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# Spatio-temporal variation of the invasive copepod *Oithona davisae* in the zooplankton community of Kavala harbour

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#### **Abstract**

Non-indigenous species (NIS) are one of the anthropogenic effects greatly threatening biodiversity and ecosystem services. Ports and harbours are among the most susceptible environments for the arrival and establishment of these species since ballast waters represent a common pathway for NIS arrivals. However, relevant studies are limited. One of the few studies dealing with the zooplankton community of a harbour and reveals the presence of the widely distributed NIS *Oithona davisae* is presented in this paper, with special mention to the spatiotemporal variation of the species. The invader, *O. davisae*, a significant contributor to the zooplankton community, confirmed its thermophilic character and showed better development in the inner part of the harbour as well as patterns of coexistence with competing native species.

Keywords: Kavala Bay; port; zooplankton; Oithona davisae; non-indigenous species.

#### Introduction

Over the past few decades, as the rate of coastal urbanization is increasing rapidly and, combined with climate change, urban marine ecosystems are experiencing highly variable conditions influenced by several types of stressors, including land-based effluents, marine transport, fishing, biodiversity losses and extreme fluctuations in water properties (Todd et al., 2019). These stressors include non-indigenous species (NIS) that are currently recognised as one of the important anthropogenic effects threatening ecosystem functioning (Molnar et al., 2008). In several cases, the disruption of food web functioning has been shown to affect the maritime industry (Raaymakers, 2007). Planktonic copepods expand their distribution and are frequently recognised outside their indigenous environments. The copepod Oithona davisae is one of the most well-known NIS; it is a nearshore species, indigenous to the temperate coastal waters of the Indo West-Pacific, inhabiting eutrophic bays and transitional waters (Ferrari & Orsi, 1984; Uye & Sano, 1998; Uriarte et al., 2016). Due to its high tolerance to temperature and salinity variations, O. davisae has successfully invaded a wide range of environments. These include the Black Sea, the Sea of Marmara and several sites across the Mediterranean Sea (e.g., Saiz et al., 2003; Altukhov et al., 2014; Razouls et al., 2015-2022; Zagami

et al., 2018; Velasquez et al., 2021;) while in the North Aegean Sea it has been recorded in the Thermaikos Gulf as well as the Kavala harbour (Dragicevic et al., 2019; Kourkoutmani et al., 2021). In some of these areas, it has established successful populations completely replacing Oithona nana (Gubanova et al., 2014), while it has even been reported to successfully compete with other larger copepods (Gubanova et al., 2019).

Ship ballast waters is the main vector recognised as the culprit for NIS introductions in the marine environment (Bailey, 2015). This makes ports and harbours among the most susceptible environments for new records of these species. However, the number of surveys relating to the structure and function of zooplankton communities in harbours is quite limited. This hampers the effective tracking of such bioinvasions, which can be further delayed when considering confusing taxonomy and small size. During the last three decades, several studies (e.g., Turner, 1994; Paffenhöfer, 1998; Zervoudaki et al., 2006) have pointed to the under-sampling of the smaller-sized fractions when using a WP-2 net, which is traditionally used for mesozooplankton sampling. This results in an underestimation of the small-sized copepods, as well as their developmental stages, which need to be collected simultaneously, using a finer meshed net.

Here, we report on the presence of *Oithona davisae* in the small-sized fraction of the metazoan zooplankton

community of Kavala harbour. The investigation of its spatio-temporal distribution, along with differences in terms of biodiversity and abundance when using nets of different size selectivity, are reported for the first time in the study area and represents one of the few studies dealing with the zooplankton community in a harbour.

