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Research Article

The Establishment of the Non-indigenous Cyclopoid Copepod *Oithona davisae* in Hamsilos Bay-Sinop, Southern Black Sea, Turkey

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ABSTRACT

Hamsilos Bay, which is located within the first–degree natural protection area, is one of the most important regions on the southern Black Sea coast. Moreover, many fish species utilize this area for breeding and rearing activities. Small copepod *Oithona davisae* has been encountered in the Black Sea for two decades and spread rapidly the overall Black Sea, and become an important component in the pelagic ecosystem of the Black Sea. In this respect, the present study aims to characterize the distribution and abundance of *Oithona davisae* in Hamsilos Bay about environmental variables. Zooplankton samples were collected monthly from seven stations from July 2015 to June 2016 by using a plankton net with a mesh size of 112 µm and a diameter mouth opening of 50 cm. *O. davisae* was observed during the whole sampling period and was the dominant species from July 2015 to December 2015. The highest mean abundance of this species was recorded in September 2015 (3533.33 ind. m^{-3}). However, the lowest mean abundance was observed between January 2016 (170.68 ind. m^{-3}) and May 2016 (0.24 ind. m^{-3}). Copepodites 5–4 were predominant in the *O. davisae* population in all months and all stations. Adult copepods and their copepodites 5–4 were more abundant between July 2015 and December 2015, whereas the abundance of copepodites 3–1 was maximum between September 2015 and December 2015. The abundance of *O. davisae* was highly correlated with chlorophyll-α and physiochemical variables (temperature, dissolved oxygen, and salinity). In conclusion, this species, which is known to be distributed only in the autumn conditions in close areas, extended its temporal distribution and prevailed in the copepod communities during warmer periods. This could be attributed to the acclimatization success of this species in Hamsilos Bay ecosystems.

Keywords: *Oithona davisae*, non-native, invasive species, abundance, developmental stages, physiochemical variables

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INTRODUCTION

Marine ecosystems are influenced by human activities such as implantation, aquacultural facilities, transoceanic shipping, and fisheries activities. These activities lead to the entrance of non-indigenous species and the change in native communities throughout the world (Leidenberger et al., 2015). Non-indigenous species have an impact on native communities, habitats, and ecosystem processes. For this reason, regular monitoring of the introduction of non-native species into the ecosystem and its ecological effects is important to follow up on the changes that may occur in the environment and respond appropriately in time to the potential effects they can create (Brog, 2009).

The Black Sea, a semi-enclosed inland sea, is connected to the Aegean Sea and the Mediterranean Sea in the southwest through the Turkish Straits System and to the Sea of Azov in the north through the Kerch Strait (Ünlüata et al., 1990). The Black Sea is a very sensitive ecosystem that can be easily affected by alien (non-native) species due to its geographic location (Selifonova, 2011a). It is known that the introduction of non-indigenous species into the Black Sea started in the 19th century and led to great problems in the following decades (Shiganova, 1998). One of the alien species that entered the Black Sea probably with ballast waters (Gubanova et al., 2014) is the cyclopoid copepod *Oithona davisae*. Known to be native to the Seas of Japan and China (Razouls et al., 2023), *Oithona davisae* has successfully settled in many different marine and estuarine ecosystems such as the Barcelona Harbor in Spain (Saiz et al., 2003), Marmara Sea (Doğan & İşinibilir Okyar, 2016), Aegean Sea in Turkey (Terbıyık Kurt & Beşiktepe, 2019), İskenderun Bay in Levantine Sea in Turkey (Terbıyık-Kurt et al., 2022), Sacramento-San Joaquin estuary in California (Ferrari & Orsi, 1984), San Francisco Bay estuary in California (Lougee et al., 2002), Bilbao estuary in Spain (Uriarte et al., 2016). *O. davisae* was first found in 2001 in Sevastopol Bay in the Black Sea. Firstly, it was misidentified as *Oithona brevicornis* (Zagorodnyaya, 2002), but later it was identified as *O. davisae* upon re-examination of zooplankton specimens (Temnykh & Nishida, 2012). The first record of *O. davisae* was in Sinop in 2009 along the Black Sea coast of Turkey (Üstün & Terbiyik Kurt, 2016). Later, *O. davisae* expanded its distribution towards the eastern Black Sea (Trabzon coast) (Yıldız et al., 2017) in 2010 and the western Black Sea (İğneada coast) in 2012-2013 (Üstün et al., 2019). It is seen that *O. davisae* become to have a higher abundance and frequency, thus expanding its range on the Turkish coasts of the Black Sea after reporting by Üstün & Terbiyik Kurt, 2016 on the Sinop coast.

