0.0789 | 3 | 3.102 | 0.105 | No |
| 40-45 vs. 45-50 | 0.0528 | 2 | 2.078 | 0.162 | Do Not Test |
| 45-50 vs. 55-60 | 0.0788 | 4 | 3.101 | 0.170 | No |
| 45-50 vs. >60 | 0.0700 | 3 | 2.752 | 0.160 | Do Not Test |

| | | | | | |
|---|---|---|---|---|---|
| 45-50 vs. 50-55 | 0.0260 | 2 | 1.024 | 0.480 | Do Not Test |
| 50-55 vs. 55-60 | 0.0528 | 3 | 2.077 | 0.333 | Do Not Test |
| 50-55 vs. >60 | 0.0439 | 2 | 1.728 | 0.241 | Do Not Test |
| >60 vs. 55-60 | 0.00888 | 2 | 0.349 | 0.808 | Do Not Test |

***Length***

Dependent Variable: Inc. Length

**Normality Test:** Passed (P = 0.476)

**Equal Variance Test:** Failed (P < 0.050)

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 2 | 0.000292 | 0.000146 | 0.273 | 0.765 |
| Size Class | 4 | 0.0188 | 0.00469 | 8.780 | <0.001 |
| Treatment x Size Class | 8 | 0.00604 | 0.000755 | 1.412 | 0.269 |
| Residual | 15 | 0.00802 | 0.000534 | | |
| Total | 29 | 0.0331 | 0.00114 | | |

Least square means for Treatment:

| Group | Mean |
|---|---|
| Control | [illegible] |
| Cl 0.0250 | [illegible] |
| Cl 0.1000 | [illegible] |

Std Err of LS Mean = 0.00731

Least square means for Size Class:

| Group | Mean |
|---|---|
| 40-45 | [illegible] |

| Group | |
|---|---|
| 45-50 | 0.06 |
| 50-55 | 0.03 |
| 55-60 | 0 |
| >60 | 0 |

Std Err of LS Mean = 0.00944

Least square means for Treatment x Size Class:

| Group | Mean |
|---|---|
| Control x 40-45 | 0.019 |
| Control x 45-50 | -0.0086 |
| Control x 50-55 | -0.017 |
| Control x 55-60 | -0.0019 |
| Control x >60 | -0.029 |
| 0.025 x 40-45 | 0.053 |
| 0.025 x 45-50 | 0.017 |
| 0.025 x 50-55 | 0.0013 |
| 0.025 x 55-60 | -0.041 |
| 0.025 x >60 | -0.056 |
| 0.100 x 40-45 | 0.067 |
| 0.100 x 45-50 | 0.0037 |
| 0.100 x 50-55 | -0.0079 |

| Group | |
|---|---|
| 0.100 x 55-60 | 0.055 |
| 0.100 x >60 | 0.011 |

Std Err of LS Mean = 0.0163

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method):

Comparisons for factor: **Size Class**

| Comparison | Diff of Means | p | q | P | P<0.050 |
|---|---|---|---|---|---|
| 40-45 vs. >60 | 0.0682 | 5 | 7.223 | 0.001 | Yes |
| 40-45 vs. 55-60 | 0.0669 | 4 | 7.091 | <0.001 | Yes |
| 40-45 vs. 50-55 | 0.0435 | 3 | 4.611 | 0.014 | Yes |
| 40-45 vs. 45-50 | 0.0344 | 2 | 3.644 | 0.021 | Yes |
| 45-50 vs. >60 | 0.0338 | 4 | 3.579 | 0.095 | No |
| 45-50 vs. 55-60 | 0.0325 | 3 | 3.447 | 0.068 | Do Not Test |
| 45-50 vs. 50-55 | 0.00913 | 2 | 0.968 | 0.504 | Do Not Test |
| 50-55 vs. >60 | 0.0246 | 3 | 2.612 | 0.189 | Do Not Test |
| 50-55 vs. 55-60 | 0.0234 | 2 | 2.479 | 0.100 | Do Not Test |
| 55-60 vs. >60 | 0.00125 | 2 | 0.132 | 0.927 | Do Not Test |

***Weight***

Dependent Variable: Inc. Weight

**Normality Test:** Passed (P = 0.471)

**Equal Variance Test:** Failed (P < 0.050)

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 2 | 0.00280 | 0.00140 | 0.714 | 0.506 |
| Size Class | 4 | 0.00637 | 0.00159 | 0.811 | 0.537 |
| Treatment x Size Class | 8 | 0.0157 | 0.00196 | 0.997 | 0.476 |
| Residual | 15 | 0.0294 | 0.00196 | | |
| Total | 29 | 0.0543 | 0.00187 | | |

