Morphological response of lagoon cockle
*Cerastoderma glaucum* (Poiret, 1789) to eutrophication in the Sea of Azov

A T Mirzoeva\(^1\) and N A Demchenko\(^1\)

\(^1\)Bogdan Khmelnitsky Melitopol State Pedagogical University, 20 Hetmanska Str., Melitopol, 72300, Ukraine

E-mail: mirzoevatonya21@gmail.com

**Abstract.** The bivalve species *Cerastoderma glaucum* (Poiret, 1789) was studied in this study. This species is allochthonous and belongs to the Mediterranean zoogeographic complex and was introduced in the Holocene. The *C. glaucum* is the dominant species among the bivalves in the Sea of Azov and has a wide range of distribution. The species is distributed in the coastal zone within 100-300 m from the shore, and it is also found in desalinated water bodies such as estuaries. The *C. glaucum* is fairly resistant to hypoxia. It is euryhaline with respect to salinity and eurybiontic with respect to soil. The species can settle on sandy, muddy or sandy-silty substrate. The aim of the study was to investigate the morphology of shells of this species in order to find out the reasons of morphological features change of *Cerastoderma glaucum* in different biotopes of the Sea of Azov. The study was conducted in early June 2021 on the northwestern coast of the Azov Sea. A total of 20 stations were investigated. *Cerastoderma glaucum* was found at all stations. The morphological variability of the bivalve *Cerastoderma glaucum* was investigated using the method of discriminant analysis. A notable morphological feature was the external alteration of the mollusc shell. A displacement of the apex to the anterior edge of the shell, lengthening of the posterior edge, and deformation of the shell shape, indicating the ecological characteristics of the study area and its inhabitants. Also, there is a difference in the ratio of shell height to shell length, indicating an increased level of siltation in the ground. Shell thickness varies in all survey areas, indicating different levels of salinity. The overall abundance of molluscs from the different biotopes indicates the factors determining the shape of cockle shells. In turn, morphological parameters indicate the general condition of the Sea of Azov. So, it can be assumed that siltation of the substrate on which benthic communities are located has increased as a consequence of massive deposition of phytoorganic residues. In addition, the hydrolytic regime has changed as a result of anthropogenic factors. As a consequence, salinity, oxygen levels are changing, etc.

1. **Introduction**

The current state of the ecosystem of the Azov Sea is in “ecological crisis” due to the process of eutrophication [1]. The depressed state of the reservoir has become as a consequence of the mass multiplication of microalgae of cyanobacteria *Nodularia spumigena*, dinophyte alga *Lingulodinium polyedra* and *Centric diatoms* called “water blooms” and decreased water transparency [2–4]. The process of “water blooms” was the result of intensive anthropogenic impact through nutrient inputs, radioactive and chemical contamination of water, dumping, and sand mining in the sea [5–8]. The long-term suppression led to the death of most...
marine organisms [9–12]. Changes in the marine ecosystem have disturbed the sustainability of inhabitants supporting the marine environment [13–15]. There are numerical reductions in populations of most shell dwellers due to a decline in the number of dominant organisms of benthic communities such as bivalve molluscs [16, 17]. In Bivalvia populations, the visible morphological changes have manifested as a result of the process of metabolism in the body, leading to changes in growth rate, abundance, and distribution in the marine environment [18].

The marine bivalve is Cerastoderma glaucum (Poiret 1789) or lagoon cockle [19–21]. A representative of the infauna of shallow sand sediments of the European coast. It belongs to the family Cardiidae; its closest relative is C. edule, originally inhabiting the Mediterranean Sea [22]. The diversion of C. glaucum from its nearest relative, C. edule, and its distribution along the European coast took place at the end of the Miocene and the beginning of the Pliocene [23]. It entered the Sea of Azov in the Holocene after the connection of the Black Sea with the Mediterranean. The physiology and evolutionary development of the species allows C. glaucum to be tolerant to physical factors of different marine ecosystems. The cockle’s euryhalinity of 5–38‰ and eurythermicity (0–25°C) allows the species to expand and diversify its habitat [23]. The cockleshell is found in lagoons, estuaries, deltas with partially saline areas, or in coastal marine shallows. In most cases, high occurrence is observed in the Mediterranean Sea and southern Europe and has been recorded off the coast of Egypt, Tunisia, Turkey, Sardinia, Italy, Greece, Portugal, Spain, France (Atlantic and Mediterranean coast), Netherlands, British Isles, Denmark, Finland, Norway, Baltic Sea, Mediterranean Sea, Adriatic Sea, Red Sea, Aegean Sea and Caspian Sea [19, 24–35]. This species is an inhabitant of shallow waters. The maximum depth of C. glaucum survival does not exceed 0.5–1 m. The depth of submergence depends on friability, tidal fluctuations of water, and hydrolytic regime. Silted sands and shells are characteristic of the Sea of Azov. The submergence of the cockleshell is 3/1 of the total body area [36]. Life expectancy depends entirely on habitat. In most of the experimental work done, the age exceeds 6 years for some populations. In conditions with increased mortality and poor growth, the figure can often be reduced to 2–3 years maximum [37–39].

