

Mediterranean Marine Science

Vol 17, No 3 (2016)



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doi: [10.12681/mms.1743](https://doi.org/10.12681/mms.1743)

To cite this article:

VIDJAK, O., BOJANIĆ, N., NINČEVIĆ GLADAN, Ž., SKEJIĆ, S., SKEJIĆ, B., & GRBEC, B. (2016). First record of small tropical calanoid copepod *Parvocalanus crassirostris* (Copepoda, Calanoida, Paracalanidae) in the Adriatic Sea. *Mediterranean Marine Science*, 17(3), 627–633. <https://doi.org/10.12681/mms.1743>

First record of small tropical calanoid copepod *Parvocalanus crassirostris* (Copepoda, Calanoida, Paracalanidae) in the Adriatic Sea

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Handling Editor: Argyro Zenetos

Received: 15 April 2016; Accepted: 26 August 2016; Published on line: 20 September 2016

Abstract

In December 2014, the adult females and copepodites of an alien paracalanid copepod *Parvocalanus crassirostris* were identified in the Central Adriatic port of Šibenik. The most probable transmission vector for this small copepod is ballast water from cargo ships that is regularly discharged at these locations. This paper focuses on the morphology of *P. crassirostris* and the state of its population in the port of Šibenik. The possible path of introduction of *Parvocalanus crassirostris* into the Adriatic Sea is also discussed.

Keywords: *Parvocalanus crassirostris*, marine copepods, alien species, ballast waters, Adriatic Sea

Introduction

Paracalanidae are common members of the copepod community in marine environments, with genera *Paracalanus*, *Calocalanus* and *Mecynocera* typically found over continental shelf waters worldwide (Boxshall & Halsey, 2004). Within Paracalanidae, the new genus *Parvocalanus* was established by Andronov (1970) to accommodate several small species formerly classified as *Paracalanus* Boeck, 1864, based on the form of the rostrum, structure of female P5 and the appearance of male cephalon. The genus originally encompassed *Parvocalanus crassirostris* (F. Dahl, 1894), *P. dubia* (Sewell, 1912), *P. scotti* (Früchtl, 1923) and *P. serratipes* (Sewell, 1912). *Parvocalanus elegans* (Andronov, 1972) and *P. latus* (Andronov, 1972) were later added by the same author (Andronov, 1972). More recently, *P. leei* (Moon *et al.*, 2014) was added to the list, while the generic position of *Paracalanus arabiensis* (Kesarkar & Anil, 2010) is under discussion (Moon *et al.*, 2014).

Despite the reported worldwide distribution, taxonomy and morphological variability of the small calanoid *P. crassirostris* are not well understood. F. Dahl's original short and incomplete description of females from an estuary on the Atlantic coast of Brazil (F. Dahl, 1894) gave rise to taxonomical uncertainties, followed by the descriptions of several different forms (Früchtl, 1923; Davis, 1944; Wellershaus, 1969). The description of the typical form (*P. crassirostris* f. *typica*) was amended by Gonzalez & Bowmann (1965) and Greenwood (1976), while all developmental stages were described in detail by Lawson & Grice (1973).

The geographical distribution of *P. crassirostris* is

extremely wide, with records obtained from subtropical and tropical coastal and estuarine environments of all three oceans (Razouls *et al.*, 2005-2016). So far, records of *P. crassirostris* from the Mediterranean Sea are rare. The species is known to reside in the NW Mediterranean, Ionian Sea, Levantine Sea and in the NE Aegean Sea (Razouls *et al.*, 2005-2016).

This paper reports on the first record of *P. crassirostris* in the Adriatic Sea, identified from the plankton samples collected at terminals used for cargo loading in the port of Šibenik, where the zooplankton community was investigated for the purpose of creating a database for port baseline surveys, within the framework of the BALMAS project (www.balmas.eu). The paper focuses on the morphology of *P. crassirostris* and the state of its population in Šibenik Bay. Considering that the European Alien Species Information Network (Katsanevakis *et al.*, 2012) classifies *P. crassirostris* as an alien species introduced to the eastern Mediterranean (Lakkis, 1976), the origin of this species in the Adriatic Sea is also discussed.

