International Journal of Biology; Vol. 6, No. 2; 2014
ISSN 1916-9671 E-ISSN 1916-968X
Published by Canadian Center of Science and Education

The Effect of Pollution on Scope for Growth in the Pearl Oyster, *Pteria aegyptiaca*, in the Gulf of Aqaba, Jordan

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Received: February 4, 2014 Accepted: March 2, 2014 Online Published: March 6, 2014

doi:10.5539/ijb.v6n2p120 URL: http://dx.doi.org/10.5539/ijb.v6n2p120

Abstract

This experiment was conducted to assess the effects of pollution on both the physiological and energetic characteristics (SFG) of *Pteria aegyptiaca* in the Gulf of Aqaba, Jordan. Four locations were chosen to represent both contaminated and uncontaminated locations. Phosphate Loading Berth (P) and the Industrial Area (I), represent contaminated locations, whilst the Hotels Area (H) and Marine Science Station (M) represent uncontaminated locations. The results of this experiment showed that contaminated regions had significant effects ($P < 0.05$) on respiration rate, clearance rate and absorption efficiency. A significant difference ($P < 0.05$) was identified between the scopes for growth of animals transplanted in contaminated regions compared with animals from uncontaminated locations. The Phosphate Loading Berth (P) and Industrial Area (I), showed the lowest SFG (18.7 ± 4.591 and 35.94 ± 7.412 Jh^{-1} respectively), reduced to only a half of SFG compared to uncontaminated locations. Results from our study indicate that oysters exposed to contaminated regions are highly compromised in both their energetics and physiology. Failure to improve the environmental conditions in these regions may have catastrophic effects on the micro and macro fauna.

Keywords: scope for growth, pollution, Gulf of Aqaba, *Pteria aegyptiaca*

1. General Introduction

The study of anthropogenic effects on the environment has been shown to impact a wide range of ecosystems. An example of which is the accumulation of heavy metals within marine environments (Luoma, 1996). The expansion of industry worldwide has resulted in an increase of chemical discharge from mining, metal refining and processing industries into coastal environments (Widdows & Donkin, 1992). Most of these pollutants have deleterious effects on the aquatic micro and macro fauna (Kennish, 1997).

The Gulf of Aqaba is highly abundant in marine organisms. Its tropical waters and diverse habitats are home of over 200 varieties of coral and more than 1000 species of subtropical fish (Atkinson et al., 2001; Alawneh, 1993). An increase in industrial activity and associated population has resulted in a flux of pollution into the estuarine and coastal environment. The pollution originates from a range of sources, including sewage, toxic materials from homes, factories, towns and cities (Phillips, 1980). Heavy metals and hydrocarbons are arguably the most toxic chemicals discharged into coastal environments. Sediments act as a large reservoir due to their ability to absorb heavy metals (Kennish, 1997). Phosphate, Diammonium phosphate fertilizer and potash are among the main sources of pollution in the Gulf of Aqaba. In 2012, the port of Aqaba exported ~ 5 × 106 tons of phosphate ore (PLC, 2012). During the phosphate loading process, the estimated quantity of phosphate ore lost as fine particulate matter is ~ 500 tons/year (Atkinson et al., 2001). It is thought that this may cause an increase in the available nutrient supply to macroalgae and cyanobacteria. It is speculated that the abundance of nutrients may cause a phase-shift, from coral to algal dominated communities (Kuffner & Paul, 2001; Done, 1992; Walker & Ormond, 1982). Many studies have shown that the rate of necrosis in coral colonies has increased significantly in in the phosphate-polluted areas (Walker & Ormond, 1982; Dunn et al., 2012).

Regions in the Gulf of Aqaba contain high levels of contaminants, which include phosphate, heavy metals and organocarbons (Youssef & El-Said, 2011; Abu-Hilal, 1987; Abu-Hilal & Badran, 1990). Pollution is detected in one of two ways. Firstly, contamination can be assessed directly by quantifying the amount of contaminants in the environment (Abu-Hilal, 1985; Abu-Hilal et al., 1988). The second method is to detect the direct effect of pollution on micro and macro fauna assemblages. Environmental stress has been found to affect the

