

## Article

# Morphological Variation between Life and Death Gastropod Populations in the Nile Delta: A Pollution-Induced Evolution

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**Abstract:** Wetland ecosystems of the Nile Delta face severe threats due to natural climatic changes and anthropogenic activities. Life and death assemblage comparisons can be implemented as a historical record to detect anthropogenic-induced environmental changes in the past few decades. A geometric morphometric approach was applied to quantify the pollution-induced morphological variation between life and death populations of the gastropod *Melanooides tuberculata*. The results indicated that life populations differ significantly from the death ones, where the first tend to be much smaller, more globular, and with a depressed aperture and whorl section. In addition, the phenetic diversity of the life populations was also decreased, and the allometric growth was shifted. These morphological changes in the life populations are well-known adaptations for reducing the cost of shell maintenance in polluted water. No distinct morphospace was found between life populations from different habitats, suggesting that habitats have no significant role in the current pollution-induced evolution.

**Keywords:** water pollution; coastal ecosystems; gastropod shells; geometric morphometrics; phenetic diversity; Nile Delta



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## 1. Introduction

Coastal habitats have been altered over the past century due to various anthropogenic influences and climatic crises [1–3]. Therefore, these habitats have been degraded, water quality was negatively impacted, and the hydraulic regimes were shifted [4–7]. According to many research studies published recently [8–12], water quality in the Nile Basin has deteriorated due to various anthropogenic factors controlling its suitability for agricultural and drinking purposes. Understanding the species–environment interactions and their potential impact on ecosystems is a primary goal for conservation studies [13]. In addition, understanding the mechanisms underlying the origin and maintenance of biodiversity is a central goal of modern ecological and evolutionary research [14]. Life and death assemblage comparisons were continuously implemented to detect environmental change and record anthropogenic stress and events over a small-time scale or when historical data are unavailable [15–21].

Comparisons usually focus on assemblage composition (community level). However, live–dead morphological variations at the species level can also detect environmental changes, although it is less explored. In contrast to shape, size comparisons received considerable investigations [22–25]. Very little research has been conducted on shape

changes [26–28]. Geometric morphometrics of live and dead molluscs indicated that morphometric differences had recorded fine-scale spatiotemporal patterns of environmental change [28].

The inexpensive geometric morphometrics approach was implemented as a potential pollution monitoring tool based on live collected specimens from separated polluted and unpolluted areas [29–36]. Few studies have used historical live-collected specimens and/or specimens from archaeological middens as controls [34,37]. Consequently, physicochemical environmental factors rather than pollution could also affect shell shape (e.g., predation intensity, wave energy, oxygen level, temperature, and water chemistry [28,38]).

Adapting populations to local environmental conditions/pollution drives natural selection, which is a central assumption of evolutionary theory. However, the ecological mechanism behind the adaptive evolutionary change remains unclear [39]. Spatial and temporal phenotypic variations will better understand the genetic and phenotypic divergence in response to habitat variation [40]. Among the primary purposes of this study is the use of the GM approach to quantify the phenotypic variation in the *Melanoides tuberculata* as a model for understanding the ecological consequences of eutrophication and environmental pollution, which may drive or shift evolutionary changes, and to answer the following research questions: (a) what variables may relate to variation in morphology over a short-term period; (b) does pollution, natural environmental variables, or both drive the morphological variations; and (c) are variations (i.e., breadth) in morphology temporarily decreased due to directional selection?

Methods adopted for assessing surface water quality based on biological indicators have been undergoing development for many years and are still being improved. Over the years, substantial biological water quality assessment methods have been developed [41]. Such methods are increasingly incorporated into technological solutions, i.e., biosensors or biologically active deposits in water purification. The advantage of biological methods is that water quality assessment is conducted in the way that a living organism would react to pollution. Research based on biological methods is often less expensive and time-consuming since some stages of sample preparation can be omitted. The advantage of such methods is that the analysis can be conducted in a laboratory and the natural habitat of the organisms used for testing. In Egypt and during the past few decades, there were downstream changes in the water quality of the Nile River, primarily due to a combination of land and water use as well as water management interventions such as (a) different hydrodynamic regimes regulated by the Nile barrages, (b) agricultural return flows, and (c) domestic and industrial waste discharges, including oil and wastes from passenger and river boats. These changes are more pronounced as the river flows through the densely populated urban and industrial centers of Cairo and the Delta region. Pollution of the surface water by agricultural drainage water, industrial wastewater, domestic solid waste, and wastewater seriously threatens the quality of irrigation and potable water supply [42].

According to the National Water Resources Plan, it also frustrates government plans for downstream reuse of drainage water, which is a critical element of its strategy to cope with water scarcity. Furthermore, the industry is a growing sector in the national economy. Further industrial development is expected to significantly affect the country's socioeconomic development, employing a large part of the growing population. Although the industrial policy is to create new cities and industrial zones outside the Nile Valley and Delta, there is still much industrial development. Moreover, through rapidly spreading informal urbanization [43], land fragmentation is increasing [44], indirectly affecting the water system. In summary, there is enormous pressure on the quality and quantity of water in the Delta, resulting from intensifying agriculture, a rapidly growing industry, and a rapidly growing population. In addition to the anthropogenic influences, climate change (sea-level rise and high temperature) is expected to intensify the water problem in Egypt in the near future.

Herein, we will test the research hypothesis by comparing gastropod populations from different habitats with different physicochemical conditions (spatial scale) and, at the same

time, collect both death assemblages that represent historical records and life populations that represent the present-day environment.