Material and Methods

Study area and sampling methods

The port of Šibenik is deeply embedded in the coast of the middle Adriatic Sea at the mouth of the Krka River estuary (Fig. 1). Due to the small tidal range and the large influx of fresh water ($30 \text{ m}^3\text{s}^{-1}$ - $170 \text{ m}^3\text{s}^{-1}$), the Krka river forms a highly stratified salt-wedge estuary, with a freshwater to brackish-surface layer that flows towards the sea and seawater in the bottom layer that flows in the opposite direction. In the lower reach, the estuary expands into Šibenik Bay, with the port located in the NE part.

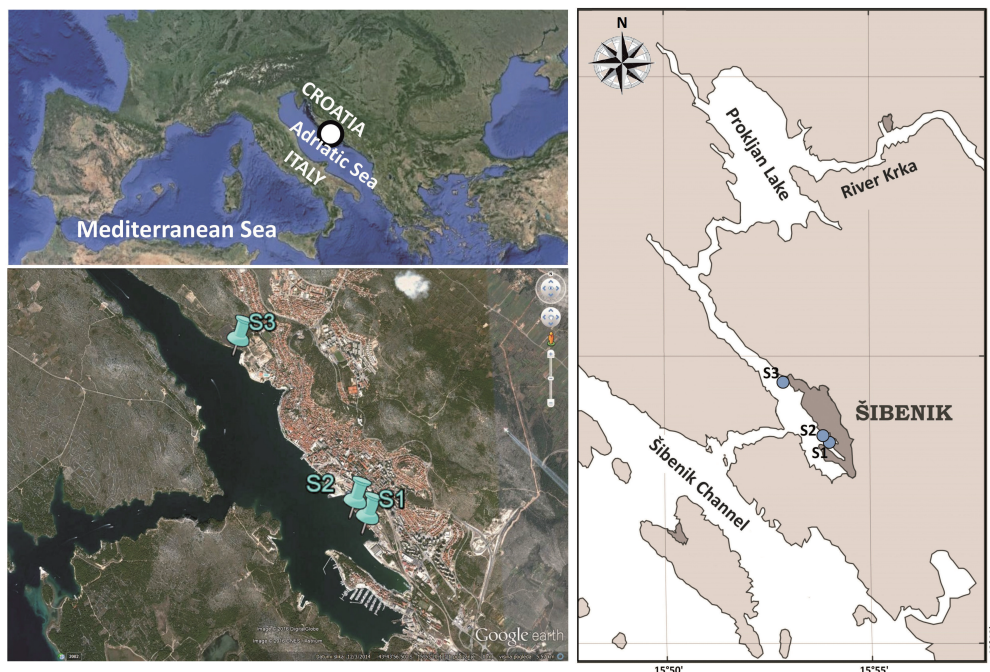


Fig. 1: Study area with the location of sampling stations.

Seawater sampling was conducted in December 2013 and April, August and December 2014, at three stations placed at terminals used for cargo loading and ballast water discharge: S1 (43.72515°N; 15.89928°E, depth 13 m), S2 (43.72673°N; 15.89757°E, depth 23 m) and S3 (43.74380°N; 15.88077°E, depth 37 m). The collection of environmental data included CTD sampling for temperature and salinity (Sea-Bird Electronic, SBE-25), determination of qualitative composition of phytoplankton by horizontal and vertical net hauls using 20 20 µm mesh size plankton net and determination of phytoplankton abundance (cells L⁻¹) using the Nansen sampler and Utermöhl sedimentation technique (Utermöhl, 1958).

Zooplankton were collected using two vertically towed Nansen nets; a 53 µm mesh size mesh size net (0.238 m² mouth area, 2.72 m total length) was used to assess microzooplankton abundance and population structure, while a 125 µm mesh size net (0.255 m² mouth area, 2.5 m total length) was used for mesozooplankton. Samples were preserved in 2.5% formaldehyde solution, previously buffered with CaCO₃.

Analysis of the samples

Counting of zooplankton organisms and species identification were performed in glass chambers using an inverted microscope at magnifications of 40-400x. The abundances were expressed as the number of organisms per cubic meter (ind.m⁻³). For morphological examination, adult specimens of *Parvocalanus crassirostris* were sorted under a stereomicroscope and cleared in 70% lactic acid before dissection and mounting in lactophenol. Slides were examined under a microscope with up to

1000x magnification. Drawings were made with the aid of a drawing tube using a microscope with differential interference contrast (OLYMPUS BH2). Body size of organisms was measured from the top of the head to the tip of the caudal rami (excluding caudal setae), using an ocular micrometer. The descriptive terminology largely follows Huys & Boxshall (1991).

