Comparative morphological analysis of diploid and triploid oysters, *Crassostrea gigas*, farmed in the Black Sea

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Abstract: Triploid organisms, in particular oysters, are widely used in marine aquaculture. However, the advantage of triploid oysters compared to diploids has not been proven in all cases. Comparative morphological analysis of Pacific oysters *Crassostrea gigas* of different ploidy levels allows evaluating the benefits of raising triploids in the Black Sea. First, the morphological characteristics of diploid and triploid Pacific oysters *C. gigas* farmed in the Black Sea were studied. Allometric ratios of weight (W, g) and shell height (H, mm) of oysters were obtained, which are described by the equations $W_{2n} = 4 \times 10^{-4} \times H_{2n}^{2.56}, R^2 = 0.86$ and $W_{3n} = 9 \times 10^{-5} \times H_{3n}^{2.90}, R^2 = 0.91$. Triploids showed positive allometry and had more biomass at smaller shell height than diploid oysters. A clear linear relationship of the height and length of oyster shells was established. Significant differences in linear parameters between 2n and 3n *C. gigas* farmed in Donuzlav Liman are not seen. Based on these results, it is assumed that the advantage of the growth of 3n mollusks is less evident in farms located at high latitudes. The environmental factors and farm technology have probably affected the growth of oysters to a greater degree than their ploidy.

Key words: *Crassostrea gigas*, triploids, allometric relationship, mariculture, Black Sea

1. Introduction

Commercial production of triploids (3n) began more than 30 years ago and became a prospect for industrial aquaculture. Triploid oysters can be produced by two general methods: chemical induction and crossing diploid (2n) mollusks with tetraploid (4n) ones. However, chemical treatment is not always reliable for producing 100% 3n populations, and crossing with tetraploid oysters is the most effective in producing triploids [1]. Nowadays, breeding lines of 4n oysters are produced in the USA, France, and Australia [2–5].

It is commonly recognized that triploids demonstrate faster growth rates, better meat conditions, partial sterility, and a higher survival rate due to resistance to disease. In general, triploids obtained by crossing 2n × 4n showed 20%–40% superior growth of shell height and 70%–80% increased whole weight compared to diploids [6,7]. Chemically induced 3n also possessed growth advantages compared to normal 2n oysters; however, this advantage was only 8% and 12%–31%, respectively [2,5].

In 3n mollusks, the growth rate, survival, and resistance to diseases may vary depending on species of oysters, cultivation conditions, environmental factors, and duration of the observation period. A number of studies demonstrated that larval stages of 3n and 2n did not differ by survival rate [6]. However, triploids of several oyster species became more vulnerable through the maturation period. For example, 3n *Crassostrea virginica* revealed higher mortality rates compared to 2n oysters [4]. Resistance to disease of polyploid oysters is also discussed. Some authors reported no influence of triploidy on survival to viral infections, such as resistance to OsHV-1, for *C. gigas*. Similarly, vibriosis caused the death of both diploids and triploids of *C. gigas* [8]. Indeed, the mortality rate of diploid oysters was greater during intensive gametogenesis, and for triploids, the peak of mortality was observed during winter months. On the other hand, the results of experiments on *C. virginica* and *S. glomerata* demonstrated a greater resistance to disease in triploids compared to diploids [6,9,10].

Abiotic factors (food supply, water temperature, and salinity) also influence the growth and survival rate of 3n oysters. Growth advantages of triploids are observed in waters of rich trophic growing areas, and in “poor” regions there are no differences in growth parameters between 2n and 3n oysters [6,11]. Geographical locations of farms also influence the cultivation of 3n oysters. The growth advantage of 3n mollusks was negligible (less than 7%).
in farms situated at higher latitudes (42–43°S), such as in Tasmania, Australia [12]. In warmer regions (33–34°N), i.e. Japan [7] and Taiwan [13], the whole weight increase was up to 81% higher for 3n Pacific oysters compared to 2n during an 8-month observation period. The difference in growth rate between 2n and 3n C. gigas was maximal at 30 °С compared to similar oysters kept at 8–15 °С [14–16].