## 2. Materials and Methods

### 2.1. Study Area and Sampling

In Egypt, Lake Manzala is a vital fisheries resource in the Nile River Delta, accounting for over 30 percent of all commercial and recreational fish landed and consumed in Egypt [45]. However, Lake Manzala has been classified as one of the most polluted lakes in Egypt [46,47]. Since Aswan High Dam was completed, the hydrodynamics and turbidity of the lakes have dramatically decreased [48]. The sediment of these lakes after Aswan High Dam, occasionally also in Manzala, is polluted mainly by Mn, Pb, Zn, and Cd, due to the direct human input from neighboring megacities, where the petrochemical industry is considered to be a significant source [48]. Aridity and low precipitation clog the flushing out of these heavy metals, as in the case of humid lakes [47].

Environmental pollution in Lake Manzala and its main drainage channels have previously been documented by measuring the concentrations of selected trace metals in fish and sediment samples [49]. Sources of contaminants in Lake Manzala include untreated sewage, atmospheric deposition, and agricultural and industrial wastes, which may impact human health when fish are consumed [50]. Freshwater inputs to the lake are insufficient to provide adequate sea flushing. Therefore, trace metals entering the system are expected to accumulate over time [45].

A sampling campaign was carried out in May 2018 on Manzala Lagoon. The water chemistry and physical properties, e.g., hydrogen level (pH) and electric conductivity (EC), were measured on the surface water. The total dissolved solids (TDSs) were calculated from the EC values at average surface water temperature using the formula  $EC/10001.0878 \times 0.4365$  (Table 1), and they were used as an approximation for the water salinity. The chemical parameters of the water were measured at each site using Xplorer GLX-PS-2002 (Pasco, Roseville, CA, USA). Using the 20 × 20 cm Ekman grab, we collected the top 10–15 cm of sediments, which, according to earlier studies [48], were deposited after the construction of the Aswan High Dam. Sampling was carried out on four sites (Figure 1). Three replicates were sampled and sieved manually using a 0.5 mm mesh. The gastropod shells were sorted in the field. The water depth reached by the grab was also recorded at each site (Table 1). All shells greater than 0.5 mm were separated and prepared for systematic identification. Living shells of the gastropod *M. tuberculata* were separated from dead ones. The examined species *Melanoides tuberculata* lives in and on the bottom sediments. The two sites yielded < 50 living shells and, therefore, were excluded from the morphometric analyses, as previous investigations [30] suggested that a small sample will result in non-significant results (i.e., not appropriate for statistical analyses, and it could provide unreliable estimates of trajectories). The final analyzed material includes four death populations and only two life populations from four sites in the Manzala lagoon (Figure 1).

**Table 1.** Physicochemical variables that were measured at the four studied sites including the water characteristics and the grain size proportion of the sediments.

Site	Depth (m)	Water			Sediments		
		pH	EC (μS/cm)	TDS	Sand (%)	Silt (%)	Mud (%)
1	0.8	6.8	20,654	11.5	63.4	10.5	30.4
2	1.2	7.1	10,248	5.6	52.0	8.8	20.0
3	4.2	9.1	42,852	24.2	98.5	0.3	0.2
4	2.3	8.9	44,133	25.1	96.3	2.3	0.8



**Figure 1.** Location of the studied populations in the Manzala lagoon, Nile Delta. (a) Map of Egypt showing the position of the Manzala Lagoon (red square) and Aswan High Dam; (b) location of the studied populations; (c) Nile Basin; (d) high-energy water at site 4; and (e) low-energy polluted water at site 2.

In addition, the sediment grain size was calculated using an electric shaker (EN 932-5, SysMo Ltd., Saint-Gilles, Belgium) for 200 g of the sediments following [51] (Table 1). In the laboratory, 0.2 g of shells, which were crushed and sieved through a 0.63 mm mesh screen, 1 mL of water aliquot, and 3 mL of a concentrated  $\text{HNO}_3$  and  $\text{HCl}$  (1:1) were mixed and diluted to 10 mL with ultra-pure water (Milli-Q). Then, the samples were filtrated for 5 min through a centrifuge. Heavy metals, including Zn, Cd, Cr, Pb, and Cu, were analyzed using inductively coupled plasma optical emission spectrometry (ICP-OES) at the Institute of Geology and Geophysics, Chinese Academy of Sciences (Beijing, China). The applied analytical method was continuously checked using a certified reference material (GSB04-1767-2004) from the National Research Center for Nonferrous Metals and Electronic Materials (Beijing, China) to ensure the quality of the performed analyses. The metals analyses' practical quantitation limits (PQLs) were in the range of  $0.01\text{--}0.05\text{ mg Kg}^{-1}$ .

## 2.2. Geometric Morphometrics

A landmark-based geometric morphometric was implemented. Images of 50 individuals from each site were captured using a Nikon  $\times 50$  digital camera attached to a LEICA stereomicroscope (Leica Microsystems, Wetzlar, Germany). To avoid errors from capturing 2D images of a 3D object, we followed the same procedure in [30], which includes identifying leveling points on the shells and rotating the tilted image to specific points. A total of 24 landmarks were chosen based on previous investigations on the shells of the *Melanoides tuberculata* [31]. Using the TPS Package, these landmarks were digitized and preserved (available from <https://www.sbmorphometrics.org/soft-dataacq.html>, accessed on 14 October 2020 [52]). Generalized Procrustes Analysis (GPA) was applied to the coordinate's data using the method provided in [53]. Landmark configurations were translated, scaled, and rotated until the distances among homologous landmarks were minimized according to least squares criteria. The resulting data were considered due to shape only.

