Effect of transplantation on heavy metal concentrations in commercial clams of Lake Timsah, Suez Canal, Egypt

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Howaida R. Gabr
Ali A-F. A. Gab-Alla

Marine Sciences Department,
Faculty of Science,
Suez Canal University,
41552 Ismailia, Egypt;

e-mail: hgabr_suezcanal@yahoo.com; aligaballa2000@yahoo.com

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Abstract

Ruditapes decussatus and *Venerupis pullastra* are commercially fished clams with a wide distribution in the shallow inshore waters of Lake Timsah, Egypt. They are usually contaminated with heavy metals. Consumption of such contaminated clams can pose a public health risk. To minimize this risk, therefore, the clams should be removed from the contaminated waters and transferred to an approved area to reduce the high levels of metals before being marketed. The aim of this work was to study the effect of transplantation on levels of heavy metals (Fe, Mn, Zn, Cu, Ni, Co, Cd, Pb) in these clams. The clams were removed from their polluted site and transplanted to a relatively clean area for a period of 120 days. Although the salinity at the transplantation site was higher than at the polluted site, it was stable and did not appear to have any adverse effect on clam growth. Heavy metals were analysed in the water, sediment and clam tissues from both the polluted and the transplantation sites. Although in both species transplantation evidently reduced heavy metal levels, these still exceeded the maximum permissible levels laid down by the WHO (1982).

The complete text of the paper is available at http://www.iopan.gda.pl/oceanologia/

1. Introduction

Contamination of bivalve shellfish (e.g. oysters, clams, mussels and cockles) is a major food safety concern (Bella & Tam 2000), so suppliers and retailers need to be sure that the products they sell are safe. Bivalves respond to changes in concentrations of contaminants in water, and they integrate contaminants from the water column over time (De Kock & Kramer 1994, Gunther & Davis 1997, Gunther et al. 1999). Shellfish contamination is caused, among other things, by the discharge of chemical substances such as metals, pesticides and organochlorine compounds from industrial and municipal treatment processes. Contaminated mollusc shellfish (oysters, clams and mussels) may cause illness in humans (Bella & Tam 2000); therefore, to protect public health, it is mandatory that shellfish be harvested from approved shellfish waters where protective standards have been met.

The risk associated with contaminated shellfish can only be reduced in two ways: by relaying (transplantation) in clean water and by depuration (Richards 1988). The practice of relaying involves moving contaminated shellfish from polluted areas to clean areas and allowing the animals sufficient time to purge themselves of pathogens or chemicals. Shellfish are collected and re-planted over existing natural beds for at least one spawning season. Depuration is the term applied to the purification, under controlled conditions, of shellfish harvested from moderately contaminated areas. The process generally involves holding the shellfish in tanks of flowing seawater for periods of 48 to 72 hours, the seawater being sterilised by ultraviolet light. Shellfish harvested from moderately contaminated areas can normally purge themselves of bacterial contaminants, but this process has not been successful in purging shellfish of heavy metals (Arnold 1991). Although the scientific literature suggests that metal depuration is possible, the mechanics of this process have not been adequately addressed (Han et al. 1993). Metal depuration rates vary widely, showing diverse ranges for the same metal within different bivalve species and for different metals within the same species (Richards 1988).

Ruditapes decussatus and *Venerupis pullastra* are clams widely distributed in high densities in the shallow inshore waters of Lake Timsah (Gabr 1991, Kandeel 1992). In Egypt, these clams are greatly appreciated by seafood consumers. In the last decade, the importance of *R. decussatus* has been increasing in terms of landing volumes, economic value and relative importance among other marine resources. The retail price for this species in 2007 was 20 LE / kg. At that price, this species is expensive for the regular Egyptian consumer but has a high value as an export commodity. *V. pullastra* is consumed in the local Egyptian market and, despite being heavily fished, is still abundant in Lake Timsah. Recently, exports of *R. decussatus* were halted owing to contamination with heavy metals and bacteria. Moreover, the clam industry has suffered a decline in sales in the local market as a result of heightened publicity being given to clam-related illnesses. Heavy metals, among other contaminants, are present in high concentrations in industrial effluents discharged into Lake Timsah (Mourad 1996). In contrast, some sites (e.g. transplantation sites) on the banks of the Suez Canal are thought to be less exposed to industrial effluents, although they have a relatively higher salinity than Lake Timsah. Therefore, the aim of this study was to test whether the salinity of such a transplantation site is suitable for growing *R. decussatus* and *V. pullastra* and to determine the effect of transplantation on heavy metal levels (Fe, Mn, Zn, Cu, Ni, Co, Cd and Pb) in the tissues of the studied clams.