The main advantage of farming triploid oysters is the absence of the spawning period. The oysters maintain their product quality through the whole year. They are also called “four-season” oysters. 2n oysters undergoing a spawning period have shell growth retardation and decrease of whole weight and soft tissue weight, whereas triploids continue their growth [7], which causes differences not only in size but also in biochemical composition. Glycogen, carbohydrates, and total protein in triploids were 45%–65% higher compared to control diploids [17,18]. Low reproductive activity and additional reserve of carbohydrates and glycogen are supposed to undermine the high survival rate of 3n mollusks under unfavorable conditions.

Recent data confirm that farms currently prefer to cultivate two main species of triploid oysters, the Pacific oyster (C. gigas) and the eastern oyster (C. virginica) [5]. Higher growth rates of weight and shell length lead to a shorter vegetation period and to reaching the market size of triploids faster than diploids. In the late 1980s C. gigas was successfully introduced into the Black Sea. Natural conditions of the Black Sea allowed the development there of marine aquaculture of bivalves and oysters in particular [19,20]. For the first time, triploid oysters obtained in French nurseries appeared in Black Sea farms in 2005 [21].

In the present study, we investigated the main morphometrical parameters, i.e. shell height and length and whole weight, in 2n and 3n oysters, Crassostrea gigas, farmed in the estuary of Donuzlav, Black Sea (western coast of the Crimean peninsula). Specific attention has been paid to the ratio of these parameters and the scaling factor b in allometric equations.

2. Materials and methods

The objects of the research were diploid and triploid pacific oysters C. gigas, which were cultivated on a farm on the western coast of the Crimean peninsula at Donuzlav Liman (45°24′36″N, 33°09′3.2″E). Donuzlav Liman is a half-closed firth of the Black Sea, situated on the western coast of the Crimean peninsula. The modern ecosystem of the estuary was formed after its connection with the Black Sea through a canal built in 1961. The temperature regime is characterized by large annual amplitude with rapid summer warming and winter cooling to the bottom in the shallow areas. Maximal average month water temperature of 26.2 °С is observed during July and August, while the minimal value of 5 °C is reached during February [22] (Figure 1). Water salinity is 17.2–18.1 ppt. Chlorophyll concentration ranged between 1.77 and 5.6 mg/m³ [23].

Mollusks were held in plastic oyster cages with density of 350–450 animals/m². The size of the oyster cage is 0.5 m × 1.00 m × 0.2 m, with mesh holes of 1.5 cm. Cages with oysters were landed at the shellfish farm and submerged 1.5–3 m from the surface of the sea. The observation period lasted from December 2015 to September 2016. Before measurements, shells were cleaned of fouling with a brush, washed with seawater, and dried with filter paper. Weighing of mollusks (defining W, g) was carried out on electronic scales with accuracy of 0.01 g. Linear parameters were measured by digital caliper with accuracy of 0.01 mm. The study was focused on the parameter of shell height, which is a criterion of marketability and cost for growing oysters. Shell height (H, mm) was estimated as a maximal distance between shell lock and growing edge. Shell length (L, mm) was measured at its widest part and was perpendicular to H [24] (Figure 2). Some authors define this parameter as shell length, or maximal linear size of the shell. This will be taken into account when discussing the results.

Based on the results obtained, the coefficients of variation, isometry equations of the relationship of the studied parameters L = m + b × H, and allometry equations of mollusk weight ratio and linear dimensions have been computed: W = a × H^b and W = a × L^b, where W is oyster weight (g), H is shell height (mm), and L is shell length (mm), with coefficient of determination R^2. All data processing was done in MS Excel.

