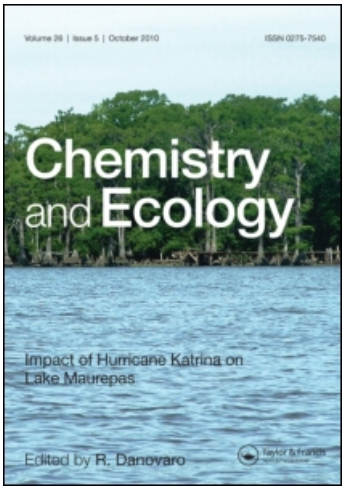

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The uptake of fluoride concentration and its effects on the growth rate of shrimps (*Palaemon elegans*, Rathke)

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The uptake of fluoride concentration and its effects on the growth rate of shrimps (*Palaemon elegans*, Rathke)

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The study was directed to monitor fluoride overload in aquatic organisms. Special attention was given to monitor fluoride accumulation as well as its effects on shrimps (*Palaemon elegans,* Rathke). Fluoride accumulation in shrimp tissues showed a growth inhibition effect, which is confirmed by the statistical analyses of the data. The higher percentage growth increment value of shrimps (132.48%) is obtained in the natural seawater of Eastern Harbor (control). Insignificant variations in % growth increments in the different fluoridated seawaters (0.5, 1, 2, 4, 8, 16, and 32 ppm) are detected. Additionally, accumulated fluoride in exoskeleton and muscles of shrimps didn't release to the surrounding seawater in which shrimps lived. Interestingly, even when the fluoridated seawater was replaced by a fresh natural one, there was no release of the accumulated fluoride from these tissues to the water.

Keywords: fluoride uptake; shrimp; growth; Eastern Harbor; Egypt

1. Introduction

Seafood is a major source of fluoride for humans. Actually, 2.5 mg F^-/mg is required to ensure optimal growth and normal incisor pigmentation [1]. However, long term exposure or intake of fluoride can lead to fluorosis with symptoms such as changes in the bone structure and it may also lead to enzyme inhibition [1]. Acute oral exposure to fluoride may produce effects including nausea, vomiting, abdominal pain, diarrhea, fatigue, drowsiness, coma, convulsions, cardiac arrest and even death [1]. Calcification of muscles, osteosclerosis and decreased production of erythrocytes may also develop as a result of long term exposure [1]. It can also affect the metabolism, growth and reproduction and other physiological process [2].

Fluoride is considered as one of the major constituents of seawater [1]. Fluoride concentrations in unpolluted seawaters range from 1.2–1.5 mg F^-/l [3]. Values of 1.36×10^{-3}, 1.36×10^{-3} and 3.10×10^{-3} g/l fluoride content were recorded at Mostafa Kamel, Eastern Harbor and El Mex sectors along the coastal seawater of the Mediterranean of Alexandria during December 1996, respectively [4]. Fluoride concentration in Lake Edku ranged between 0.00 and 10.28×10^{-4} g/l during 1996–1997 [5]. The ranges of fluoride content in macrophytes, algae and muscles of

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fish samples collected from the Egyptian coast of the Mediterranean Sea during 2000–2001 were 29.79–34.30, 19.17–53.70 mg/g and 1.875–3.379 μg/g, respectively [6]. Similarly, the ranges of fluoride concentration in crustacean, gastropod, bivalve, echinoderm and byrozoa samples ranged between 80.59–120.46, 4.45–7.62, 4.50–5.38, 11.56–97.17 and 10.57–74.85 mg/g, respectively [7].

Fluoride tends to accumulate in the exoskeleton and bone tissue of aquatic animals [3]. Its levels in terrestrial biota are higher in areas of natural and anthropogenic origin [2]. Environmental pollution affects commercially shrimps and mollusca. They spend most of their life in the upper reaches of the estuaries where they become closer to the discharged water containing sewage, industrial, and agricultural wastes [1]. Actually, fluoride is documented as a toxic harmful pollutant to a variety of animals when it exists in high concentrations [1].

It seems advisable to study the accumulation effects of fluoride in the *Palaemon elegans* as a marine organism that is supposed to be major source for human consumption.

2. Materials and methods

2.1. *Collection area*

The Eastern Harbor of Alexandria is a semicircular basin with an area of 2.53 km^2 and an average depth of 6 m (Figure 1). The Harbor is mainly used in fishing as well as the hatching processes. It is subjected to different discharged waters containing sewage, and garbage wastes.

2.2. *Biological background of* Palaemon elegans

Palaemon elegans is widely distributed in both the river estuaries and the upper shore pools. Paolucci described the species from Adriatic near Italy [8]. It usually inhabits rock pools and shallow sub-littoral zones. Heldet listed the species among the edible shrimps of Tunisia and also