To confirm identification, the morphology of *P. crassirostris* females from Šibenik Bay was compared to the published descriptions and illustrations of this species in the following sources: Davis (1944), Tanaka (1960), Gonzalez & Bowmann (1965), Lawson & Grice (1973), Greenwood (1976), Ramirez (1976), Hiromi (1981), Zheng *et al.* (1982), Bradford-Grieve (1994), Al-Yamani & Prusova (2003) and Vives & Shmeleva (2007).

Results

Description of the *Parvocalanus crassirostris* females from the port of Šibenik

Body small and stout, tapering towards the frontal part of the cephalosome (Fig. 2A). Total body length in the range of 480 µm - 520 µm (0.497±0.02 µm, N=9). Rostrum short and blunt (Fig. 2F). Cephalosome slightly humped in lateral view (Fig. 2B). Prosome about 2.5 times longer than wide and 3 times longer than the urosome including caudal rami. Fourth and fifth pedigerous somites partially separated. Antennules extending approximately to second urosomite. Urosome consisting of 4 somites, urosomite 2 and urosomite 3 narrower than the preceding and successive somites (Fig. 2C). Genital somite approximately 1.4 times wider than long with paired genital pores on the sides and operculum

positioned midventrally (Fig. 2D). Caudal rami short and cylindrical, twice as long as wide and armed with 5 caudal setae (Fig. 2C, D).

Armature formula of the first to fourth pair of swimming legs (P1-P4) as in Table 1. Posterior surfaces ornamented with rows of denticles on exopodal segments of P1-P3 (Fig. 2H, 3A-C) and endopodal segments of P2-P4 (Fig. 3A-C). Proximal outer margin serrated in third exopodal segment (Exp3) of P2 (4 spines), P3 (7 spines) and P4 (9 spines), as shown in figures 3A-C. Fifth legs (P5) symmetrical, two-segmented, proximal segment unarmed. Distal segment elongated and 2.3 times longer than wide, devoid of spinules along the distal edge, and apically armed with 2 unequal spines, the inner spine without serration and 2.25 times longer than outer spine (Fig. 2E).

Population status of *Parvocalanus crassirostris* and environmental settings in the port of Šibenik

P. crassirostris was found in the samples collected with the 125 μm mesh size Nansen net in December 2014 at three investigated stations in the port of Šibenik (Fig. 1); the calculated combined abundances of adults and juveniles being 26.5 ind. m^{-3} (S1), 128.0 ind. m^{-3} (S2) and 8.5 ind. m^{-3} (S3). All identified adults were females. Copepodites with total body length in the range of 330 μm - 400 μm were also recorded. The percentage of juveniles in the population was high at S1 and S2 (80% and 60%, respectively), while the number of adult females at S3 was low. The contribution of this species to total copepods was low (0.2-3.2%), but increased considerably when only the calanoid population was considered (2.3-54.0%).

At all sampling stations, collected mesozooplankton was highly dominated by copepods (>90%) (Fig. 4A). The contribution of calanoids (5-8%) and the numerical dominance of small non-calanoid species (*Oithona nana*, *Oncaea waldemari*, *Monothula subtilis*, *Euterpina acutifrons*) was relatively poor for all sites. Protozooplankton collected with 53 μm net on average attained 1343.6 \pm 352.24 ind. m^{-3} and was dominated by tintinnids (62.5 \pm 2.1%), with *Codonellopsis schabi* and *Tintinnopsis fracta* as the most abundant species. Taxopodid species *Sticholonche zanclea* and juvenile radiolarians contributed with 10.9 \pm 5.1% and 24.1 \pm 2.7%, respectively (Fig. 4B).