3. Results

3.1. Morphological parameters of diploid and triploid oysters

Variation series of shell height showed that the majority of 2n oysters possessed H 25–35 mm (16%) or 60–80 mm
More than half of 3n oysters were characterized with shell height of 55–75 mm (55.4%). Biometric parameters (shell height and length) and whole body weight of diploid and triploid oysters are shown in Table 1.

The obtained data demonstrate that variability of linear parameters was in the range of 0.32–0.35. For other species of oysters, for example *C. corteziensis* in the Gulf of California, CV_L may reach 0.38 and CV_H 0.41 [25]. In our study, body weight of *C. gigas* varied widely (CV_W_2n: 0.77; CV_W_3n: 0.63), which was two times higher than the variability of linear parameters (Table 1). Some authors suggest that high coefficients of variation of morphological features may be caused by high heterozygosity of the organism [26]. In triploid mollusks obtained by crossing, CV_W and CV_H reached 0.85 and 0.55, respectively, which was significantly higher than in diploids [5]. CV is also influenced by various factors, i.e., genetic diversity of growing mollusks, environmental conditions, and methods for the selection of the objects studied and their further examination.

### 3.2. Allometric relationship of biometric parameters and whole weight of diploid and triploid oysters

Shell growth in bivalves is usually determined by the substrate quantity, population density (overpopulation), tidal and wave intensity, current speed, depth, feeding spectrum, etc. [24,27,28]. For cultivating oysters, additional factors are the method of growing (construction of oyster cages, their location in the sea, etc.) [29] and density of mollusks in cages [21,30]. In *C. gigas* shell shape possesses high individual plasticity due to disproportional and uneven increase of shell height and length. Shell length grows much slower than height [24,27]. In juvenile oysters up to 25 mm in size the flap height is equal to the length, and the shell has a rounded shape. As it grows, the shell of the Pacific oyster becomes first oval (in size group 35–55 mm), and then for larger mollusks the shell length is approximately 3/4 the height and shell shape becomes elongated (Figures 2 and 4).

The H-L relationship of the studied oysters is well described by linear equations L_{2n} = 0.58 × H_{2n} + 0.95, R^2 = 0.83 and L_{3n} = 0.50 × H_{3n} + 4.82, R^2 = 0.72. Similar linear relations between shell height and length were obtained in other works: for *C. gigas*, L_{2n} = 0.96 × H_{2n} – 1.01; *C. commercialis*, L_{2n} = 0.65 × H_{2n} – 0.11 [24]; *C. virginica*, L_{2n} = 0.50 × H_{2n} + 18.82, L_{3n} = 0.48 × H_{3n} +22.65; and *C. ariakensis*, L_{3n} = 0.54 × H_{3n} + 33.35 [31].

The ratio of linear dimensions (H/L) allows assessing shell shape. Disk-shaped and round specimens usually possess H/L close to 1, whereas an elongated shape of mollusks is associated with 1.5–2; H/L > 2 indicates long
and narrow shells. In our study, the average ratio was 1.71–1.73, varying in the range of 1.21–2.48 for diploids and 1.14–2.6 for triploids. Thus, 3n oysters possessed higher variations of the ratio. More than 64% of mollusks were characterized by ellipsoidal shape; 12%–15% of specimens were elongated.

The relationship between shell height and whole body weight of living oysters was nonlinear but represented a power function (Table 2). The power coefficient $b$ is a universal parameter for allometric equations, because its value does not depend on the type of mollusk weight used (wet, dry, or whole body weight) [28]. According to our data, for 2n $C. gigas$ in the Donuzlav estuary $b$ was 2.56, which was lower than for 3n oysters (2.90) (Table 2). The estimated value of $b$ for bivalves varies in the range of 2.5–3 [28]. It has been determined that species of the family Ostreidae possess sagittally compressed shell shape, presuming a low power coefficient $b$ in allometry equations of 1.5–2.5, or $b \approx 2$. For example, for $C. columbiensis$ the estimated $b$ is 2.35 [32]; for $C. madrasensis$, 1.7–2.0 [33] and 2.49–2.92 [34]; for $C. virginica$, 1.86 [35], 2.15 [36], 2.17 [1], and 2.26–2.39 [27]; for $C. iridescens$ and $C. angulata$, 1.48 [35]; and for $C. gigas$, 1.12–2.79 [37], 1.87 [35], 2.39 [21], 2.43–2.52 [36,39], and 2.81 [40]. It may vary depending on environmental conditions [28].