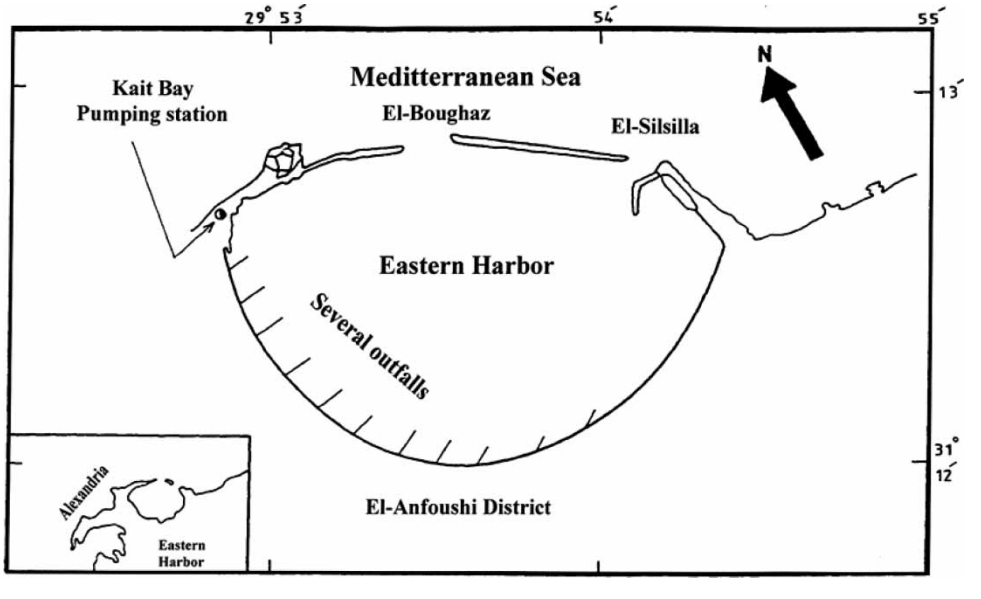

Figure 1. Sampling location during June to November 2006.

the North of Africa [9]. It was recorded also in Egypt by Holthuis & Gottlib along El-Maadiah region [10] and was captured from the Eastern Harbor of Alexandria [11].

Palaemon elegans lay eggs within a few hours of copulation. The eggs are then fertilised on extrusion by the semen attached to the exterior of the females' body and transferred to the brood chamber of the underside of the abdominal region of the female. The life cycle is divided into three distinct phases (egg, post larval and adult) [12]. The larvae are planktonic. They are recorded in the spawning season with a maximum abundance during April to December and size range of cm 3–7 cm. Females spawn about three to four times in their first year of life. They start to mature and lay their eggs at 2.5 cm [12].

2.3. *Collection of shrimps*

Palaemon elegans were collected from the Eastern Harbor of Alexandria by using a hand net with a mesh size of 100 μ during June 2006 to November 2007 (Figure 1). They were kept at a constant temperature (22–25 °C) in an aerated plastic chamber containing fresh seawater during their transfer to the lab.

2.4. *Experiment setup*

Ten shrimps were placed in each aquarium. Eight aquariums were used, containing 1.5 liters of filtered seawater (as control, Table 1) collected from Eastern Harbor (Figure 1). Seawater was filtered by super pumps 1.5 HP/220 V connected with both sandy 211 (200 μ) and back filters (50 μ) (Mazzei Injector Corp., Bakersfield, CA, Spain).

Shrimps were fed an artificial meal (≈15% of their weight) everyday (Table 2). Any unconsumed food was removed to prevent water fouling. The seawater in each aquarium was changed weekly with a fresh filtered one. The water quality of the seawater was determined (Table 1). Each aquarium was aerated continuously to obtain an adequate oxygen concentration.

Table 1. The water quality of Eastern Harbor seawater (control) during June 2006 to January 2007.

Parameter	Value ± SD
Temperature	22.5 ± 2.5°C
Salinity	29 ± 1 psu
pH	8.2 ± 1
Ca^{+2}	581.2 ± 20 mg/l
Mg^{+2}	1452.5 ± 42 mg/l
SO_4^{-2}	1380 ± 202 mg/l
F^{-}	8.5 ± 0.5 ppm
P	0.158 ± 0.006 μg at P/l

Table 2. The component items and approximate analysis for the artificial meal.

Item	gm	Approximate analysis	%
Fish meal	55	Dry matter	91
Soybean meal	27	Crude protein (CP)	48
Wheat bran	7	Crude lipid (CL)	8.05
Yellow corn	4	Crude fiber (CF)	3.05
linseed oil	5	Ash	15.7
Vitamin & minerals	2	Nitrogen free extract (NFE)	26.42

Two experiments were performed to determine the growth rate and fluoride content in the different tissues (head, exoskeleton and muscles) of examined *Palaemon elegans* in control and fluoridated seawaters.

Experiment (1): The shrimps during the first segment of study (June 2006 to November 2006) were maintained in control seawater

Experiment (2): The shrimps examined during the second segment of investigation (1–30 November 2006) were place in eight aquariums. A series of different fluoride contents (0.5, 1, 2, 4, 8, 16, and 32 ppm, as fluoridated natural seawaters) were added to the seven aquariums, respectively. The eighth aquarium was filled with control seawater (as a blank).

The different fluoridated seawaters were replaced by control one after one month. However, the remaining organisms are subjected to fresh control seawater during December 2006 to January 2007.

Total length; T. L. (taken from the tip of rostrum to the end of telson, measured in cm) was determined. Similarly, carapace length; C. L. (taken from the edge of the rostrum to the end of carapace, measured in cm) and the total wet weight; T. wt. (in grams), were measured in the two experiments. The fluoride content in the different tissues (head, exoskeleton, and muscles) of the shrimps was also determined.

2.5. *Isolation, preparation, and digestion of different tissues*

The shrimps were dissected to the different tissues (head, exoskeleton and muscles). These tissues were homogenised to make a composite sample. Each composite sample was weighed separately in clean, labeled Petri dishes and dried for several days at 50°C to a constant weight. Pulverisation and homogenisation were achieved by grinding the tissue samples.

An exact 0.1 gm (triplicate) of each crushed dried samples was placed in Teflon vessels followed by the addition of 3 ml of concentrated HNO_3 (Merck) at room temperature in fit closed Teflon cubs. The vessels were tightly covered and left until the samples were digested at room temperature. Samples were diluted with distilled water to 10 ml. The resulting solutions were stored in plastic vials until further analysis.