2. Material and methods

2.1. Study area and experimental design

The clam species *Ruditapes decussatus* and *Venerupis pullastra* were collected from their location in Lake Timsah for transplantation to a pond belonging to a private commercial hatchery located 11 km north of Ismailia, near El Qantara (Figure 1). The water is supplied from the Suez Canal. The seawater temperature in the pond varies seasonally from a maximum of 30°C in August to a minimum of 14°C in January, and salinity ranges from 43 PSU in September to 47 PSU in August. It has an average pH of 7.9 and an average dissolved oxygen content (DO) of 10.03 mg dm^{-3} (unpublished data). By comparison, the temperature in Lake Timsah varies seasonally from a maximum of 28°C in August to a minimum of 15°C in January, and the salinity ranges from 36 PSU at some sites to 44 PSU at others, depending on the freshwater input. It has an average pH of 7.9 and an average DO of 8.03 mg dm^{-3} (Madkour et al. 2006).

The collected samples were transferred to the transplantation site within 24 hours. The experiment started on 9 December 2006. *R. decussatus* ranged in size from 1.41 to 2.2 cm, *V. pullastra* from 1.47 to 2.46 cm. The clams were sampled at time $T = 0$ (the beginning of transplantation) and harvested on 6 April 2007, $T = 120$ days (the end of transplantation). Immediately after harvesting, the total weight (shell + meat) of each individual clam was measured to the nearest 0.1 g, and the shell length (SL, the longest shell dimension) of each individual clam was determined to the nearest 0.1 mm using Vernier callipers. The soft tissues of 30 individuals from each site were carefully removed by deshelling the bivalves with a plastic knife, then freeze-dried (O'Connor 1996) and pulverised to a uniform particle size before analysis (Vale et al. 1985).

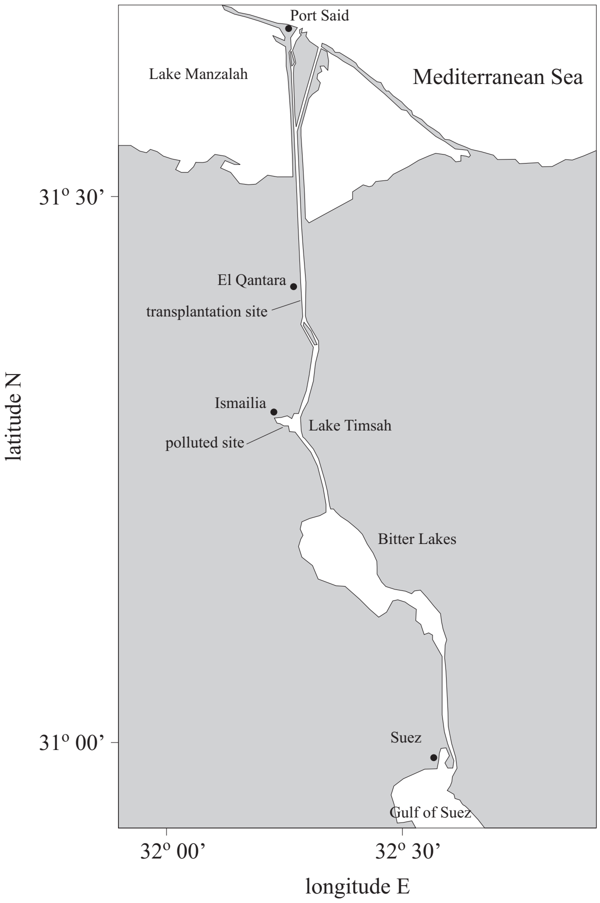

Figure 1. Map showing the polluted and transplantation sites on the Suez Canal

The length-weight relationship for the two species was determined according to the equation $W = a\,L^b$, where W is the total body weight [g] and L is the body length [cm], and a and b are constants.