The Procrustes residual data were projected to Principal Component Analysis (PCA), which compares inter and intraspecific variation among the variables/groups (landmarks coordinates/different populations). Thin Plate Spline (TPS) was also implemented, which represents the shape changes when one specimen is deformed into another. Furthermore, the Relative Warp (RW) scores of each specimen were used to map their location on RW axes [54,55]. The distribution of the individuals examined in the RW axes/morphospace represents shape changes among individuals and populations and may reflect the speciation process or phylogenetic relationship [56].

Size data were extracted before performing the Procrustes transformation to compare size among different samples separately. The shell height/width, whorl height/width, aperture length/width, spire height, and body whorl height were all calculated from the landmarks prior to the Procrustes to characterize the shell shape of the *M. tuberculata*. All specimens' shell height and width determined the geometric mean. Frequency histograms were plotted using a size-class interval, reducing empty spaces as much as possible. Size-frequency distributions were compared to a normal distribution using a Shapiro–Wilk test and the QQ plot [57]. All morphometrics and statistical analyses were conducted using PAST V. 2.17c [58].

## 3. Results

### 3.1. Environmental Factors

The four sites have different physicochemical conditions: sites 1 and 2 have muddier (20–30%) bottom sediments, while sites 3 and 4 are more sandy (96–98%, Table 1). In addition, sites 1 and 2 have lower pH levels, low salinity (5–11%), and shallower water depths. Furthermore, sites 1 and 2 have higher Cu, Pb, and Zn concentrations. Abdelhady et al. [45] classified the lagoon into highly polluted and unpolluted areas based on Sediment Quality Guidelines (SQGs) and pollution indices including the pollution load index (PLI), enrichment factor (EF), contamination factor (CF), and the geo-accumulation index (Igeo). The metals measured herein agree with Abdelhady et al. [31], where living populations at sites 1 and 2 have higher concentrations of Cu, Pb, and Zn (classified herein as polluted), while sites 3 and 4 have lower concentrations of these metals (classified herein as unpolluted (Table 2)). Note that as both life and death populations are collected from the same site; the physicochemical variables of both types of populations are the same.

**Table 2.** Metals' concentration on the shells of the studied populations compared to limits given in the literature [59–61].

Population	Metals Concentration ( $\mu\text{g}/\text{Kg}$ )				
	Cd	Cr	Cu	Pb	Zn
1	1.5	47.16	45.4	162.5	91.06
2	1.508	6.2	16.31	2.788	15.37
3	1.43	5.78	12.33	2.514	14.24

Table 2. Cont.

Population	Metals Concentration (µg/Kg)				
	Cd	Cr	Cu	Pb	Zn
4	1.272	4.66	10.02	0.196	11.35
5	1.06	8	13.75	10.18	36.24
6	0.888	4.13	7.39	0	9.49
Goher et al. [59]	0.2	30.8	21.8	10.9	35.4
Earth crust [60]	0.15	100	55	12.5	70
ERL [61]	1.2	81	34	46.7	150
ERM [61]	9.6	370	270	218	410

3.2. Quality of the Data

To assess the sufficiency of the included specimens and to exclude probable sampling bias, cumulative plots for the average centroid sizes, i.e., the square root of the sum of the squared distances of the measured landmarks to their common centroid [62], were calculated prior to the Procrustes to be used as estimates of the overall sizes of the specimens of each population of the six populations. The cumulative plot from all sites indicates that after including 220–225 specimens, there will be no or only minor changes in the cumulative average (i.e., additional specimens will not add or change the population average and hence the intended analyses (Figure 2)).