Hamsilos Bay had great significance on the southern Black Sea coast due to its location within the first–degree natural protection area (Anonymous, 2018). Furthermore, Hamsilos Bay is a breeding and rearing area for many fish species. In the present study, the temporal and spatial distribution of the abundance values of *O. davisae* in Hamsilos Bay, a protected area, and its relationship with environmental parameters have been first examined.

MATERIALS AND METHODS

Sampling area

Our study area, Hamsilos Bay, is 11 km from Sinop, a natural harbor located at the northernmost point of Turkey. While it looks like a fjord, it is a small ria formed by the submersion of a valley underwater (Akkan, 1975). The importance of Hamsilos Bay is demonstrated by its status as a natural area of protection, the absence of residential areas, and no dumping of any pollutants in this area, therefore is no stress factor harming the flora and fauna living here. Additionally, as it is an enclosed and protected area in terms of winds (mistral–northbound) and covered with *Zostera* facies in its deep structure (Ersoy Karaçuha, 2006), it provides shelter for various benthic and demersal living beings and makes the Bay a significant reproduction and shelter area for fish (Uygun, 2015).

Sampling and laboratory studies

The study was conducted monthly at seven stations in Hamsilos Bay from July 2015 to June 2016 (Table 1 and Figure 1). Environmental variables were simultaneously obtained after zooplankton samplings. The physical parameter (temperature, dissolved oxygen, and salinity) values of the surface seawater were recorded with the YSI 6600 MDS model multiparameter (YSI Incorporat-

Table 1. Information about sampling stations.

Station name	Information of Coordinate	Sampling Depth (m)
A	42°03'38"N – 35°02'31"E	2
B	42°03'36"N – 35°02'36"E	4
C	42°03'40"N – 35°02'36"E	9
D	42°03'45"N – 35°02'40"E	13
E	42°03'52"N – 35°03'14"E	30
F	42°04'5"N – 35°02'59"E	30
G	42°04'12"N – 35°02'45"E	30

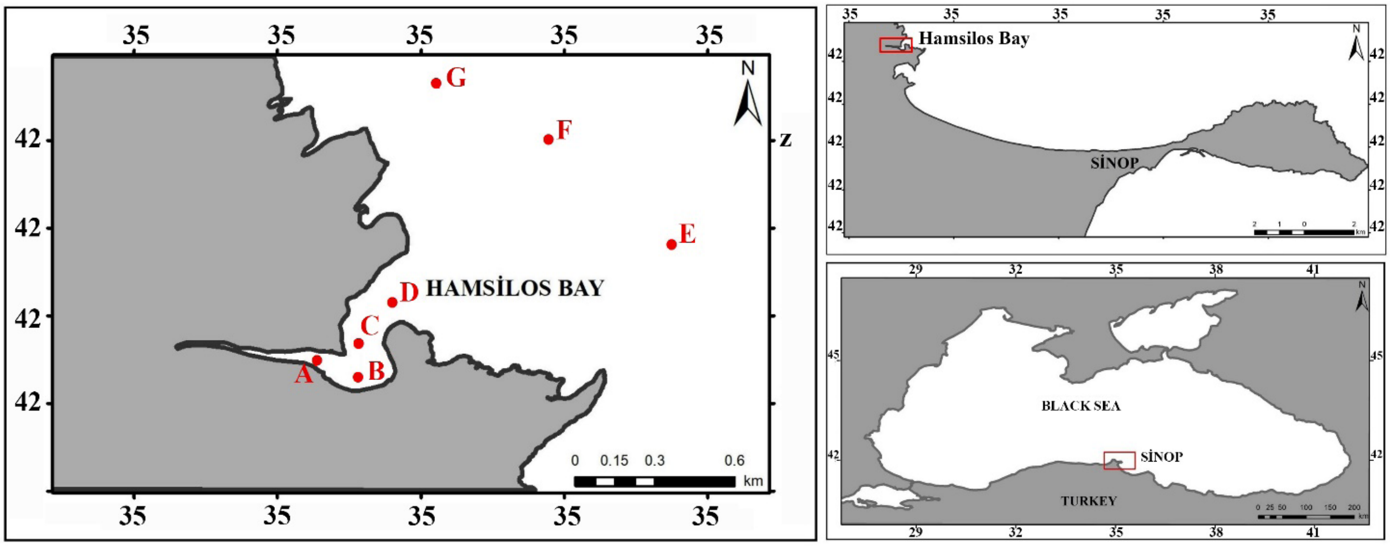

Figure 1. Location of sampling stations.

ed, Ohio, USA). The concentration of chlorophyll-*a* (µg. l^{-1}) was estimated according to Parsons et al. (1984).

