STUDY ON RECENT TRENDS IN NUTRIENTS LEVELS IN THE UPPER ADRIATRIC SEA AND HOW TRENDS ARE LINKED TO THE SEA PRIMARY PRODUCTION

Final Version of December 2022

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Final Report

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FOREWORD

This study was carried out in the Marano and Grado Lagoon, one of most important wetland areas of the Mediterranean.

The Marano and Grado Lagoon (Fig. 1), bounded on the west by the river Tagliamento and on the east by the river Isonzo, extends parallel to the northernmost coast of the Adriatic Sea for a length of about 32 km, with an average distance between the coastline and the islands of the coastline of about 5 km, which corresponds to a total area of about 160 km^2.

Fig. 1 Map of the Marano and Grado Lagoon (courtesy of ARPA FVG).

In the framework of the Water Framework Directive (WFD/2000/60/CE), the Marano and Grado Lagoon was divided into water bodies. For classification, some specific descriptors such as geographic location, geomorphology, tides and surface salinity were analyzed. Previous studies (Bettoso et al. 2010) conducted in the lagoon indicated the presence of three types (mesohaline, polyhaline, euhaline) and 13 river mouths. The final types were assessed by applying the DPSIR model, taking into account the main pressures (e.g., nutrients and organic matter enrichments, presence of priority substances, aquaculture activities). On this basis, 17 water bodies were identified (4 heavily modified) (Fig. 2). The abbreviations TEU, TPO and TME refer to the aline classification of the water bodies. In particular, the so-called eualine water

bodies belong to the TEU type, where salinity is usually between 30 and 40. In the TPO or polyaline water bodies, the salinity is between 20 and 30, while the mesoaline or TME type includes water bodies with values between 5 and 20 (Bettoso et al. 2010). The water bodies marked with the abbreviation FM are those classified as highly modified, due to the presence of fishing valleys or the bridge connecting Grado and Aquileia, which severely restricts the hydraulic regime of the water bodies located east of this artificial barrier.

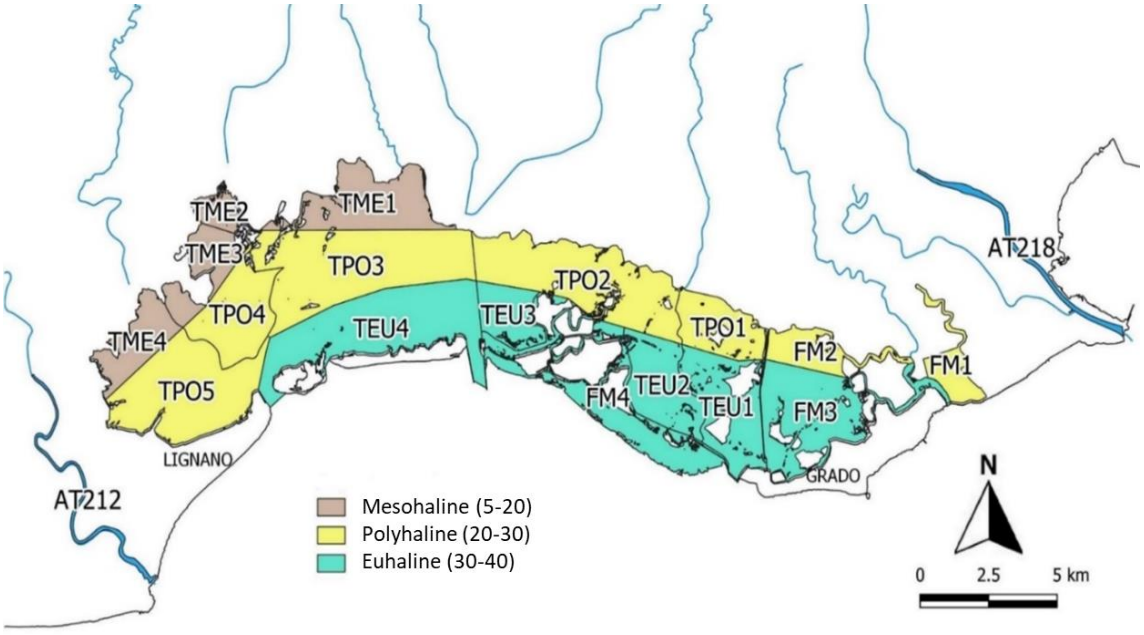


Fig. 2 Classification of the Marano and Grado Lagoon in water bodies (courtesy of ARPA FVG).

The Marano and Grado Lagoon has been designated as a site of the Natura 2000 network, i.e. the network of sites of the European Union that have priority due to their naturalistic value and the protection of biodiversity itself. According to the Habitats Directive 92/43/EEC, this lagoon is a Special Area of Conservation (SAC - IT3320037) for the protection of habitats and important species of flora and fauna at European level, and according to the Birds Directive 2009/147 / EC, it is a Special Protection Area (SPA - IT3320037) for the protection of wild bird species and their habitats. It also includes two Regional nature reserves established under Regional Law No. 42/96: the Valley of the Canal Novo (121 hectares) and the Foci dello Stella (1,377 hectares). Due to the close interaction between natural processes and human activities, this lagoon is an example of conflict between conservation needs and human uses, as this basin also plays an important role for fishery, fish and shellfish farming.

Moreover, since 2016 the non-indigenous species *Mnemiopsis leidyi* - considered one of the 100 most dangerous aquatic invasive species due to the significant negative impacts it can have on ecosystem functioning and on fishery- makes summer blooms in the Marano and Grado Lagoon (Malej et al. 2017).
Since its first massive appearance in the Marano and Grado Lagoon (summer 2016), *Mnemiopsis* was immediately reported by fishermen as a major disturbance factor for the small scale fishery carried out by fyke nets (*cogolli*). Indeed, when present in large numbers, ctenophores can clog the meshes of fyke nets: the ctenophores, driven by the current, can pile up near the opening of fyke net or inside the net, causing the gear to become clogged - with the consequence that it is no longer possible to catch fish species of commercial interest - and the gear becomes so heavy that it cannot be recovered on board. Although the fyke net is vigorously shaken in the water to clear it of ctenophores, the large mass of these organisms present in nets still causes significant additional physical effort for the operator and often causes the structural components of the fyke nets to break when the gears are retrieved.
Observations in Marano and Grado Lagoon in 2018 and 2019 (NOCE di MARE project) estimated that in July, when *Mnemiopsis* abundance was not yet maximal, the weight of ctenophores found in a fyke net could be 5-7 times the amount caught. It was also noted that the time required to separate the catch from the gelatinous mass significantly slowed down the fishing activities. In 2018-2020, many of the lagoon fishermen using gillnets were forced to stop their activities during the peak presence of *Mnemiopsis leidyi*. Similar observations were also made in 2020 in the Venice lagoon (Piccardi, 2020), where in a sample of 45 fyke nets, on average between 22 and 69% of the nets surveyed were occupied by ctenophores, and 70% of the fishermen interviewed reported that the decrease in their income (estimated by them to be around 75-100%) was caused by the massive presence of sea nuts. Comparable difficulties were also highlighted by fishermen in Sardinian lagoons (Diciotti et al., 2016) and in Berre Lagoon in France, where fishermen suffered annual losses of 50% of their income (Marchessaux, 2020).
In addition to mechanically clogging fishing gear, *Mnemiopsis* can cause great harm to the fishing industry by heavily predating zooplankton, which as a direct result causes a decline in food for many planktivorous fish species, such as *Atherina boyeri*.