physiological function of many organisms (Díaz-Jaramillo et al., 2013; Dunn et al., 2012). Bivalve molluscs appear to be one of the most suitable groups to study the environmental stress, and have been the subject of many studies (Phillips, 1980; Navarro et al., 2013; Oyarzún et al., 2013). Bivalves are typically used to assess environmental conditions because they are widespread, easily obtained in large quantities, and they are sedentary. In addition to this, bivalves are filter feeders, which means they pass large volumes of water, resulting in the accumulation of many pollutants. However, it is difficult to measure a bivalve molluscs' growth and production directly because a large proportion of the total production can be lost in the form of gametes (Rueda & Smaal, 2004). This issue is overcome by calculating the "scope for growth". The term scope for growth (SFG) was proposed and used by Warren and Davis (1967) to describe the energy retention and storage component in physiological energy balance studies (Warren & Davis, 1967). Scope for growth (SFG) has been employed throughout many studies (Widdows, 1990; Navarro et al., 2013; Oyarzún et al., 2013; Prieto et al., 2012).

The current study investigates the effects of contaminated sediment on the physiology of organisms living on or within sediments. We also aim to compare the scope for growth of *Pteria aegyptiaca* (Dillwyn, 1817) transplanted to regions contaminated with phosphate, heavy metals, compared with non-contaminated habitats. In order to calculate SFG, clearance rate, absorption efficiency and respiration was recorded for both treatments.

2. Material and Methods

2.1 Sample Preparation

The winged pearl oyster *P. aegyptiaca* byssally attaches to rocks, corals, and other hard objects. It dwells from low tide levels to a depth of 30 m (Kent, 1998; Mastaller, 1987). This species of oyster is collected as a food resource and is also used for pearl production (production of half-pearls or mabe) in many countries around the world (Alagarswami et al., 1989). Two hundred adult *P. aegyptiaca* with anteroposterior shell sizes of 150 - 200 mm were randomly collected from the Gulf of Aqaba. The animals were cleaned with filtered sea water (FSW) using a brush to remove epiphytic growths and particulate matter from the shell surface. The shells were tagged using super glue and shellfish ID badges. After the glue had dried, animals were placed in an aquarium tank with 60 litres seawater for three days to recover from handling.

2.2 Study Location and Experimental Design

The Gulf of Aqaba in the northern Red Sea is a warm water body, approximately 160 km (99 mi) long and 24 km (15 mi) wide, and attains a depth of about 1850 m in its central area. Four locations were selected to be representative of a contamination gradient (Figure 1). They were selected based on previous studies of the sources of contamination and chemical data (Al-Rousan et al., 2012; Al-Najjar et al., 2011). Phosphate Loading Berth (P) and Industrial Area (I) were chosen to represent contaminated locations. Hotels Area (H) and Marine Science Station (M) were chosen to represent uncontaminated locations. All these locations were chose to reflect the natural habitat of *P. aegyptiaca*. A nested sampling design was used in this study. To ensure sufficient replication, two sites were randomly selected within 20 meters of each other, and two cages were placed at each site.

2.3 Cages and Cockles Deployment

A total of sixteen plastic cages were deployed in July 2013. Cages were constructed of polypropylene mesh (mesh size 5×5 mm) and were barrel-like in structure with a diameter and height of 20 cm. The cages were embedded into the benthic sediment and fixed into place using tent pegs. Once the cages were deployed, 10 animals were placed in each cage. The cages were then secured to prevent the entry of any predators.

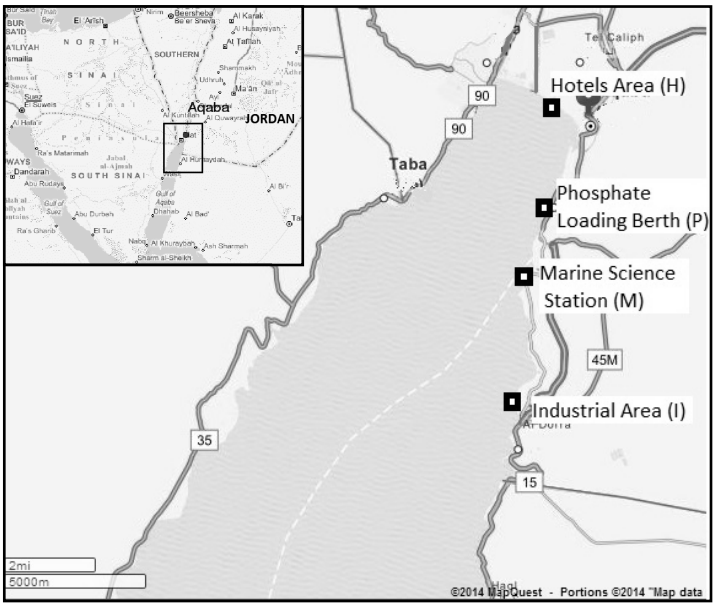

Figure 1. Map of four locations selected to be representative of a contamination gradient in the Gulf of Aqaba. Hotels Area (H), Marine Science Station (M), Phosphate Loading Berth (P), Industrial Area (I)