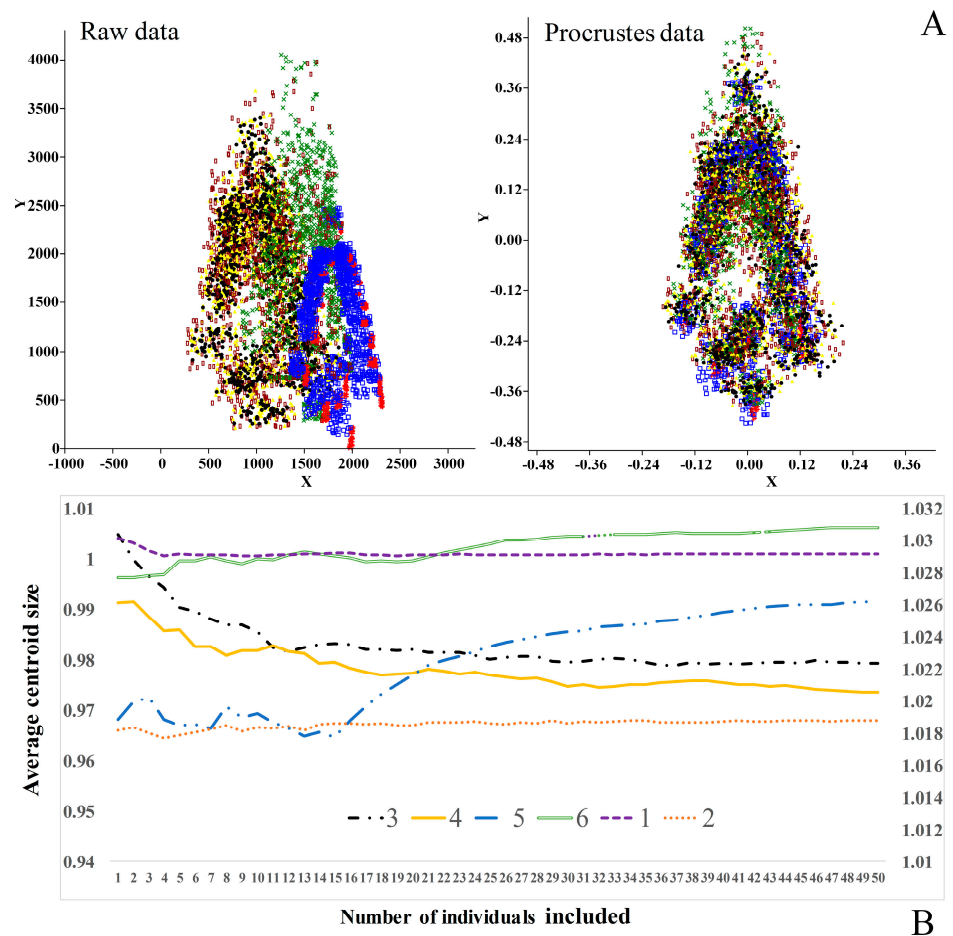
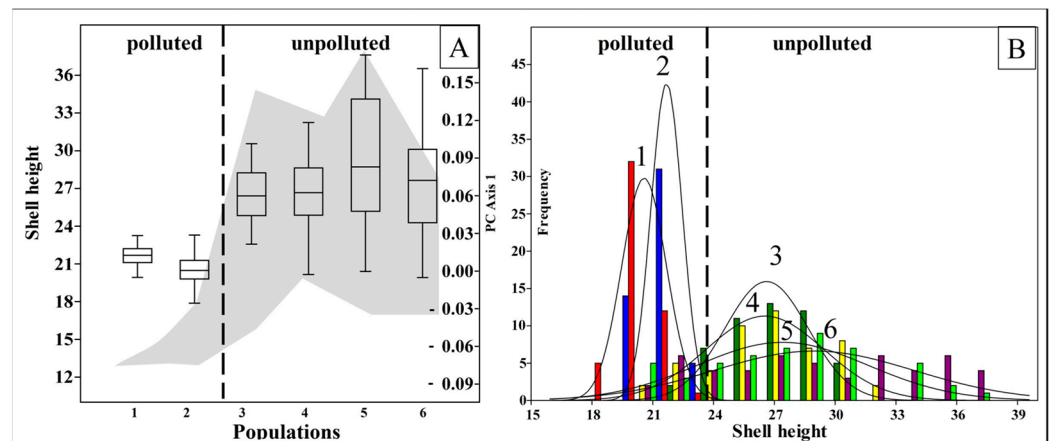


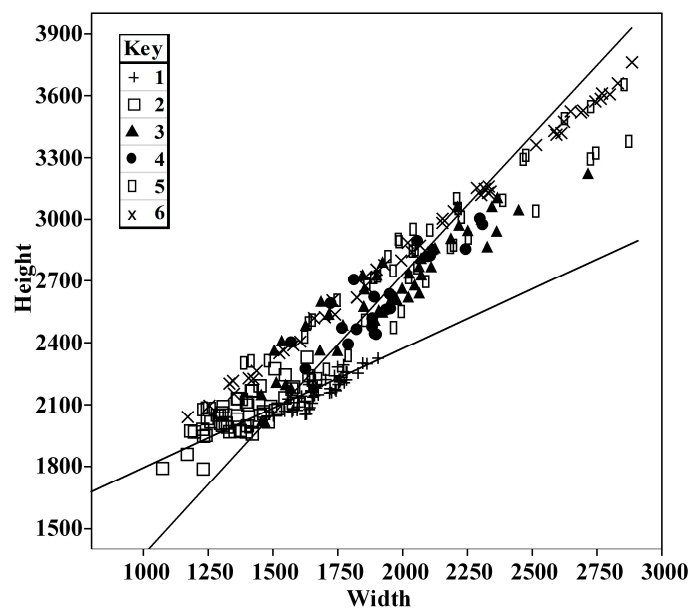
Figure 2. Landmark plot of the raw data and the Procrustes transformation (A) and the cumulative curves of the centroid size for each population (B). The cumulative curve was used to check the sufficiency of the individual number included in the shape analyses. The change in the mean centre size stabilized with 20–25 individuals.

### 3.3. Size Variation

The centroid size, calculated prior to the Procrustes, was used to estimate the overall size. The shells from the life populations (1 and 2) have less height than the dead ones (Figure 3). Populations 4–6 show a higher frequency of small individuals than samples 1–2. The size-frequency distribution of the shells from the life populations is skewed strongly toward the smaller sizes (<24 mm). In contrast, the death populations (3–6) have a more uniform distribution. In addition, there is less size variation for the life populations compared to a higher variability for the death ones (Figure 3). Both the Box plot (Figure 3A) and the histograms (Figure 3B) indicate these patterns. The height/width plots of all taxa show a distinct linear correlation (Figure 4). However, a distinct separation between the life (1 and 2) and the death populations is quite evident based on the height/length diagrams, where the life populations have a lower height/width ratio (lower y-values; Figure 4). The latter suggests that the allometric growth pattern has been altered in populations living in polluted water.



**Figure 3.** Box plots of size variation in the examined populations (A). The shaded area (axis to the right) shows the variation of PC Axis 1. Note the smaller shell height and the less variation on the PC axis of the populations from polluted sites. (B) Size-frequency histograms of the studied population. Life populations have narrower curves and slightly right-skewed histograms (smaller size).

