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ZEKİYE BİRİNCİ ÖZDEMiR

EYLEM AYDEMİR ÇİL

SÜLEYMAN ÖZDEMİR

HÜNKAR AVNİ DUYAR

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An important species for Black Sea biodiversity: Some population characteristics of invasive sea vase (*Ciona intestinalis* Linnaeus, 1767) distributed on the Sinop shores

Zekiye BİRİNCİ ÖZDEMİR[1,*], **Eylem AYDEMİR ÇİL**[2], **Süleyman ÖZDEMİR**[3], **Hünkar Avni DUYAR**[3]

[1]Department of Marine Biology, Fisheries Faculty, Sinop University, Sinop, Turkiye
[2]Department of Environmental Engineering, Engineering and Architecture Faculty, Sinop University, Sinop, Turkiye
[3]Department of Fishing Technology and Processing Technology, Fisheries Faculty, Sinop University, Sinop, Turkiye

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Abstract: This study aimed to provide a comprehensive summary of the fundamental biological and certain population characteristics of *Ciona intestinalis* found along the Black Sea coasts of Türkiye. The research was conducted between September 2022 and April 15th, 2023, off the Sinop coast of the Black Sea, utilizing samples collected by commercial fishing vessels in the region. A total of 103 specimens of *C. intestinalis* were examined during the study. The average total length, width, and weight of *C. intestinalis* were 3.614 ± 0.089 cm, 1.967 ± 0.054 cm, and 2.004 ± 0.067 g, respectively. The length-weight relationship (LWR) and length-width relationship (LW_dR) of *C. intestinalis* were established as $W = 0.3996L^{1.2424}$ (R = 0.9389), $W_d = 0.5362L + 0.0237$ (R = 0.8897), and $W = 0.9443W_d^{1.0889}$ (R = 0.8786), respectively. This study presents, for the first time, the LWR, LW_dR, and W_dWR for *C. intestinalis*, a species of significant importance in the benthic ecosystem of the Black Sea. It is anticipated that this research will lay the groundwork for future investigations into the population dynamics and biological aspects of this species.

Key words: Tunicate, invasive species, benthic, distribution, central Black Sea

1. Introduction

The Black Sea stands out as one of the vital seas in the Mediterranean Basin, characterized by its closed nature and connectivity to other seas and oceans (such as the Marmara Sea, Aegean Sea, Mediterranean Sea, Gibraltar Strait, and Suez Canal) through the İstanbul Bosporus. A distinctive feature of the Black Sea is the presence of an oxygen-depleted layer, referred to as anoxia, covering its bottom region (Talley et al., 2011; Sergeeva et al., 2012). This anoxic condition limits eukaryotic life to only 10% of the total depth of the Black Sea, resulting in low species diversity, particularly among benthic organisms (Kideys, 2002; Bat et al., 2007). Despite its rich nutritive characteristics, the Black Sea experiences high organic and inorganic nutrient input from numerous rivers, which plays a crucial role in sustaining life within its food chain and ecosystem.

The biodiversity of the Black Sea is dynamic, undergoing constant changes that impact various components of its ecosystem, including benthic and pelagic organisms, marine mammals, fish, crustaceans, arthropods, phytoplankton, zooplankton, and benthic creatures (Üstün and Birinci-Özdemir, 2019; Özdemir et al., 2023; Radulescu, 2023).

Historically, the introduction of nonindigenous species has led to significant disruptions, destruction, and imbalances in the Black Sea ecosystem (Bat et al., 2007, 2011; Radulescu, 2023). Invasive species tend to seek refuge in estuaries, deltas, and brackish water regions, particularly in rivers such as the Danube, Dnieper, Dniester, Kızılırmak, Yeşilırmak, and Sakarya. This intrusion can impact local species and potentially lead to the loss of ecological niches. Euryhaline and eurytherm species exhibit enhanced adaptability to the brackish waters of the Black Sea. Recent years have witnessed significant alterations in the Black Sea ecosystem, notably affecting fish species, with fish biomass decreasing by more than two times in the last 25 years (Kideys, 2002; Bat et al., 2011).