A plankton net with a mouth opening of a diameter of 50 cm and a mesh size of 112 µm was vertically towed from the bottom to the surface of the water during the daytime. After sampling, the collected material was transferred into a bottle and preserved in a solution of borax-buffered 4% formaldehyde in seawater. *O. davisae* individuals were quantitatively examined using subsamples (two replicates) with a Stempel pipette (1 ml). Subsamples varied from 1/1 to 1/100 (usually 1/25 and 1/50) depending on the abundance of individuals in the samples. The abundance results of *O. davisae* were given in individual (ind.) m^{-3}. The consequences were presented as mean ± standard deviation.

In a zooplankton counting chamber, the development stages of *Oithona davisae* were identified and counted under a stereomicroscope. The present study did not include naupliar stages of this species because the mesh size of our zooplankton was inappropriate (Uye & Sano, 1995). According to Ferrari & Orsi (1984) and Temnykh & Nishida (2012), *O. davisae* was identified.

Analyses of Spearman correlation (SPSS 21, IBM Corp., Armonk, NY, USA) were applied to determine the correlation between the abundance of *O. davisae* and abiotic and biotic variables. The difference in abundance values of developmental stages of *O. davisae* between stations and months was tested by One-way ANOVAs with post hoc Tukey tests (SPSS 21, IBM Corp., Armonk, NY, USA).

RESULTS

Environmental parameters

A detailed description of the hydrological status of the sampling site was given by Üstün (2019) and Üstün & Birinci Özdemir (2019). During the sampling period, a clear seasonal cycle was observed in the mean temperature of surface seawater and fluctuated from 8.44 ± 0.04 °C (February 2016) to 25.71 ± 0.16 °C (August 2015) in Hamsilos Bay. The surface seawater means salinity ranged between 17.74 ± 0.07‰ (September 2015) and 18.96 ± 0.03‰ (May 2016). The dissolved oxygen values in surface seawater varied from 7.56 ± 0.36 mg. l^{-1} (July 2015) to 9.88 ± 0.13 mg. l^{-1} (February 2016). The mean concentration of chlorophyll-*a* fluctuated between 0.15±0.10 µg. l^{-1} (March 2016) and 0.71 ± 0.16 µg. l^{-1} (October 2015) in the surface seawater (Figure 2).

Seasonal dynamics of population abundance of *Oithona davisae*

The clear seasonal distribution pattern of *Oithona davisae* abundance was observed in the study area and ANOVA showed that differences between the abundance of *O. davisae* were statistically important in sampling months (F=14.961; p<0.05), but not at sampling stations (F=0.195; p>0.05). *O. davisae* had greater mean abundance values during the summer-autumn periods. The highest value for mean abundance was observed in September 2015 (3533.33 ± 1194.89 ind. m^{-3}). A small–scale decrease in mean abundance was observed in the period from September 2015 to December 2015 (1715.05 ± 938.67 ind. m^{-3}). In January 2016, mean abundance values started to decrease sharply and reached 170.68 ± 52.74 ind. m^{-3}. After January 2016, the decreasing trend continued gradually until May 2016 (0.24 ± 0.58 ind. m^{-3}) and reached its lowest level in May 2016 (Figure 3).

O. davisae concentrated in the inshore of the bay and the abundance of this species decreased at stations located outer of the bay. The maximum abundance value of *O. davisae* was noted in September 2015 at station B (5550 ind. m^{-3}) and the minimum abundance value was determined in May 2016 at station D (1.67 ind. m^{-3}). While there were high abundance values at stations inside the bay during July–August–September 2015, the abundance of this species at stations outside of the bay reached high values during October–November–December 2015. *Oithona davisae* was found only at station D in May 2016 (Figure 3, Table 2).