Since 2010, the *Agenzia Regionale per la protezione dell'ambiente della Regione Autonoma Friuli Venezia Giulia* (hereafter ARPA FVG) carried out monthly (from 2010 to 2015) and seasonally (2016 –today) monitoring of some environmental and biological variables that are important to assess the trophic status of the lagoon

(temperature, salinity, concentration of nitrates, silicates and phosphates, phytoplankton). Results obtained from these studies have been presented at the ARGOS -Scientific Conference on Fishery - “Status and Perspectives of the Fishery Sector in the Adriatic Sea” (26th May 2022, AQUAFARM -Pordenone, Italy) and will be hereafter summarized.

Physical and biogeochemical parameters showed an extreme heterogeneity in terms of spatial and seasonal distribution. The occidental side of the lagoon (Marano basin) receives the major river inputs and as consequence, it is characterized by higher level of nutrients and lower salinities (Fig. 3). Phytoplankton communities were mainly dominated by nanoflagellates (Fig. 4 C) in almost all monitored sites. Any significant trend was observed nor for nutrients than for phytoplankton (Fig. 4 A, B, D) but nitrate inputs still represent a concern that deserves attention.

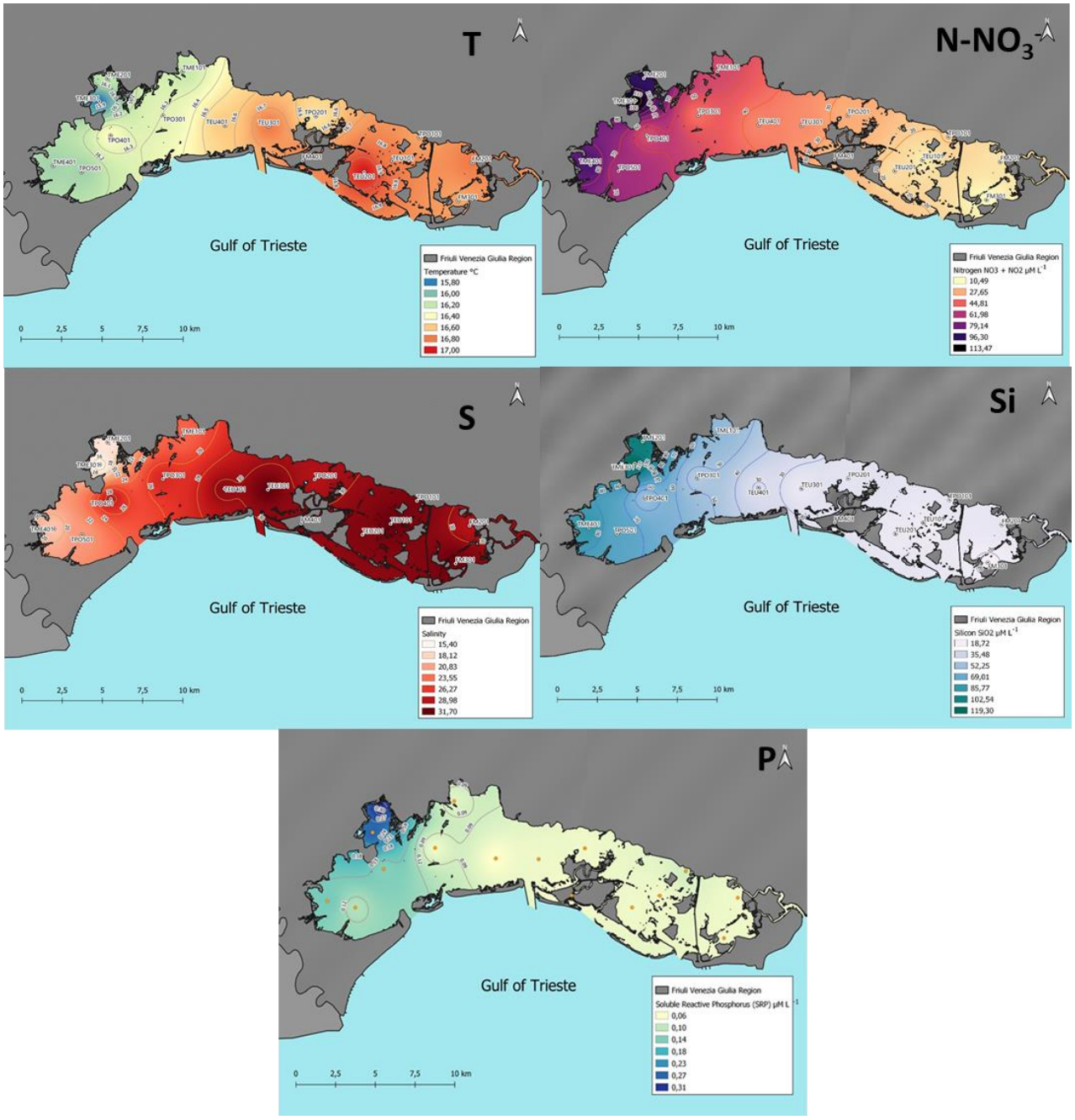


Fig. 3 Average distribution of physical parameters (T: temperature; S: salinity) and nutrients (N-NO_3^-: nitrates; Si: silicates; P: phosphates). Data presented by A. Acquavita (ARPA FVG) at the ARGOS - Scientific Conference on Fishery - "Status and Perspectives of the Fishery Sector in the Adriatic Sea" (26th May 2022, AQUAFARM - Pordenone, Italy) (courtesy of ARPA FVG).