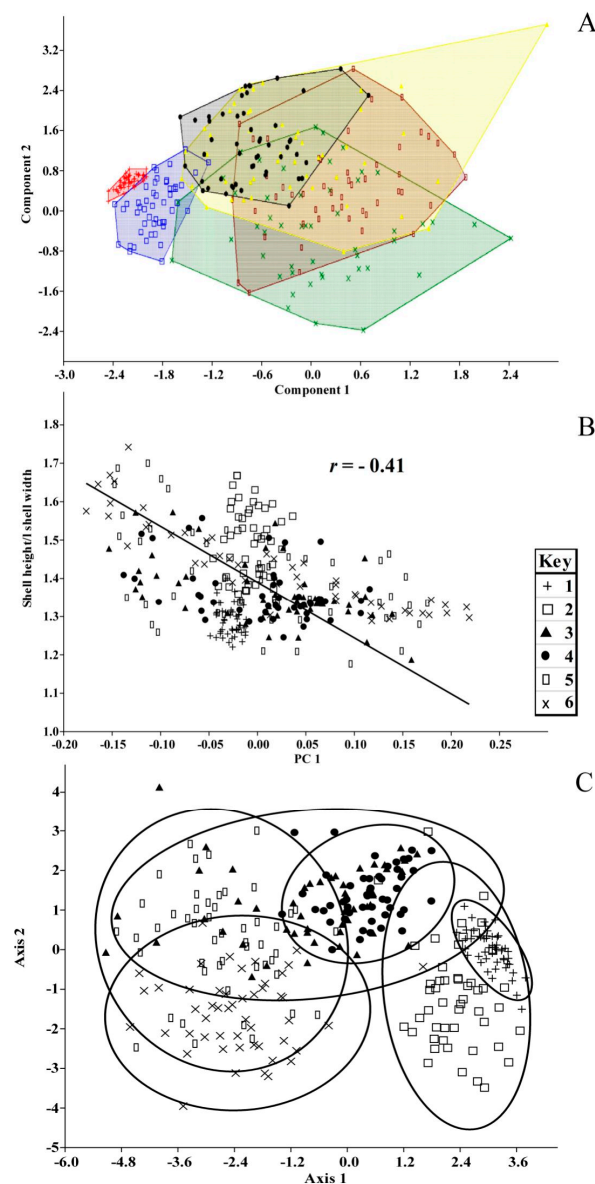


**Figure 4.** Linear regression model between the shell height and the shell width. Populations 1 and 2 have a gentle curve and can be separated from the death populations.

### 3.4. Shape Variation

#### 3.4.1. Ordination

The morphological variability within and between the shells from different samples is described by the shell shape. The PCA plots illustrate the morphological variability of life versus death populations (Figure 4). PC Axis 1 represents an evident gradient in the ordination of specimens. PC 1 (49%) is strongly influenced by relative shell height, where individuals become slenderer from left to right. This is clearer from the RMA linear regression between PC Axis 1 and the shell height/shell width ratio, where a significant negative correlation was found ( $p = 0.001$ ,  $r = 0.41$ ; Figure 5B). Although PC 2 (17%) is difficult to link to specific shell parameters, whorl shape and inclination represent a relative trend toward more inclined sutures.



**Figure 5.** (A) 2D-PCA plot that shows a clear distinctness between life and death populations. Note the low variability for the shell shape in the polluted sites. (B) Linear regression model between PCA axis 1 and shell height/shell width ratio. (C) CVA scatter plot of the same data. There is a slight gradient in the distribution of the populations, where the life populations are plotted at the most right. Populations 3 and 4 overlapped with 1 and 2, suggesting that they include some phenotypes of the life populations.

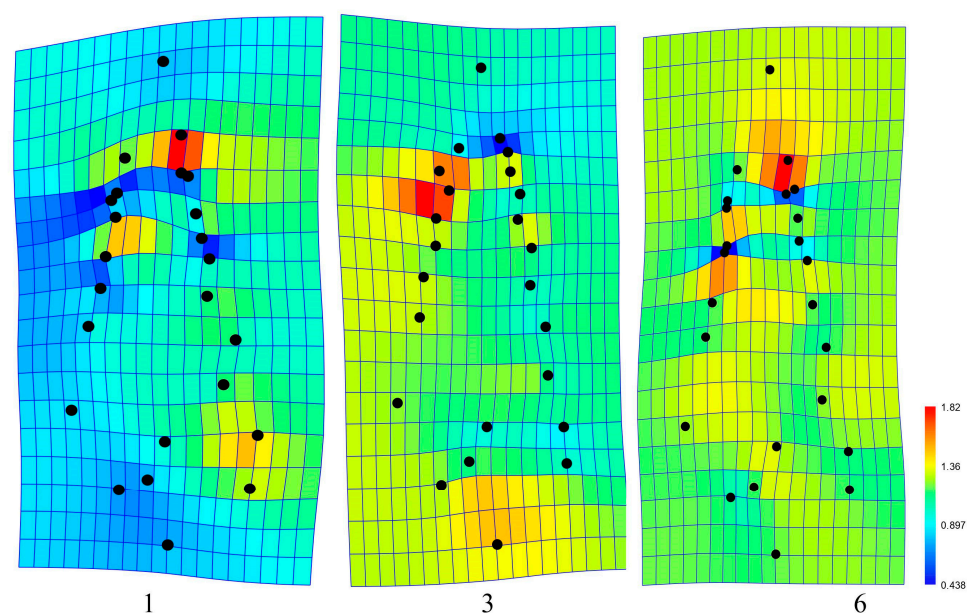


The PCA plot shows weak morphological overlap between the life populations (1–2) and the death populations (samples 3–6). Furthermore, a substantial overlap among life populations 1 and 2 suggests that environmental conditions have no significant impact on phenotypic variations. Low PC Axis 1 scores are obtained when the shell has a globular body whorl and a relatively low spire, whereas high scores are associated with a shell with a narrow body whorl and narrow aperture (Figure 5A). This means that individuals from the death populations have a more slender and narrow shell shape. Note that variation decreases in life populations, where they are plotted very closely (Figure 5A; see also Figure 3A).