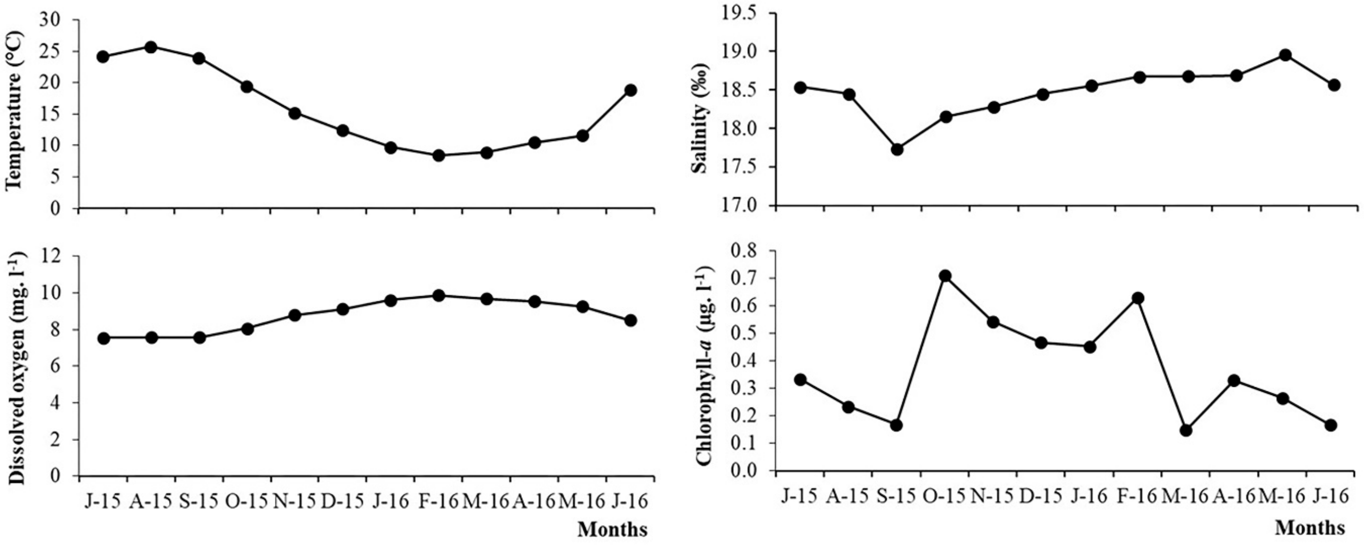

Figure 2. The monthly mean variation in environmental factors on surface seawater in Hamsilos Bay, Sinop.

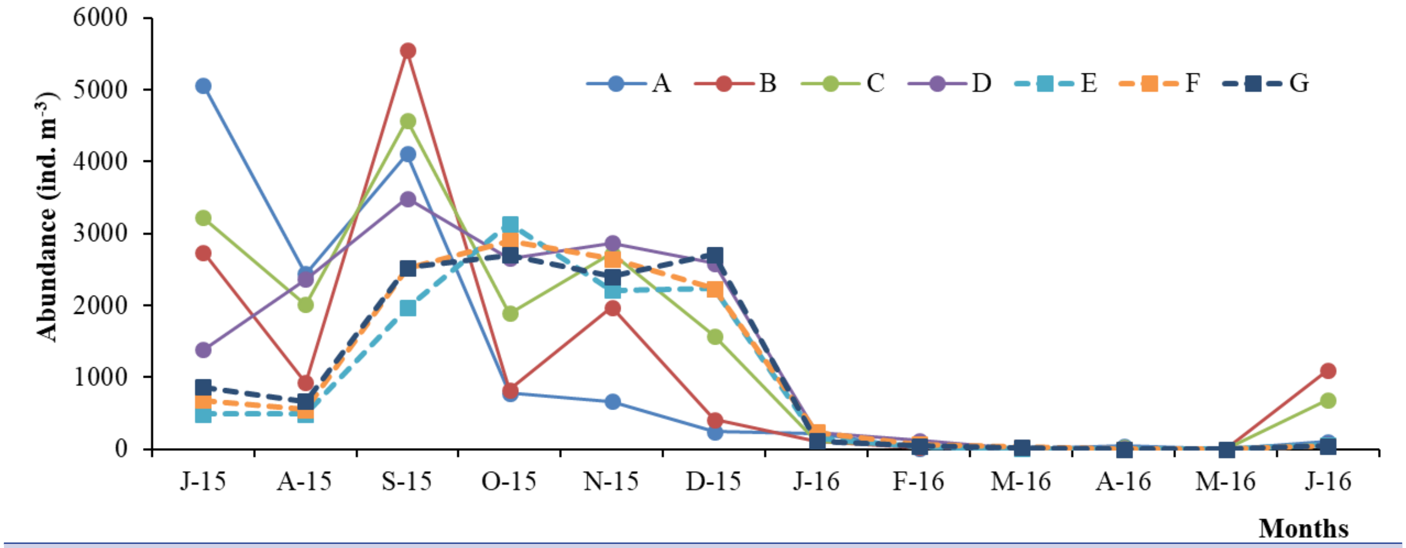

Figure 3. Spatial and temporal distributions in abundance (ind. m^{-3}) of *O. davisae* in Hamsilos Bay.

Table 2. Minimum, maximum, and mean of abundance values (ind. m^{-3}) of *O. davisae* according to stations in Hamsilos Bay.