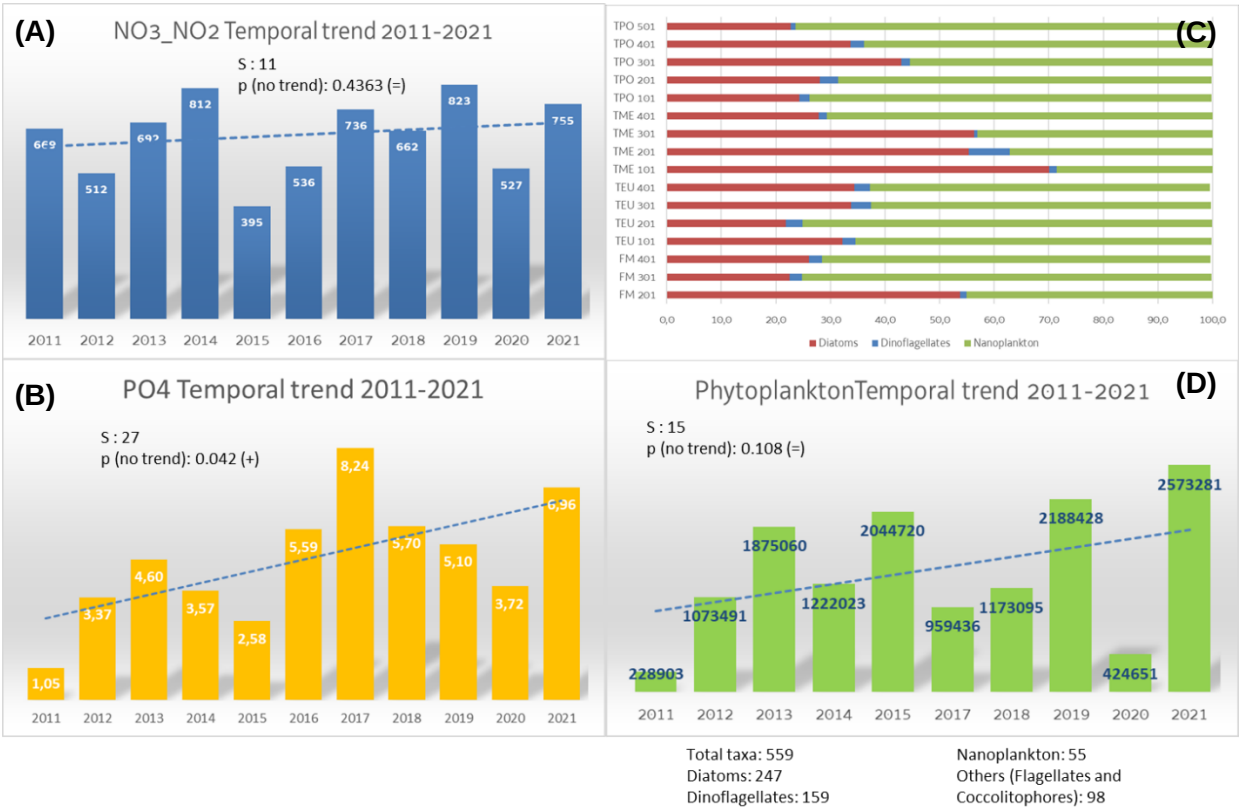


Fig. 4 Data presented by A. Acquavita (ARPA FVG) at the ARGOS -Scientific Conference on Fishery – "Status and Perspectives of the Fishery Sector in the Adriatic Sea" (26th May 2022, AQUAFARM - Pordenone, Italy) on temporal trends from 2011-2021: (A) nitrate concentration, (B) phosphate concentration, (C) phytoplankton composition, (D) abundance of phytoplankton (courtesy of ARPA FVG).

The Water Framework Directive 2000/60/EC (WFD) and its implementing decree, third part of Legislative Decree 152/2006, in order to define the ecological status of surface water bodies, require its classification on the basis of four Biological Quality Elements (BQEs): phytoplankton, macrophytes, benthic macroinvertebrates and fish fauna (trophic role of some BQEs is presented in Figure 5). Despite zooplankton plays a relevant ecological role, it is currently not included among the BQEs required by the WFD. Therefore, there is currently no institutional monitoring that tracks the qualitative-quantitative evolution of zooplankton in transitional waters, as it is done in marine waters for the Marine Strategy activities (Framework Directive 2008/56/EC).

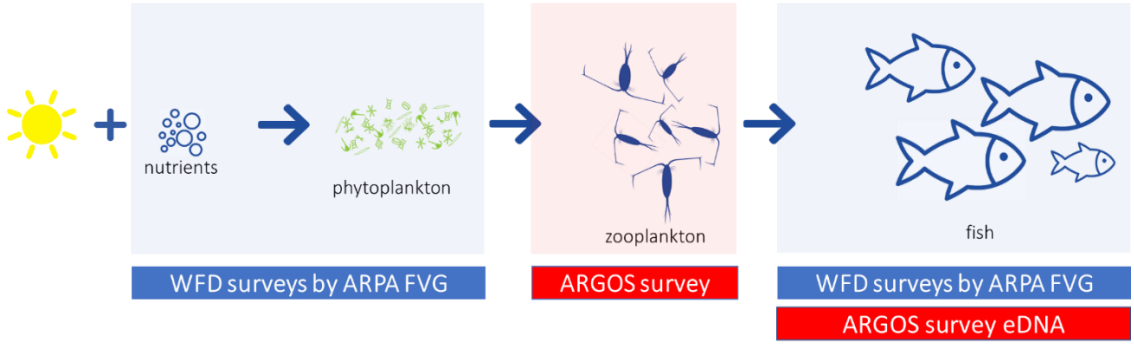


Fig. 5 Diagram of trophic chain and related monitoring activities. WFD: Water Framework Directive; ARPA FVG: Agenzia Regionale per la protezione dell'ambiente della Regione Autonoma Friuli Venezia Giulia; ARGOS survey: present study.

Zooplankton play a fundamental ecological role transferring energy from primary producers to higher trophic levels. However, the trophic habits of zooplankton are far from uniform: although herbivores often dominate, many zooplankters are first- and second-order carnivores (i.e., their diet consists of both herbivores and other carnivores), while others are detritivores and omnivores.

The term zooplankton refers to a large number of organisms belonging to numerous zoological groups and represented, if not by adult individuals, at least by their larval stages. In fact, we can distinguish between organisms that complete their entire life cycle in the plankton (holoplankton) and those that spend only a short time there (meroplankton). The meroplankton, or temporary plankton, consists mainly of eggs and larvae of adult animals belonging to the nekton or benthos. The composition of this plankton, which is particularly abundant in shallow waters, is related to the reproductive period of the species it represents.

Few studies focused on zooplankton in Italian lagoons, although it has been recognized that the study of mesozooplankton (i.e., zooplankton with a size between 0.2 and 2 mm) can provide important information on the trophic state of these transitional areas (Acri et al. 2004; Bianchi et al. 2003). The Marano and Grado Lagoon is not an exception, although it represents one of the largest and most characteristic natural areas of the Autonomous Region of Friuli Venezia Giulia (Italy). Therefore, ARGOS's study focused in filling this important gap (Fig. 5).

Monitoring fish fauna is an essential but challenging activity, particularly in large areas like the Marano and Grado Lagoon. To overcome this difficulty, ARGOS tested for the first time the application of environmental DNA analysis (eDNA) to assess lagoon biodiversity (Fig. 5). eDNA is the DNA released by an organism into the environment

(water, sediment, soil) in the form of skin cells, faeces, urine, hair, mucus, excretions, saliva, blood, and gametes and left behind after death. eDNA can remain for up to several weeks in water and up to years or decades in soil and sediment, from which it can be collected and analyzed. This technique allows detection of cryptic, rare, and endangered species, early detection of alien and invasive species, and assessment of overall ecosystem health. ARGOS, for the first time, applied this approach to monitor fish biodiversity in the Marano and Grado Lagoon. Moreover, a species-specific (*M. leidyi*) and more general (metazoans) manner were applied to invertebrate fauna.

ZOOPLANKTON COMMUNITY

Materials and Methods

Sampling

From May to November 2021, zooplankton samples were collected monthly in the Marano and Grado Lagoon at stations monitored for nutrients and phytoplankton by the Regional Agency for Environmental Protection of Friuli Venezia Giulia (ARPA FVG) as part of the Water Framework Directive surveys (WFD/2000/60/ EC). Zooplankton was collected at 6 stations, each located in a different water body of the Marano and Grado Lagoon and identified with the following abbreviations: TME4, TME3, TPO5, TPO4, TEU3, TPO2. The location of the sampled stations is shown in Figure 6 and Table 1.

Fig. 6 Marano and Grado Lagoon: location of the stations where the zooplankton was sampled.

Tab. 1 Geographical coordinates of the zooplankton sampling stations.

Station	Latitude	Longitude
TME401	45° 42.768' N	13° 5.537' E
TPO501	45° 42.211' N	13° 6.484' E
TME301	45° 44.567' N	13° 7.508' E

TPO401	45° 43.988' N	13° 13.141' E
TEU 301	45° 43.929' N	13° 15.093' E
TPO204	45° 43.513' N	13° 23.188' E

Sampling was performed with a Bongo net equipped with a 330-micron net and floats were attached to support the net collectors (Fig. 7). This allowed the net to be towed horizontally without dragging the bottom in shallower sites. The net was towed at low speed (<1 m/second) for about 5 minutes, allowing an average of about 6000 liters of water to be sampled by each tow. Immediately after returning to the laboratory, samples were fixed in 96% alcohol and stored in the refrigerator until analysis under the microscope. Dates of sampling are reported in Table 2.