## **Materials and Methods**

# Study site

Kavala harbour is an important terminal for passenger ships in Greece, and since 2016 a number of floating platforms have been installed that provide numerous docking sites. It is located northwest of Kavala Bay (40.88056° N, 24.41667° E), a semi-enclosed area on the continental shelf of the northern Aegean Sea in the eastern Mediterranean Sea (Fig. 1). At the eastern part of the Bay, a commercial port, "Philip II" in New Karvali (40.95649° N, 24.50639° E), has been operating since 2002, serving mainly general cargo and bulk carrier vessels (www. marinetraffic.com). Kavala Bay is one of the most wellknown fishing and nursery grounds in the northern Aegean Sea (Stergiou et al., 1997), which is particularly important for small pelagic fish populations (Stergiou et al., 2007; Sylaios et al., 2010; Karagianni et al., 2019). Furthermore, due to its location, Kavala Bay is influenced by the Black Sea Waters (BSW) since the surface current flowing through the Dardanelles along the Thracian Sea enters Kavala Bay (Sylaios et al., 2005).

#### Sampling and microscopic analysis

Sampling was conducted monthly, from February 2017 to January 2018 (except October), from the floating platforms of the Kavala harbour basin (Fig. 1). Zooplankton samples were collected by vertical hauls throughout the water column, using both a 50  $\mu$ m and a 200  $\mu$ m mesh size plankton net for better representation of the different zooplankton size classes. All samples were preserved in 4% formalin-seawater solution for further microscopic analysis.

Laboratory analysis included species identification and abundance estimation under a microscope (Leica, Leitz Laborlux S). Taxonomic identification of individuals was based on appropriate taxonomic keys (Tregouboff & Rose, 1957; Conway, 2012a,b), as well as the classification system proposed by Razouls et al. (2005-2022). Adults, when possible, were identified all the way down to species level, whereas copepodites were grouped together and were not further identified. In the 50 µm net samples, female adult individuals of the genus Oithona were identified based on morphological features (Fig. 2) of the rostrum, mandible, maxillule and urosome as well as the number of exopod spines of the swimming legs, as presented in Dragicevic et al. (2019). In the 200 µm net samples, the presence of O. davisae was confirmed but O. davisae and O. nana adults were counted together; the number of males also includes all Oithona species. Quantitative samples were analysed according to Harris et al. (2000).

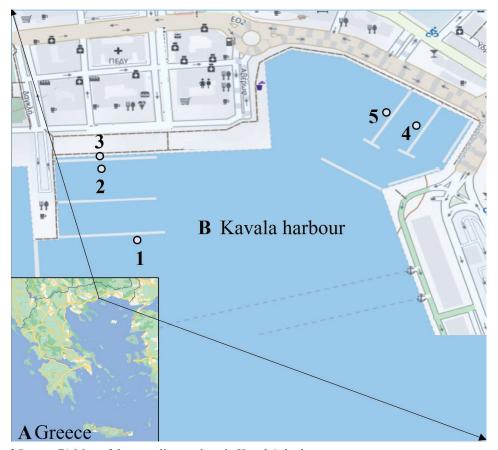


Fig. 1: A) Map of Greece, B) Map of the sampling stations in Kavala's harbour.

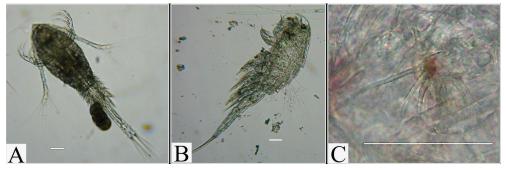


Fig. 2: Oithona davisae collected from Kavala's harbour A) dorsal view, B) side view, C) maxillule (scale 50 μm).

### Data analysis

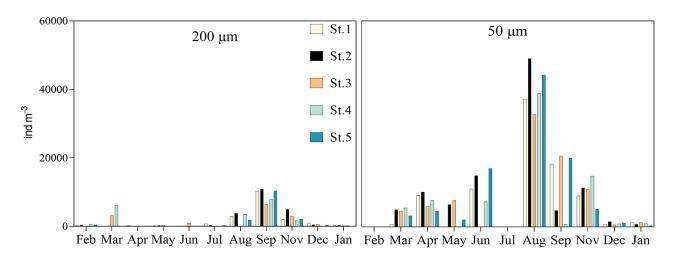
In order to identify the similarity of the zooplankton community during the sampling period, Hierarchical Cluster Analysis (CLUSTER) based on the Bray-Curtis similarity index was performed on the copepod's (nauplii, copepodites, adult copepods) abundance matrix, using group-average linking; abundance data were log (x+1) transformed in order to reduce bias due to highly abundant groups. Non-Metric Multidimensional Scaling (NMDS) was performed on the same Bray-Curtis similarity matrix in order to further visualize the clustering of the copepod's community. The similarity analysis routines, Analysis of Similarity (ANOSIM) and Similarity Percentage Analysis (SIMPER), were used to test the significance levels and sources of variance between the zooplankton assemblages of the different groups derived by hierarchical cluster analysis. The above analyses were performed using the Plymouth Routine in Multivariate Ecological Research (PRIMER) v.6 software package (Clarke & Gorley, 2006).