It was demonstrated that water temperature, chlorophyll concentration, tidal currents speed, and cultivation method used influence the ratio of shell height to weight in $C. virginica$ [42]. Previously, we obtained the opposite results for oysters in the open coastal area of the Black Sea (the Blue Gulf) (Table 2), which may be caused by the peculiarities of the farm's location.

### Table 1. Statistical analysis of the biometric and weight characteristics of cultivated $C. gigas$.

<table>
<thead>
<tr>
<th></th>
<th>Diploids</th>
<th>Triploids</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of specimens</td>
<td>485</td>
<td>484</td>
</tr>
<tr>
<td>Shell height, mm</td>
<td>$M \pm SD$ 61.17 ± 19.88</td>
<td>60.23 ± 17.38</td>
</tr>
<tr>
<td>Min–max</td>
<td>23.0–99.1</td>
<td>23.0–98.9</td>
</tr>
<tr>
<td>CV</td>
<td>0.32</td>
<td>0.29</td>
</tr>
<tr>
<td>Shell length, mm</td>
<td>$M \pm SD$ 36.53 ± 12.76</td>
<td>35.11 ± 10.27</td>
</tr>
<tr>
<td>Min–max</td>
<td>14.0–67.4</td>
<td>14.0–64.6</td>
</tr>
<tr>
<td>CV</td>
<td>0.35</td>
<td>0.29</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>$M \pm SD$ 20.45 ± 15.73</td>
<td>19.50 ± 12.33</td>
</tr>
<tr>
<td>Min–max</td>
<td>0.98–65.48</td>
<td>1.33–53.57</td>
</tr>
<tr>
<td>CV</td>
<td>0.77</td>
<td>0.63</td>
</tr>
</tbody>
</table>

![Figure 4](image-url). The ratio of the height and length of diploid and triploid oysters $C. gigas$.

### Table 2. Parameters of allometry equation $W = a \times H^b$ and $W = a \times L^b$ of diploid and triploid oysters $C. gigas$.

<table>
<thead>
<tr>
<th>Area</th>
<th>Oysters' ploidy</th>
<th>$a$</th>
<th>$b$</th>
<th>$R^2$</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donuzlav</td>
<td>Diploids</td>
<td>$4 \times 10^{-4}$</td>
<td>2.56</td>
<td>0.86</td>
<td>Present data</td>
</tr>
<tr>
<td></td>
<td>Triploids</td>
<td>$9 \times 10^{-3}$</td>
<td>2.90</td>
<td>0.91</td>
<td>Present data</td>
</tr>
<tr>
<td>Blue Gulf</td>
<td>Diploids</td>
<td>$8 \times 10^{-4}$</td>
<td>2.34</td>
<td>0.94</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td>Triploids</td>
<td>$9 \times 10^{-4}$</td>
<td>2.18</td>
<td>0.95</td>
<td>[21]</td>
</tr>
<tr>
<td>W = $a \times L^b$</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donuzlav</td>
<td>Diploids</td>
<td>$9 \times 10^{-3}$</td>
<td>2.7</td>
<td>0.81</td>
<td>Present data</td>
</tr>
<tr>
<td></td>
<td>Triploids</td>
<td>$1.8 \times 10^{-3}$</td>
<td>2.56</td>
<td>0.82</td>
<td>Present data</td>
</tr>
</tbody>
</table>