Stations	A	B	C	D	E	F	G
Minimum	10 (March 2016)	4 (April 2016)	12.5 (March 2016)	1.67 (May 2016)	4.17 (April 2016)	4.17 (April 2016)	2.5 (April 2016)
Maximum	5062.5 (July 2015)	5550 (September 2015)	4575 (September 2015)	3487.5 (September 2015)	3137.5 (October 2015)	2900 (October 2015)	2712.5 (December 2015)
Mean±STD	1142±1683.7	1569±1568.6	1408±1459.5	1315±1321.7	901±1094.7	994±1145.6	1008±1147.1

Population age structure

The majority of the population of *O. davisae* consisted of copepodite 5–4 (Figure 4). The abundance values of females varied from 0.83 ind. m^{-3} (June 2016; St F and G) to 625 ind. m^{-3} (July 2015; St A). The presence of females was more pronounced in September 2015 and October 2015 (Figure 5). The female abundance values among months differed significantly (F=11.850; p<0.05). Between stations, there were no statistically significant variations in female abundance (F=0.217; p>0.05). Abundance values of the male were lowest in January 2016 (2.5 ind. m^{-3}; St C) and highest in July 2015 (781.25 ind. m^{-3}; St A). Three peak periods (July 2015, September 2015, and December 2015) were observed in males (Figure 5). Male abundance significantly differed between months (F=5.899; p<0.05). In terms of male abundance, there were no statistical differences between stations (F=0.600; p>0.05). The mean sex ratio (female/male) values of *O. davisae* varied between 0.56 and 3.79. Female individuals dominated the community from August 2015 to February 2016 (Figure 6).

Copepodit 5–4 was present all over the research period (in May 2016 only in station D), with its maximum abundance of 4825 ind. m^{-3} at station B in September 2015 and its minimum abundance of 1.67 ind. m^{-3} at station D in May 2016. Copepodit 5–4 was dominated during the July 2015–December 2015 periods (Figure 5). Abundance values of copepodit 5–4 showed statistically significant differences among months (F=15.077; p<0.05). There were no statistically significant differences in copepodit 5–4 abundance among the stations (F= 0.255; p>0.05) (Figure 5).

Copepodit 3–1 abundance values ranged between 1 ind. m^{-3} (February 2016; St B) and 650 ind. m^{-3} (November 2015; St F). It contributed significantly to the *O. davisae* population between September 2015 and December 2015 (Figure 5). Abundance values of copepodit 3–1 showed statistically significant differences among months (F=13.723; p<0.05). No significant differences in copepodit 3–1 abundance were observed between stations (F=0.151; p>0.05).

Female, male, and copepodite 3–1 individuals were not encountered in March 2016 and May 2016. In April 2016, female and copepodit 3–1 were determined only at station A, and male at station C (Figure 5). Minimum and maximum mean abundance values were in the range of 0.71 ± 1.75 (April 2016) to 317.26 ± 48.40 (September 2015) ind. m^{-3}in female, 0.71 ± 1.75 (April 2016) to 262.5 ± 246.52 (July 2015) ind. m^{-3}in male, 0.24 ± 0.58 (May 2016) to 2818.75 ± 1163.20 (September 2015) ind. m^{-3}in copepodit 5–4, 1.43 ± 3.5 (April 2016) to 441.55 ± 176.88 (November 2015) ind. m^{-3} in copepodit 3–1 (Figure 5).

The abundance of developmental stages of *O. davisae* was well correlated with trophic elements and physiochemical variables. It was found that the abundance and distribution of developmental stages of *O. davisae* correlated negatively with salinity and