Samples of water (0.5 m depth) and sediment (grain size fraction < 63 μm) were collected three times from the original location and the transplantation site to determine background levels of metals in the two habitats. The total metal content in seawater was determined following the method of Brewer et al. (1969). The metals were pre-concentrated from seawater using ammonium pyrrolidine dithiocarbamate (APDC) as chelating agent and after extraction were dissolved in methyl isobutyl ketone (MIBK). The organic extract was then aspirated directly to a flame atomic absorption spectrophotometer to determine the metal concentrations.

The sediments were dried at room temperature, then stored in plastic bags until analysis. Sediment samples of 0.5 g were digested with a mixture of concentrated acids (HNO_3, $HClO_4$ and HF) in Teflon vessels for 2 h according to the method described by Origioni & Aston (1984).

The powdered clams were digested with concentrated HNO_3 in high-pressure vessels in a microwave oven (FAO 1976). The metals – Cd, Cu, Zn, Fe, Mn, Ni, Co and Pb – were analysed in a flame atomic absorption spectrophotometer (Perkin Elmer Atomic Absorption Analyst 100). Deuterium background correction was used when necessary.

3. Results

3.1. Monthly variation in growth

Bivalve growth rate may be the best indicator of physico-chemical effects at the present transplantation site. Tables 1 and 2 set out the respective monthly variations in mean total length and mean total wet weight for *Ruditapes decussatus* and *Venerupis pullastra*. For both species, total length and total weight increased slightly from December to February, but there were more pronounced increases in March and April. The average monthly increment over five months was marginally higher (73.4%) for *R. decussatus* than for *V. pullastra* (72.95%).

Table 1. Monthly variations in mean total length [cm] and mean total wet weight [g] in the clam *Ruditapes decussatus* during the transplantation period

Month	Mean length ± SD	Mean weight ± SD
December	1.99 ± 0.20	1.48 ± 0.15
January	2.05 ± 0.35	1.64 ± 0.10
February	2.29 ± 0.27	1.80 ± 0.17
March	2.43 ± 0.30	2.35 ± 0.20
April	2.71 ± 0.28	3.50 ± 0.25

SD – standard deviation.

Table 2. Monthly variations in mean total length [cm] and mean total wet weight [g] in the clam *Venerupis pullastra* during the transplantation period

Month	Mean length ± SD	Mean weight ± SD
December	1.99 ± 0.47	1.25 ± 0.12
January	2.10 ± 0.19	1.41 ± 0.13
February	2.17 ± 0.21	1.51 ± 0.10
March	2.30 ± 0.25	2.35 ± 0.19
April	2.73 ± 0.25	2.47 ± 0.20

SD – standard deviation.

3.2. Length-weight relationships

Figures 2 and 3 illustrate the relationship between shell length and total wet weight for *R. decussatus* and *V. pullastra*. The regression formula for *R. decussatus* was $W = 0.1719L^{2.83}$, indicating that body weight was a negative allometric function of the total length, as the value of b (2.83) was < 3. In contrast, the regression formula for *V. pullastra* was $W = 0.1463L^{3.15}$, indicating that body weight was a positive allometric function of the total length, as the value of b (3.15) was > 3. The respective correlation coefficients for *R. decussatus* and *V. pullastra* were high: $r^2 = 0.85$ and 0.97 at $p \leq 0.001$.

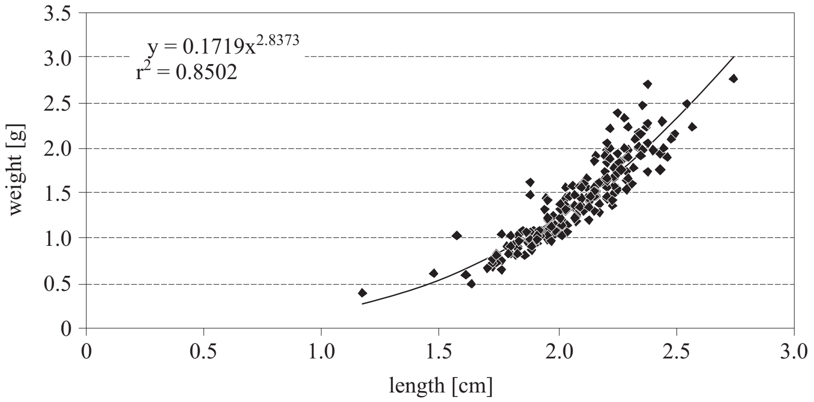

Figure 2. Relationship between shell length and total wet weight of transplanted *Ruditapes decussatus*