![Graph](image-url).
4. Discussion

The morphology of the *C. gigas* shell is quite plastic; therefore, investigating the shell's morphological parameters allows assessing the conditions of bivalves at various stages of growth. The main criteria for commercial oysters are their size and weight. Controlling the formation of shell and biomass allows obtaining quality seafood. Shell shape depends on environmental factors (temperature, salinity) and habitat conditions (population density, depth, substrate type, etc.) [29,31]. The height to length ratio, in turn, influences oysters' shell surface area, internal volume, and soft tissue weight. Several authors demonstrated that the H-L ratio in *C. gigas* may be linear or nonlinear, which for the most part depends on habitat conditions or cultivation methods [24]. It is known that the H-L ratio was greater in mollusks inhabiting soft substrates compared to hard substrates. A high density of oyster populations also substantially influenced shell shape and increased H-L values [31].

Notably, not all the studies published demonstrated growth advantages of triploid oysters. In areas with unfavorable environments or intensive marine culturing the advantage of triploids was negligible or was not observed. Previous studies have shown that triploids respond to environmental conditions (low salinity, high water temperature) differently than diploids. Callam et al. [43] showed that at low salinity (6–13 ppt) linear and weight growth of diploid and triploid *C. virginica* did not substantially differ. It was reported that 4% slower shell growth was seen in triploid *C. gigas* but there was a more rapid increase of body weight at water temperatures of 17.2–26.5 °C compared to diploids [18]. Besides environmental factors, the cultivation method also influences the conditions of diploids and triploids. Indeed, intensive technological manipulation during the growing period, removing fouling organisms from oyster cages, and mollusk sorting may reduce the differences in size and weight in diploid and triploid mollusks [44]. Construction of oyster carriers also influences the growth of bivalves. Rapid increase of soft tissue weight was observed in 3n oysters growing in plastic cages or cages submerged at defined depths on rope lines compared to oysters raised in mesh bags [29].

Thus, peculiarities of growth of 3n and 2n mollusks are probably determined by a complex influence and/or interaction of abiotic factors (salinity, water temperature, currents, tides, diversity and amount of phytoplankton food, etc.) and biotic components (species, age, maturation stage, the origin of oysters, etc.). The advantage of triploids becomes most noticeable when oysters reach a certain size. Hand et al. [45] reported significant differences between 2n and 3n Sydney rock oysters *S. commercialis* after they reached weights of 5–10 g or shell heights of 20–40 mm, which corresponds to initiation of gametogenesis and spawning in diploid mollusks. In eastern oyster, *C. virginica*, farmed in the Chesapeake Bay, a similar phenomenon was observed several months after transplanting the mollusks in the sea, starting with size <25 mm or weight <10 g [45].

In the present work, we did not observe significant differences in linear parameters between polyploid and diploid mollusks farmed at Donuzlav (Table 1, Figure 4). Obtained results confirmed the assumption that the advantage of the growth of 3n mollusks is less evident in farms located at high latitudes, the coordinates of the Donuzlav estuary being 45°24′36″N, 33°9′3.2″E. Environmental conditions probably influenced the growth of oysters to a greater extent than their ploidy. The absence of expected advantages in the growth of 3n oysters compared to 2n ones may be caused by the method of culturing in the suspended cages and seasonal wave events in the area, which can damage the growing edge of the shell and reduce the linear dimensions of mollusks.

Comparative analysis of the height-to-length ratio in diploid and triploid *C. gigas* revealed substantial differences depending on the area of cultivation. According to the value of power coefficient *b*, the weight of oysters from the Donuzlav estuary was greater than that for similarly sized mollusks in the Blue Gulf. It is quite difficult to predict the mass of an oyster by its height, since the mass is more variable than shell size. Mollusk weight depends on various factors, including the quality and availability of phytoplankton, filtration rate, maturation stage, age, and season. It is also known that in bivalves the proportion of the shell itself in the total raw weight gradually increases due to age-related shell thickening [38,39].

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References


