

*Recent Observations on the Size Structure of *Donax trunculus* Linnaeus, 1758 and *Chamelea gallina* (Linnaeus, 1758) in the Bulgarian Black Sea as Status Indicators of Commercially Exploited Shellfish under the Marine Strategy Framework Directive (MSFD)*

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Abstract. Healthy stock of commercially exploited fish and shellfish are determined by MSFD as one of the marine environmental status descriptors (D3). The clams *Donax trunculus* and possibly *Chamelea gallina* have become commercially exploited shellfish in the Bulgarian Black Sea since 2012. Mixed catches due to habitat range partial overlap and lumped landings statistics create uncertainty about the catch's species composition and ratio but personal communication with clam catchers suggests predominant harvest of *D. trunculus*. Rapidly increasing landings to a maximum of 819 t in 2017 dropped to 506 t as soon as 2019. This study examines the wild population status of *Donax trunculus* in front of Chernomorets Beach (Varna) by investigating the size and weight structure, and the condition index as observed in February 2020. The predominant size class is 22 mm (37% of the sample), as the smallest and the largest observed specimens were 14.69 mm and 38.81 mm respectively. The b-value of the length – weight relationship was 2.82 ($p < 0.0001$), which was indicative of a negative allometric growth. The good status thresholds of the indicators 95th percentile of the Length (L95) and Height (H95) defined under MSFD D3 Criterion 3 were not reached with values calculated at 28.26 mm and 18.00 mm, respectively. The average condition index was 15.5. Overall deterioration of the population status is possibly associated with harvesting pressure. Year-round monthly surveys are planned to study the annual population dynamics with the objective to devise improved indicators and thresholds for better assessment of the population status.

Key words: *Donax trunculus*, commercially exploited species, size structure, length – weight relationship, Bulgarian Black Sea, MSFD.

Introduction

The clams *Donax trunculus* Linnaeus, 1758 and *Chamelea gallina* (Linnaeus, 1758) represent dominant species in the marine benthic habitat type “infralittoral sand” across the world. *D. trunculus* is distributed from Senegal to the northern Atlantic coast of France (Tebble, 1966), the Mediterranean and the Black Sea (Bayed & Guillou, 1985), as well

as along the Marmara Sea (Deval, 2009). Whereas *Chamelea gallina* is found from the Lofoten Isles, south to the Iberian Peninsula, the Mediterranean and the Black Sea, along the Atlantic coast of Morocco and to Madeira and the Canary Isles (Marine Species Identification Portal, 2020).

In the Bulgarian Black Sea *D. trunculus* dominates the exposed to wave action upper

infralittoral medium and fine sands usually between 1 and 6.5 m (but is also observed from 0.9 to 9 m), where wide fluctuations in abiotic factors are present. This limits the species diversity, but the characteristic species can reach a high density (Todorova, 2017; Todorova et al., 2015). *Donax* physiological and behavioural adaptations, in particular its excellent burrowing, enable the bivalve to maintain its position in the surf zone and avoid the risk of stranding and desiccation (Gaspar et al., 1999; Neuberger-Cywiak et al., 1990).

On the contrary *Ch. gallina* lacks such adaptations and prefers greater depths from 5 to 18 m (rarely till 25+ m), of the infralittoral medium and fine sands and muddy sands in the Bulgarian Black Sea, (Todorova, 2017; Todorova et al., 2015).

Both species have become commercially exploited shellfish in the Bulgarian Black Sea since 2012. Mixed catches due to habitat range partial overlap and lumped landings statistics create uncertainty about the catch's species composition and ratio but personal communication with clam catchers suggests predominant harvest of *D. trunculus*. Rapidly increasing landings to a maximum of 819 t in 2017 dropped to 506 t as soon as 2019.

Healthy stock of commercially exploited species are determined by the EU Marine Strategy Framework Directive – MSFD (EC, 2008) as one of the total 11 marine environmental status descriptors (D3), according to which: “Populations of all commercially exploited fish and shellfish are within safe biological limits, exhibiting a population age and size distribution that is indicative of a healthy stock”. A key criterion for assessment of the progress towards achieving good environmental status is the population age and size structure to include a high proportion of adults/large individuals and limited adverse effect of exploitation on genetic diversity.