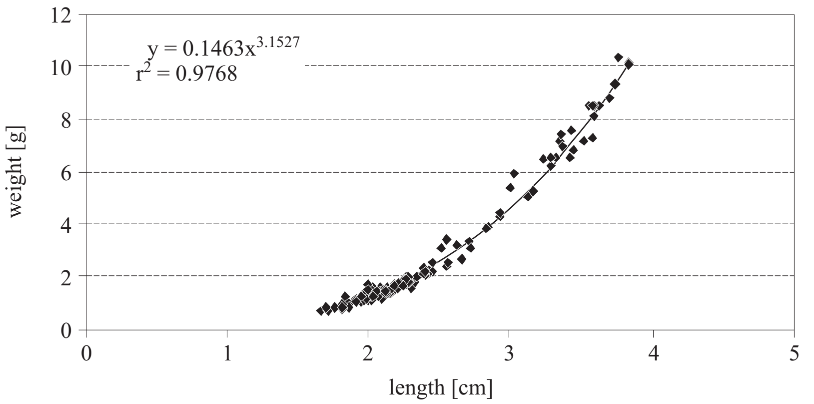

Figure 3. Relationship between shell length and total wet weight of transplanted *Venerupis pullastra*

3.3. Measurement of heavy metals

As expected, concentrations of all the heavy metals in water were significantly higher ($p < 0.001$) at the original site than at the transplantation site (Tables 3 and 4). Metal levels in the sediments displayed the same trend, being significantly higher ($p < 0.001$) at the original site than at the transplantation site for the same metals (Tables 3 and 4).

Table 3. Mean concentrations of heavy metals ± standard deviation (ppm dry weight, $n = 3$) in clams, sediment and water from the contaminated and transplantation sites

Element	Contaminated site			
	Ruditapes decussatus	*Venerupis pullastra*	sediment	water
Fe	961.5 ± 7.0	1896.5 ± 5.0	6.5 ± 0.5	1.27 ± 0.01
Mn	24.0 ± 1.0	26.5 ± 1.0	14 ± 0.5	0.09 ± 0.01
Zn	155.6 ± 2.0	132.2 ± 5.0	62.75 ± 2.0	0.17 ± 0.02
Cu	23.8 ± 1.0	23.2 ± 1.7	8.9 ± 0.5	0.10 ± 0.01
Ni	21.5 ± 2.0	27.5 ± 1.8	20.5 ± 1.0	0.54 ± 0.03
Co	23.5 ± 2.0	30.5 ± 1.5	27 ± 1.0	0.45 ± 0.02
Cd	4.5 ± 0.5	8.7 ± 0.5	1.3 ± 0.1	0.15 ± 0.001
Pb	15.5 ± 1.0	18.5 ± 0.5	14.5 ± 1.5	0.31 ± 0.01
	Transplantation site			
	Ruditapes decussatus	*Venerupis pullastra*	sediment	water
Fe	609.5 ± 6.0	600.5 ± 4.0	3.73 ± 0.3	0.96 ± 0.10
Mn	18 ± 1.7	24.5 ± 3.0	14.0 ± 1.3	0.07 ± 0.01
Zn	103 ± 5.0	120.7 ± 4.0	58.3 ± 2.3	0.09 ± 0.01
Cu	15.9 ± 1.7	16.65 ± 1.5	8.8 ± 1.1	0.06 ± 0.01
Ni	18 ± 1.5	21.5 ± 1.3	14.5 ± 1.5	0.28 ± 0.10
Co	23.5 ± 1.2	23.5 ± 2.0	23 ± 2.0	0.27 ± 0.10
Cd	2.35 ± 0.3	1.9 ± 0.3	1.25 ± 0.2	0.03 ± 0.01
Pb	14.5 ± 1.0	17.0 ± 0.6	13.5 ± 1.5	0.17 ± 0.03

Table 4. One-way analysis of variance for heavy metals (in water, sediment, *Ruditapes decussatus* and *Venerupis pullustra*) recorded between the polluted and transplantation sites (degree of freedom, $df = 7$)

p-value	F-ratio	Source of variation
		Heavy metals
< 0.0001	16.658	in water
< 0.0001	137.563	in sediment
< 0.0001	18.284	in *R. decussatus*
< 0.05	3.532	in *V. pullastra*

p-value ≤ 0.05 – significance level.