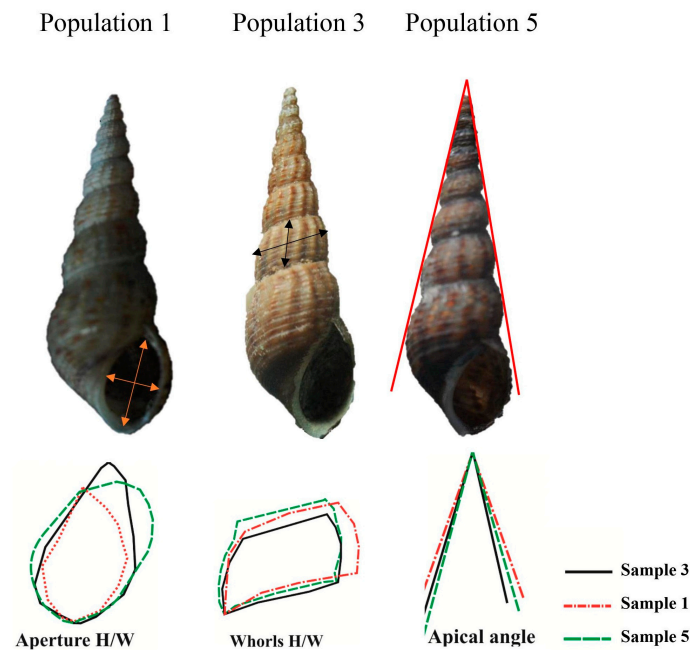
The results from CVA retained the same pattern, where populations 1 and 2 do not overlap with samples 5 and 6. Furthermore, samples 4 and 5 have wide variation and overlap with preceding samples 5 and 6 and succeeding samples 1 and 2 (Figure 5C). The latter may result from mixing different generations in death populations (time-averaged populations), where modern specimens living under current environmental conditions (polluted water) are mixed with older ones. In contrast, there is minimal variation within life populations, indicating a decrease in phenotypic breadth, and this may be attributed to the fact that they represent the same generation.

### 3.4.2. Thin Plate Spline

The TPS grids show a similar topology to ordinations, where populations 1 and 2 contain less calendrical and more globular species. However, the complicated grid pattern suggests that the variation is not limited to single-shape variables (Figure 6). To test the latter further, and as there is a variation on more than two shape characters, a representative individual from the populations was selected, and the shapes of specific characters were redrawn on these individuals with simplified geometric lines. Shell height/width was simplified as a dimension's rectangle, while the apical angle was redrawn as a triangle connecting the two tangents of the spire connected at the apex. In addition, whorl sections were redrawn as simplified perpendicular lines (Figure 7). To visualize the shape variation among the different populations, the simple redrawn shapes were moved one above another and aligned at specific corners/edges (first-order landmark (Figure 7)). The simplified lines indicate that life populations have more globular shells with depressed whorl sections and smaller apertures (Figure 7).



**Figure 6.** Heat maps show the shape change in *Melanoides tuberculata* shells as depicted by thin-plate spline deformation of the mean shape to populations 1 (life), 3, and 6 (death).



**Figure 7.** The main phenotypes dominating in life (1) and death populations (3 and 6) with simplification of the main shape differences.

## 4. Discussion

### 4.1. Sexual versus Asexual Reproduction

There are two main results of the geometric morphometric analyses: (1) a significant difference in shell shape between life and death assemblages and (2) a decreasing morphologic breadth in life populations. The variation among life and death assemblages could be attributed to many variables. Decreasing morphologic breadth can be attributed to a mixture of sexual and asexual reproduction of *M. tuberculata*, where an asexual reproduction may have resulted in less morphological diversity in contrast to a sexual one. *M. tuberculata* is capable of both sexual and asexual reproduction [63]; however, asexual reproduction is the more common one [64], which may result in rapid population growth but probably less genetic variability. If this is the case, why does *M. tuberculata* change its reproduction strategy on life and not death assemblages?

### 4.2. Directional Selection

Another possibility is directional selection, a mechanism for natural selection, in which allele frequency within a population shifts over time toward an extreme phenotype because of differences in the survival and rate of different phenotypes [62]. The changing environment will determine which phenotype will survive. Any species consists of a variety of distinct morphs, where the generalist nature makes it able to inhabit a wide range of aquatic environments regardless of resource availability [63]. The morphological breadth decreased in life populations 1 and 2 (Figure 5A). In addition, smaller globular shells became the dominant phenotype in the same direction (Figure 7), suggesting that directional selection is the prevailing natural selection mechanism in the studied populations. Ref. [64] indicated that environmental perturbations restrict niche availability and may decrease the phenotypic diversity, where a single or a few morphs can survive, which is the case in life populations.

All specimens of populations 1 and 2 have smaller specimens characterized by a relatively small height and large width. The size differences among the studied populations are well illustrated by the boxplot (Figure 3A), where life populations have a smaller mean shell height with a slight variance relative to death populations. Similarly, the size-frequency distributions differ significantly between life and death populations (Figure 3B).

Similarly, the variability of populations from the polluted sample on the histogram is also minimal (few size classes and narrower curve). The distinctive differences in shell size between life and death populations may suggest a directional selection toward smaller individuals in the current environment. The less size-variability of the populations from the polluted samples resembles the less variability on the PC Axis 1 (shaded area in Figure 5A).