2.6. *Determination of fluoride content*

Fluoride concentration was measured applying the modified colorimetric procedure [13,14]. Since, 1.5 ml of 1 mol/l NaOH solution was added to each sample before the addition of Zr-ARS reagent to avoid the interferences of some metals especially calcium and magnesium. The samples were warmed and filtered after cooling. 1 ml of the filtered sample was treated with 1.5 ml (0.004 mol/l) ARS, 1 ml of standardised $ZrCl_4$ (0.004 mol/L) and then diluted to 25 ml by distilled water. The pH of samples was adjusted as that of the blank. The absorbance was measured at $\lambda = 420$ nm after two hours where Zr-ARS was used as a blank with Shimadzu Double-Beam spectrophotometer UV-150-02. The concentration of the unknown sample (mg/l) was obtained using the calibration curve in which the standard NaF was of 10 μ F^-/ml. The concentration of fluoride is expressed as ppm.

2.7. *Water quality analysis*

Salinity of samples was calculated from measuring their electrical conductivity using the inductive salinometer (Beckman model RS10). The salinometer was standardised against standard seawater from Copenhagen, Denmark, salinity 35‰. The conductivity ratio was measured to the nearest 0.01‰ after making corrections using the tables of salinity/conductivity conversions. Temperature

of seawater samples was measured by dipping the bucket thermometer (range 0–200°C) into the upper water layer for few minutes.

pH values are determined by a calibrated digital pH-meter (model 607). The readings were made three times and the mean value was recorded. Total hardness of the seawater samples were determined using EDTA titrimetric method, in the presence of NH_3-NH_4Cl buffer solution (pH = 10) and Eriochrome black T as an indicator [15]. Calcium concentration was determined by EDTA titration in presence of murexide indicator [15]. Magnesium content was measured using, mg/l Mg^{2+} = [total hardness (as mg/l $CaCO_3$) – calcium hardness (as mg/l $CaCO_3$)] × 0.244 equation [15]. Sulphate concentration was determined using turbidimetric method by measuring the absorbance of barium sulphate suspension in the presence of the condition reagent (glycerol mixed with conc. HCl, distilled water, ethyl alcohol and NaCl), using Perkin Elmer Lambda/UV/visible single beam spectrophotometer at $\lambda = 420$ nm [15]. The sample was diluted to a suitable dilution then was stirred. A spoonful of $BaCl_2$ crystals was added to the diluted sample while it's stirring for 1 min. Some of this solution is poured into the absorption cell, measured the turbidity at 30 sec intervals for a minute. The maximum absorption obtained in 4 minutes intervals was recorded. The required concentration was determined using a standard curve. Phosphorus content was determined using a colorimetric method [15]. The samples were treated with an acidified ammonium molybdate containing ascorbic acid and a small amount of potassium antimony tartarate. The developed blue color was measured spectrophotometrically at $\lambda = 885$ nm and the concentration was expressed by μg at P/l. The water quality of seawater of the Eastern Harbor is illustrated in Table 1.

2.8. *Statistical analysis*

The collected data were analysed using the Statistica computer software. Correlation matrix and multiple regression analyses were used to determine the relation among total and carapace lengths and total wet weight in all shrimp samples in the control and in different fluoridated seawaters. Also, these two analyses are performed to refer to the relation among the fluoride content in the different tissues (head, exoskeleton and muscles). However, r (correlation coefficient) and R (multiple regression coefficient) values measure the strength between these variables. All variables were analyzed at significance level of $\alpha = 0.05$.

3. Results

3.1. *The growth increment percentage of shrimps in control and fluoridated seawaters*

External body dimensions (carapace length, C. L. and total length, T. L.) and total wet weight, T. wt., are used to determine the criteria for growth. These dimensions are measured once each week in both segment cases. The % increments in these cases are given in Table 3 and Figure 2.

The high percentage increment value (132.48%) for control seawater was detected in the first experiment. Insignificant percentage increments were recorded in the different fluoridated seawaters (second experiment).

The correlation matrix is performed for average of initial and final total lengths and % increment values in the first and second experiments (Table 3). It gives a high significant reverse relation between the average initial total length of shrimps and % increment values ($r = -0.9663$, $p < 0.000$).

In the second experiment, a good direct relation between the average of both initial and final total length of the shrimps ($r = 0.9268$, $p < 0.003$) was detected.

Table 3. The total length (T.L.) and % increment of shrimp (*Palaemon elegans*) samples in the two experiments during June 2006 to January 2007.

Experiment	Range (cm) initial T.L.	Average of initial T.L. ± SD	Range (cm) final T.L.	Average of final T.L ± SD	% increment (cm)
First	1.35 – 1.80	1.57 ± 0.32	3.10 – 4.20	3.65 ± 0.78	132.48
Second					
0.5 ppm	3.00 – 4.00	3.50 ± 0.71	3.50 – 4.50	4.00 ± 0.71	14.29
1 ppm	3.00 – 3.50	3.25 ± 0.35	3.40 – 4.00	3.70 ± 42	13.85
2 ppm	3.00 – 3.50	3.25 ± 0.35	3.30 – 3.80	3.55 ± 0.35	9.23
4 ppm	3.40 – 3.90	3.65 ± 0.35	3.60 – 4.40	4.00 ± 0.57	9.59
8 ppm	3.50 – 4.00	3.75 ± 0.35	4.00 – 4.1.0	4.10 ± 0.07	9.33
16 ppm	2.70 – 4.00	3.35 ± 0.92	3.20 – 4.10	3.65 ± 0.57	8.97
32 ppm	3.30 – 4.00	3.65 ± 0.49	3.70 – 4.20	3.95 ± 0.35	8.22