However, heavy metal levels in the tissues of *R. decussatus* and *V. pullastra* were significantly lower ($p < 0.001$ and $p < 0.05$ respectively) at the transplantation site than at the contaminated one (Tables 3 and 4).

4. Discussion

This research has demonstrated that spats of the clams *Ruditapes decussatus* and *Venerupis pullastra* can be grown successfully in water with a salinity range between 43 PSU and 47 PSU, a temperature range between 14 and 28°C, an average pH of 7.9 and an average DO of 10 mg dm^{-3} . Most of the growth (increases in length and wet weight) in both *R. decussatus* and *V. pullastra* took place in March and April. These observations are in agreement with those of Paillard (1992) and Soudant et al. (2004) on the growth of *V. philippinarum*, and those of Laruelle et al. (1994) on the growth of *V. philippinarum* and *V. decussatus* in Brittany. Temperature and food availability are the two most important parameters having a direct influence on bivalve mollusc growth (Sastry 1979, Maitre-Allain 1982, Beninger & Lucas 1984, Bodoy et al. 1986, Laing et al. 1987). The temperatures recorded in the current study are considered optimum for the culture of *R. decussatus* according to Laing et al. (1987) and Jara-Jara et al. (1997), who found that the growth-rate coefficients for *R. decussatus* juveniles were greatest at 15–25°C. Other factors such as salinity and oxygen can affect growth, but being very stable, they do not appear to have had any significant effect on our results.

As expected, heavy metal concentrations in the sediments greatly exceeded those in the surrounding water. In aquatic environments, heavy metals discharged from industrial or sewage effluents or from atmospheric deposition may be rapidly removed from the water column and transported to the bottom sediments (Förstner & Wittmann (eds.) 1981, Fung & Lo 1997). Consequently, metal concentrations in sediments are often several orders of magnitude higher than those in the ambient seawater (Luoma 1990, Kit Chong & Wang 2000). The results of the heavy metal analyses in water and the species under study illustrate the process of biomagnification across the trophic levels, where the concentrations of the contaminated elements in water are lower than those in the tissues of *R. decussatus* and *V. pullastra*.

The change in metal levels between the contaminated and transplanted clams was clearly associated with the change in water and sediment metal levels. Concentrations were evidently lower in the tissues of the transplanted clams, which is indicative of the effect of the levels of metals in water on their accumulation. Because shellfish have a filter feeding mechanism, they can accumulate chemical and/or bacteriological pollutants and naturally occurring toxins from the surrounding waters even at a considerable distance from pollution sources (Gunther et al. 1999).

Heavy metal concentrations in whole soft tissues of the clams *R. decussatus* and *V. pullastra* from Lake Timsah are consistent with previous studies (Mourad 1996). Nevertheless, the levels of some metals in the transplanted species, namely, Zn, Cu, Cd and Pb, exceeded the maximum permissible levels (MLPs) of 100 μg g^{-1} Zn, 10 μg g^{-1} Cu, 2 μg g^{-1} Cd and 5 μg g^{-1} Pb laid down by the WHO (1982) (see also Wang et al. (2005)). However, since every state has specific legislation regarding bivalves, heavy metal concentrations should also be subject to legal control in Egypt.

In conclusion, Suez Canal clams have a high commercial value locally and as an export commodity. Therefore, to ensure the ongoing viability and future prosperity of this clam fishery, a food safety programme needs to be implemented. This should provide sanitary surveys of each shellfish harvesting area prior to its approval as a source of clams for the consumer. The purpose of such a sanitary survey is to identify and evaluate the factors affecting the sanitary quality of shellfish harvesting areas. These factors may include sources of potential and actual pollution, the bacterial quality of the water and shellfish, and the hydrographic characteristics of the harvesting areas. New areas along the Suez Canal could be opened for shellfish transplantation, including those with a higher salinity but lower heavy metal concentrations than in Lake Timsah. The recorded salinity does not seem to have had any great effect on the growth of *R. decussatus*, and *V. pullastra*, though further studies are required to confirm this. Smaller sizes and more time are needed to allow the clams to self-cleanse, thereby minimising heavy metal levels.

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