2. Research aim and objectives

The stability of C. glaucum to these water body habitats allows it to play a dominant role in the most benthic communities of the northeastern coast. This species is often found in a community with Abra nitida (Müller, 1776), Capitella capitata, Alitta succinea, Spio filicornis, Tubificoides sp. [40,41]. Or as a subdominant in a community with an allochthonous, fairly recently colonized species, A. kagoshimensis [42].

In this connection, the purpose of this work was to study the morphological variability of the lagoon cockle Cerastoderma glaucum in different areas of the Azov Sea.

3. Material and methods

An analysis of morphometric traits was performed to reveal the patterns of morphological adaptations in C. glaucum populations in the different areas of the Sea of Azov. Mollusc shells sampling was conducted in early June 2021 on the coast of the Sea of Azov. The average water temperature was 18.5°C. The material was collected at 5 sites (table 1).

In the past century, the benthic communities of the study areas have been exposed to commercial fishing and baitfish digging, including fisheries based on manual rake. In this study, we need to find out what are the impacts now experienced by modern benthic communities at the head of the lagoon cockle. To do this, fourteen morphometric features were selected for each C. glaucum, by which the condition of the species inhabiting the Sea of Azov will be evaluated. Measurements of the shell were made to an accuracy of 0.01 mm using a caliper. Was measured H – shell height, mm; B/2 – shell width, mm; L – shell length, mm; a – ligament length, mm; b – length of the upper edge of the shell, mm; c – length from anterior edge of the ligament to
Table 1. Geographic data of the sample collection site.

| Location of collection | Latitude     | Longitude      |
|------------------------|--------------|----------------|
| Station 1              | 46°13’00.0”N | 35°14’00.0”E  |
| Station 2              | 45°59’34.7”N | 34°49’40.8”E  |
| Station 3              | 45°59’33.3”N | 34°49’29.8”E  |
| Station 4              | 45°59’33.3”N | 34°49’29.8”E  |
| Station 5              | 46°00’00.3”N | 34°49’25.2”E  |

the apex, mm; \(d\) – length from anterior edge of the shell to the apex, mm; \(e\) – distance between the anterior end of the shell and the apex, mm; \(f\) – distance from the mantle edge to the shell edge, mm; \(h\) – width of the posterior adductor scar, mm; \(i\) – width of the anterior adductor scar, mm; \(j\) – height of umbo, mm; \(k\) – length of umbo, mm; \(l\) – thickness of the bivalve, mm; \(m\) – ribs (figure 1). Measurement error in the use of these methods does not contribute significantly to the variation of the characters. We chose the characters previously used by the authors in the morphological diagnosis of mollusc shells [43, 44]. The descriptive statistics of morphometric characters and the degree of compliance with the laws of distribution were performed in the Excel program.

Figure 1. Shell traits measured for each individual clam in *Cerastoderma glaucum*: \(H\) – shell height, mm; \(B/2\) – shell width, mm; \(L\) – shell length, mm; \(a\) – ligament length, mm; \(b\) – length of the upper edge of the shell, mm; \(c\) – length from anterior edge of the ligament to the apex, mm; \(d\) – length from anterior edge of the shell to the apex, mm; \(e\) – distance between the anterior end of the shell and the apex, mm; \(f\) – distance from the mantle edge to the shell edge, mm; \(h\) – width of the posterior adductor scar, mm; \(i\) – width of the anterior adductor scar, mm; \(j\) – height of umbo, mm; \(k\) – length of umbo, mm; \(l\) – thickness of the bivalve, mm; \(m\) – ribs.
4. Results