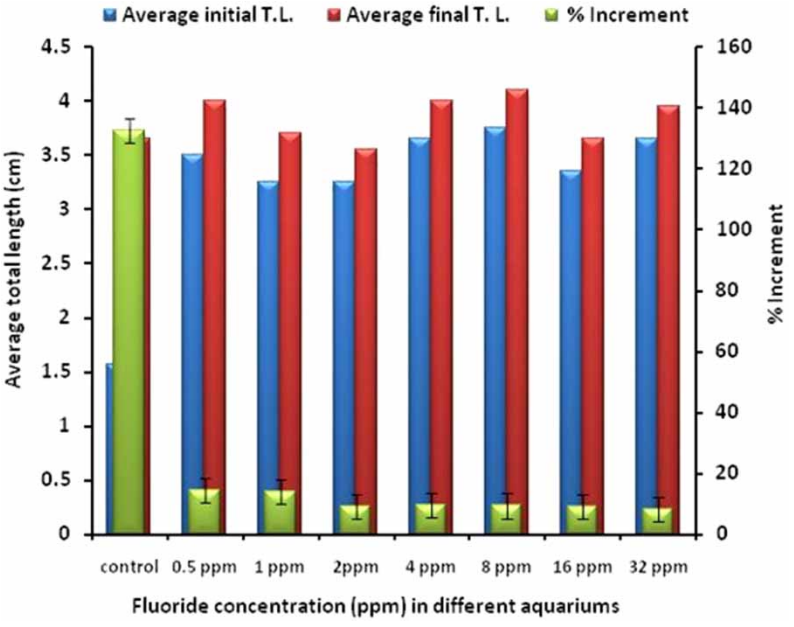

Figure 2. The average of initial and final total lengths, T.L. (cm) and % increment and its standard error of *Palaemon elegans* samples in each fluoride level during June 2006 to November 2006.

3.2. *Fluoride content (mg/g) in the different tissues of shrimps in control seawater*

The fluoride contents in dried samples of head, exoskeleton, muscles and total body of shrimps after capture from Eastern Harbor are of 51.76, 43.13, 10.59 and 105.47 mg/g, respectively (Figure 3). Head tissue shows a higher fluoride concentration than the others. Also, fluoride accumulates in the exoskeleton tissue.

3.3. *Total length-total weight and total length-carapace length relations in control and fluoridated seawaters*

3.3.1. *Control seawater*

Total length-total wet weight and total length-carapace length relations in control seawater along the first experiment are illustrated in Figures 4 and 5.

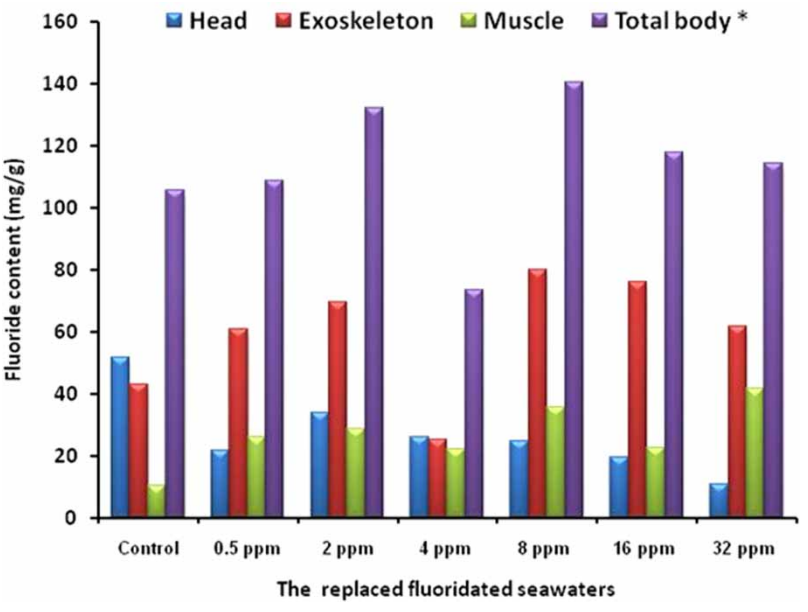

Figure 3. Fluoride content (mg/g) in different dried tissues of *Palaemon elegans* in control and replaced fluoridated seawaters. *Where, total body weight is obtained from the summation of fluoride content in each dried tissue (head, exoskeleton and muscles).

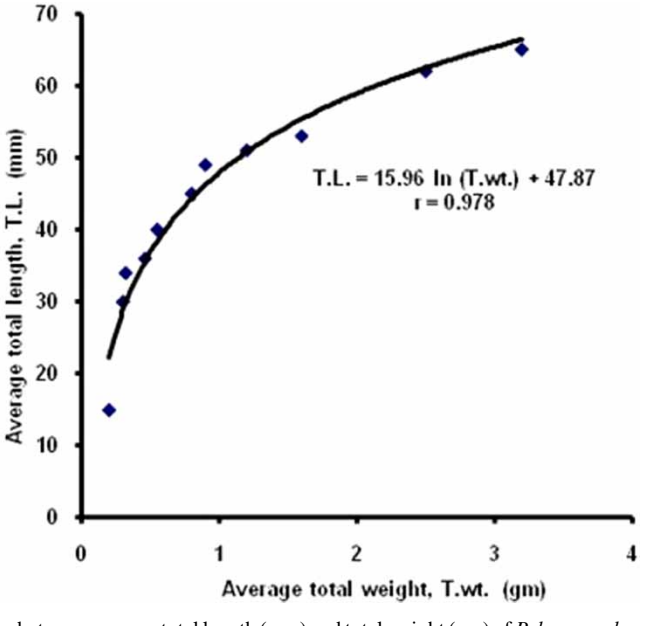

Figure 4. The relation between average total length (mm) and total weight (gm) of *Palaemon elegans* in control seawater during June 2006 to November 2006.

The correlation matrix analysis among average contents of total length (T.L.), carapace length (H.L.), and total weight (T. wt.) gives:

- High positive significant relations among T.L. and each of C.L., T. wt. and progressed time ($r = 0.9542$, 0.8802 and 0.9544, $p = 0.000$).