In the Black Sea, which is rapidly affected by changes in its ecosystem, recent research activities have observed that the ascidian species *Ciona intestinalis* is frequently found in nets, as reported by personal observations and fishermen. This species, whose native origin is from the Northeast Atlantic, has become invasive in mild climate regions globally since the mid-1800s (Therriault and Herzborg, 2008; Carman et al., 2010). Characterized by rapid growth, development, and high reproduction, *C.*

* Correspondence: zekbiroz@gmail.com

intestinalis exhibits opportunistic behavior and poses a threat as an invasive species. The introduction of this nonnative species has negatively affected the ecosystems, reducing species richness in benthic ecosystems through competition for food and restricting the habitat of native species (Carver et al., 2006; Blum et al., 2007; Lutz-Collins et al., 2009). Commonly found in harbors or semienclosed basins in extensive communities, *C. intestinalis* is known as the Sea Vase and is distributed along the coasts of the Black Sea (Palomares and Pauly, 2023)[1].

The species can survive at 0–27 °C (Kenworthy et al., 2018) and reproduces at 8–22 °C (Dybern, 1965). It has wide salinity tolerance and lives in the range of 9–35 PSU. *C. intestinalis* is a hermaphrodite (Millar, 1970) and reproduces by external fertilization. Eggs and sperm are released into the surrounding water column where fertilization takes place. Fertilized eggs hatch into larva endowed with a set of adhesive papillae. The larvae spend one to five days planktonic swimming, before settling on hard substrates and undergoing metamorphosis (Berrill, 1947; Dybern, 1965; Svane and Havenhand, 1993). The larval period may be longer at low temperatures (Dybern, 1965). Once settled, the tunicates begin to feed by filtering (Barnes, 1983). Larvae possess six advanced organ systems (tunicate, epidermis, notochord, tail muscles, adhesive organ, and nervous system) and four rudimentary organ systems, as well as a primordial branchial sac (Katz, 1983). The adhesive papillae located at the anterior end of their body are used to attach to substrates during settlement (Millar, 1970).

C. intestinalis is a phlebo-branchiate ascidian species with a short life cycle. It also exhibits tolerance to changes in salinity (Petersen and Riisgård, 1992; Mazouni et al., 2001). Researchers have identified that individuals in Nova Scotia, Canada, live for 12 to 18 months, from the beginning of spring to the end of autumn, and enter a dormant state during the winter (Carver et al., 2003). If tunicates survive the winter, they may reinitiate gamete production and reproduce again in early spring before senescence and death (Millar, 1952; Dybern, 1965).

Ascidian species also contain chemical compounds with potential applications in the medical sector, particularly in the production of cancer drugs (Popov et al., 2002). Their economic significance is increasing due to their role as raw materials for medicinal drugs and as a source for various chemicals (Pisut and Pawlik, 2002; Aslan, 2006; Miroğlu and Yalçın, 2020).

Although there have been numerous systematic studies on ascidians in the seas of Türkiye (Demir, 1952, 1954; Kiseleva, 1961; Geldiay and Kocataş, 1972; Uysal, 1976; Mavili, 1987; Koukouras et al., 1995; Dinçaslan and Öber, 2004, 2005; Aslan, 2006; Dinçaslan et al., 2007; Çınar, 2014, 2016; Aydin-Onen, 2018; Mutlu, 2021), information regarding their distribution in the Black Sea remains limited. The presence of *C. intestinalis* in the Black Sea was first reported by Miroğlu and Yalçın (2020). This study aimed to provide an overview of the basic biology and some population characteristics of the sea vase, *C. intestinalis*, which is also found along the Sinop coasts of the Black Sea.