Least square means for Treatment:

| Group | Mean |
|---|---|
| Control | -0.0555 |
| Cl 0.0250 | -0.0726 |
| Cl 0.1000 | -0.0499 |

Std Err of LS Mean = 0.0140

Least square means for Size Class:

| Group | Mean |
|---|---|
| 40-45 | [illegible] |
| 45-50 | [illegible] |
| 50-55 | [illegible] |
| 55-60 | [illegible] |
| >60 | [illegible] |

Std Err of LS Mean = 0.0181

Least square means for Treatment x Size Class:

| Group | Mean |
|---|---|
| Control x 40-45 | -0.0682 |
| Control x 45-50 | -0.0770 |
| Control x 50-55 | -0.0594 |
| Control x 55-60 | -0.0496 |
| Control x >60 | -0.0234 |
| 0.025 x 40-45 | -0.0428 |
| 0.025 x 45-50 | -0.0932 |
| 0.025 x 50-55 | -0.0461 |
| 0.025 x 55-60 | -0.0829 |
| 0.025 x >60 | -0.0981 |
| 0.100 x 40-45 | -0.00609 |

| Group | Mean |
|---|---|
| 0.100 x 45-50 | -0.0515 |
| 0.100 x 50-55 | -0.0543 |
| 0.100 x 55-60 | -0.0248 |
| 0.100 x >60 | -0.113 |

Std Err of LS Mean = 0.0313

***Width***

Dependent Variable: Inc. Width

**Normality Test:** Passed (P = 0.423)

**Equal Variance Test:** Failed (P < 0.050)

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 2 | 0.000156 | 0.0000780 | 0.0836 | 0.920 |
| Size Class | 4 | 0.0155 | 0.00386 | 4.138 | 0.019 |
| Treatment x Size Class | 8 | 0.0152 | 0.00190 | 2.031 | 0.113 |
| Residual | 15 | 0.0140 | 0.000934 | | |
| Total | 29 | 0.0448 | 0.00154 | | |

Least square means for Treatment:

| Group | Mean |
|---|---|
| Control | 0.05 |
| Cl 0.0250 | 0.09 |
| Cl 0.1000 | 0.06 |

Std Err of LS Mean = 0.00966

Least square means for Size Class:

| Group | Mean |
|---|---|
| 40-45 | |
| 45-50 | |
| 50-55 | |
| 55-60 | |
| >60 | |

Std Err of LS Mean = 0.0125

Least square means for Treatment x Size Class:

| Group | Mean |
|---|---|
| Control x 40-45 | 0 |
| Control x 45-50 | |

| Group | |
|---|---|
| Control x 50-55 | |
| Control x 55-60 | |
| Control x >60 | |
| 0.025 x 40-45 | |
| 0.025 x 45-50 | |
| 0.025 x 50-55 | |
| 0.025 x 55-60 | |
| 0.025 x >60 | |
| 0.100 x 40-45 | 0 |
| 0.100 x 45-50 | |
| 0.100 x 50-55 | |
| 0.100 x 55-60 | |
| 0.100 x >60 | |

Std Err of LS Mean = 0.0216

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method):

Comparisons for factor: **Size Class**

| **Comparison** | **Diff of Means** | **p** | **Q** | **P** | **P<0.050** |
|---|---|---|---|---|---|
| 40-45 vs. >60 | 0.0647 | 5 | 5.189 | 0.017 | Yes |
| 40-45 vs. 55-60 | 0.0540 | 4 | 4.328 | 0.036 | Yes |
| 40-45 vs. 50-55 | 0.0363 | 3 | 2.910 | 0.133 | No |
| 40-45 vs. 45-50 | 0.0246 | 2 | 1.969 | 0.184 | Do Not Test |
| 45-50 vs. >60 | 0.0402 | 4 | 3.220 | 0.148 | No |
| 45-50 vs. 55-60 | 0.0294 | 3 | 2.359 | 0.249 | Do Not Test |
| 45-50 vs. 50-55 | 0.0117 | 2 | 0.942 | 0.516 | Do Not Test |
| 50-55 vs. >60 | 0.0284 | 3 | 2.279 | 0.272 | Do Not Test |
| 50-55 vs. 55-60 | 0.0177 | 2 | 1.417 | 0.332 | Do Not Test |
| 55-60 vs. >60 | 0.0107 | 2 | 0.861 | 0.552 | Do Not Test |