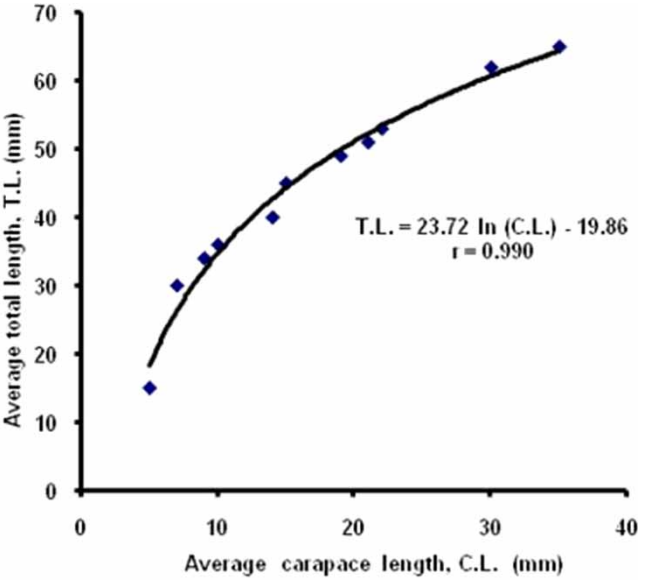

Figure 5. The relation between total and carapace lengths (mm) of *Palaemon elegans* in control seawater during June 2006 to November 2006.

- Good significant correlations between C.L. and both T. wt. and time (r = 0.9704 and 0.9895, $p = 0.000$).
- Total weight shows a high relation with time (r = 0.9643 and $p = 0.000$).

Similarly, the multiple regression equation shows the same good correlation as follows:

$$\text{T.L.} = 1.59 + 1.11\ \text{C.L.} - 0.83\ \text{T. wt} + 0.65\ \text{days}\ (\text{R} = 0.97733,\ p < 0.000044).$$

3.3.2. *Fluoridated seawaters*

The T.L., C.L. and T. wt. of shrimps in all different fluoridated seawaters in the second experiment are recorded. Insignificant growth is obtained in all fluoridated seawaters.

Correlation matrix among these parameters shows a good relation between average values of C.L. and both T.L. (r = 0.9466, $p < 0.001$) and T. wt. (r = 0.8970, $p < 0.006$) of shrimps, respectively. Furthermore, it gives a high significant relation between average contents of T.L. and T. wt. of shrimps (r = 0.9543, $p < 0.001$).

Multiple regression analysis among average values of T.L., C.L. and T. wt. is given as follows:

$$\text{T.L.} = 2.50 + 0.95\ \text{T. wt.}\ (\text{R} = 0.95431,\ p < 0.0084).$$

3.4. *Fluoride content in different shrimp tissues (head, exoskeleton, and muscle) in the second experiment*

3.4.1. *Head*

Fluoride content in the head of the shrimps in the control and all the different fluoridated seawater levels (0.5, 1, 2, 4, 8, 16, and 32 ppm) was determined (Figures 6 and 7).