Fig. 7 Bongo net used for zooplankton sampling.

Tab. 2 Dates of zooplankton sampling.

25 May 2021
21 June 2021
21 and 26 July 2021
25 August 2021
28 September 2021
26 October 2021
12 November 2021

Sampling took place from morning (around 9 A.M.) to early afternoon (1 P.M.) to standardize as much as possible sample collection and allow the time for laboratory treatment of the samples.
Water temperature and salinity were measured at the surface (first 50 cm) at each station using a Hydrolab MS5 probe and presented in Table 3.

Tab. 3 Physical sampling data in the Marano and Grado Lagoon.T: temperature; S: salinity.

Station	Date	Depth [m]	T [°C]	S	Tide
TEU 301	25/05/2021	3	16,92	19,75	flood

Station	Date	Depth [m]	T [°C]	S	Tide
TME 301	25/05/2021	1,2	16,02	3,16	flood
TME 401	25/05/2021	1,2	16,82	12,1	flood
TP0 401	25/05/2021	2	16,52	10,41	flood
TP0 501	25/05/2021	1,4	15,99	10,06	flood
TPO 204	25/05/2021	NR	17,5	22,79	flood
TEU 301	21/06/2021	2	24,7	20,79	ebb
TME 301	21/06/2021	1,3	24,5	7,76	ebb
TME 401	21/06/2021	1	27,03	15,85	ebb
TP0 401	21/06/2021	1,3	27,36	18,94	ebb
TP0 501	21/06/2021	1,2	27,2	21,63	ebb
TPO 204	21/06/2021	1	28,51	30,06	ebb
TEU 301	26/7/2021	3	26,67	30,05	flood
TME 301	21/7/2021	1,5	27,14	22,41	flood
TME 401	21/7/2021	1,1	26,30	18,68	flood
TP0 401	21/7/2021	1,4	25,84	20,74	flood
TP0 501	21/7/2021	1,1	27,29	25,81	flood
TPO 204	26/7/2021	0,9	27,71	31,6	flood
TEU 301	25/08/2021	4,4	23,84	35,16	flood
TME 301	25/08/2021	1	21,73	20,89	flood
TME 401	25/08/2021	1,1	22,06	20,84	flood
TP0 401	25/08/2021	1,3	22,65	27,39	flood
TP0 501	25/08/2021	1,2	21,92	22,07	flood
TPO 204	25/08/2021	1,3	23,69	34,55	no tidal change
TEU 301	28/09/2021	3,6	22,08	31,81	flood
TME 301	28/09/2021	1,1	19,65	6,06	flood
TME 401	28/09/2021	1	19,78	6,32	flood
TP0 401	28/09/2021	1,2	20,39	12,29	flood
TP0 501	28/09/2021	1,1	20,1	13,43	flood
TPO 204	28/09/2021	1,2	22,84	31,52	flood
TEU 301	26/10/2021	3,2	15,53	36,42	flood
TME 301	26/10/2021	1,4	11,28	8,18	flood
TME 401	26/10/2021	1,3	11,45	14,45	flood
TP0 401	26/10/2021	1,4	12,79	25,73	flood
TP0 501	26/10/2021	1,4	12,05	20,43	flood
TPO 204	26/10/2021	1,4	14,05	35,06	ebb
TEU 301	12/11/2021	3,4	13,24	32,2	ebb
TME 301	12/11/2021	1,1	11,39	8,67	ebb

Station	Date	Depth [m]	T [°C]	S	Tide
TME 401	12/11/2021	1,1	11,04	8,71	ebb
TP0 401	12/11/2021	1,2	12,54	17,3	ebb
TP0 501	12/11/2021	1,2	10,09	11,47	ebb
TPO 204	12/11/2021	0,9	12,64	27,86	ebb

Qualitative and quantitative analysis

Qualitative and quantitative analysis were performed on aliquots obtained by splitting the original fixed sample or on the whole sample, until enumerating and identifing at least 1000 individuals. The analysis was performed using two stereomicroscopes: Leica 165C (120x) and Leica 205 C (160x). To calculate abundance, expressed as the total number of individuals present in one cubic meter of water (n ind. m^{-3}), the individuals contained in the fraction were related to the total sample and then divided by the number of cubic meters filtered for each tow. Rare species (not found in the counted aliquot) were identified in the rest of the samples and their abundance was arbitrarily assigned as 1 and then divided for the filtered volume. The filtered water volume was calculated using a flow meter (HYDRO-BIOS) placed at the mouth of the net and calculated as follows:

filtered volume (m^3) = number of revolutions x area of the mouth of the net (m^2) x k

where k (k = 0.3) is a constant characteristic of the type of flowmeter used.

Identification was made at species level or at the lowest possible taxonomical level, using the following texts: Avancini et al. (2006), Boltovskoy (1999), Castellani and Edwards (2017), Nishida (1985), Razouls et al. (2016), Rose (1933), Tregouboff and Rose (1957). The nomenclature of the identified taxa was prepared in accordance with the World Register of Marine Species (WoRMS).

Selected specimens Sanger sequencing

A molecular approach (Sanger sequencing) was performed for selected specimens (such as fish, crab, and bivalve larvae) whose identification was not possible using the traditional morphological approach (microscopic observation).

DNA was extracted with EZNA® Mollusc DNA Kit (Omega Biotek) following manufactures' instructions and quantified by a Qubit Fluorometer (Thermo Fisher Scientific).
For the DNA barcoding, mitochondrial cytochrome c oxidase subunit I (COI) was amplified using LCO1490 forward (5′- GGTCAACAAATCATAAAGATATTGG -3′) and HCO2198 reverse (5'- TAAACTTCAGGGTGACCAAAAAATCA -3') primers (Leray et al. 2013). PCR amplifications were performed in a total volume of 50 µl with 0.5 µM of each primer, 1 U of HiProof HF Master Mix (Bio-Rad), and 5 µl of DNA. The thermal cycling profiles started with 98 °C for 30 s, followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s, with a final extension at 72 °C for 7 min.
PCR products were checked on a 1.5% agarose gel and purified with QIAquick PCR Purification Kit (Qiagen). Sanger sequencing of PCR products was performed with an ABI 3730xl DNA Analyzer (Thermo Fisher Scientific) at BMR Genomics S.r.l., Padua, Italy (www.bmr-genomics.it).

Trophic group assignment

The trophic group describes the primary food source of a species and provides information about its role in food web (Pomerleau et al. 2015). In this study, the assignment of identified taxa to a trophic group was based on information from previous studies and online sources (supplementary material of Benedetti et al. 2015, Ge et al. 2022 and citations therein, http:// www.eol.org; http://copepodes.obs-banyuls.fr/en). On the basis of trophic regime, taxa were classified into 7 trophic groups: carnivore, carnivore- detritivore, herbivore, omnivore, omnivore- carnivore, omnivore- detritivore, omnivore- herbivore. **Herbivores** refers primarily to herbivorous species; **omnivore-herbivores** refer primarily to herbivorous species that occasionally feed on other small organisms or occasionally organic detritus; **Carnivores** are predatory zooplankton that feed on small zooplankton, eggs, and larvae; **Carnivore-detritivores** refers to organisms that prey on zooplankton and also feed on organic detritus; **omnivore-carnivores** refer primarily to carnivorous species that sometimes eat phytoplankton and organic detritus; **Omnivore-detritivores** refers to species that feed primarily on organic detritus and sometimes phytoplankton; **Omnivores** groups species with a broad dietary regime and species whose food source was not clear. This latter group was added to place taxa of unknown trophic regime.