## 6.4 Appendix C: Physiological performance

***Concentration of oxygen in the different respirometer T0***

**Dependent Variable:** T0

**Normality Test:** Passed (P = 0.152)

**Equal Variance Test:** Failed (P < 0.050)

| Source of Variation | DF | SS | MS | F | P |
|---|---|---|---|---|---|
| Treatment | 2 | 0.886 | 0.443 | 0.421 | 0.666 |
| respirometer | 3 | 6.308 | 2.103 | 1.999 | 0.168 |
| Treatment x respirometer | 6 | 1.001 | 0.167 | 0.159 | 0.983 |
| Residual | 12 | 12.625 | 1.052 | | |
| Total | 23 | 20.820 | 0.905 | | |

Least square means for Treatment:

| Group | Mean |
|---|---|
| 0.1000 | 18.638 |
| 0.0250 | 18.675 |
| control | 18.250 |

Std Err of LS Mean = 0.363

Least square means for respirometer:

| Group | Mean |
|---|---|
| 1.000 | 19.317 |
| 2.000 | 18.617 |
| 3.000 | 18.150 |
| blank | 18.000 |

Std Err of LS Mean = 0.419

Least square means for Treatment x respirometer:

| Group | Mean |
|---|---|
| 0.100 x 1.000 | 19.350 |
| 0.100 x 2.000 | 18.600 |
| 0.100 x 3.000 | 18.200 |
| 0.100 x blank | 18.400 |
| 0.025 x 1.000 | 19.500 |
| 0.025 x 2.000 | 18.700 |
| 0.025 x 3.000 | 18.150 |
| 0.025 x blank | 18.350 |
| control x 1.000 | 19.100 |
| control x 2.000 | 18.550 |
| control x 3.000 | 18.100 |
| control x blank | 17.250 |

Std Err of LS Mean = 0.725

***Concentration of oxygen in the different respirometer T1***

Dependent Variable: T1

**Normality Test:** Passed (P = 0.194)

**Equal Variance Test:** Failed (P < 0.050)

| **Source of Variation** | **DF** | **SS** | **MS** | **F** | **P** |
|---|---|---|---|---|---|
| Treatment | 2 | 0.0258 | 0.0129 | 0.0111 | 0.989 |
| respirometer | 3 | 15.017 | 5.006 | 4.309 | 0.028 |
| Treatment x respirometer | 6 | 2.351 | 0.392 | 0.337 | 0.904 |
| Residual | 12 | 13.940 | 1.162 | | |
| Total | 23 | 31.333 | 1.362 | | |

Least square means for Treatment:

| **Group** | **Mean** |
|---|---|
| 0.1000 | 14.500 |
| 0.0250 | 14.438 |
| control | 14.512 |

Std Err of LS Mean = 0.381

Least square means for respirometer:

| Group | Mean |
|---|---|
| 1.000 | 14.750 |
| 2.000 | 14.267 |
| 3.000 | 13.367 |
| blank | 15.550 |

Std Err of LS Mean = 0.440

Least square means for Treatment x respirometer:

| Group | Mean |
|---|---|
| 0.100 x 1.000 | 14.550 |
| 0.100 x 2.000 | 14.850 |
| 0.100 x 3.000 | 12.800 |
| 0.100 x blank | 15.800 |
| 0.025 x 1.000 | 14.900 |
| 0.025 x 2.000 | 13.850 |
| 0.025 x 3.000 | 13.600 |
| 0.025 x blank | 15.400 |
| control x 1.000 | 14.800 |
| control x 2.000 | 14.100 |
| control x 3.000 | 13.700 |
| control x blank | 15.450 |

Std Err of LS Mean = 0.762

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method):

Comparisons for factor: **respirometer**

| **Comparison** | **Diff of Means** | **p** | **Q** | **P** | **P<0.050** |
|---|---|---|---|---|---|
| blank vs. 3.000 | 2.183 | 4 | 4.962 | 0.020 | Yes |
| blank vs. 2.000 | 1.283 | 3 | 2.917 | 0.140 | No |
| blank vs. 1.000 | 0.800 | 2 | 1.818 | 0.223 | Do Not Test |
| 1.000 vs. 3.000 | 1.383 | 3 | 3.144 | 0.107 | No |
| 1.000 vs. 2.000 | 0.483 | 2 | 1.098 | 0.453 | Do Not Test |
| 2.000 vs. 3.000 | 0.900 | 2 | 2.045 | 0.174 | Do Not Test |