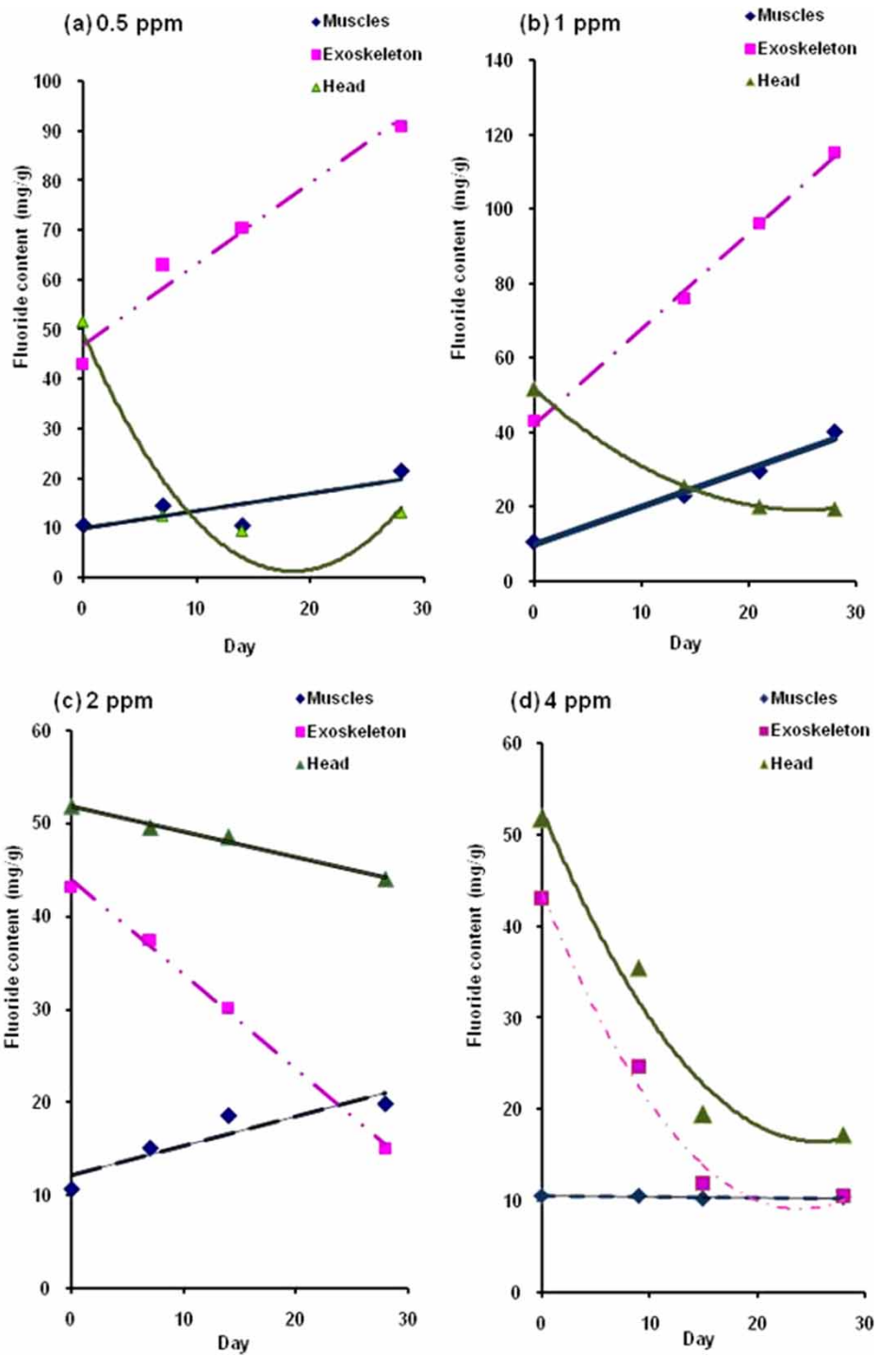

Figure 6. The fluoride content (mg/g) in head, exoskeleton and muscles of *Palaemon elegans* during November 2006. (a) 0.5 ppm, (b) 1 ppm, (c) 2 ppm, (d) 4 ppm.

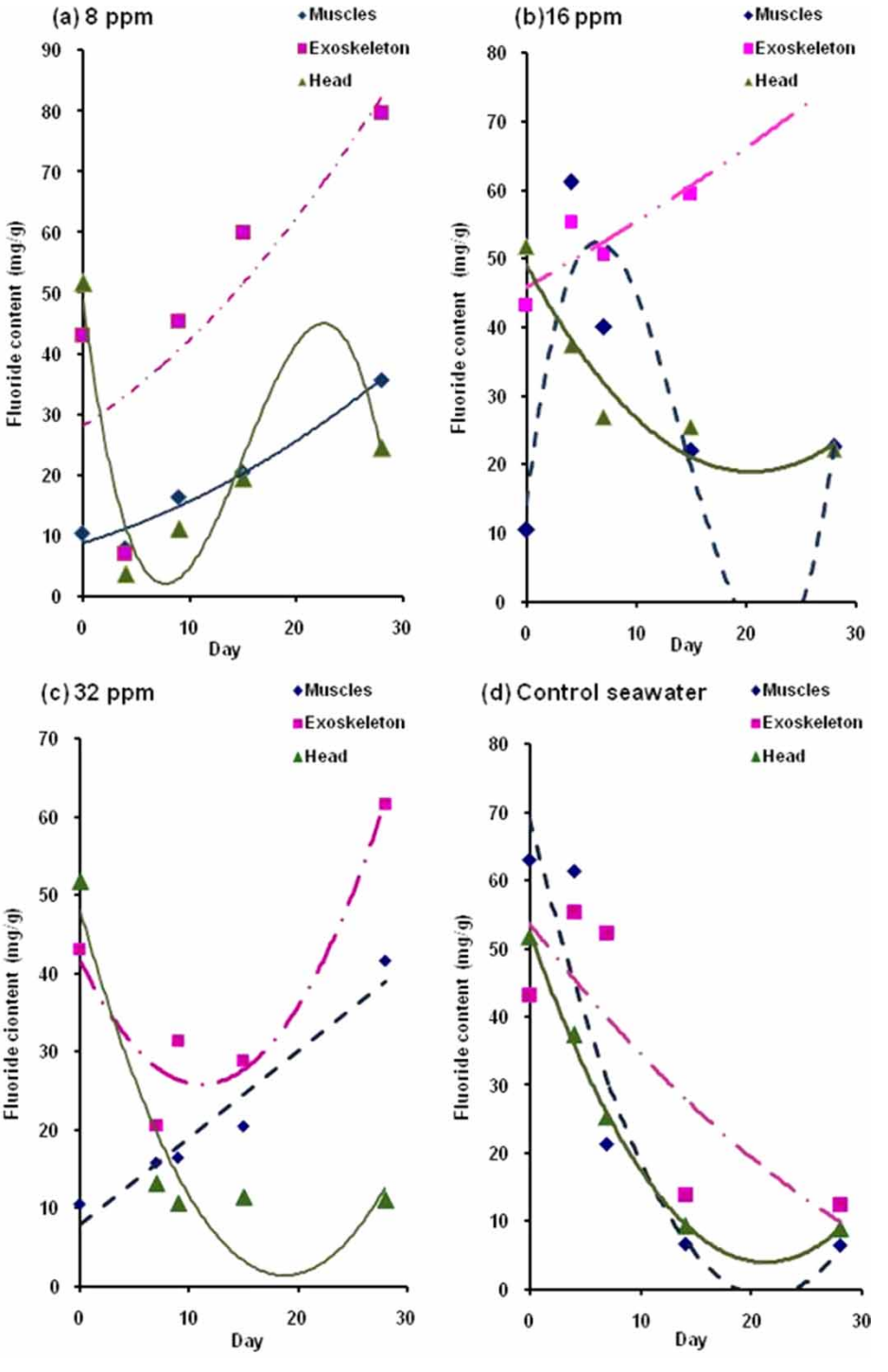

Figure 7. The fluoride content (mg/g) detected in the head, exoskeleton and muscles of *Palaemon elegans* during November 2006. (a) 8 ppm, (b) 16 ppm, (c) 32 ppm, (d) control seawater.

Table 4. Regression equations belong to fluoride content (mg/g) in different tissues of shrimps and the progressed time (day) in control and different fluoridated seawaters.