2. Materials and methods

The study was conducted between September 2022 and April 15th, 2023, off the Sinop coasts of the Black Sea (Figure 1). *C. intestinalis* individuals were collected by commercial fishing vessels operating at depths ranging from 15 to 80 m. Sea trials were carried out using the beam and demersal trawl methods. *C. intestinalis* individuals, which entered the fishing gears from the seabed, were immediately cleaned with seawater on the deck and placed in sample storage containers (Figure 2).

Subsequently, the samples brought to the laboratory were washed with distilled water, initially diagnosed using a stereo microscope, and photographed (Figure 3). The specimens were then preserved in 70% alcohol. For the diagnostic process, the colonial and solitary samples were first separated. Species identification procedures followed the protocols of Monniot et al. (1991), Dinçaslan and Öber (2005), Dinçaslan et al. (2007), and Turon et al. (2016). Measurements of the total body length and width of the specimens were recorded in centimeters. Additionally, they were weighed, and the length-weight relationships (LWRs) were predicted by fitting an exponential curve ($W = aL^b$) to the data (Pauly, 1984).

Parameters a and b of the exponential curve were estimated through linear regression analysis over log-transformed data using the equation below:

$$\mathrm{Log}W = \mathrm{Log}a + b\mathrm{Log}L \qquad (1)$$

Here, the total weight (W) is in grams, the total body length (L) is in centimeters, b is the slope, and a is the intercept, determined using the least-squares method. The association-degree between the variables of W and L was assessed using the specification coefficient (R). Additionally, 95% confidence limits of parameter b were established. Pauly's t test was performed, and the statistic was calculated using the formula below:

$$t = \frac{SD_{logL}}{SD_{logW}} \frac{|b-3|}{\sqrt{1-r^2}} \sqrt{n-2} \qquad (2)$$

Here, SdlogTL is the standard deviation of the log TL values, SdlogW is the standard deviation of the log W values, and n is the number of specimens used in the computation. If the calculated t value is greater than the tabulated t values for n −2° of freedom, the value of b is considered different than b = 3 (Pauly, 1984).

[1]Palomares MLD, Pauly D (2023). Sea Life Base (Version 08/2023). Website www.sealifebase.org [Accessed 10 November 2023].

Figure 1. Chart of the *C. intestinalis* sample collection areas. DT: Demersal trawl areas, BT: beam trawl areas.

Figure 2. Sediment sample collected from the study area.

Figure 3. Samples of *C. intestinalis* examined in the study.

3. Results and discussion

A total of 103 *C. intestinalis* specimens were examined. The body exhibited a cylindrical structure with soft, translucent tissues, and its color varied from pale greenish/yellow to orange. The species morphology was consistent with the description provided by Carver et al. (2006).

In recent years, *C. intestinalis* has emerged as a model chordate in various fields of biology, including comparative genomics, evo-devo, and developmental biology. Molecular studies have indicated the possible existence of different cryptic species under the name *C. intestinalis* (Zhan et al., 2010; Sato et al., 2012; Brunetti et al., 2015) demonstrated that groups morphologically identified as types A and B in molecular identification represent two distinct species, namely *Ciona robustra* and *C. intestinalis*, respectively. They highlighted the presence of tubercular protrusions on the tunic as a crucial morphological feature distinguishing these two species, with *C. robustra* exhibiting these protrusions, while *C. intestinalis* does not. The absence of tubercular protrusions in the examined samples aligned with the findings of Brunetti et al. (2015).

The average total body length, width, and weight of the individuals were 3.614 ± 0.089 cm, 1.967 ± 0.054 cm, and 2.004 ± 0.067 g, respectively. The maximum and minimum weights were recorded as 3.669 and 0.685 g, respectively. The biological measurements of the species are presented in Table 1.

The total body length of the individuals varied between 1.8 and 5.7 cm. The highest number of individuals was observed in the 3.5 cm total length (TL) group, accounting for 25.24%, while the lowest number was in the 6 cm TL (0.97 %) group. In the width frequency distribution, the majority of individuals were in the 2 cm group (39.81%), with the least number in the 1 cm group (0.97 %). The weight frequency distribution exhibited prominent peaks at 3.5 cm (Figure 4).