2.4 Physiological Measurements

Animals were collected for physiological measurement at the 29th of November 2012. Every second day animals from the four groups were collected and transferred under standard conditions to the laboratory. The oysters were housed in an aquarium containing 60 litres of recirculating sea water and were fed a mixture of algal cells. The mixture contained two species of algae, *Nannochloropsis* sp. and *Tetraselmis* sp. (Reed Mariculture Inc., USA). A twenty four hour recovery from aerial exposure, handling, and transport effects was allowed. Animals were carefully cleaned from fouling organisms. Five animals from each group were used for physiological measurement. Animals were only used in one measurement per day.

2.5 Measurements of Physiological Components of Scope for Growth

2.5.1 Clearance Rate and Consumed Energy

Clearance rate (CR) represents the volume of water cleared from particles per time unit measured in organisms from four locations. Seven plastic buckets each containing with 1 liter of filtered sea water (FSW) and a single oyster were used. A control bucket (without any animals) representing "treatment" was also used. The initial algal concentration in the bucket was relatively low, ~ 30000 cells / ml, in order to avoid the formation of the pseudo faeces. Water was gently aerated to keep the algae in suspension. Two samples were collected from each bucket every 30 minutes for 150 minutes. Cell concentrations were recorded as the average of two counts using a cell counter (hemocytometer). Clearance rate or the volume of water cleared of particles per unit of time (Lh^{-1}) by the animal was calculated using the following the equation (Coughlan, 1969):

$$CR = V (\ln C_1 - \ln C_2) / t$$

Where CR is the clearance rate, V is the volume of water used, C and C_2 are the cell concentrations between two sampling times, and t is the time in hours (h).

The amount of consumed energy (C) can be calculated for each animal by multiplying the clearance rate (CR) by the amount of organic material (POM) per litre in each bucket at the beginning of each run and with the energy content of algal material, 23 J/Mg/l (Widdows et al., 1995). After the clearance rate was measured, the animals were returned to the aquarium to collect faeces for the absorption efficiency determination.

The amount of particulate organic material (POM) per 10,000 algae was determined according to Oyarzún et al. (2013).

2.5.2 Respiration Rate and Respired Energy (R)

Respiration rate is often used to indicate stressful conditions and can be accurately measured. Rates of oxygen consumption were measured for each animal in closed and sealed respirometers of 600 ml. The decline in the oxygen rate within the chamber was recorded with a calibrated oxygen microelectrode inserted in the respirometer and connected to an Oxygen meter (Professional Dissolved Oxygen meter HD3030, Trans). Control measurements (without animal) were performed to ensure no drifting was caused by other factors. Chambers were completely filled with FSW and provided with magnetic stirring to ensure even distribution of oxygen. Oxygen run lasted for about 1.5 h. Each run contained a random mixture of animals (treatment and control) collected from different sites. The rate of oxygen consumption was then calculated using the following equation:

$$R = [Ct_0 - Ct_1] \times V \times 60 / (t_1 - t_0)$$

Where R is the rate of oxygen uptake ($\mu MO_2 l^{-1}$), Ct is the concentration of oxygen in the water ($\mu MO_2 l^{-1}$) at time t, V is the volume of the water in the respirometer and t0 and t1 the initial and the end times (in minutes) of the measurement period.

2.5.3 Absorption Efficiency

The absorption efficiency (AE) represents the efficiency with which organic material is absorbed from ingested food material. To calculate the AE, the organic content of food and faeces need to be determined. The faeces of each animal were collected at the end of each experiment using 5 ml syringe. The collected faeces were moved to the centrifuge tubes and centrifuged at 6000 rpm for 10 minutes. The faecal matter was then washed with ammonium formate (38%) to remove salt. The collected faeces were then centrifuged at 6000 rpm for 10 minutes. The pellets were transferred to pre-weight (W_1) platinum crucibles and dried for 48 hours at 80 °C and transferred to a desiccator to cool to meet the room temperature and weighed (W_2). The samples were burnt overnight at 500 °C and weighed (W_3) after they cooled to room temperature. The amount of organic material [(W_2-W_3) / (W_2-W_1)] was calculated. The absorption efficiency (AE) was calculated using the method of Conover (1966):

$$AE = (F - E) / [(1 - E) \times F]$$

Where F is the organic content of the food ingested and E is the organic content of the faeces.