The investigated populations of *C. glaucum* had their own morphological features. The coefficient of variation of morphometric characteristics of the shell of *C. glaucum* in the Site 1 was in the range of 0.09 to 0.35%. The shell of the cockleshell of the Site 4 ranged from 0.07 to 0.43%. These small differences in values indicate similar conditions of the cockleshell populations. The smallest sizes were the cockleshells collected at Site 3 and Site 5. The coefficient of variation of Site 3 was in the range of 0.06 to 0.55%. The value (CV) for Site 5 was in the range of 0.09–0.32%. In the Site 2 population, the coefficient of variation ranged from 0.07 to 0.41%. The ratio of cockleshell sizes showed a trend of variation depending on the habitat. Analogous in appearance shells were found at sites 1, 3, and 5. The shells were rounded, the contour of the shells globularly swollen, the crowns wide, usually strongly protruding. The symmetry of the form of shells in a ratio of height to length was observed. The slightly elongated hind margin of shells of the *C. glaucum*. At the Site 2 and 4 the shells also possessed strongly swollen roundish form. Asymmetry in relation to height to length and with more or less smoothed angles at the transition of dorsal margin to anterior and posterior, if there is an angle of posterior shoulder, it is usually equal. The Site3 population differed from all other populations, showing the lowest values.

The morphological distinction of the cockle shells in the Azov Sea was the arrangement of the ribs. The cross-section was shaped, of different height and length. Usually the flaps in the front part of the shell were found with rounded ribs in cross section, in the middle trapezoidal, or in the second half of the middle part triangular with the triangular apex shifted to the lateral posterior facet. Most of the sample data on the number of ribs ranged between 17 and 27. The mean number of ribs was high for Site 1 populations, 21.19 ± 0.29 having a relationship with shell size. Only Site 4 showed a similarly high number of shell ribs (table 2). Once again confirming the similarity of habitat conditions.

The analysis of the histograms showed that the value of the index H (shell height) varies depending on the shell collection area. The highest index in the area of the Site 3, the lowest in the area of Site 5. In terms of the B/2 shell thickness, the values are off the charts at the Site 4. The value L is the length of the shell, corresponding to the height. A slight elongation of the posterior end of the shell indicates the depth of burrowing into the ground. The normal location of the clam in the ground is 3/1 of the entire length of the shell of individuals. The highest values are at the Site 1 and 4. The index a - ligament length varies insignificantly compared to the variability of this trait. The dependence of ligament length as well as ligament shape on shell contour is practically not traced. The ligament length predominates in the largest individuals of Site 1 and 4, respectively. At the Site 3, the shortest shell ligaments were found, which belong to mollusks with abnormalities in the structure. The characters b, c, d, e, f, h, i, j correspond to the proportions of shells of mollusks. The number of ribs index - m, varied depending on the size and age peculiarities of cockles in individual study areas. Trait - l, hypothetical suggests that shell thickness indicates water salinity. The highest index at Site 1 was 1.03 ± 0.05 (figure 2).

5. Discussion

Each morphological trait describes the environment in which the cockleshell exists. Indicating which environmental factors are the main in the formation and growth of *C. glaucum*. It is a known fact that the growth of the cockleshell is 5–250 mcm daily during the first years of life. The stoppage of growth is observed in winter, during breeding and in old age [45]. The formation of the mollusc shell occurs as a result of extracellular growth. The epithelium of the outer surface of the mantle extrudes shell-building products into the extrapallial fluid. The ratio of height to length of the ravine indicates normal growth. Deviations are not significant. The total maximum height of the Azov Sea shells was 29.3 mm, a figure rather insignificant compared to data from southern studies, where the maximum shell size was 53.2 mm [19], and
in some areas 29 mm Tunisia Laguna Bugrara [26], 32 mm Egypt Lake Timsah [46], 37 mm at the Gabyes coast [25].