The LWR, length-width relationship (LW_dR), and width-weight relationship (W_dWR) of *C. intestinalis* were $W = 0.3996L^{1.2424}$, $W_d = 0.5362\ L + 0.0237$, and $W = 0.9443W_d^{\ 1.0889}$ respectively. The regressions for the LWR, LW_dR, and W_dWR were 0.9389, 0.8897, and 0.8786, respectively. The regression of the LWR exhibited values greater than 0.90, while those for the LW_dR and W_dWR were lower than that. The LWR and LW_dR graphics of *C. intestinalis* are presented in Figure 5.

Parameters a and b for the LWR of *C. intestinalis* were 0.3996 and 1.242, respectively. Additionally, parameters b and a for the LW_dR and W_dWR were 0.9368 and 0.5863, and 1.0889 and 0.9443, respectively. Table 2 provides details on the sample size, a and b parameters, standard error of the slope, 95% confidence intervals for b and a, regression (R), and p-value for *C. intestinalis*.

The studies conducted on species in the Turkish Seas, similar to those worldwide, have predominantly focused on morphology, anatomy, reproduction, life history, feeding habits, and habitat characteristics (Petersen et al., 1997; Carver et al., 2006; Dinçarslan et al., 2007; Brunetti et al., 2015; Petersen, 2016; Harris et al., 2017; Miroğlu and Yalçın, 2020; Clutton et al., 2021). However, comprehensive investigations into the population parameters of *C. intestinalis* are notably lacking.

A singular study on the length-weight relationship of *C. intestinalis*, conducted in the North Sea, examined 147 individuals with total body lengths ranging from 1.4 to 7.1 cm. The calculated b, a, and "R" values were 2.485, 0.15595, and 0.869 ($R < 90$), respectively (Robinson et al., 2010). The study results exhibited slight discrepancies from this research, possibly attributed to the distinct seas and habitats from which the *C. intestinalis* specimens were obtained. Temperature variations caused by climate change, particularly extreme temperatures, have significant effects on chemical toxicity for individual organisms. These effects, in turn, influence population size and structure, species composition within communities, and the overall structure and functioning of ecosystems (Cairns et al., 1975, 1978; Lau et al., 2014; Zhou et al., 2014; Kazmi et al., 2022). The initiation and duration of reproduction in marine invertebrates is affected by a combination of endogenous factors (e.g., internal cycles of growth and development) and external factors (e.g., temperature, salinity, food availability) that cause variation in growth and development (Giese, 1958). Researchers have reported that in warmer waters, *C. intestinalis* individuals reach the critical size for maturation more quickly and at a smaller size. Additionally, it has been emphasized that the duration of the breeding season increases with decreasing latitude (Millar, 1952; Dybern, 1965; Yamaguchi, 1975). In another study, an extended reproductive window and increased recruitment were observed in areas with warmer waters within the same region (Reinhardt et al., 2013).

Table 1. Some biological features of *C. intestinalis*.

Parameters	Total body length (cm)	Width (cm)	Weight (g)
Minimum	1.8	1.4	0.685
Maximum	5.7	3.2	3.669
Mean and StdE*	3.614 ± 0.089	1.967 ± 0.054	2.004 ± 0.067

*StdE: Standard error

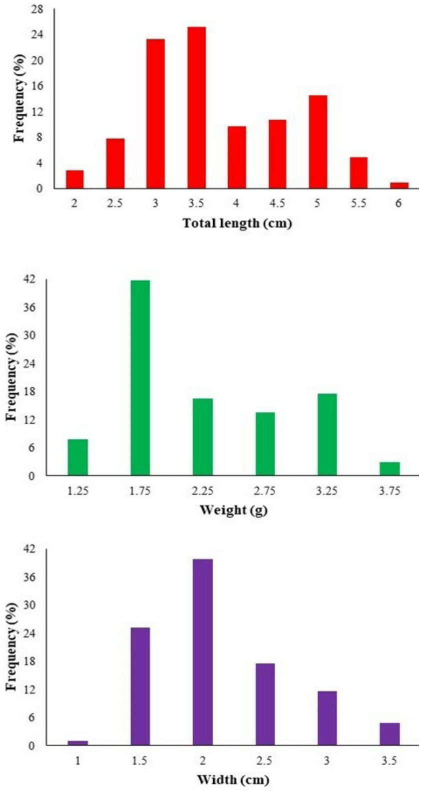

Figure 4. Frequency distributions of the total body length, width, and weight of *C. intestinalis*.