Results

From May to November 2021, 42 zooplankton samples were collected and analysed: 72 taxa were identified, 30 of which at species level. In terms of abundance (Fig. 8), decapod larvae (especially Brachyura (crab larvae)) were the predominant taxon (46%), followed by copepods belonging to the non-native species *Acartia (Acathacartia) tonsa* (20%), juvenile copepods (copepodites) of the genus *Acartia* (9%) and cladocerans of the species *Penilia avirostris* (7%), while only 5% of the collected organisms belonged to the copepod species *A. (Acartiura) clausi*. Both harpacticoids of the family Metidae and individuals of the invasive ctenophore *Mnemiopsis leidyi* contributed for 2% of the analyzed community, respectively. The remaining taxa (e.g., echinoderm larvae, amphipods, copepod *Centropages ponticus*, other harpacticoid copepods, cladoceran *Pleopis polyphemoides*, etc.) were detected only in small amount (< 2%). The complete list of taxa is presented in Table 4 while the ten main taxa found in each station are listed from Table 5 to 11.

A total of 6 non-indigenous species (NIS) were identified in zooplankton samples using traditional taxonomic or molecular techniques: the copepods *A. tonsa*, *Pseudodiaptomus marinus* and *O. davisae*, the ctenophore *M. leidyi* and the crab species *Rhithropanopeus harrisii* and *Dyspanopeus sayi*. All copepods were first records for the study area (Fig. 9).

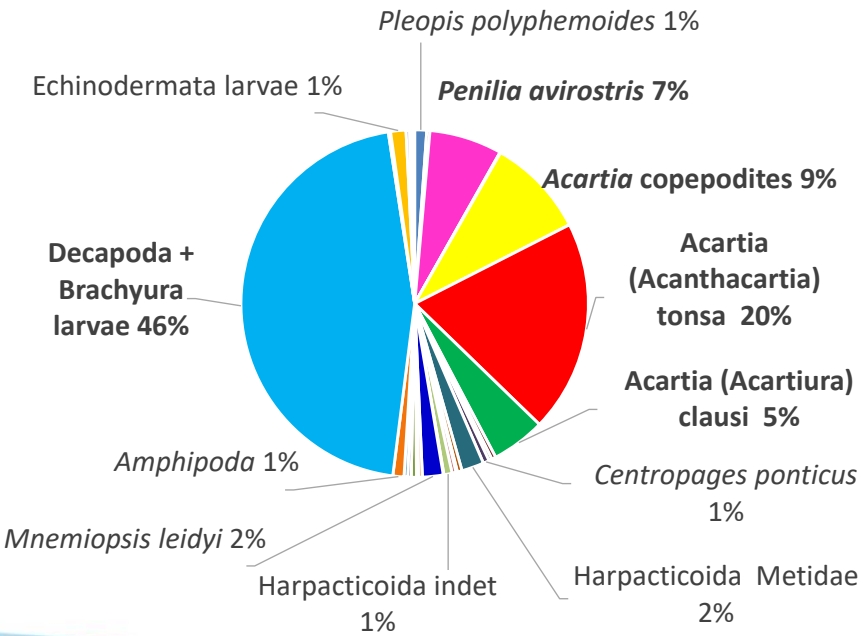


Fig. 8 Relative abundance of identified taxa in the Marano and Grado Lagoon from May to November 2021.

Tab. 4 List of taxa identified in the samples collected in the Marano and Grado Lagoon (May-November 2021).

CNIDARIA	HYDROZOA	Hydrozoa
		Anthoathecata
		Siphonophorae
	HYDROZOA - Leptothecata	*Obelia* spp.
	SCYPHOZOA	ephyra larvae
CTENOPHORA		cyddipid larvae
		Mnemiopsis leidyi
PLATYHELMINTHES		Müller larvae
PHORONIDA		actinotrocha larvae
MOLLUSCA		Bivalvia larvae
		Gastropoda larvae
ANNELIDA		Polychaeta
		Polychaeta *Lanice* larvae
		Polychaeta larvae
		Polychaeta *Magelona* larvae
CRUSTACEA	OSTRACODA	Ostracoda
	CLADOCERA	*Evadne nordmanni*
		Evadne spinifera
		Penilia avirostris
		Pleopis polyphemoides
		Podon intermedius
		Podonidae
		Pseudevadne tergestina
	COPEPODA - Calanoida	Calanoida
		Calanoida copepodites
		Acartia copepodites
		Acartia (Acanthacartia) tonsa
		Acartia (Acartiura) clausi
		Acartia (Acartiura) margalefi
		Calanipeda aquaedulcis
		Calanipeda aquaedulcis copepodites
		Calanus helgolandicus copepodites
		Centropages copepodites
		Centropages ponticus
		Centropages typicus
		Clausocalanus copepodites

	Clausocalanus spp.
	Labidocera brunescens
	Labidocera wollastoni
	Paracalanus copepodites
	Paracalanus parvus s.l.
	Paracartia latisetosa
	Paracartia latisetosa copepodites
	Pontellidae
	Pseudodiaptomus marinus
	Pseudodiaptomus marinus copepodites
	Temora longicornis
	Temora longicornis copepodites
	Temora stylifera
	Temora stylifera copepodites
COPEPODA - Cyclopoida	Corycaeidae
	Cyclopoida (excluding genus *Oithona*)
	Ditrichocorycaeus brehmi
	Oithona copepodites
	Oithona davisae
	Oithona nana
	Oithona plumifera
	Oithona similis
	Oithona spp.
	Oncaea copepodites
	Oncaea curta
	Onychocorycaeus giesbrechti
COPEPODA - Harpacticoida	Harpacticoida
	Metidae
	Microsetella norvegica
	Euterpina acutifrons
COPEPODA - Monstrilloida	Monstrilloida
COPEPODA - Siphonostomatoida	Siphonostomatoida
COPEPODA	Copepoda nauplius
CIRRIPEDIA	Cirripedia cypris
	Cirripedia nauplius
ISOPODA	*Idotea* spp.
	Isopoda

		Munnidae
	AMPHIPODA	Amphipoda
		Caprellidae
		Gammarida
	DECAPODA	Brachyura larvae
		Decapoda larvae
	MYSIDA	Mysida
CHELICERATA		Acari
		Pycnogonida
CHAETOGNATHA	Sagittidae	*Sagitta* spp.
	Spadellidae	*Spadella* spp.
ECHINODERMATA	Echinoidea	Echinoidea plutei
	Holoturoidea	Holoturoidea larve auricolaria
	Ophiuroidea	Ophiuroidea
		Ophiuroidea plutei
CHORDATA	Appendicularia	*Oikopleura* spp.
	Asciadiacea	Asciadiacea larvae
VERTEBRATA	Teleostei	*Engraulis encrasicolus* eggs
		Teleostei eggs
		Teleostei larvae

Tab. 5 Top ten taxa observed in samples collected in May 2021 in the Marano and Grado Lagoon.