At national level the Bulgarian monitoring program for commercially exploited fish and shellfish established the 95th percentile of the observed species size

structure during marine scientific surveys (L95 for the length parameter and H95 for the height respectively) as a quantitative indicator for their environmental status (Todorova, 2017).

This study examines the wild population status of *Donax trunculus* and *Chamelea gallina* in front of Chernomorets and Pasha Dere Beaches (Varna) by investigating the size and weight structure, and the condition index as observed in February 2020. The obtained size structure values are interpreted according to the thresholds in the above-mentioned national monitoring program. Additionally, a comparison between all examined parameters and those reported for other populations at different geographical locations is made.

Material and Methods

Sampling methods

In February 2020 around 200 specimens of *D. trunculus* and *Ch. gallina* were sampled by dredging with a fishing dredge. Its metal frame was 80 cm wide, 60 cm long with mouth of 18 cm height and 30 cm long teeth spaced by 0.8 cm. The attached net was with 1 cm mesh size. The dredge was operated from boat in front of Chernomorets and Pasha Dere Beaches, located south of Galata Cape, near Varna (Fig. 1). The tows were performed parallel to the shoreline in depths from 2 to 6 m with a duration of 5 minutes each. For each individual, the shell length (maximum distance along the anterior posterior axis) and shell height (maximum distance from the umbo to the ventral margin) were measured to the nearest 0.01 mm accuracy with a digital caliper. The total weight (TW) of each individual, together with shell weight (SW), tissue wet weight (TWW) and tissue dry weight (TDW), obtained after oven drying at 60°C for 48 hours were determined by a digital balance with a precision of 0.0001 g.

Statistical analysis

Length-frequency distribution was calculated per 2 mm size-classes. Statistical analyses (95th percentile and regressions)

were performed using MS Office-Excel 365 ProPlus® software.

The relationship between length and weight was estimated by regression analysis, using the logarithmic model:

$$\log Y = b \cdot \log X + \log a,$$

which could easily be turned into a power curve type:

$$Y = a \cdot X^b,$$

where Y is the total weight or the tissue dry weight and X is the shell length, a is the intercept and b is the slope.

The regression line was calculated using the least squares method to test the existence of an isometry or allometry between the correlated variables. Comparing the value of the slope of the fitted line with a theoretical value, three cases are possible: $b=3$ (isometric), $b>3$ (positive allometry) and $b<3$ (negative allometry).

The coefficient of determination R^2 together with Significance F and P -value were used as indicators of the quality of the regression.

The condition index (CI) was calculated through two different approaches:

$CI = \text{tissue dry weight} / \text{shell weight} * 100$ (Beninger, 1984; Walne & Mann, 1975)

$CI = [\text{tissue dry weight} / (\text{total weight} - \text{shell weight})] * 100$ (Crosby & Gale, 1990)
total weight - shell weight = shell cavity weight.

Results and Discussion

We consider as relevant only the collected data for *D. trunculus*, taking into account that the sample of *Ch. gallina* was taken from a site which was not representative habitat for the species. It turned out to be a difficult task to find a representative place with enough living specimens, even at greater depths. Nevertheless, the estimated values for the 95th percentile of the length and height data distribution, length-weight relationship and condition index were given at the end of this section.

Length-frequency distribution

The predominant size class for *D. trunculus* was 22 mm (37% of the sample), as the smallest and the largest observed specimens were 14.69 mm and 38.81 mm respectively (Fig. 2).