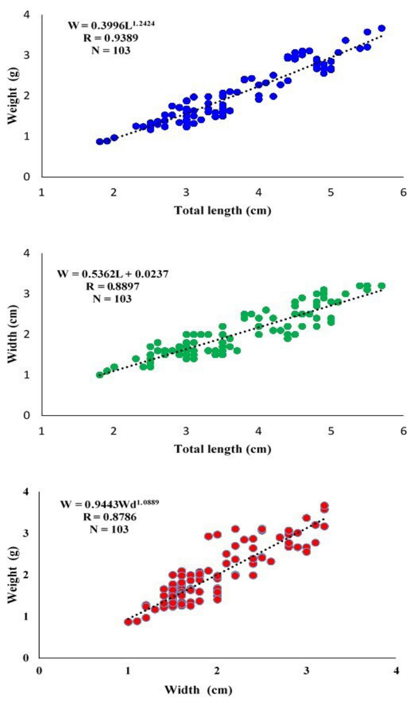

Figure 5. LWR, LW_dR, and W_dWR relationship graphics for *C. intestinalis.*

Table 2. LWR, LW_dR, and W_dWR parameters for *C. intestinalis*.

Parameters	LWR	LW_dR	W_dWR
n	103	103	103
a	0.3996	0.5362	0.9443
b	1.2424	0.9368	1.0889
Standard error of b	0.0390	0.0465	0.0589
95% confidence interval of a	0.3562–0.4483	0.5152–0.6672	0.8707–1.0241
95% confidence interval of b	1.1524–1.3323	0.8357–1.0379	0.9721–1.2057
R	0.9389	0.8897	0.8786
p-value	<0.05	<0.05	<0.05

Limited studies on the growth and population parameters of *C. intestinalis* underscore the necessity for more comprehensive ecological and population dynamics research on Ascidian species. Given their substantial impact on ecosystem dynamics, these studies become imperative for understanding and managing the biological diversity these species contribute to our seas.

The encrustation of fish farm cages and nets by *C. intestinalis* can lead to equipment failure, resulting in substantial cleaning costs. Severe infestations compromise water flow rates, diminishing oxygen exchange, and ultimately jeopardizing fish health and yield. Reports from fish farms in Africa, New Zealand, Chile, and Canada highlight an increased risk of fish diseases associated with *C. intestinalis* presence.

It was reported that tunicates reduced mussel growth rates in a shellfish farming facility in Nova Scotia, Canada, and this had serious economic impacts (Ramsay et al., 2008). Another negative effect is that *C. intestinalis* and other tunicates can retain and carry live cells and cysts of toxic phytoplankton when they contaminate aquaculture equipment and vessels (Rosa et al., 2013). However, it also fulfils their nutritional needs by filtering seawater and accumulated toxic substances that contribute to environmental pollution. They act as a natural filter system, regulating water balance (Miroğlu and Yalçın, 2020).

Considering this information, it seems likely that *C. intestinalis* will negatively affect the aquaculture sector in the Black Sea economically, although it contributes to reducing pollutants in water. In previous studies, concerns were expressed about the negative effects of nonnative *C. intestinalis* on aquaculture and commercial fishing equipment, and it was emphasized that this potentially invasive species could pose a significant threat to native species and ecosystems (Mazouni et al., 2001; Carver et al., 2003; 2006; Harris et al., 2017).