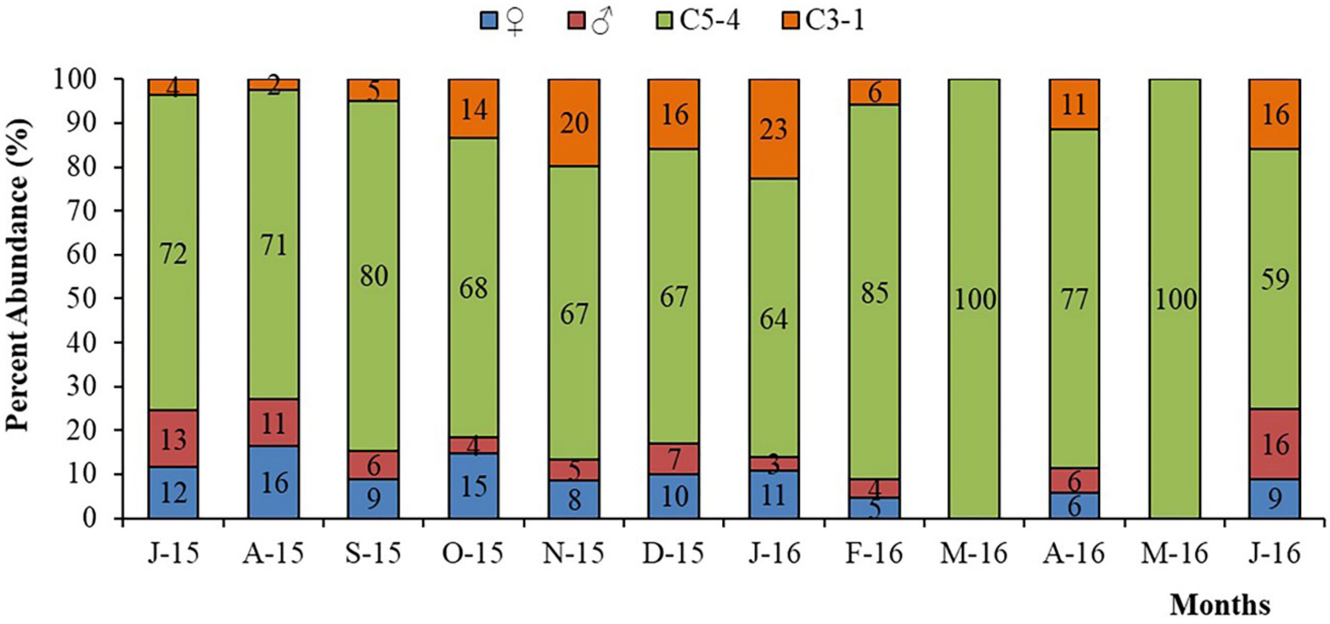

Figure 4. The percentage mean abundance contributions of different developmental stages of *O. davisae* in Hamsilos Bay.

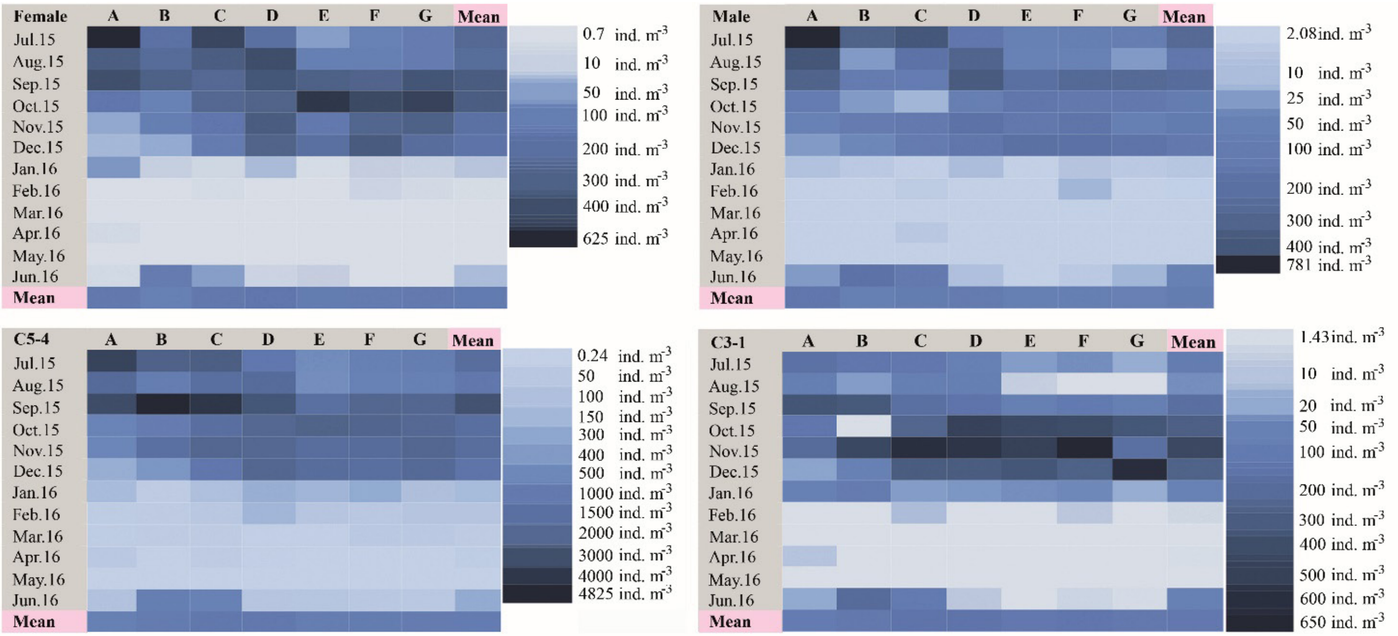

Figure 5. Temporal changes in mean abundance distributions of different developmental stages of *O. davisae* in Hamsilos Bay.

dissolved oxygen, but showed a positive correlation with chlorophyll-*a* and temperature. No correlation was found between the abundance of males and chlorophyll-*a* (Table 3).