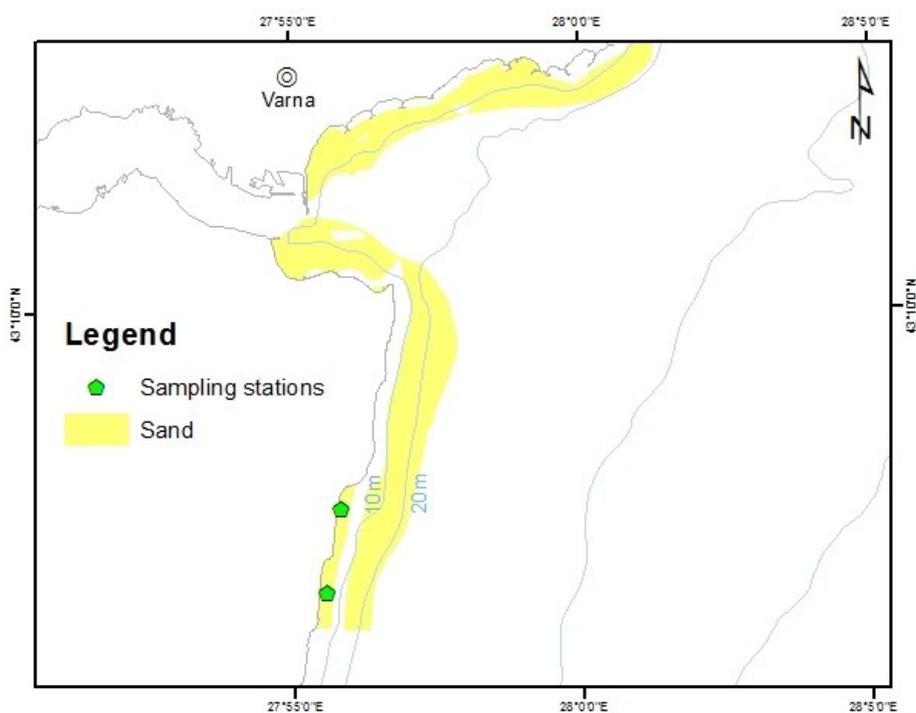


Fig. 1. Indicative map of the sampling area.

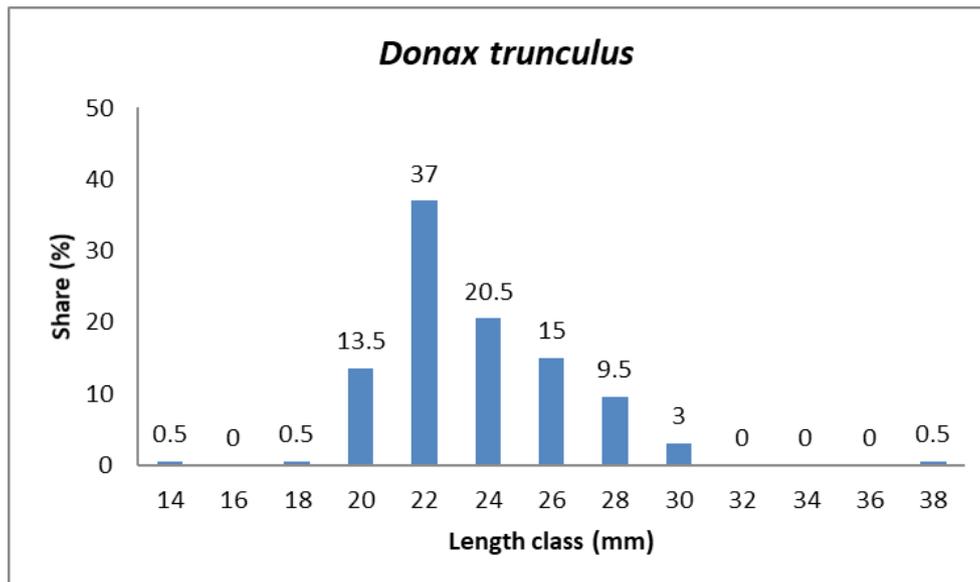


Fig. 2. *Donax trunculus* length-frequency distribution in February 2020.