	Rejuession equations					
	Head		Exoskeleton		Muscles	
FLUorwo love l	Equation	r value	Equation	r value	Equation	r value
Control	$F = 0.11\ Day^2 - 4.56\ Day + 52.47$	0.985	$F = 0.02\ Day^2 - 2.11\ Day + 53.72$	0.820	$F = 0.15\ Day^2 - 6.57\ Day + 69.21$	0.939
0.5 ppm	$F = 0.14\ Day^2 - 5.17\ Day + 49.06$	0.962	$F = 1.62\ Day + 47.04$	0.982	$F = 0.\ 35\ Day + 9.96$	0.815
1 ppm	$F = 0.05\ Day^2 - 2.59\ Day + 51.75$	1.000	$F = 2.57\ Day + 42.10$	0.998	$F = 1.02\ day + 9.69$	0.992
2 ppm	$F = -0.27\ Day + 51.76$	0.992	$F = -1.02\ Day + 43.88$	0.998	$F = 0.32\ Day + 12.09$	0.917
4 ppm	$F = 0.06\ Day^2 - 2.83\ Day + 52.77$	0.983	$F = 0.06\ Day^2 - 2.9\ Day + 43.71$	0.993	$F = -0.01 + 10.53$	0.676
8 ppm	$F = -0.03\ Day^2 + 1.18\ Day^2 - 13.64\ Day + 48.99$	0.943	$F = 0.03\ Day^2 + 1.14\ Day + 28.12$	0.806	$F = 0.02\ Day^2 + 0.53\ Day + 8.99$	0.983
16 ppm	$F = 0.07\ Day^2 - 2.94\ Day + 49.08$	0.952	$F = 0.01\ Day^2 + 0.897\ Day + 45.92$	0.961	$F = 0.03\ Day^3 - 1.30\ Day^2 + 13.07\ Day + 14.26$	0.879
32 ppm	$F = 0.13\ Day^2 - 4.97\ Day + 48.14$	0.999	$F = 0.13\ Day^2 + 0.\ 90\ Day + 45.92$	0.958	$F = 1.00\ Day + 7.89$	0.972

3.4.2. *Exoskeleton*

Fluoride accumulation in the exoskeleton was observed at 0.5 and 1 ppm fluoridation levels (Figure 6a and b). The fluoride content in the exoskeletons of the shrimps in both 0.5 and 1 ppm fluoridation levels in the second experiment showed higher values than those in the control one, whereas Figure 6 (c and d), shows a gradual decrease in fluoride content in the exoskeleton of shrimps in 2 and 4 ppm fluoridation levels. The increased fluoride concentrations of the exoskeleton of shrimps in 8, 16 and 32 ppm fluoridation levels are observed in Figure 7 (a,b and c).

Figure 7 (d) represents the gradual release of fluoride concentration from different tissues of shrimps living in natural seawater (as a blank).

3.4.3. *Muscle*

Generally, fluoride content in muscles reflected an increase during the study (Figures 6 and 7), except that extracted from shrimps living in 4 ppm fluoridation level where the concentration remained constant (Figure 6d).

Regression equations of fluoride content (mg/g) in the different tissues of shrimps and the progressed time (day) in control and different fluoridated seawaters (0.5, 1, 2, 4, 8, 16, 32 ppm) obtained from Figures 5 and 6 are shown in Table 4.

3.5. *Fluoride content in different tissues (head, exoskeleton and muscles) of the shrimps after the replacement of fluoridated seawaters*

After a month of exposure to the different fluoridated seawaters (second experiment), the remaining shrimps of each aquarium were placed in the control one. The average fluoride contents (mg/g) in the different dried tissues and total body of the shrimps after the replacement are illustrated in Figure 3. High accumulation of fluoride in the muscles (soft) and exoskeleton tissues (hard) is detected while head tissues show minimum fluoride content (Figure 3).

The statistical analyses show a reverse relation between the fluoridated seawaters (ppm) and fluoride content (mg/g) in head tissues. However, correlation matrix gives $r = -0.8280$ at $p < 0.042$. Also, the multiple regression equation is given as follows:

$$\text{Fluoridated seawater} = 41.08 - 0.85\ \text{Head}\ (R = 0.8493,\ p < 0.03235).$$

Interestingly, low fluoride contents in the head, exoskeleton, muscles, and total body of the shrimps living in 4 ppm (26.29, 25.27, 22.08, and 73.64 mg/g, respectively) are detected (Figure 3).

4. Discussion

4.1. *The growth increment percentage of shrimps in control and fluoridated seawaters*

The high % increment in the first experiment (132.48%) for control seawater shows the ideal growth of shrimps in natural seawater. The minimum % increments in the second experiment reflect the growth inhibition effect of fluoride (Table 3 and Figure 2). Moreover, mortality in control and fluoridated seawater is ranged between 10% and 30%, respectively. On the other hand, the % growth increment decreases by 118.19 % in 0.5 ppm fluoridated seawater from that of the control one during one month. Furthermore, an insignificant growth in the subsequent yield of shrimps also might occur at fluoride levels greater than 10 ppm. Actually, the localised fluoride pollution problems may be caused by the fishing activities as well as the drainage waters coming from the

outfalls in the Eastern Harbor. Accordingly, this pollution may destroy the shrimps' growth and their population. This conclusion leads us to imagine the huge damage in shrimps' fishing along Eastern Harbor. However, it will not be economically feasible to fish this shrimp's size.

Interestingly, the correlation matrix analysis shows the growth inhibition. However, the high significant reverse relation between initial T.L. and % growth increments reflects the same result. Similarly, the good direct relation between initial and final T.L. of shrimps shows the insignificant growth [1–3].