# Results

In total, 60 zooplankton taxa were identified in samples obtained with both nets (50 and 200  $\mu$ m) during the sampling period in Kavala harbour; 32 Copepoda, 6 Cladocera, 6 gelatinous taxa, and 16 meroplanktonic taxa (Table S1).

Total 50 µm net abundance ranged from 387.26 to 49037.09 ind m<sup>-3</sup> while the 200 µm net abundance ranged from 1.27 to 10908.62 ind m<sup>-3</sup> (Fig. 3). The mean abundance of the 200 µm net (1876.13 ind m<sup>-3</sup>) during the sampling period was at least five times lower than the mean 50 µm net abundance (10763.52 ind m<sup>-3</sup>) (t-test; p = 0.01). However, at Station 4 in March, the 200 μm net abundance was higher than that of the 50 μm net due to the higher numbers of Bryozoa and Pleopis polyphemoides in the 200 µm net (Fig. 3, Table S1). The maximum differences between the numbers of the 200 and 50 µm net were observed for copepodites of cyclopoida and nauplii, which showed 98 times higher values when collected with the 50 µm net, while almost identical abundances for adult copepods were recorded for both nets. Also, other smaller-sized fractions, e.g., rotifer taxa, were captured only when using the 50 µm plankton net (Table S1). During the sampling period, zooplankton communities collected with both nets presented similar distributions, exhibiting substantial temporal variability with abundance values being higher in late summer-early autumn (Fig. 3).

The developmental stages of copepods, nauplii and copepodites, dominated the 50 µm net samples throughout the sampling period (range 53-98%) contrary to the 200 µm net samples where their contribution was considerably lower, averaging 18% of the total community (Fig. S1). Within the copepod's community of the 50 µm net, *Oithona nana* and *Oithona davisae* dominated, contribut-



*Fig. 3:* Abundance (individuals m<sup>-3</sup>) of the zooplankton community, grouped by sampling stations, during the sampling period 2017-2018 in Kavala harbour.

ing cumulatively up to 90% to adult copepod abundance, in contrast to the community collected with the 200 µm net where their contribution among adult copepods was rather low (<10%) (Fig. 4). Regarding their temporal distribution, *Oithona nana* showed a persistent occurrence throughout the sampling period while *O. davisae* was more abundant from late spring to early winter, although they both exhibited their peak abundance during the summer-autumn period. The native *O. nana* exhibited extended spatial presence in the sampling area, contrary to *O. davisae*, which reached maximum density at the stations situated further away from the open sea and closer to the area where ships dock (Stations 4 and 5) (Fig. 5).

Cluster analysis based on copepod (nauplii, copepodites, adult copepods) abundance data revealed three major clusters. Group 'a' consisted of only one sampling (September 2017; St.4). The samplings of September, November and August formed the cluster of Group 'c', whereas the rest of the samplings made up the cluster of Group 'b'. The dissimilarity between Groups 'b' and 'c'

(ANOSIM, R = 0.819, p = 0.001; Table S2) was mainly due to the contribution of the invasive copepod *Oithona davisae* (SIMPER average dissimilarity 9.78 %) (Fig. 6, Table S2).