**Table 2.** Descriptive statistics of shell morphometric traits: shell height, mm; B/2 valve width, mm; L – shell length, mm; a – ligament, H – length, mm; l – thickness of the bivalve shell; m – ribs.

| Traits   | H       | B/2     | L       | a      | l     | m       |
|----------|---------|---------|---------|--------|-------|---------|
| Site 1, N = 42 | 18.26±0.78 | 7.50±0.30 | 19.41±0.80 | 4.05±0.25 | 1.03±0.05 | 21.19±0.29 |
| Maximum     | 29.3    | 12.10   | 31.90   | 7.30   | 1.62  | 27.00   |
| Minimum     | 11.2    | 4.40    | 12.30   | 1.20   | 0.51  | 19.00   |
| CV, %       | 0.28    | 0.26    | 0.27    | 0.35   | 0.30  | 0.09    |
| Site 2, N = 42 | 16.34±0.32 | 6.65±0.21 | 18.27±0.36 | 2.45±0.19 | 0.47±0.04 | 19.98±0.20 |
| Maximum     | 23.40   | 12.70   | 24.80   | 6.20   | 1.06  | 23.00   |
| Minimum     | 11.90   | 5.20    | 14.60   | 1.20   | 0.11  | 18.00   |
| CV, %       | 0.13    | 0.20    | 0.13    | 0.41   | 0.23  | 0.07    |
| Site 3, N = 52 | 13.65±0.37 | 6.24±0.28 | 15.26±0.41 | 1.04±0.15 | 0.62±0.23 | 20.90±0.17 |
| Maximum     | 24.10   | 10.90   | 26.50   | 3.60   | 1.34  | 24.00   |
| Minimum     | 8.50    | 3.20    | 8.70    | 1.20   | 3.65  | 19.00   |
| CV, %       | 0.19    | 0.32    | 0.19    | 0.33   | 0.55  | 0.06    |
| Site 4, N = 127 | 18.43±0.39 | 8.82±0.18 | 21.7±2.40 | 3.37±0.35 | 0.72±0.03 | 21.64±0.14 |
| Maximum     | 28.90   | 19.70   | 34.5    | 7.6    | 1.29  | 26.00   |
| Minimum     | 8.40    | 3.80    | 10.70   | 1.30   | 0.11  | 17.00   |
| CV, %       | 0.24    | 0.43    | 0.26    | 0.37   | 0.41  | 0.07    |
| Site 5, N = 41 | 14.56±0.35 | 7.30±0.34 | 16.56±0.39 | 3.19±0.30 | 0.59±0.04 | 21.37±0.32 |
| Maximum     | 21.50   | 12.90   | 23.90   | 4.20   | 1.23  | 25.00   |
| Minimum     | 10.00   | 3.60    | 11.90   | 1.90   | 0.38  | 17.00   |
| CV, %       | 0.15    | 0.30    | 0.15    | 0.25   | 0.32  | 0.09    |

Inferring from the size of the shells from the Sea of Azov, it is possible to assume the age of the cockles. The literature indicates that the maximum life span of cockles of *C. glaucum* is 5 years. Depending on the environmental conditions with regard to mortality and poor growth, this figure can often be reduced to 2 – 3 years [38, 39]. It is worth assuming that life expectancy varies in the Sea of Azov from 1 to 3 years except for single individuals. Following the morphological differences in the shells of *C. glaucum* from different collection areas, it should be assumed that the change in their size depends on environmental factors in different geographical collection sites. Increased temperature, salinity and oxygen levels contribute to favorable growth and longevity of cockles at Site 1 and 4. Conditions at Site 5 show a difference in reduced growth, abundance, and shell size. From the other side, the environmental conditions of Sites 2 and 3 [47] showed that low average salinity and lower water temperature have a positive effect on the growth of the *C. glaucum* shell. Challenging assumptions about the factors affecting viability in the literature is a contradiction and indicated that elevated temperatures and high levels of phytoplankton in Gabes Bay [25] promote rapid growth.
Figure 2. Histograms of morphometric traits of *Cerastoderma glaucum* shells. The red line indicates the histogram normal distributions: $H$ – shell height, mm; $B/2$ – shell width, mm; $L$ – shell length, mm; $a$ – ligament length, mm; $b$ – length of the upper edge of the shell, mm; $c$ – length from anterior edge of the ligament to the apex, mm; $d$ – length from anterior edge of the shell to the apex, mm; $e$ – distance between the anterior end of the shell and the apex, mm; $f$ – distance from the mantle edge to the shell edge, mm; $h$ – width of the posterior adductor scar, mm; $i$ – width of the anterior adductor scar, mm; $j$ – height of umbo, mm; $k$ – length of umbo, mm; $l$ – thickness of the bivalve, mm; $m$ – ribs.
On the other hand, [21] showed that low mean salinity negatively affects the growth of \textit{C. glaucum} from the Baltic Sea. [32, 48] described respectively isometric and positive relative
growth in \textit{C. glaucum} from several Mediterranean, Atlantic and Baltic sites. This wide variation
in the relative growth of the species is certainly due to genotype [49], but also various local
specific environmental factors can strongly influence the variations in shell shape present in
bivalves. It is worth noting that the allometric growth of \textit{C. glaucum} are a functional response
to different habitat typologies. Thus, allometry was evident in Site 2 and 4. Indeed, bivalves
are known for their remarkable morphological diversity and phenotypic plasticity, which is
perceived as the main algorithm that allows organisms to survive under changing environmental
conditions [50, 51] especially for \textit{C. glaucum}, which proved its ability to withstand extreme and
unstable environmental conditions in different areas of the Azov Sea.