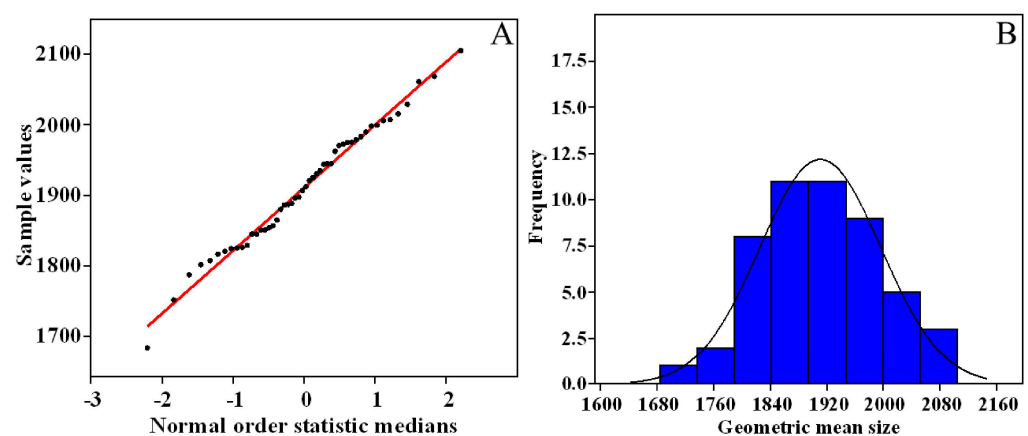
#### 4.3. Predation Intensity

Ref. [5] found an upward-increased abundance of *M. tuberculata* in the core sediments. This increase in population density can comment on the smaller size of life populations. The smaller body size is faster and more agile, and it needs less food and thus is better at surviving when food is limited. The variability of population structures among samples is more likely a result of variations in growth and mortality rates, possibly related to differences in the physical and chemical environment and/or biotic variables (high density and competition for food/habitats).

Furthermore, temporal variability of predation pressure by fishes could directly influence the population structure by reducing intraspecific competition [65]. However, according to [66–68], reducing intraspecific competition for resources is one of this species' most successful invasion strategies. Ref. [69] found that gastropods in fish-free ponds had narrow shells with a well-developed spire, whereas snails that coexisted with fish had more rotund shells with a low spire (a shell morphology known to increase the survival rate from shell-crushing predators). Can predation drive the morphologic variation in Manzala lagoon? There is no evidence of a change in predation intensity in the past few decades, so the answer will be negative. Moreover, Ref. [70] found that fish diversity (potential predators) is declining in the lagoon, suggesting that they cannot be the driving force behind the morphologic changes in life populations. Therefore, the small life populations of *Melanoides tuberculata* may be attributed to abiotic rather than biotic variables.

#### 4.4. Taphonomic Distortion

The very distinct and relatively more minor shell size may be physical (i.e., size sorting by waves or currents) rather than biological. In order to assess the origin of the smaller size at site 1, a histogram based on the geometric-mean size and QQ plot was drawn (Figure 8), and the population was tested for normality. As there was no evidence to exclude that the population can be normally distributed ( $p < 0.001$ ; Figure 8), the population may represent an autochthonous community without post-mortem or taphonomic distortion, and the relatively smaller size is biologic [60].



**Figure 8.** QQ probability plot (A) and size-frequency histogram of the geometric mean size of population (B). There is no evidence to reject the hypothesis that the population is normally distributed.

#### 4.5. Allometric Growth

Allometry, the change of a quantitative character with variation in overall body size, may affect the results of shape variation in the studied populations. Individuals of different sizes also have different shapes in almost all organisms [71]. A linear regression model between the shell height and shell widths indicated a clear linear correlation ( $r = 0.96$ ; Figure 4). However, a separation among the different populations on the linear plot is still evident, where the linear trend of the polluted areas is less steep (wider shells (Figure 4)), suggesting that the allometric growth pattern was shifted in life populations. Gastropod shell shapes depend on size (i.e., the shape differences between the taxa are partly an indirect consequence of the selection of body size [72]). However, the linear correlation between the life and death populations is highly similar, even when only the same size classes are compared. Therefore, the shape variation may be related to other environmental or ecological traits rather than allometry.

#### 4.6. Pollution-Induced Evolution

The reactions of living organisms to pollution are manifested by physiological, morphological, and behavioral changes [73]. Dunithan et al. [74] suggest that morphological variation ensures a wide distribution and maximizes local success for an aquatic gastropod in various environments. Abdelhady et al. [31] found that water pollution has induced morphologic variation between populations living in polluted and non-polluted habitats (i.e., pollution-induced natural selection). The size decrease in more eutrophic Polish lakes has been previously observed [75]. As there are no variations between the two life populations from habitats with different settings (silt vs. sand-dominated substrate, low vs. high salinity, and low vs. high wave energy), habitats have no or a minor role in driving the morphologic variations. In contrast, water pollution mainly drives the observed morphologic variations among life and death populations.

Freshwater gastropods were continuously used as model organisms for analyzing phenotypic plasticity [38]. Abiotic factors such as temperature, current, and salinity were found to influence the shell morphology based on significant laboratory and field observations [76,77]. In contrast to our results, [74] examined the covariation of morphology in North American freshwater gastropod *Elimia livescens* with regional environmental variables and found that the shape of *E. livescens* varied significantly with flow intensity, water temperature, conductivity, and substrate type. They found that individuals with smaller apertures and streamlined shapes occurred in smaller watersheds in the southeastern regions of Indiana and local habitats with low water flow and conductivity. The latter suggests a significant role for habitats, which is not seen here in the Manzala lagoon. Similarly, Ref. [78] indicated that the morphology of molluscs *U. delphinus* shells might be influenced by the water flow, where estuarine shells are more elongated, while riverine shells are more subovated. Based on molecular and morphological data, Márquez et al. [79] found that the gastropod *Trophon geversianus* shell polymorphisms represent habitat-specific constraints (e.g., intertidal vs. subtidal habitat). Furthermore, [80] suggested that geographic isolation is the main controlling factor of the morphological variation, while evolution and living habitat are less significant.