#### **Discussion**

In our study we recorded the well-established fact that the small-sized fraction of crustacean zooplankton, is vastly underestimated due to the traditional sampling methods (Calbet, 2001; Gallienne & Robins, 2001; Turner, 2004; Zervoudaki *et al.*, 2006), with copepod developmental stages (nauplii and copepodites) (cumulative contribution reached up to 98%) mainly contributing to this difference.

In total, copepods dominated the zooplankton community of Kavala's harbour collected using a 50 μm net and contributed up to 98%, with the small-sized oithonoids (*O. nana* and *O. davisae*) dominating in terms of

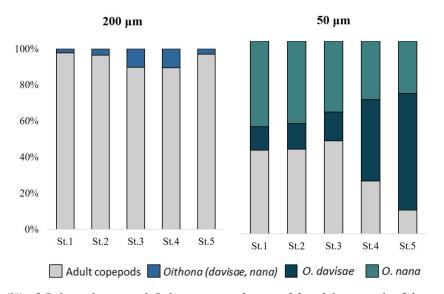


Fig. 4: Contribution (%) of Oithona davisae and Oithona nana to the rest of the adult copepods of the zooplankton community collected with the 50 and 200 μm net.

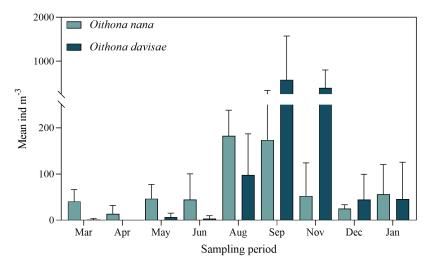
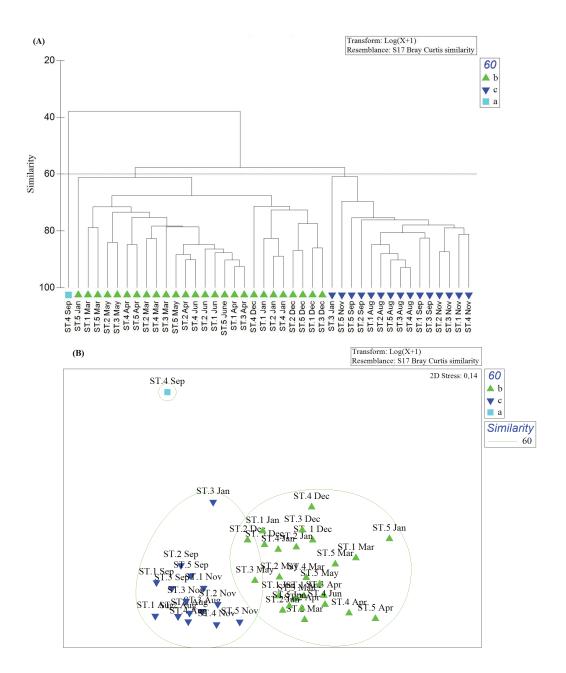


Fig. 5: Abundances (ind m<sup>-3</sup>) (mean  $\pm$  SD) of Oithona davisae and Oithona nana during the sampling period 2017-2018 in Kavala harbour.



*Fig.* 6: (A) Cluster Analysis and (B) Non-metric Multi-dimensional Scaling configuration (NMDS) on copepod's abundance data (50 µm net). The groups were delineated at a 60% similarity level (dashed line).

adult copepod abundance. This is of no surprise considering that oithonoids have been described as the most widespread and abundant copepods worldwide (Gallienne & Robins, 2001). Among them, Oithona davisae and Oithona nana have been overlooked in zooplankton studies because, due to their small size, they escape from the classical sampling equipment used for zooplankton collection (Gallienne & Robins, 2001; Tseng et al., 2007). Therefore, in many cases there is no clear picture of their distribution patterns or the time of O. davisae invasion in the newly established areas. In the Mediterranean Sea, it has so far been reported in the coastal lakes Faro & Ganzirri in Italy (Zagami et al., 2018), Venice Lagoon (Vidjak et al., 2019; Pansera et al., 2021), Barcelona port in Spain (Saiz et al., 2003), Thermaikos Bay in Greece (Dragicevic et al., 2019), the Turkish coasts of the Aegean Sea (Terbiyik Kurt & Beşiktepe, 2019) and the Levan-