The minimum and maximum shell length values empirically reported to the whole distribution area ranges from 3 to 45 mm [Northern Atlantic: (Ansell & Lagardère, 1980; Bayed & Guillou, 1985; Gaspar et al., 2002; Mazé & Laborda, 1990); Mediterranean: (de la Huz et al., 2002; Hafsaoui et al., 2016; Mouëza & Chessel, 1976); Adriatic: (Zeichen et al., 2002); Marmara Sea: (Çolakoğlu, 2014; Çolakoğlu & Tokaç, 2011; Deval, 2009); Black Sea: (Aydın et al., 2020).

The minimum shell length values obtained from the present study were higher than those reported by Aydın et al. (2020) for the Southeastern Black Sea coast due to the fact that a different sampling gear was used. The larger mesh size of the fishing dredge that was used did not allow an appropriate interpretation of this parameter, which made the maximum shell length more indicative for the population structure. Its estimation was comparable with the reports from the Mediterranean and Adriatic where the values were between 37 and 40 mm (Mouëza & Chessel, 1976; Zeichen et al., 2002), while larger specimens 42-45 mm were observed from the Atlantic and the Marmara Sea (Çolakoğlu, 2014; Delgado et al., 2017; Deval, 2009). From the Turkish Black Sea coast

lower maximum length of 35.5 mm was reported, despite the large number of the sample taken from 0-1.5 m depth (Aydın et al., 2020). This dissimilarity could be caused by the different-sized vertical distribution pattern described by Zeichen et al. (2002) for the Adriatic, where the ecological conditions are relatively closer to those in the Black Sea. Juveniles from the Adriatic population were distributed mainly at least depth and down to 1 m with decreasing densities. Adults were conspicuously found in the deepest bathymetric layers from 0.7 m down to the distribution limit of the species. A possible explanation is the 'sink-source' diffusion model in *D. trunculus*, with the least depths functioning as source and the greatest ones as sinks. This phenomenon could be explained by currents and hydrodynamism passively pushing larvae to colonize the shallower areas on the shore. As they increase in size and in their capacity for active movement, individuals migrate deeper, possibly in order to decrease food competition and population density (Ansell & Lagardère, 1980). Observations from the Thyrrenian Sea (Voliani et al., 1990) and the Atlantic coast populations (Ansell & Lagardère, 1980) confirmed the increase in size with depth trend and it has been

explained as due to the intraspecific competition between juveniles and adults.

D. trunculus in the Bulgarian Black Sea is distributed mainly between 1 and 6.5 m (but is also observed from 0.9 to 9+ m) (Todorova, 2017; Todorova et al., 2015). In the present study the species was sampled from 2.5 m depth.

The shell length of 38.8 mm could be to an individual aged from 2 to 4 years (Mazé & Laborda, 1990; Mouëza & Chessel, 1976; Zeichen et al., 2002), but a further investigation on the growth rate in the specific conditions of the Black Sea is needed. Various factors such as temperature, trophic conditions and population density (Ansell et al., 1980; Ansell & Bodoy, 1979; Neuberger-Cywiak et al., 1990) seem to regulate growth processes.

The good status thresholds of the indicators 95th percentile of the Length (L95≥33.78 mm) and Height (H95≥20.91 mm) defined under MSFD D3 Criterion 3 were not reached with values calculated at 28.26 mm and 18.00 mm, respectively.

The majority of the individuals from the sample (95.5%) fell into 5 size classes from 20 to 30 mm shell length. The very low percent of the smaller size classes was due to the selectivity of the sampling gear, which was purposed to a commercial use. Furthermore, the absence of the larger size classes could be indicative for the impact of the intensive fishing pressure on the species in the last years.

Length-weight relationship

The b-values of the length-weight relationships were 2.82 for the total weight and 2.68 for the tissue dry weight, ($p < 0.0001$). That was indicative of a negative allometric growth (Fig. 3-a,b).

Differences in allometry allow life-history comparisons between populations from different habitats. Intersite differences depicted in weight-length relationships could be related to differences observed in growth (Tlili et al., 2010) and reproduction between the sites of origin of bivalves.

The estimated values of the slope were slightly lower than that reported by Aydın et

al. (2020) for the Southeastern Black Sea population, as similar negative allometry was observed.