Recently, considerable research was conducted to test the impact of water pollution on species morphology, and there is increasing evidence supporting a pollution-induced evolution (phenotypic differentiation). Pollution may influence the molluscan macrobenthos in a variety of ways. In addition to causing the local extirpation of taxa within the lagoon [45,81], pollution-induced stress is hypothesized to suppress molluscan growth rates [82,83]. Marshal et al. [30] found that gastropod *Planaxis sulcatus* and *Echinolittorina nodosus* have more globular shells and smaller apertures in polluted water. Similarly, and based on a shape comparison between present-day specimens of the gastropod *B. globulosus* from the harbor zone and those collected before the increase of maritime activity and pre-Hispanic archaeological Middens, Ref. [37] demonstrated that pollution produces a globular shell shape. This effect probably allows gastropods to isolate themselves from the

external adverse environment. Only individuals that can tolerate higher metal concentrations and/or eutrophication can survive (survival of the fittest), while other morphs will go extinct. This can explain the general decrease in the morphological breadth of the *M. tuberculata* life populations in the studies' sites.

The size decrease in more eutrophic Polish lakes has been observed previously [75]. Ref. [84] indicated that eutrophication led to the dominance of small-bodied taxa. Vonlanthen et al. [85] found that eutrophication has resulted in genetic and functional distinctiveness among fish species. Anthropogenic eutrophication may intensify dissolved oxygen depletion, affecting the benthic communities [86]. According to Ali et al. [72], the small body size and aperture in gastropods from the Western Desert of Egypt could be attributed to aridity, where low precipitation and higher temperatures might limit food resources. As many gastropod shapes depend on size, the shape differences between the taxa are partly an indirect consequence of the selection of body size. In contrast, the reduction of aperture size may directly affect the temperature. Furthermore, the shell size, which depends on the shell calcification rate, is mainly controlled by atmospheric CO<sub>2</sub> concentration and water chemistry. The supersaturated carbonate water has higher calcification rates, producing larger and thicker shells. Finally, although morphologic variations are expected to occur naturally, anthropogenic activities may also impact the rate at which these changes occur. This view is supported by higher estimates of the rate of change/evolution in populations influenced by human activity compared to populations that are less influenced in such a way [87].

#### 4.7. Taphonomy versus Ecology

Differences in the size of dead and live shells can indicate different factors [88]: (1) post-mortem distortion due to physical agents such as waves or currents; (2) mass mortality due to environmental crises (e.g., anoxia or water pollution), (3) limitation of nutrients and competition for food, and (4) aging. Herein, life and death populations were collected from the same environment; therefore, the first factor (post-mortem distortion) is excluded. A skewed size-frequency distribution toward larger shells can indicate that the population is aging [89]. However, calculated life expectancies of gastropods indicated 0.74, 0.9, and 0.5 years for the first, second, and third years, respectively, confirming a normal size distribution frequency [90] under natural environmental conditions. Normal size frequency distribution is indicative of natural mortality [91], while uneven distribution is indicative of anthropogenic environmental deterioration [60] or human exploitation [92,93]. In normal or low-stress environments, symmetric, unimodal, bell-shaped curves, and slightly left-skewed due to the higher mortality of adults, are expected [94]. Moreover, empty shells of *M. tuberculata* collected from freshwater pools in Jordan have normal distribution [95]. Therefore, a normal distribution was expected in the study lagoon, and, therefore, we have assessed the normal distribution of the size-frequency data herein using the Shapiro-Wilk test and the QQ plot. Population heterogeneity in size structure is attributed to human-induced environmental degradation, where a decrease in population size over the past 50 was a marked feature in the Chinese plateau lakes [89]. Strongly right-skewed histograms generally indicate higher rates of juvenile mortality [96]. Manzlala Lake is classified as mesotrophic to eutrophic, with higher organic matter and nutrient enrichment due to agricultural discharge into the lagoon [97,98], which indicates that competition for nutrients is not a main factor. Therefore, we have attributed the decrease in size and morphologic breadth in the life population to the pollution by heavy metals.

## 5. Conclusions

The morphological breadth decreased significantly in the life populations of the study area compared to the death assemblages, and both have significant shape differences. Life populations in the area studied have smaller and more globular shells with smaller apertures, which are well-known morphological adaptations for reducing shell maintenance costs in polluted water. No distinct morphological variations were found among the death

populations from different habitats, suggesting that habitats have played no significant role in the phenotypic variations. As pollution in the lagoon water is the main difference between life and death populations, the phenotypic differentiation was attributed to pollution, i.e., a pollution-induced evolution. The morphologic variation between life and death populations can be attributed to time, but time only cannot comment on the marked variations (e.g., the decrease in both morphologic breadth and shell size). Water pollution after the construction of the AHD (1970) may accelerate the evolutionary process. As gastropod shells are used mainly for protection against predators, these pollution-induced changes in shell morphology will weaken their functions and may reduce gastropod survival and fitness. The findings highlight the possible impact of marine pollution in the evolution process and contribute to our understanding of how natural selection mechanisms work in real-world conditions.

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