2.5.4 Correction for Body Weight

Body weight is one of the important variables that affect most physiological responses. This effect can be removed by transplanting animals of similar body size and weight. After the physiological variables were measured, the animals employed in the experiments were dissected and the soft tissue were dried at 70 °C for 48 hours and then cooled and weighed. After that, they were burnt to ash overnight at 500 °C and weighed again.

All physiological rates were standardised to 1 gram dry weight following the formula of Bayne et al. (1987).

$$Y_s = (W_s / W_e)^b \times Y_e$$

Where Y_s is the physiological rate of an animal of standard weight, W_s is the standard weight of animal, Y_e is uncorrected (measured) physiological rate, W_e is the observed weight of the animal, and *b* is the exponent of allometric relationship between body mass And the rate of the physiological parameter. *b* = 0.66 was used for respiration rate and clearance rate.

2.5.5 Calculation of Scope for Growth

The measured physiological rates were converted into energy equivalents ($J.h^{-1}$) and used in the balanced energy equation to calculate the energy available for growth and reproduction (SfG). Energy available for growth and reproduction was calculated by using the following equations:

(C) is the energy consumed or ingested

$$C = CR \times (mgPOMl^{-1}) \times (23\ J\ mg^{-1}\ POM)$$

(A) is the energy absorbed A= (C) × absorption efficiency (R) is the energy respired where R = (µmoles O_2 $g^{-1}h^{-1}$) × 0.456

Where the heat equivalent of oxygen uptake is 0.456 J µmoles^{-1} O_2

(U) is the energy excreted

$$U = (\mu moles\ NH_4\text{-}N\ h^{-1}) \text{ is equivalent to an energy of } 0.349\ Jh^{-1}$$

The rate of ammonia excretion is usually closely coupled to the respiration rate and forms a small proportion of metabolic energy expenditure. Therefore it was omitted from physiological energetic measurements and the calculation of scope for growth: (P) is the Scope for Growth

$$P = A - R$$

2.6 Statistical Analyses

One way analysis of variance (ANOVA) were carried out to test for treatment or location within treatment effects. The Newrnan-Keuls test was used to investigate which treatments were different and result in significant differences. No data transformation was used because the Cochran's test was not significant for all the data.

3. Results

3.1 Clearance Rate

As shown in Figure 2, the clearance rates of animals from the Phosphate Loading Berth (P) and the Industrial Area (I) (contaminated locations) were ~38% lower than the rates of animals from Hotels Area (H) Marine Science Station (M) (uncontaminated locations). This reduction was statistically significant (Figure 2) compared to (H), (M) and (I). The (P) was found to have the lowest feeding rate (0.6505 ± 0.2025).

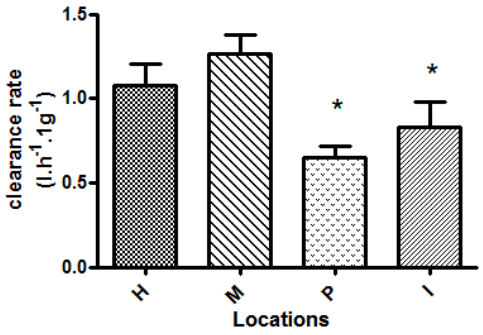

Figure 2. Clearance rate (mean ± SE) of *Pteria aegyptiaca* caged in four locations in the Gulf of Aqaba. Hotels Area (H), Marine Science Station (M), Phosphate Loading Berth (P), Industrial Area (I). n = 5, bars = SEM. (*) indicates a significant difference from the uncontaminated locations (H) and (M) ($P < 0.05$)

3.2 Absorption Efficiency

Absorption efficiencies of animals in the four locations were not significantly different (Figure 3). In addition, no significant effects were identified in the absorption efficiency of the "treatment" oysters. The average AE value was ~ 0.595 ± 0.017 (mean ± SE). But the animals from the (P), showed the lowest AE (0.4068 ± 0.056).