DISCUSSION

Seven oithonid species were found in the Black Sea until now (Razouls et al., 2023). Most of these recorded oithonid species were known as questionable and had insufficient resources (Gubanova et al., 2014). Although *Oithona similis* are commonly distributed overall Black Sea (Gubanova et al., 2014), *Oithona davisae* expanded its distribution and became one of the most important components in the coastal community in the Black Sea (Gubanova et al., 2014), after the first observation of this species in Sevastopol Bay in the early 2000s (Zagorodnyaya, 2002). The abundance of this species in Sevastopol Bay already exceeded 40,000 ind. m^{-3} in October 2005 (Gubanova & Altukhov, 2007) and reached almost 100,000 ind. m^{-3} in October 2016 (Svetlichny

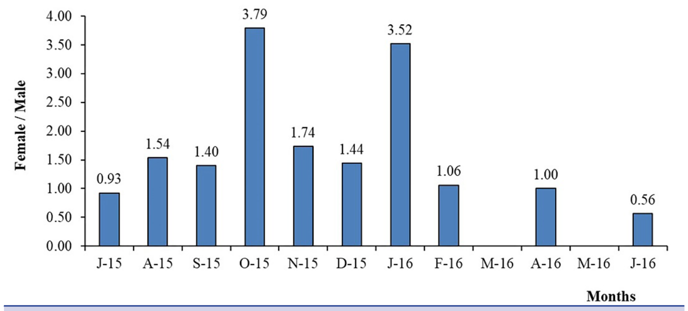

Figure 6. Mean sex ratio (male/female) values of *O. davisae* in Hamsilos Bay.

et al., 2018). Only eight years after the first appearance in the Black Sea, the evidence of the distribution of *Oithona davisae* was first observed on Turkey's coast in the Black Sea (Üstün & Terbiyik Kurt, 2016). It is seen that the presence of *O. davisae* is observed in the plankton every month and its abundance value increased when compared with the present study conducted in 2015–2016 in Sinop in respect of the species that were found to be present only in September–December period in Sinop in 2009. It was observed in other studies (Yıldız et al., 2017; Üstün et al., 2019; Yildiz & Feyzioğlu, 2020) carried out on the Turkish coasts of the Black Sea that the species reached values of maximum in the July–December (summer–autumn) period and demonstrated the lowest values in the January–May (winter-spring) period as is the case with the present study. Similar results were also revealed in the studies carried out on the coasts of Sevastopol Bay, Novorossiysk Bay, Romania, Bulgaria, and Georgia (Gubanova & Altukhov, 2007; Selifonova, 2011b; Timofte & Tabarcea, 2012; Mihneva & Stefanova, 2013; Seregin & Popova, 2016; Shvelidze, 2016; Svetlichny et al., 2018). Although the different plankton net sizes and sampling periods and depths do not allow us to compare the abundance values obtained from different studies, the studies demonstrated that the species has expanded its abundance and both temporal and spatial distribution since it was first found in the Black Sea.

In 2009, *O. davisae* was only distributed autumn period (Üstün & Terbiyik Kurt, 2016), however, it seems that *O. davisae* expanded its temporal distribution and was observed the following year on the Sinop coast. *O. davisae* to successfully inhabit and spread to new marine areas most probably due to having bioecological features, such as thermophilic (Ferrari & Orsi, 1984; Uye & Sano, 1995; Mihneva & Stefanova, 2013; this study), euryhaline (Svetlichny & Hubareva, 2014; Hubareva & Svetlichny, 2016), reproduction style (Svetlichny et al., 2016), food source (principally feeds on ciliates and heterotrophic dinoflagellates) (Uchima, 1988; Saiz et al., 2003; Khanaychenko et al., 2018), locomotor activity and energy metabolism (body mass density, sinking, and swimming speeds) (Svetlichny et al., 2016).

Table 3. Correlation between the abundance of *O. davisae* and developmental stages and environmental variables.