Table 1. Armature formula for swimming legs of *Parvocalanus crassirostris* females

♀ Legs	Coxa	Basis	Exopod	Endopod
P1	0-0	0-0	0-1; 0-1; II, I, 4	0-1; 1,2,2
P2	0-1	0-0	I-1; I-1; II, I, 5	0-1; 0-2; 2, 2, 3
P3	0-1	0-0	I-1; I-1; II, I, 5	0-1; 0-2; 2, 2, 3
P4	0-1	0-0	I-1; I-1; II, I, 5	0-1; 0-2; 2, 2, 3

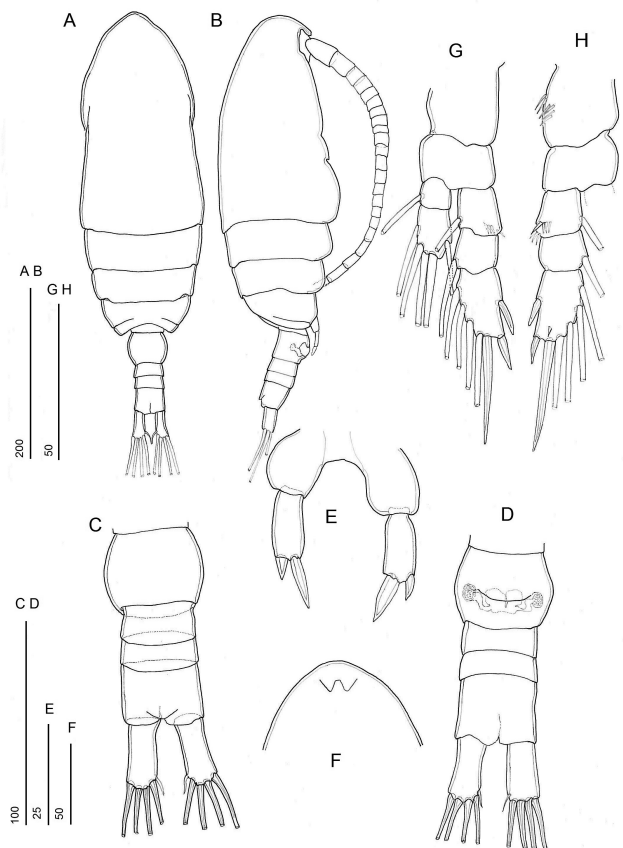


Fig. 2: *Parvocalanus crassirostris*, adult female: A, habitus dorsal; B, habitus lateral (A1 not shown in full length); C, urosome dorsal; D, Urosome lateral; E, P5; F, rostrum; G, P1 (anterior surface); H, P2 (posterior surface, endopod not shown). All scales in micrometers.

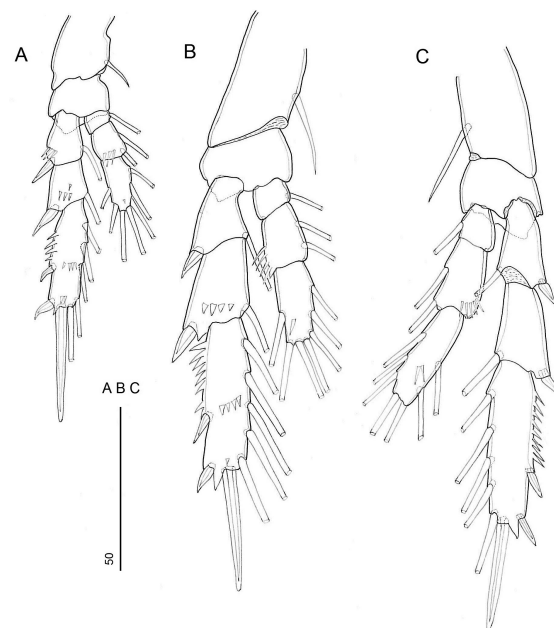


Fig. 3: *Parvocalanus crassirostris*, adult female: A, P2; B, P3; C, P4; posterior surfaces. All scales in micrometers.