TME 401	%
Decapoda Brachyura larvae	30,45
Acartia copepodites	26,28
Acartia (Acartiura) clausi	18,27
Acartia (Acanthacartia) tonsa	10,26
Gastropoda larvae	3,21
Acartia (Acartiura) margalefi	2,56
Decapoda larvae	1,92
Cirripedia nauplii	1,60
Paracalanus copepodites	0,96
Centropages copepodites	0,64
Centropages ponticus	0,64
Harpacticoida	0,64
Teleostei larvae	0,64

TME 301	%
Decapoda Brachyura larvae	69,70
Acartia (Acanthacartia) tonsa	10,00
Acartia (Acartiura) clausi	8,48
Acartia copepodites	5,45
Teleostei larvae	2,12
Decapoda larvae	1,82
Centropages copepodites	0,61
Amphipoda Gammarida	0,61
Penilia avirostris	0,30
Acartia (Acartiura) margalefi	0,30
Calanipeda aquaedulcis	0,30
Teleostei eggs	0,30

TPO 501	%
Acartia (Acartiura) clausi	55,08
Acartia copepodites	19,88
Decapoda Brachyura larvae	11,23
Acartia (Acanthacartia) tonsa	5,16
Decapoda larvae	3,19
Paracalanus copepodites	0,91
Centropages ponticus	0,76
Penilia avirostris	0,61
Teleostei larvae	0,61
Centropages copepodites	0,46
Oikopleura spp.	0,46

TPO 401	%
Decapoda Brachyura larvae	64,09
Acartia (Acartiura) clausi	17,68
Acartia (Acanthacartia) tonsa	6,63
Acartia copepodites	3,87
Decapoda larvae	2,76
Amphipoda Gammarida	1,10
Teleostei larvae	1,10
Acartia (Acartiura) margalefi	0,55
Euterpina acutifrons	0,55
Paracalanus parvus s.l.	0,55
Cirripedia nauplii	0,55
Gastropoda larvae	0,55

TPO 204	%
Acartia copepodites	43,64
Acartia (Acartiura) clausi	39,90
Decapoda Brachyura larvae	4,34
Decapoda larvae	2,93
Gastropoda larvae	1,52
Pleopis polyphemoides	1,11
Amphipoda Gammarida	1,01
Harpacticoida	0,71
Paracalanus copepodites	0,61
Centropages ponticus	0,51
Cirripedia cypris	0,51

TEU 301	%
Acartia (Acartiura) clausi	47,38
Acartia copepodites	22,64
Decapoda Brachyura larvae	11,16
Centropages ponticus	5,15
Decapoda larvae	3,98
Penilia avirostris	2,26
Centropages copepodites	1,72
Gastropoda larvae	1,25
Pleopis polyphemoides	0,94
Amphipoda Gammarida	0,78

Tab. 6 Top ten taxa observed in samples collected in June 2021 in the Marano and Grado Lagoon.

TME401	%
Decapoda Brachyura larvae	63,38
Acartia (Acanthacartia) tonsa	25,70
Amphipoda Gammarida	7,55
Acartia copepodites	1,06
Ostracoda	0,50
Engraulis encrasicolus eggs	0,44
Decapoda larvae	0,37
Mysida	0,31
Harpacticoida	0,19
Acari	0,12
Isopoda *Idotea* spp.	0,12

TME301	%
Decapoda Brachyura larvae	99,89
Acartia (Acanthacartia) tonsa	0,02
Decapoda larvae	0,02
Isopoda *Idotea* spp.	0,02
Amphipoda Gammarida	0,02
Ostracoda	0,008
Cyclopoida (excluding genus *Oithona*)	0,004
Harpacticoida	0,004
Ophiuroidea plutei	0,004
Engraulis encrasicolus eggs	0,004

TPO501	%
Decapoda Brachyura larvae	99,28
Acartia (Acanthacartia) tonsa	0,26
Engraulis encrasicolus eggs	0,15
Decapoda larvae	0,13
Amphipoda Gammarida	0,09
Acartia copepodites	0,04
Centropages copepodites	0,02
Gastropoda larvae	0,02

TPO401	%
Decapoda Brachyura larvae	51,16
Acartia (Acanthacartia) tonsa	40,02
Acartia copepodites	5,66
Amphipoda Gammarida	0,93
Teleostei larvae	0,37
Engraulis encrasicolus eggs	0,37
Pleopis polyphemoides	0,28
Decapoda larvae	0,19
Ostracoda	0,19
Calanipeda aquaedulcis	0,09
Centropages copepodites	0,09
Centropages ponticus	0,09
Harpacticoida	0,09
Paracalanus copepodites	0,09
Siphonostomatoida	0,09
Acari	0,09
Hydrozoa	0,09
Isopoda *Idotea* spp.	0,09

TPO204	%
Amphipoda Gammarida	23,64
Ostracoda	20,77
Decapoda Brachyura larvae	8,95
Acartia copepodites	6,39
Harpacticoida	6,39
Decapoda larvae	4,79
Gastropoda larvae	4,79
Acartia (Acanthacartia) tonsa	3,83
Mysida	3,83
Spadella spp.	3,19

TEU301	%
Decapoda Brachyura larvae	57,75
Ophiuroidea plutei	7,49
Amphipoda Gammarida	6,15
Ostracoda	5,08
Acartia (Acanthacartia) tonsa	4,55
Penilia avirostris	3,48
Pleopis polyphemoides	2,14
Decapoda larvae	2,14
Acartia copepodites	1,60
Harpacticoida	1,60

Tab. 7 Top ten taxa observed in samples collected in July 2021 in the Marano and Grado Lagoon.

TME401	%
Acartia (Acanthacartia) tonsa	30,28
Acartia copepodites	11,04
Decapoda Brachyura larvae	11,04
Decapoda larvae	9,78
Ctenophora cyddipid larvae	6,31
Ostracoda	5,36
Calanipeda aquaedulcis	3,15
Acartia (Acartiura) clausi	3,15
Amphipoda Gammarida	2,52
Centropages ponticus	2,21
Harpacticoida	2,21

TME301	%
Acartia (Acanthacartia) tonsa	43,88
Acartia copepodites	27,66
Harpacticoida Metidae	9,04
Decapoda Brachyura larvae	6,38
Decapoda larvae	2,39
Harpacticoida	2,13
Acartia (Acartiura) clausi	1,33
Cirripedia nauplii	1,33
Ostracoda	0,80
Pseudevadne tergestina	0,53
Centropages ponticus	0,53
Paracartia latisetosa	0,53
Ctenophora cyddipid larvae	0,53
Mnemiopsis leidyi	0,53
Amphipoda	0,53

TPO501	%
Decapoda larvae	40,45
Acartia (Acartiura) clausi	14,61
Ophiuroidea plutei	12,36
Decapoda Brachyura larvae	6,74
Teleostei eggs	5,62
Pseudevadne tergestina	4,49
Acartia copepodites	3,37
Centropages copepodites	2,25
Pseudodiaptomus marinus	2,25
Acartia (Acanthacartia) tonsa	2,25
Amphipoda Gammarida	2,25

TPO401	%
Decapoda Brachyura larvae	27,91
Acartia (Acanthacartia) tonsa	25,58
Acartia copepodites	11,63
Calanipeda aquaedulcis	4,65
Harpacticoida Metidae	4,65
Centropages ponticus	2,33
Calanipeda aquaedulcis copepodites	2,33
Acartia (Acartiura) clausi	2,33
Paracartia latisetosa	2,33
Paracartia latisetosa copepodites	2,33
Mnemiopsis leidyi	2,33
Gastropoda larvae	2,33
Ostracoda	2,33
Amphipoda Gammarida	2,33
Decapoda larvae	2,33
Teleostei larvae	2,33

TPO204	%
Harpacticoida	34,44
Centropages copepodites	7,78
Pseudevadne tergestina	6,67
Decapoda larvae	5,56
Calanoida copepodites	4,44
Acartia (Acanthacartia) tonsa	4,44
Decapoda Brachyura larvae	4,44
Spadella spp.	4,44
Centropages ponticus	3,33
Cyclopoida (excluding genus *Oithona*)	3,33
Harpacticoida Metidae	3,33

TEU301	%
Decapoda larvae	50,00
Acartia (Acanthacartia) tonsa	12,50
Paracartia latisetosa	12,50
Anthoathecata	12,50
Isopoda *Idotea* spp.	12,50

Tab. 8 Top ten taxa observed in samples collected in August 2021 in the Marano and Grado Lagoon.