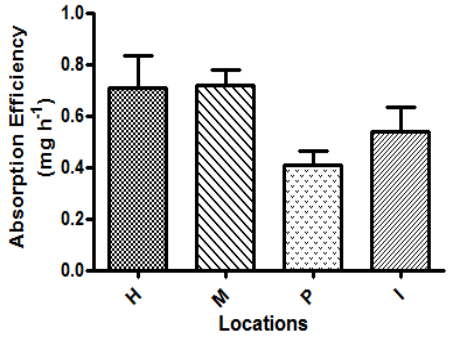

Figure 3. Absorption rate (mean ± SE) of *Pteria aegyptiaca* caged in four locations in the Gulf of Aqaba. Hotels Area (H), Marine Science Station (M), Phosphate Loading Berth (P), Industrial Area (I). n = 5, bars = SEM. (*) indicates a significant difference from the uncontaminated locations (H) and (M) ($P < 0.05$)

3.3 Respiration Rate

Animals which were collected from (H) and (M) area showed similar rate of respiration (Figure 4). The difference between the controls vs. the treatment groups was significant. Animals at (I) showed the lowest respiration rates 12.96 ± 1.602 (mean ± SE).

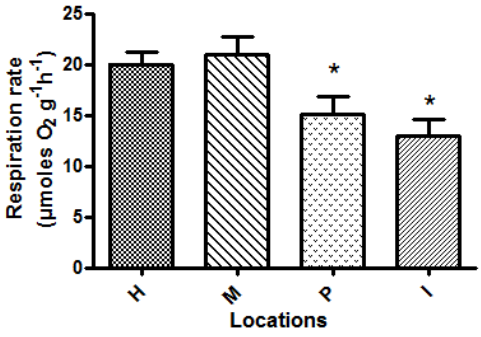

Figure 4. Respiration rate (mean ± SE) of *Pteria aegyptiaca* caged in four locations in the Gulf of Aqaba. Hotels Area (H), Marine Science Station (M), Phosphate Loading Berth (P), Industrial Area (I). n = 5, bars = SEM. (*) indicates a significant difference from the uncontaminated locations (H) and (M) ($P < 0.05$)

3.4 Scope for Growth (SFG)

Figure 5 demonstrates that positive values of SFG were recorded in all locations ranging between 18.7 to 77.25 J h^{-1}. In this study, a significant difference ($P < 0.05$) was found between the SFG of animals from (H) and (M) area and the animals from (P) and (Table 1). Even though the SFG was positive for all groups, oysters from the uncontaminated locations had SFG values twice as high as animals from contaminated locations.

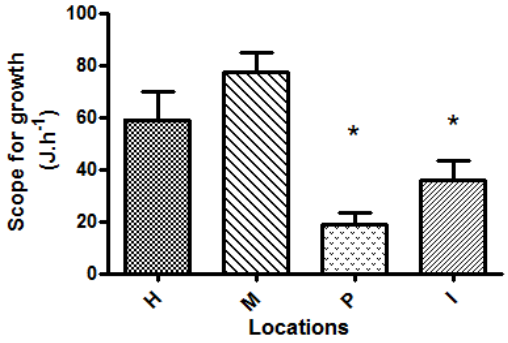

Figure 5. Scope for growth (mean ± SE) of *Pteria aegyptiaca* caged in four locations in the Gulf of Aqaba. Hotels Area (H), Marine Science Station (M), Phosphate Loading Berth (P), Industrial Area (I). n = 5, bars = SEM. (*) indicates a significant difference from the uncontaminated locations (H) and (M) ($P < 0.05$)

Table 1: Newman-Keuls Multiple Comparison Test

Newman-Keuls Multiple Comparison Test	Mean Diff.	Q	Significant? P < 0.05?	Summary
P VS M	-58.55	7.397	Yes	***
P VS H	-40.38	5.101	Yes	**
P VS I	-17.24	2.178	No	ns
I VS M	-41.31	5.219	Yes	**
I VS H	-23.14	2.923	Yes	*
H VS M	-18.17	2.296	No	ns

Results of Newman-Keuls Multiple Comparison Test, testing the effects of pollution, Scope for growth (SFG) of *Pteria aegyptiaca* caged in four locations in the Gulf of Aqaba. Hotels Area (H), Marine Science Station (M), Phosphate Loading Berth (P), Industrial Area (I). (*) indicates a significant difference.

4. Discussion

This experiment was conducted to assess how heavy metal stressors affect the physiological and energetic functions in *P. aegyptiaca*. Results from this study indicate that animals transplanted to contaminated locations have lower SFG compared to the uncontaminated locations. The animals at (M) were found to have faster clearance rates than animals from (H), (P) and (I). Both the Phosphate Loading Berth (P) and (I) had the lowest clearance rates. The clearance rate is an important component in the calculation of the energy budget and is very sensitive to a wide range of chemical contaminants and stressors (Echevarria et al., 2012; Sanders et al., 2013; Widdows et al., 1990; Sarà et al., 2000). It has been demonstrated that the reduction in energy acquisition is primarily due to a reduced clearance rate and enhanced respiration rate (Widdows & Page, 1993; Widdows et al., 1990).