Negative allometric growth of *Donax trunculus* was commonly reported across its distribution area (Aydın et al., 2020; Çolakoğlu, 2014; Çolakoğlu & Tokaç, 2011; Deval, 2009; Mazé & Laborda, 1990).

The obtained zero b-value for the shell length-condition index (estimated by both ways) relationship showed that no relationship was found, which indicated that the condition of the individuals was not related to their size (Fig. 3-c,d).

Condition Index

The average condition index estimated by the second method as a ratio between tissue dry weight and shell cavity weight was 15.5 ± 2.5 (SD). Its value calculated using the first method (ratio between tissue dry weight and shell weight) was 9.5 ± 1.5 (SD).

Condition indices are usually regarded as useful measurements of the nutritive status of bivalves (Crosby & Gale, 1990) and are influenced by many factors including food availability, temperature, and most importantly the gametogenic cycle (Boscolo et al., 2003).

A similarity in the patterns of variation of the condition index and the degree of gonadal development was reported. Aydın et al. (2020) observed a steady increase in the condition index after the initiation of the gametogenesis in February. This increasing trend continued until the beginning of spawning. The condition index decreased sharply as the percentage of spawning individuals reached maximum in May (121.3 ± 18.2 SD). During the inactive stage the condition of specimens increased slightly until August (42.8 ± 19.82 SD), when there was a decrease in the index. Since the spawning period showed parallelism with the period of maximum condition index value, it could be assumed that the reproduction season in the Black Sea is between May and August. Such trend was also identified for the adjacent Marmara Sea

(Deval, 2009). Researches conducted in other areas also reported that the reproduction season is between May and August (Gaspar et al., 1999; Zeichen et al., 2002). Therefore *D. trunculus* was

categorized as an opportunistic species in which growth and reproductive activities are restricted to the favourable environmental conditions of the spring-summer season (Delgado et al., 2013).