***Concentration of oxygen in the different respirometer T2***

Dependent Variable: T2

**Normality Test:** Passed (P = 0.264)

**Equal Variance Test:** Failed (P < 0.050)

| **Source of Variation** | **DF** | **SS** | **MS** | **F** | **P** |
|---|---|---|---|---|---|
| Treatment | 2 | 1.651 | 0.825 | 0.736 | 0.499 |
| respirometer | 3 | 73.395 | 24.465 | 21.828 | <0.001 |
| Treatment x respirometer | 6 | 10.743 | 1.790 | 1.597 | 0.230 |
| Residual | 12 | 13.450 | 1.121 | | |
| Total | 23 | 99.238 | 4.315 | | |

Least square means for Treatment:

| **Group** | **Mean** |
|---|---|
| 0.1000 | 13.188 |
| 0.0250 | 13.438 |
| control | 12.800 |

Std Err of LS Mean = 0.374

Least square means for respirometer:

| Group | Mean |
|---|---|
| 1.000 | 13.033 |
| 2.000 | 12.883 |
| 3.000 | 10.867 |
| blank | 15.783 |

Std Err of LS Mean = 0.432

Least square means for Treatment x respirometer:

| Group | Mean |
|---|---|
| 0.100 x 1.000 | 13.050 |
| 0.100 x 2.000 | 13.650 |
| 0.100 x 3.000 | 9.450 |
| 0.100 x blank | 16.600 |
| 0.025 x 1.000 | 13.150 |
| 0.025 x 2.000 | 13.200 |
| 0.025 x 3.000 | 11.950 |
| 0.025 x blank | 15.450 |
| control x 1.000 | 12.900 |
| control x 2.000 | 11.800 |
| control x 3.000 | 11.200 |
| control x blank | 15.300 |

Std Err of LS Mean = 0.749

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method):

Comparisons for factor: **respirometer**

| **Comparison** | **Diff of Means** | **p** | **Q** | **P** | **P<0.050** |
|---|---|---|---|---|---|
| blank vs. 3.000 | 4.917 | 4 | 11.376 | <0.001 | Yes |
| blank vs. 2.000 | 2.900 | 3 | 6.710 | 0.001 | Yes |
| blank vs. 1.000 | 2.750 | 2 | 6.363 | <0.001 | Yes |
| 1.000 vs. 3.000 | 2.167 | 3 | 5.013 | 0.011 | Yes |
| 1.000 vs. 2.000 | 0.150 | 2 | 0.347 | 0.810 | No |
| 2.000 vs. 3.000 | 2.017 | 2 | 4.666 | 0.007 | Yes |

***Concentration of oxygen in the different respirometer T3***

**Normality Test:** Passed (P = 0.322)

**Equal Variance Test:** Failed (P < 0.050)

| **Source of Variation** | **DF** | **SS** | **MS** | **F** | **P** |
|---|---|---|---|---|---|
| Treatment | 2 | 4.676 | 2.338 | 2.342 | 0.138 |
| respirometer | 3 | 124.742 | 41.581 | 41.650 | <0.001 |
| Treatment x respirometer | 6 | 23.641 | 3.940 | 3.947 | 0.021 |
| Residual | 12 | 11.980 | 0.998 | | |
| Total | 23 | 165.038 | 7.176 | | |

Least square means for Treatment:

| **Group** | **Mean** |
|---|---|
| 0.1000 | 11.775 |
| 0.0250 | 12.413 |
| control | 11.337 |

Std Err of LS Mean = 0.353

Least square means for respirometer:

| Group | Mean |
|---|---|
| 1.000 | 11.583 |
| 2.000 | 10.900 |
| 3.000 | 9.350 |
| blank | 15.533 |

Std Err of LS Mean = 0.408

Least square means for Treatment x respirometer:

| Group | Mean |
|---|---|
| 0.100 x 1.000 | 11.300 |
| 0.100 x 2.000 | 11.900 |
| 0.100 x 3.000 | 7.800 |
| 0.100 x blank | 16.100 |
| 0.025 x 1.000 | 11.150 |
| 0.025 x 2.000 | 12.200 |
| 0.025 x 3.000 | 10.250 |
| 0.025 x blank | 16.050 |
| control x 1.000 | 12.300 |
| control x 2.000 | 8.600 |
| control x 3.000 | 10.000 |
| control x blank | 14.450 |