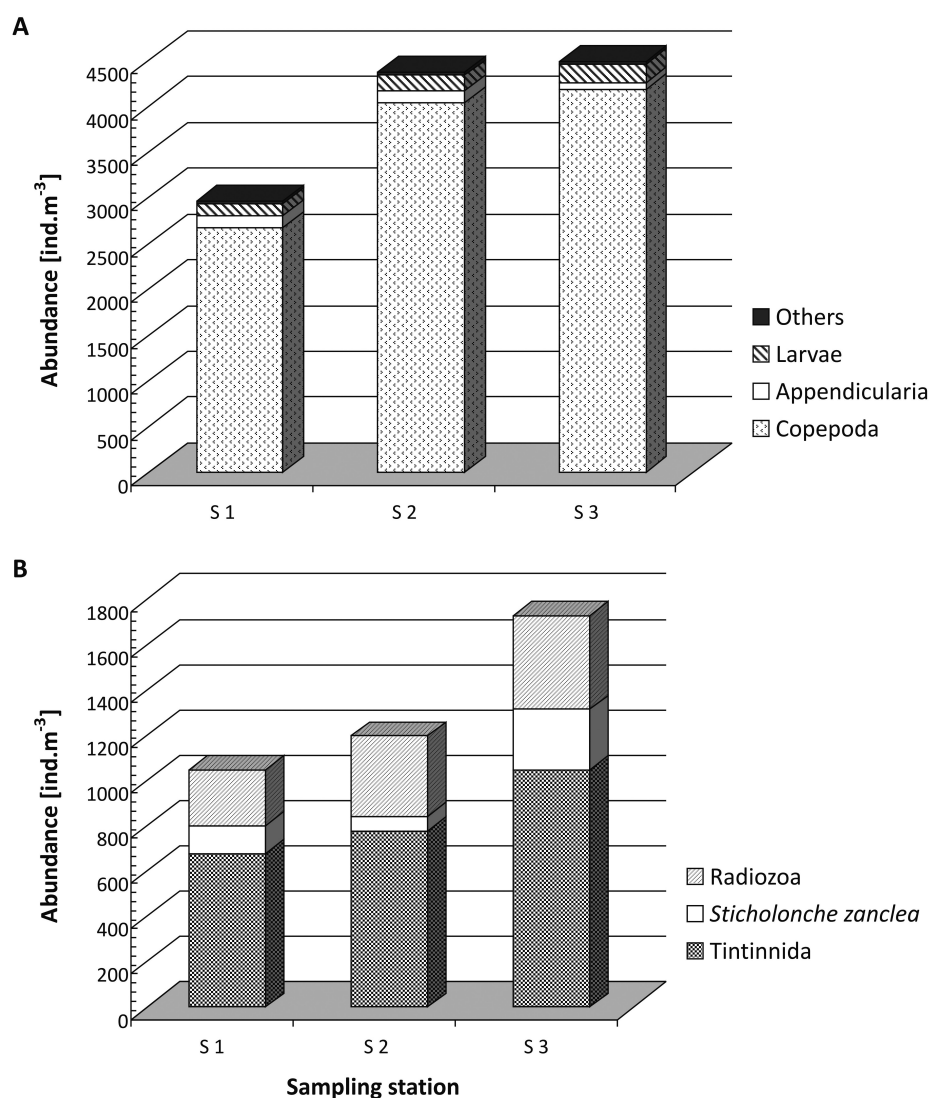


Fig. 4: Zooplankton population structure at the sampling stations in December 2014: A, Mesozooplankton; B, Protozooplankton.

Phytoplankton was numerically dominated by nanoplankton cells. The most abundant taxon at all stations was *Chrysochromulina* sp., with an average of $1.7 \times 10^5 \pm 69202.9$ cells L⁻¹ at the surface of all three stations. Other characteristic species were the coccolithophorid *Ophiaster hydroideus* and the dinoflagellate *Gymnodinium* sp. as well as diatoms *Pseudonitzschia delicatissima* group and *Thalassionema nitzschioides*.

Vertical profiles of physical parameters were very similar among sampling sites. The temperatures in the water column varied between 10.2°C - 18.8°C, while salinity ranged between 4.42-38.22, with cold and less saline water at the surface and sharp increases in temperature and salinity observed at about 4 m depth (Fig. 5).

Discussion

As regards general body morphology and size, as well the structure of P5, female *Parvocalanus crassirostris* from Šibenik port closely resemble *P. crassirostris*

f. *typica* (as described in Greenwood, 1976). Some differences were evident in the number of spines on the proximal outer margin of the third exopodal segment and in the ornamentation of the denticles on the exopodal segments of P2–P4. However, these characters reportedly vary between individuals of *Parvocalanus* species, even from the same locations - e.g. in *P. crassirostris* (Hiromi, 1981), *P. crassirostris* f. *cochinensis* (Wellershaus, 1969) or *P. leei* (Moon *et al.*, 2014). Our specimens can be clearly distinguished from the *P. crassirostris* var. *nudus* described from Chesapeake Bay (Davis, 1944) by the well-developed spiny ornamentation on the posterior faces of the rami of swimming legs P1-P4, which is lacking in Davies's specimens. Similarly, the morphology of P5, with unserrated inner spine and the distal end of the second segment devoid of spinules clearly distinguish our specimens from *P. crassirostris* f. *cochinensis* described from Indian waters (Wellershaus, 1969).