The most significant difference in physiological responses recorded was the clearance rate. Scope for growth showed the pattern similar to that of clearance rate; therefore measuring SFG is occasionally simplified to measuring clearance (Ostroumov & Widdows, 2006; Donkin et al., 1989; Donkin et al., 1997). However, the determination of clearance rate as a single physiological parameter does not have the same power of discrimination as the more complete and integrated measure termed SFG. Many studies indicate that clearance rate does not depend on the changes in many environmental factors including water temperature and salinity (Sarà et al., 2000). In addition to that, these studies have shown that CR is influenced by seasonal changes in terms of the quality and quantity of available food (Sarà et al., 2000).

A significant decrease in respiration rate observed in oysters from the (P) and (I). This decrease may result of reduction in feeding activity. A similar pattern of respiration rate has been described in many bivalve species, such as Cerastoderma edule (Nilin et al., 2012). However, the animal response may vary depending on species, pollutant type and concentration (Bayne, 1980; Widdows et al., 1990). Results of this study do not agree with those of Burt et al. (2007) who showed an increase in the oxygen consumption after 90 days of exposure in

Anadara trapezia that were transplanted in metal contaminated location (Burt et al., 2007). Wang et al. (2005) also showed that respiration rate is less sensitive than other physiological responses (Wang et al., 2005).

The feeding response provides an initial indication of physiological stress which may lead to growth retardation (Sheehan, 1984). The treatment sites were shown to have a significant effect on the absorption efficiency. The efficiency with which food material is absorbed by the digestive system can be altered by many environmental factors, the type of and condition of the animal, the quantity and organic content, and the quantity and quality of food (Newell & Bayne, 1980; Bayne et al., 1993). Animals at all locations had a similar absorption efficiency, which can be explained by the insensitivity of absorption efficiency for the pollutant. The absorption efficiency was found relatively high (H: 0.7095 ± 0.1256, M: 0.7178 ± 0.06241, P: 0.4068 ± 0.05622, I: 0.5397 ± 0.09113 mg h^{-1}) in this experiment in all locations. These values can be interpreted by a good quality and quantity of food in the Gulf of Aqaba. Several bivalve species have also demonstrated that absorption is independent of seston concentration; however, it is dependent on the seston quality, or percentage of organics (Bayne et al., 1993; Denis et al., 1999). These results are not comparable to results obtained in other studies, where the absorption efficiency decreased in the presence of contaminants (Widdows et al., 1987).

The values for SFG shown in this study were relatively high when compared with those published by others (Navarro & Gonzalez, 1998; Navarro & Contreras, 2010). Negative SFG values indicate that the animals are under severe stress conditions where they do not gain enough energy to survive (Bayne et al., 1985). The mean value for SFG was 47.75 ± 10.03 $J.h^{-1}$. It can be concluded that the animals were under mild stress in all the locations and we can explain that by the low level of the contaminants in all the locations. The SFG of animals living at (M) and (H) were significantly higher than animals at (P) and (I). This may be due to the relatively high levels of phosphate and heavy metals in (P) and (I). The observed decline in SFG of animals transplanted in the contaminated locations was mainly due to a slower clearance rate. Differences between treatments were significant (Figure 5). These differences may be explained by the low value of SFG for the oysters at (P). The value was low in comparison with those at other locations. That may be due to the presence of disturbance; at this location there was high concentration of phosphate and also a large number of ships, and as a result, two cages were lost at this location but not all the animals were found dead but embedded in the sediment.

5. Conclusions

Physiological responses can be used as general indices to demonstrate the effects of pollution as they are directly associated with the health of individual animals (Bayne, 1980). The results of this experiment showed that contaminated regions had significant effects ($P < 0.05$) on respiration rate, clearance rate and absorption efficiency However, it would be useful to examine the effect of physical disturbances on the SFG. Concentrations of contaminants in the tissues of the animals should also be examined to determine how they correlate to Scope for Growth.

Acknowledgements

This research was funded by Al-Hussien Bin Talal University in Jordan. I would like to express my deep gratitude to Dr. Saber Al-Rousan for the invaluable assistance in collecting and processing specimens.

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