It is hypothesized that shell thickness indicates the level of salinity. Thickening of the shell
is observed with growth and age. The thickening process occurs uniformly along the entire
axis height, from the apex to the ventral edge. The variation in the thickness of Azov Sea
cockleshells indicates variations in salinity. Following from the indices of thickness of cockle
shells of Site 1 the indices of water salinity should prevail over the rest of the study area.
Slightly lower is the thickness of the shells of Sites 4 and the thinnest shells of Site 2, 3, and
5. Referring to [30] the shell thickness of 0.76 mm indicates that the shells are two years old.
The measurements obtained in this work indicate a suppression of growth on this trait at Sites
2, 3 and 5. According to literature sources, a sharp increase in the salinity of the Sea of Azov
was observed from 2007 – 2008, annually adding 0.25 ppm on average. In 2020, more than 15
ppm, that is, 15 grams of salt per liter of water. In the Black Sea, in the surface layer, where
it adjoins the Sea of Azov, 17 ppm [52]. The salinity is explained by the low water exchange
between the Black Sea and the Sea of Azov.

An important morphological trait is the number of ribs. Their number is directly related to
salinity. The number of ribs in the Sea of Azov varies from 17 to 27. In the Black Sea from 18 to
28 [47]. In the Mediterranean near Tunisia, 19 to 21; La Skhira beach 23; Lac Ichkeu; 19 to 24
ribs; [26]; Near Greece, 17 to 28 [19]; In northwestern Europe, 18 to 20 [53]; On the Irish coast,
22 to 28 [54–56]. Another presumed factor influencing the morphological changes of \textit{C. glaucum}
shells is the process of sea eutrophication. Many factors of abiotic and biotic type influence on the
starting mechanism of mass development of nutrients. The greatest harm is caused by irrational
use of a water body. Human activity destroys natural habitat of marine organisms, affecting
it by aggressive factors. All wastes of industrial, economic activities are discharged into drains
and utilized in the sea with high content of organic and inorganic substances. From factories
and iron and steel works: mercury, lead, phenols, oil products, phosphates, nitrates and various
organic substances. Huge amounts of agricultural products: pesticides, detergents, ammonium
salts and nitrates. Subsequent over-fertilization of the Azov Sea was followed by an increase in
the number and biomass of phytoplankton. Massive growth of the diatom-cyanobacterial algal
complex resulted in sea blooms, changes in color, transparency, and increased CO$_2$ levels [57].
As a result, affecting the number, diversity, and distribution of marine life.

6. Conclusion

During the morphological measurements of cockleshells of \textit{Cerastoderma glaucum} in the five
studied sites of the Sea of Azov revealed differences. According to the complex of morphological
features, the most distinguishable were the size and allometric shape of the shell, its thickness,
and the number of ribs. The result of the work showed that the most favorable conditions
for the full development and life of cockles are the areas of Sites 1 and 4 based on their size,
and therefore the age of the mollusc. Although small deviations from the norm were observed.
As for the rest of the study areas, the area is subject to constant natural fluctuations in the
environment. From what was observed uneven morphological and physiological development of
individuals, early mortality. The supposed hypothesis that eutrophication of the Sea of Azov affects the morphological development of cockles is not confirmed. For more exact statement it is necessary in the further researches to carry out a number of researches connected with physiology of mollusc *Cerastoderma glaucum*.

ORCID iDs
A T Mirzoeva https://orcid.org/0000-0002-4924-8248
N A Demchenko https://orcid.org/0000-0001-6469-760X

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