In plankton communities, a small indigenous species can remain undetected for a long time due to use of

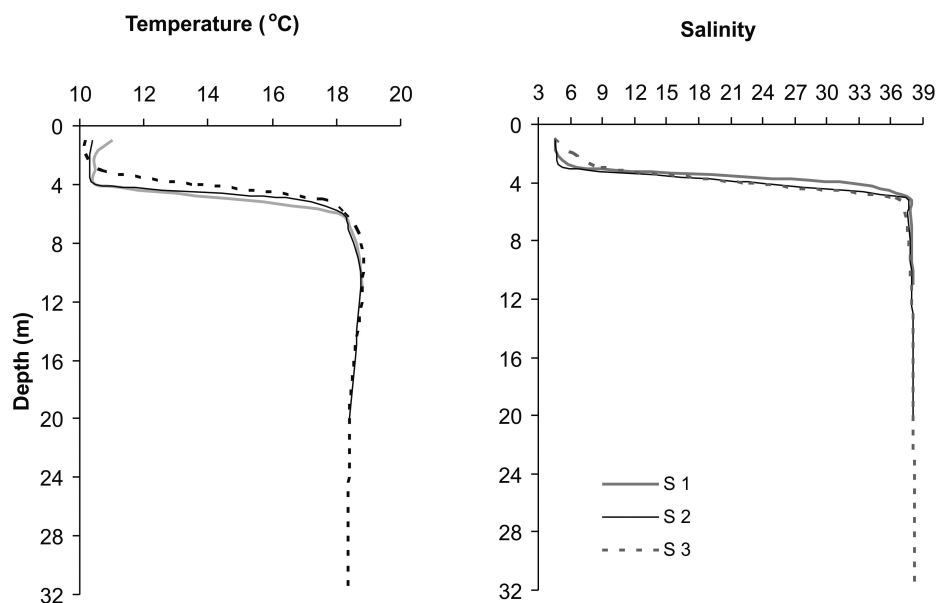


Fig. 5: Vertical profiles of temperature and salinity at the sampling stations in December 2014.

plankton nets with coarse mesh sizes (Davis, 1944; Greenwood, 1976). However, this is not likely in the case of the Šibenik Bay area, which has been investigated for zooplankton parameters for decades, using various types of plankton nets and water bottles, suitable for the collection of a wide range of plankton size classes (Regner, 1977; Kršinić, 1987; Vidjak *et al.*, 2009, 2012). The specific localities of this discovery, namely the same cargo terminals, were repeatedly sampled for zooplankton in 2013 and 2014, yet *P. crassirostris* was not recorded until December 2014. Considering that water ballast discharge is regularly performed at the investigated sites during ship cargo loading, it is very likely that the ballast water from the ships acted as the transmission vector for this small copepod. The analysis of ballast water from ships sailing from the eastern Pacific Japanese coast into the port of Vladivostok revealed the presence of this small copepod in ballast water on several occasions (Kasyan, 2010). Because of its ecology, *P. crassirostris* is a highly suitable candidate for this path of introduction: it is associated almost exclusively with coastal waters (Björnberg, 1963) and commonly found in brackish environments (Eskinazi-Sant'Anna, 2013; Jungbluth & Lentz, 2013), which is consistent with the usual location of the ports that are the main sources of ballast water. This species is also extremely eurythermic and euryhaline, capable of supporting a salinity range of 3.4-55.0 and temperatures of up to 30°C (Björnberg, 1963), which is necessary for the survival in harsh conditions during this inadvertent transport.