TME 401	%
Acartia (Acanthacartia) tonsa	54,92
Acartia copepodites	18,31
Harpacticoida Metidae	7,94
Penilia avirostris	3,70
Centropages ponticus	2,22
Acartia (Acartiura) clausi	1,90
Decapoda larvae	1,27
Calanipeda aquaedulcis copepodites	1,16
Mnemiopsis leidyi	1,06
Cirripedia nauplii	0,95

TME 301	%
Acartia (Acanthacartia) tonsa	67,83
Acartia copepodites	18,01
Harpacticoida Metidae	7,19
Calanipeda aquaedulcis copepodites	0,96
Centropages ponticus	0,82
Penilia avirostris	0,74
Centropages copepodites	0,67
Calanipeda aquaedulcis	0,59
Harpacticoida	0,52
Decapoda larvae	0,52

TPO 501	%
Acartia (Acanthacartia) tonsa	40,14
Acartia copepodites	14,05
Acartia (Acartiura) clausi	13,21
Penilia avirostris	11,53
Decapoda larvae	4,51
Harpacticoida Metidae	2,32
Mnemiopsis leidyi	2,32
Calanipeda aquaedulcis	1,80
Centropages ponticus	1,42
Pseudevadne tergestina	1,29

TPO 401	%
Acartia (Acanthacartia) tonsa	65,29
Acartia copepodites	20,59
Harpacticoida Metidae	4,83
Ostracoda	1,21
Penilia avirostris	1,15
Cirripedia nauplii	1,09
Decapoda larvae	0,75
Calanipeda aquaedulcis	0,63
Acartia (Acartiura) clausi	0,58
Centropages ponticus	0,58
Decapoda Brachyura larvae	0,58

TPO 204	%
Penilia avirostris	18,20
Decapoda larvae	16,56
Acartia (Acartiura) clausi	12,95
Harpacticoida Metidae	8,36
Acartia (Acanthacartia) tonsa	7,05
Paracartia latisetosa	4,59
Decapoda Brachyura larvae	4,26
Acartia copepodites	3,93
Paracalanus copepodites	3,93
Centropages ponticus	3,61

TEU 301	%
Penilia avirostris	32,04
Acartia copepodites	20,56
Acartia (Acanthacartia) tonsa	17,38
Acartia (Acartiura) clausi	12,18
Harpacticoida Metidae	7,84
Decapoda larvae	2,72
Pseudevadne tergestina	1,55
Decapoda Brachyura larvae	1,16
Centropages ponticus	1,01
Amphipoda Gammarida	0,78

Tab. 9 Top ten taxa observed in samples collected in September 2021 in the Marano and Grado Lagoon.

TME 401	%
Penilia avirostris	68,87
Pleopis polyphemoides	7,55
Acartia (Acanthacartia) tonsa	6,79
Acartia copepodites	2,45
Acartia (Acartiura) clausi	1,89
Paracalanus parvus s.l.	1,51
Harpacticoida Metidae	1,32
Centropages copepodites	1,13
Centropages ponticus	1,13
Harpacticoida	1,13

TME 301	%
Acartia (Acanthacartia) tonsa	36,38
Acartia copepodites	20,19
Ostracoda	14,74
Penilia avirostris	6,41
Amphipoda Gammarida	4,01
Gastropoda larvae	3,53
Calanipeda aquaedulcis	2,72
Harpacticoida	1,92
Pleopis polyphemoides	1,76
Harpacticoida Metidae	1,44
Mysida	1,44

TPO 501	%
Penilia avirostris	76,89
Pleopis polyphemoides	6,79
Acartia (Acartiura) clausi	5,62
Acartia copepodites	1,56
Gastropoda larvae	1,33
Decapoda larvae	1,01
Pseudevadne tergestina	0,86
Paracalanus parvus s.l.	0,86
Ophiuroidea plutei	0,86
Centropages ponticus	0,78

TPO 401	%
Acartia (Acanthacartia) tonsa	48,59
Acartia copepodites	26,19
Penilia avirostris	15,06
Pleopis polyphemoides	2,33
Harpacticoida Metidae	1,84
Acartia (Acartiura) clausi	0,98
Gastropoda larvae	0,98
Harpacticoida	0,61
Decapoda Brachyura larvae	0,61
Decapoda larvae	0,49

TPO 204	%
Amphipoda Gammarida	28,24
Isopoda Munnidae	23,66
Pleopis polyphemoides	8,40
Ophiuroidea plutei	8,40
Ctenophora cyddipid larvae	4,58
Acartia (Acartiura) clausi	3,05
Amphipoda Caprellidae	3,05
Decapoda larvae	3,05
Penilia avirostris	2,29
Acartia copepodites	2,29

TEU 301	%
Ophiuroidea plutei	28,48
Amphipoda Gammarida	17,41
Acartia (Acanthacartia) tonsa	9,18
Penilia avirostris	8,86
Acartia copepodites	6,65
Amphipoda Caprellidae	3,80
Isopoda Munnidae	3,48
Ostracoda	3,16
Oikopleura spp.	2,53
Acartia (Acartiura) clausi	1,90
Asciadiacea larvae	1,90

Tab. 10 Top ten taxa observed in samples collected in October 2021 in the Marano and Grado Lagoon.

TME 401	%
Penilia avirostris	25,11
Ophiuroidea plutei	19,31
Acartia copepodites	16,80
Acartia (Acartiura) clausi	14,37
Acartia (Acanthacartia) tonsa	7,62
Pleopis polyphemoides	6,15
Harpacticoida Metidae	1,39
Ctenophora cyddipid larvae	1,39
Bivalvia larvae	1,30
Oikopleura spp.	1,21

TME 301	%
Acartia copepodites	44,77
Acartia (Acanthacartia) tonsa	19,55
Ctenophora cyddipid larvae	8,92
Acartia (Acartiura) clausi	6,52
Pleopis polyphemoides	3,09
Ophiuroidea plutei	2,92
Harpacticoida Metidae	2,57
Penilia avirostris	1,89
Paracalanus copepodites	1,72
Mnemiopsis leidyi	1,20

TPO 501	%
Penilia avirostris	27,54
Acartia (Acartiura) clausi	21,41
Ophiuroidea plutei	21,33
Acartia copepodites	10,40
Pleopis polyphemoides	7,68
Bivalvia larvae	1,32
Decapoda larvae	1,24
Gastropoda larvae	1,16
Acartia (Acanthacartia) tonsa	1,01
Oikopleura spp.	0,93