tine Sea (Terbiyik Kurt et al., 2022). Besides recording its presence, few of these studies investigated its seasonal and spatial variations. In every case, including the current study, O. davisae has confirmed its thermophilic character by exhibiting higher abundance during the period from summer to autumn (Ueda, 1991), which is the warm period in the Mediterranean. Its dominance in autumn could probably provide a niche partitioning in time, allowing for coexistence with the native competing Oithona nana. This could be a successful strategy at the beginning of its invasion phase that could later lead to a full overtake of O. davisae against O. nana as is the case recorded in the Black Sea where it occupied the niche of O. nana and resulted in its complete disappearance (Gubanova et al., 2014). This cannot be confirmed herein since there is no clear time frame as regards the beginning of its invasion due to lack of proper monitoring. Of course, differences

in spatial distribution can also provide niche partitioning in space. In the studies mentioned above, including the current study, reporting spatial data, there was a clear spatial differentiation between *O. davisae* and *O. nana*. *Oithona davisae* was clearly in favour of the inner part of the harbour while *O. nana* was more abundant at the stations of the outer part (Fig. 5). *Oithona davisae* seems to find local conditions that promote its settlement and distribution, especially in the inner and more trophic sites (Pansera *et al.*, 2021). Future monitoring of the evolution of the non-indigenous *O. davisae* population will allow investigation of potential consequences of its introduction, such as exclusion of congeneric species and competition with other copepods for limited resources and microhabitats.

It is evident that underestimation of the small-sized fraction of zooplankton can result in erroneous estimates of biomass and secondary production since taxa of the metazoan microzooplankton community have a higher impact on the trophic coupling's effectiveness between primary producers, protozooplankton and higher trophic levels than larger taxa (Turner, 2004; Calbet & Saiz, 2005). This is of great importance for ecosystem functioning of ports and harbours that, apart from being susceptible to the arrival and establishment of NIS, are highly affected by nutrient effluents and subject to eutrophication. In eutrophic coastal areas, the link between the traditional food web and the microbial loop in eutrophic coastal areas can determine the energy flow and final trophic level reached (Sommer et al., 2018). The developmental stages of copepods and small-sized oithonoids can contribute to this; and the same applies to rotifers, a group of taxa erroneously considered absent from nearshore/coastal environments (Fontaneto et al., 2006) mainly because the majority of studies focus on the mesozooplankton community. Therefore, within the framework of the current study, we recorded the presence of Synchaeta spp., which had a notable contribution in the summer-autumn period community collected with a 50 µm net.

The current study is the first study on the zooplankton community in the Kavala's harbour region and one of the few on harbour areas in Greece and worldwide. We followed the approach of combined mesh size nets in order to investigate the under-represented size fraction of the smaller metazoan zooplankton community, reporting on the presence and distribution patterns of the invasive copepod Oithona davisae for the first time in the study area. The results of our study confirm the thermophilic character of O. davisae as well as the fact that it finds local conditions in harbours that promote its settlement and distribution, especially in the inner part of harbours. At the current point O. davisae exhibited coexistence patterns with the competing native species. However, since the time of its arrival is unknown, future monitoring of the harbour area will clearly frame the effects of its arrival on the structure and functioning of the zooplankton community in Kavala harbour.

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# **Supplementary Data**

The following supplementary information is available online for the article:

**Table S1.** List of recorded taxa in Kavala harbour. Mean abundance values (individuals m<sup>-3</sup>) of all samplings per station. Adults include males and females.

**Table S2.** Results of ANOSIM and SIMPER analysis applied on Bray-Curtis similarity matrix of log(x+1) transformed copepods abundance data, collected with 50  $\mu$ m plankton net.

Fig. S1: Contribution (%) of the main groups to the 200 μm and 50 μm net abundance in the Kavala harbour during 2017-2018. a) Contribution (%) of nauplii and copepodites on the rest zooplankton community. b) Contribution (%) of dominant mesozooplankton groups to total abundance.