The exact path of introduction of *P. crassirostris* into the port of Šibenik is difficult to reconstruct at this time, considering its worldwide distribution; however, certain hypotheses can be put forward. The total volume of discharged ballast water in the port of Šibenik in 2014 was 27603 m³, with the largest share (80%) originating

from inter-Adriatic vessel transport between the Italian ports of Ancona, Marghera and Ravenna, and the rest mainly from the Baltic Sea, the Black Sea and the Mediterranean Sea (Antolić *et al.*, 2015). There is no recorded presence of *P. crassirostris* in the Baltic Sea (Razouls *et al.*, 2005-2016); thus, the possibilities are narrowed down to the Black Sea, the Mediterranean Sea or inter-Adriatic translocation, all non-native environments of *P. crassirostris*. The Black Sea appears to be the least likely possibility given that *P. crassirostris* is considered an alien species with rare sporadic occurrence and that information on population establishment is scarce (Gubanova *et al.*, 2014). In the Mediterranean Sea, as a consequence of Lessepsian migration through the Suez Canal (Lakkis, 1976), the species is widely distributed along the Levantine coast. Considering the origin and quantities of ballast water discharged in the port of Šibenik in 2014, inter-Adriatic translocation also seems very likely. Although this small calanoid has not so far been included on the list of Adriatic invaders (Pećarević *et al.*, 2013), the existence of an already established population originating from prior ballast water discharge in numerous and much larger ports along the western and eastern Adriatic coasts cannot be excluded.

The status of the investigated population in Šibenik Bay was characterized by low abundances (mean value between stations <60 ind.m⁻³) and low contribution to total zooplankton (<4%), and thus not indicative of an excessively successful establishment. Conversely, in the Greek waters of the NE Aegean Sea, the population of *P. crassirostris* reached an annual mean of 9906 ind.m⁻³ and contributed to total zooplankton by up to 14.3% (Papantoniou & Fragopoulou in Eleftheriou *et al.*, 2012). Furthermore, in the native tropical Pacific coastal environments, *P. crassirostris* is usually among the dominant species (Calbet *et al.*, 2000;

Hwang *et al.*, 2006). The absence of males in the samples from Šibenik Bay is presumably conditioned by the heavily biased sex ratio (Alajmi & Zeng, 2015).

Both field data and laboratory cultures indicate that *P. crassirostris* thrives in environments that are rich in nanophytoplankton (Calbet *et al.*, 2000; McKinnon *et al.*, 2003). In addition, SEM analysis of *P. crassirostris* faecal pellet content showed that larger diatoms (*Chaetoceros* sp., *Thalassiotrix frauenfeldii*, *Skeletonema costatum* and *Thalassionema nitzschioides*) and protozoan *Sticholonche zancelea* considerably contribute to diet diversity, providing an important source of energy and micronutrients (Eskinazi-Sant'Anna, 2013). In December 2014, the plankton community in the port of Šibenik represented a favourable trophic environment for *P. crassirostris*, being abundant in nanophytoplankton and *Sticholonche zancelea*. Although the current assessment of the invasive potential of *P. crassirostris* indicates a low environmental impact risk (Katsanevakis *et al.*, 2012), it is important to monitor further spreading and potential influence on native paracalanid populations in Šibenik Bay. Laboratory experiments on the biology and reproduction of *P. crassirostris* have demonstrated its ability to attain very high culture densities under optimal conditions (Alajmi & Zheng, 2015) that, in combination with the resilience to physical fluctuations of the environment can significantly contribute to its invasive potential in enclosed coastal locations.

Acknowledgements

We thank the crew and technicians of r/v “BIOS DVA” for their assistance during fieldwork. All the comments and suggestions of the reviewers are greatly appreciated. We are also thankful to Michelle J. Jungbluth (University of Hawaii) for valuable advice concerning the identification of *P. crassirostris*. This study was supported with the financial assistance of the IPA Adriatic Cross-border Cooperation Programme 2007-2013, within the framework of the strategic project “Ballast waters management system for Adriatic Sea protection” (BALMAS). The contents of this publication are the sole responsibility of the authors and can under no circumstances be regarded as reflecting the position of the IPA Adriatic Cross-Border Cooperation Programme Authorities. Additional support was provided by the Croatian Science Foundation as part of research projects IP-2014-09-3606 “Marine plankton as a tool for assessment of climate and anthropogenic influence on the marine ecosystem” (MARIPLAN) and IP-2014-09-4143 “Marine microbial food web processes in global warming perspective” (MICROGLOB).

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