	O. davisae	Female	Male	Copepodit 5–4	Copepodit 3–1
Chlorophyll-a	.253 (*)	.232 (*)	.151	.246 (*)	.331 (**)
Temperature (°C)	.669 (**)	.752 (**)	.754 (**)	.685 (**)	.488 (**)
Salinity (‰)	-.771 (**)	-.795(**)	-.730(**)	-.810 (**)	-.734 (**)
Dissolved oxygen (DO mg. l^{-1})	-.710 (**)	-.763 (**)	-.770(**)	-.738 (**)	-.509 (**)

*Correlation is significant at the 0.05 level (2-tailed); **Correlation is significant at the 0.01 level (2-tailed)

Oithona davisae is a long–lived species and demonstrates considerable variation in population size depending on temperature changes affecting food resources and the development of planktonic predators (Svetlichny et al., 2016). It is either absent (Takahashi & Uchiyama, 2007) or very scarce (Uye & Sano, 1995) during the winter–spring seasons among the planktons of the coastal waters of East Asia, its original habitat. The abundance of this cyclopoid copepod is low in the Black Sea during winter and spring and increases from summer to autumn with the increasing temperature (Gubanova &Altukhov, 2007; Selifonova, 2011b; Altukhov et al., 2014; Seregin & Popova, 2016). Capable of surviving the cold seasons in the Black Sea, fertilized females reproduce the next generation under favorable spring conditions (Svetlichny et al., 2016).

In the coastal waters of southeastern Japan, the original habitat of *O. davisae*, the species has been found to inhabit in seasonal temperature variations between 8.9 and 28.2 °C (Uye & Sano, 1995). The present study observed the highest abundance values between July 2015 and September–December 2015. The water temperature varied between 12–24 °C during this period. The lowest abundance values were recorded between February (8.4 °C–54 ind. m^{-3}) and May (11.6 °C–0.24 ind. m^{-3}). The results of this study agree with findings acquired in the Black Sea previously (Mihneva & Stefanova, 2013; Üstün & Terbiyik Kurt, 2016; Yıldız et al., 2017; Yildiz & Feyzioğlu, 2020). It was observed in the Black Sea that an intense increase in the population size of this species started when the surface water temperature reached 18–20 ℃ (Seregin & Popova, 2016). The analysis of seasonal variations of *O. davisae* recorded in samples collected from the coastal areas of Sinop suggested that temperature has a remarkable influence on the development time and abundance of the species. This study detected a strong positive correlation between temperature and *O. davisae* abundance. A negative correlation was calculated between *O. davisae* abundance values and surface seawater temperature in the seasonal study managed in İğneada (Üstün et al., 2019).

Exotic species that can successfully colonize new habitats are considered to have a wide salinity tolerance (Lee et al., 2003). *O.*

davisae has been detected in the salinity range of 28.6–32.3‰ in the Sea of Japan (Uye & Sano, 1995), in the salinity range of 12–19‰ in the San Francisco estuary (Ferrari & Orsi, 1984) and in the salinity range of 15.8–17.2‰ in the western Black Sea (Mihneva & Stefanova, 2013). In their experimental studies, Svetlichny & Hubareva (2014) and Hubareva & Svetlichny (2016) have shown that *O. davisae* can adapt well to high salinity and the species can live in the salinity range of 3–55‰ by keeping its body mass density constant and that *O. davisae* is (partly, 12–40‰) osmoregulatory species. The salinity ranged between 17.74–18.54‰ (mean: 18.27 ± 0.27‰) during the period when the values of the maximum abundance of the species were observed in our study. This study determined a negative correlation between salinity and abundance values of *O. davisae*. A positive correlation was calculated between the abundance values of *O. davisae* and surface seawater salinity in the seasonal study conducted in İğneada (Üstün et al., 2019). This contradictory report could be related to the joint and complex effect of the environmental variables.

In its original habitat, Asian waters, there is a congruence between the months with the highest abundance of the species and the months with the highest concentration of chlorophyll-*a* (Uye & Sano, 1995). It was revealed in our study that chlorophyll-*a* values ranged between 0.17–0.71 μg. l^{-1} (mean: 0.41 ± 0.19 μg. l^{-1}) during the period when the highest abundance values of the species were observed. A positive correlation was detected between abundance values of *Oithona* and chlorophyll-*a* in this study, however, such a case was not detected in the study conducted in Trabzon (Yıldız et al., 2017).

CONCLUSION

O. davisae has a high tolerance to different environmental conditions and physiological structures (osmoregulatory systems, high egg production rate, high metabolic activity, etc.) which facilitate to survival and reproduction of *Oithona davisae* in the Black Sea. In our study, it seems that *O. davisae* has expanded its spatial and temporal distribution along the Sinop coast in the Black Sea and has been inhabited by increasing its population, and biotic and abiotic factors affected the temporal distribution of this species. This study suggests that *O. davisae* has been successful in inhabiting and establishing a constant population in the southern Black Sea.

Note: This study was presented orally at the "13th International Conference on Copepoda" held in Los Angeles, California, from the 16th to the 21st of July 2017.

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Compliance with Ethical Standard: Authors declare that ethical approval is not required for this type of study.

Conflict of Interests: Authors declare that they have no financial interests or personal relationships that could affect this work, hence no conflict of interest.

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