As the introduction of non-Black Sea species into the ecosystem continues to be a source of concern (Aydın and Gül, 2021; Aydemir-Çil et al., 2023; Bilecenoğlu et al., 2023), this underscores the importance of protecting, monitoring, and researching the biodiversity of the Black Sea.

In the Black Sea, which is rapidly affected by changes in its ecosystem, recent research activities have observed that the ascidian species *C. intestinalis* is frequently found in nets, as reported by personal observations and fishermen.

The species is found in both anthropogenically impacted and undisturbed environments (Brunetti et al., 2015). Reaching densities of 5 individuals/cm^2, it poses a serious challenge to aquaculture producers in Atlantic Canada (Ramsay et al., 2008, 2009). In another study, heavy infestations of *C. intestinalis* on PVC panels added tremendous weight to marine structures and aquaculture gear in the Damariscotta River, Maine, USA. The species increased the pollution surface, and an average of 1.048 kg was found on 100 cm^2 of PVC panels (Bullard et. al., 2013). In Prince Edward Island, Canada, during August (2006), when the surface sea water temperature was 48.4 °C, *C. intestinalis* the settlement rate on cage equipment was recorded at a peak value of 34.571/m^2/day (Ramsay et al., 2009). The spread of *C. intestinalis* depends on the competence of its larvae and their settlement ability (Rius et al., 2014). The early life stages of *C. intestinalis* are sensitive to heat stress and exhibit a thermal response within the sea water temperature range of 8–22 °C (Dybern, 1965). In filter-feeding organisms like *C. intestinalis*, water filtration rates are influenced by external factors such as the sea water temperature (Xu et al., 2024). With climate change, given the notable reproductive capability of *C. intestinalis*, its ability to adapt to environmental variation, and its global success story (Therriault and Herborg, 2008; Zhan et al., 2010; Rocha et al., 2017; Kenworthy et al., 2018), the past environmental conditions affecting the resistance of this species to heat stress should be further investigated. In this case, their morphological and physiological responses to temperature changes in the future can be predicted and the precautions that should be taken in the Black Sea can be discussed.

To ensure the balance and sustainability of the Black Sea ecosystem:

- Mediterranean-origin and nonnative species entering the Black Sea should be identified, and measures to address these issues should be developed. Monitoring studies should be conducted across the entire ecosystem and within local areas of the Black Sea.

• Increased maritime transport facilitates the intersea transfer of nonnative species; therefore, biological treatment of ship ballast and discharge water is necessary.

• More effective deterrent measures should be implemented for endangered and protected species. This invasive species, which has settled in the Black Sea and shown a recent increase in population, is anticipated to have negative impacts, particularly on mussel and fish farming facilities within the ecosystem.

In the adaptation processes of previously established alien and invasive species in the Black Sea, changes in body size and morphology have been observed due to environmental factors such as temperature and salinity. Accordingly, similar changes may occur within this species, and its interactions with counterpart species in the ecosystem should be carefully monitored. Future research should focus on tracking the morphological and physiological changes of *C. intestinalis* over time, allowing for a more comprehensive analysis of its impact on the Black Sea ecosystem. By doing so, the potential impacts of the species on similar marine ecosystems can be predicted, providing valuable insights for managing the health and stability of the Black Sea and other marine environments amidst ongoing climate change.

4. Conclusion

Some population parameters of *C. intestinalis*, which is increasing in the Black Sea, were evaluated herein. *C. intestinalis* is known for its ability to filter water and thus could aid in controlling pollution levels by removing particulates and contaminants. However, its status as an invasive species also means it could negatively impact local aquaculture and disrupt existing benthic communities. The balance between its filtration capacity and potential ecological harm is now uncertain for the Black Sea ecosystem.

Given these complexities, there is a clear need for comprehensive research into the effects of *C. intestinalis* on the Black Sea ecosystem. Such research would help determine the extent to which this species influences ecological dynamics and could guide management strategies for both conserving native biodiversity and addressing challenges posed by invasive species.

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