Std Err of LS Mean = 0.707

All Pairwise Multiple Comparison Procedures (Student-Newman-Keuls Method):

Comparisons for factor: **respirometer within 0.1**

| **Comparison** | **Diff of Means** | **p** | **Q** | **P** | **P<0.05** |
|---|---|---|---|---|---|
| blank vs. 3.000 | 8.300 | 4 | 11.748 | <0.001 | Yes |
| blank vs. 1.000 | 4.800 | 3 | 6.794 | 0.001 | Yes |
| blank vs. 2.000 | 4.200 | 2 | 5.945 | 0.001 | Yes |
| 2.000 vs. 3.000 | 4.100 | 3 | 5.803 | 0.004 | Yes |
| 2.000 vs. 1.000 | 0.600 | 2 | 0.849 | 0.560 | No |
| 1.000 vs. 3.000 | 3.500 | 2 | 4.954 | 0.005 | Yes |

Comparisons for factor: **respirometer within 0.025**

| **Comparison** | **Diff of Means** | **p** | **Q** | **P** | **P<0.05** |
|---|---|---|---|---|---|
| blank vs. 3.000 | 5.800 | 4 | 8.209 | <0.001 | Yes |
| blank vs. 1.000 | 4.900 | 3 | 6.935 | 0.001 | Yes |
| blank vs. 2.000 | 3.850 | 2 | 5.449 | 0.002 | Yes |
| 2.000 vs. 3.000 | 1.950 | 3 | 2.760 | 0.167 | No |

| Comparison | Diff of Means | p | Q | P | P<0.05 |
|---|---|---|---|---|---|
| 2.000 vs. 1.000 | 1.050 | 2 | 1.486 | 0.314 | Do Not Test |
| 1.000 vs. 3.000 | 0.900 | 2 | 1.274 | 0.386 | Do Not Test |

Comparisons for factor: **respirometer within control**

| Comparison | Diff of Means | p | Q | P | P<0.05 |
|---|---|---|---|---|---|
| blank vs. 2.000 | 5.850 | 4 | 8.280 | <0.001 | Yes |
| blank vs. 3.000 | 4.450 | 3 | 6.299 | 0.002 | Yes |
| blank vs. 1.000 | 2.150 | 2 | 3.043 | 0.053 | No |
| 1.000 vs. 2.000 | 3.700 | 3 | 5.237 | 0.008 | Yes |
| 1.000 vs. 3.000 | 2.300 | 2 | 3.255 | 0.040 | Yes |
| 3.000 vs. 2.000 | 1.400 | 2 | 1.982 | 0.187 | No |

Comparisons for factor: **Treatment within 1**

| Comparison | Diff of Means | p | Q | P | P<0.05 |
|---|---|---|---|---|---|
| control vs. 0.025 | 1.150 | 3 | 1.628 | 0.503 | No |
| control vs. 0.100 | 1.000 | 2 | 1.415 | 0.337 | Do Not Test |
| 0.100 vs. 0.025 | 0.150 | 2 | 0.212 | 0.883 | Do Not Test |

Comparisons for factor: **Treatment within 2**

| **Comparison** | **Diff of Means** | **p** | **Q** | **P** | **P<0.05** |
|---|---|---|---|---|---|
| 0.025 vs. control | 3.600 | 3 | 5.095 | 0.010 | Yes |
| 0.025 vs. 0.100 | 0.300 | 2 | 0.425 | 0.769 | No |
| 0.100 vs. control | 3.300 | 2 | 4.671 | 0.006 | Yes |

Comparisons for factor: **Treatment within 3**

| **Comparison** | **Diff of Means** | **p** | **Q** | **P** | **P<0.05** |
|---|---|---|---|---|---|
| 0.025 vs. 0.100 | 2.450 | 3 | 3.468 | 0.073 | No |
| 0.025 vs. control | 0.250 | 2 | 0.354 | 0.807 | Do Not Test |
| control vs. 0.100 | 2.200 | 2 | 3.114 | 0.048 | Do Not Test |

Comparisons for factor: **Treatment within blank**

| **Comparison** | **Diff of Means** | **p** | **Q** | **P** | **P<0.05** |
|---|---|---|---|---|---|
| 0.100 vs. control | 1.650 | 3 | 2.335 | 0.263 | No |
| 0.100 vs. 0.025 | 0.0500 | 2 | 0.0708 | 0.961 | Do Not Test |
| 0.025 vs. control | 1.600 | 2 | 2.265 | 0.135 | Do Not Test |