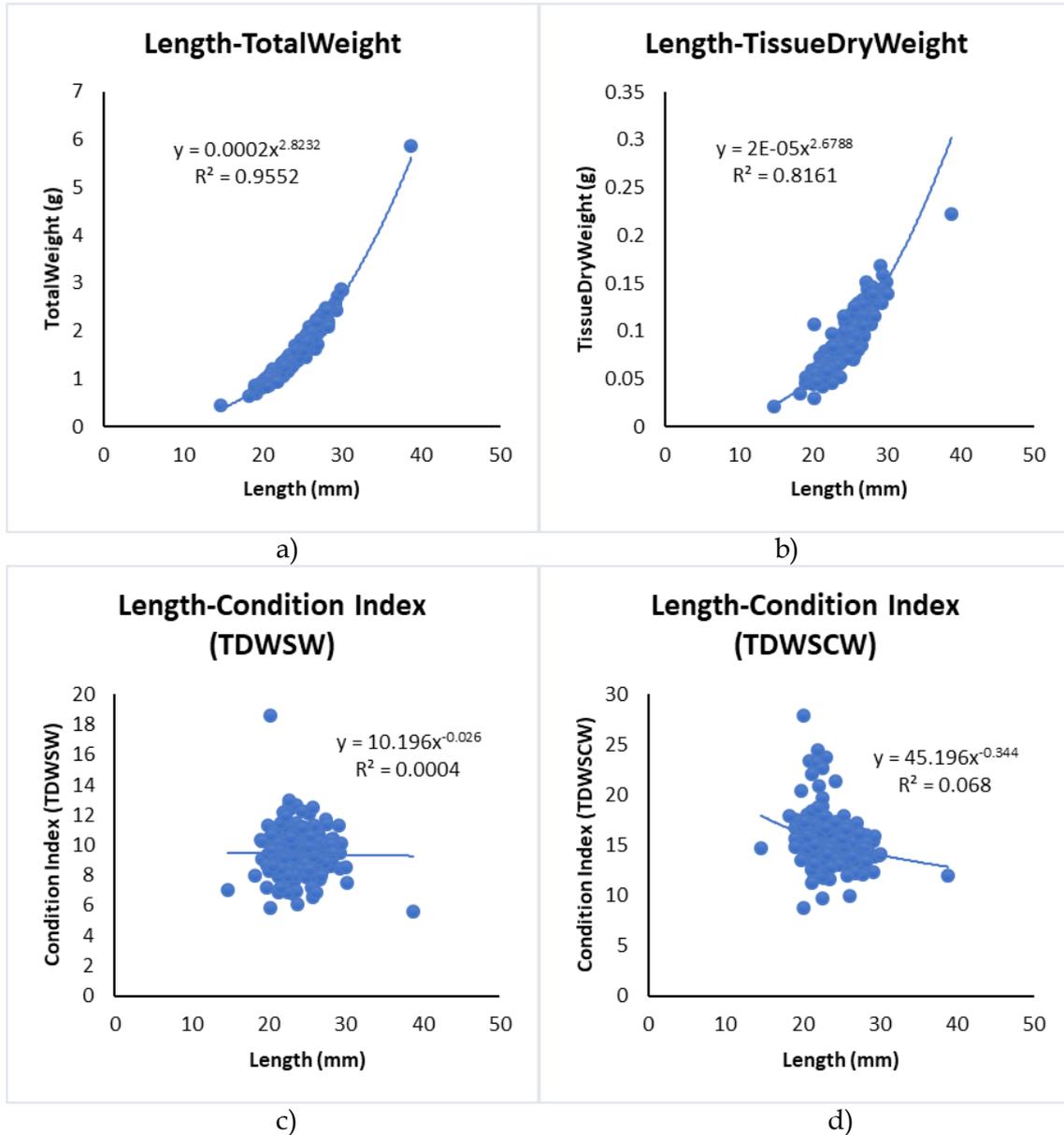


Fig. 3. Estimated relationships of *Donax trunculus* for: a) Length-Total weight; b) Length-Tissue dry weight; c) Length-Condition index (as a ratio between TDWSW-Tissue dry weight and Shell weight); d) Length-Condition index (as a ratio between TDWSCW-Tissue dry weight and Shell cavity weight).

In the present study an analogy could be examined between the estimated by Aydın et al. (2020) values of the condition index for

February (average 53.8, min. 11.5 and max 260.0) in Southeastern Black Sea and those from the population in front of

Chernomorets Beach, Western Black Sea. The average condition index value 15.5 obtained from the Western Black Sea was 3.5- fold lower than that from the southeastern part and its value was relatively close to the calculated minimum border. This contrast could be mainly caused by a methodological difference between both studies and precisely the imported value in the formula for tissue weight, which could have accounted for different results. Other factors that influence the condition index of the bivalves are the food availability and temperature. Several authors highlighted the importance of beach morphodynamics, and particularly, the sediment grain-size as main factor controlling the distribution of *D. trunculus* populations (de la Huz et al., 2002; La Valle et al., 2011).

The predominant size class for *Ch. gallina* was 14 mm (38,5% of the sample), as the smallest and the largest observed specimens were 10.47 mm and 19.32 mm respectively. The b-values of the length-weight relationships were 2.62 for the total weight and 3.27 for the tissue dry weight, ($p < 0.0001$). That was indicative of a negative allometric growth of the total weight with increasing body length. On the contrary a positive allometric growth of the tissue dry weight with increasing body length was reported. The obtained 0.76 and 0.36 b-values for the shell length-condition index relationship (estimated by both ways) showed that no relationship was found, which indicated that the condition of the individuals was not related to their size.

The good status thresholds of the indicators 95th percentile of the Length ($L_{95} \geq 23.92$) and Height ($H_{95} \geq 22.22$) defined under MSFD D3 Criterion 3 were not reached with values calculated at 18.06 mm and 16.53 mm, respectively. The average condition index was 10.3 ± 1.4 (SD).

Conclusions

Overall deterioration of the population status of *Donax trunculus* is possibly associated with harvesting pressure since

2012. Year-round monthly surveys are planned to study the annual population dynamics and biological processes with the objective to devise improved indicators and thresholds for better assessment of the population status.

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