4.2. *Fluoride content (mg/g) in the different tissues of shrimps in control seawater*

The high fluoride concentration in head tissue may indicate the accumulation mechanism on gills during the continuous circulation of seawater through the shrimp's chamber (Figure 3) [2]. This high content in head and exoskeleton tissues reflects the accumulation in the hard tissues [2,3]. It was found that fluoride can play an important role in the hardening of hard tissues. The combination of fluoride with calcium and phosphorus lead to formation of fluorapatite ($Ca_5(PO_4)_3F$) [2,3]. Many studies were performed to determine the fluoride content in different marine organisms [1,3]. For example, the reported fluoride content in Krill species ranges from 1000–6000 mg/Kg dry weight [16]. The previous studies estimated that its concentration in the exoskeleton of *Euphausia crystallorophias* and the mouthparts of *E. supera* of Antarctic were of 5977 μg/g and 12876 μg/g dry weights, respectively [3]. Also, it ranged between 80.59 and 120.46 mg/g in the dried total body of *Trachypenaeus curvirostris* (shrimp) and *Palaemon* sp. (prawn) in the Egyptian coast of the Mediterranean Sea [7]. Generally, the fluctuation in fluoride content in the different species around the world may be controlled by the environmental conditions (sediment, ambient levels in seawater, temperature, etc.) as well as the rate of feeding and molting, the population size, etc. [1].

4.3. *Total length-total weight and total length-carapace length relations in control and fluoridated seawaters*

4.3.1. *Control seawater*

These relations indicate the gradual increase in growth along the first experiment (Figures 4 and 5). Similarly, the correlation matrix and multiple regression analyses among T.L., C.L., T. wt and progressed time show the ideal growth of *Palaemon elegans* in control seawater.

4.3.2. *Fluoridated seawater*

Shrimps show small growth increments. This result reflects the inhibition of growth in shrimps despite the fluoridation level. Similarly, the statistical analyses indicate the same result.

4.4. *Fluoride content in different shrimp tissues (head, exoskeleton, and muscles) in the second experiment*

4.4.1. *Head*

The release of fluoride from the head of shrimps in the control seawater (Figures 6 and 7) goes in harmony with previous studies [1,2]. The low fluoride content in all head samples in fluoridated seawaters may be linked with the decrease in both respiration and subsequently, the formation of fluorapatite ($Ca_5(PO_4)_3F$) [2,3,16]. Also, this decrease possibly reflects the regulation of osmotic

pressure [16]. The same result was estimated in the fluoride uptake in blue crab, *Callinectes sapidus* in Pamlico River estuary, North Carolina since the fluoride content departed significantly from linearity and they showed a gradual decrease in their accumulation in gills [2].

Interestingly, it was deduced that fish need to accumulate minerals in their bodies in fresh water. In contrast, in seawater or brackish water they excrete extra amounts of minerals to adjust their osmotic pressure [16].

4.4.2. *Exoskeleton*

The fluoride accumulation in exoskeleton in 0.5 and 1 ppm fluoridation levels (Figure 6a and b) is expected since its normal level (Table 1) in the Eastern Harbor approximately equals these two fluoridation levels. This may relate to the combination of fluoride with calcium and magnesium ions (as cofactors by different enzymes). This process might be a primary mechanism in many instances [3]. The gradual decrease in fluoride content in the exoskeleton of shrimps in 2 and 4 ppm fluoridated seawaters is shown in Figure 6 (c and d). This phenomenon is not expected. However, it was revealed that the tissues having the highest fluoride content rapidly accumulate [2,3]. It seems that the minimum fluoride content in exoskeleton in these fluoridation levels relates to the uptake mechanism [3]. Also, this fact may accompany with the adjustment of osmotic pressure in shrimps [16]. The maximum fluoride concentrations in the exoskeleton of shrimps in 8, 16 and 32 ppm fluoridation levels is determined (Figure 7a, b and c). This increase is in agreement with previous results [1–3,16]

The gradual release of fluoride from the different tissues of shrimps living in natural seawater (as a blank) possibly refers to the uptake mechanism of shrimps (Figure 7d) [3].

4.4.3. *Muscles*

Generally, fluoride content in muscles reflects an increase during the study (Figures 6 and 7). The muscles of shrimps living in 4 ppm fluoridation level give constant fluoride content along the progressed time of the experiment (Figure 6d). This probably reflects the equal fluoride uptake and release rates [2] and the osmotic pressure regulation [16]. It can be represented as the ideal case for muscles of shrimps. Since, below and above this fluoride level the muscles cannot defend against the fluoride uptake.

4.5. *Fluoride content in different tissues (head, exoskeleton and muscles) of the shrimps after the replacement of fluoridated seawaters*

The high accumulation of fluoride in exoskeleton (hard) is confirmed by the documented data [1–3,16] (Figure 3) where it was estimated that fluoride tends to accumulate in the exoskeleton in invertebrates and in the bone of fishes [1–3]. This may confirm the defense mechanism against fluoride intoxication and its removal from body circulation [3]. On the other hand, the same result was estimated in the fluoride uptake in blue crab, *Callinectes sapidus* in Pamlico River estuary, North Carolina [2]. The decrease in fluoride content in head tissues may be due to regulation of osmotic pressure (Figure 3) [16]. This decrease goes parallel with the statistical analyses. Interestingly, the low fluoride content in all tissues of the shrimps living in 4 ppm possibly relates to the fluoride uptake mechanism [2]. However, the rate of uptake of fluoride equals the release one. This represents the ideal case.

5. Conclusions

The study on *Palaemon elegans* reveals that there is a significant growth inhibition effect of fluoride. Fluoride accumulates in exoskeleton and muscles tissues of shrimps even though the different fluoridated seawaters are replaced by the control one. Moreover, the study of the effects of fluoride uptake and its accumulation in marine organisms that is supposed to be a major source for human consumption is a necessity. In addition the study points out that, it's not recommended economically to use shrimps of small size for feeding purposes. Interestingly, studies on the biotransference of fluoride as well as its biomagnification have to be carried out among the aquatic food chain organisms (producers and consumers). The aim may be further extended to study the impact of using such organisms on humans.

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