TPO 401	%
Penilia avirostris	27,18
Acartia copepodites	17,12
Ophiuroidea plutei	15,92
Acartia (Acartiura) clausi	15,17
Acartia (Acanthacartia) tonsa	6,91
Pleopis polyphemoides	6,01
Harpacticoida Metidae	2,55
Sagitta spp.	1,80
Ctenophora cyddipid larvae	1,50
Oikopleura spp.	1,05

TPO 204	%
Acartia (Acartiura) clausi	17,20
Acartia copepodites	17,06
Penilia avirostris	14,14
Ophiuroidea plutei	13,85
Pleopis polyphemoides	13,56
Oikopleura spp.	5,83
Sagitta spp.	4,52
Cirripedia nauplii	2,33
Ctenophora cyddipid larvae	1,31
Echinoidea plutei	1,17

TEU 301	%
Acartia copepodites	21,79
Pleopis polyphemoides	16,51
Acartia (Acartiura) clausi	11,31
Penilia avirostris	10,70
Ophiuroidea plutei	9,33
Oikopleura spp.	7,95
Sagitta spp.	5,81
Ctenophora cyddipid larvae	4,82
Acartia (Acanthacartia) tonsa	1,83
Gastropoda larvae	1,22

Tab. 11 Top ten taxa observed in samples collected in November 2021 in the Marano and Grado Lagoon.

TME 401	%
Acartia copepodites	33,33
Acartia (Acartiura) clausi	23,70
Penilia avirostris	7,41
Harpacticoida Metidae	7,41
Acartia (Acanthacartia) tonsa	4,44
Bivalvia larvae	3,70
Harpacticoida	2,96
Paracalanus copepodites	2,96
Pleopis polyphemoides	2,22
Ophiuroidea plutei	2,22

TME 301	%
Mnemiopsis leidyi	53,70
Ctenophora cyddipid larvae	33,45
Acartia (Acanthacartia) tonsa	5,82
Acartia copepodites	3,52
Acartia (Acartiura) clausi	0,73
Oikopleura spp.	0,73
Penilia avirostris	0,36
Anthoathecata	0,36
Paracalanus copepodites	0,24
Harpacticoida Metidae	0,12
Oithona copepodites	0,12
Oithona nana	0,12
Oithona similis	0,12
Paracalanus parvus s.l.	0,12
Temora longicornis copepodites	0,12
Bivalvia larvae	0,12
Ophiuroidea plutei	0,12
Sagitta spp.	0,12

TPO 501	%
Acartia (Acartiura) clausi	22,35
Acartia copepodites	21,18
Penilia avirostris	20,59
Acartia (Acanthacartia) tonsa	6,47
Harpacticoida Metidae	5,29
Pleopis polyphemoides	4,71
Paracalanus copepodites	4,71
Oikopleura spp.	3,53
Paracalanus parvus s.l.	2,35
Ophiuroidea plutei	1,76

TPO 401	%
Mnemiopsis leidyi	20,83
Acartia copepodites	19,32
Ctenophora cyddipid larvae	18,18
Acartia (Acanthacartia) tonsa	16,67
Acartia (Acartiura) clausi	13,26
Penilia avirostris	2,65
Harpacticoida Metidae	1,52
Oikopleura spp.	1,52
Oithona similis	1,14
Anthoathecata	0,76

TPO 204	%
Harpacticoida	45,40
Isopoda Munnidae	28,89
Amphipoda Gammarida	9,01
Mysida	2,63
Acartia (Acartiura) clausi	2,44
Acartia copepodites	1,88
Pseudodiaptomus marinus copepodites	1,50
Ostracoda	1,31
Polychaeta	0,94
Penilia avirostris	0,75
Pseudodiaptomus marinus	0,75

TEU 301	%
Ctenophora cyddipid larvae	42,47
Penilia avirostris	24,71
Oikopleura spp.	7,72
Mnemiopsis leidyi	4,63
Acartia copepodites	3,47
Ophiuroidea plutei	2,70
Acartia (Acartiura) clausi	1,93
Paracalanus parvus s.l.	1,93
Bivalvia larvae	1,93
Gastropoda larvae	1,93

Acartia (Acanthacartia) tonsa **(NIS)**

A. (Acartiura) margalefi (left) and *A. (Acartiura) clausi* (right)

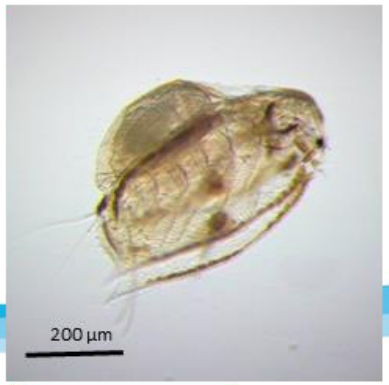
Pseudodiaptomus marinus **(NIS)**

Oithona davisae **(NIS)**

Harpactocoida Metidae

Brachyura larvae (crab larvae)

Mnemiopsis leidyi **(NIS)**



Penilia avirostris

Fig. 9 Images of the main taxa found in Marano and Grado Lagoon (May-November 2021). Non-indigenous species (NIS) are highlighted in red.

As for total abundance, the community showed values between the minimum of 33.78 ± 28.42 ind. m^{-3} (mean ± standard deviation) in July and the maximum of 861.95 ± 1036.55 ind. m^{-3} in June. The mean total abundance was 266.49 ± 482.64 ind. m^{-3} (mean ± standard deviation) (Fig. 10). The total abundance observed in each month at each station is shown in Figure 11.

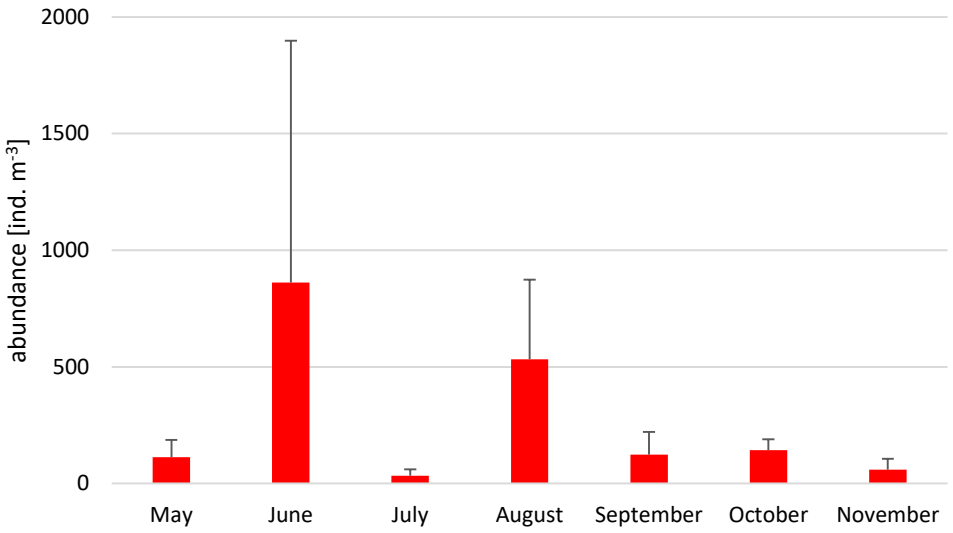


Fig. 10 Average monthly abundance (mean ± standard deviation) of the zooplankton community in the Marano and Grado Lagoon during May-November 2021.

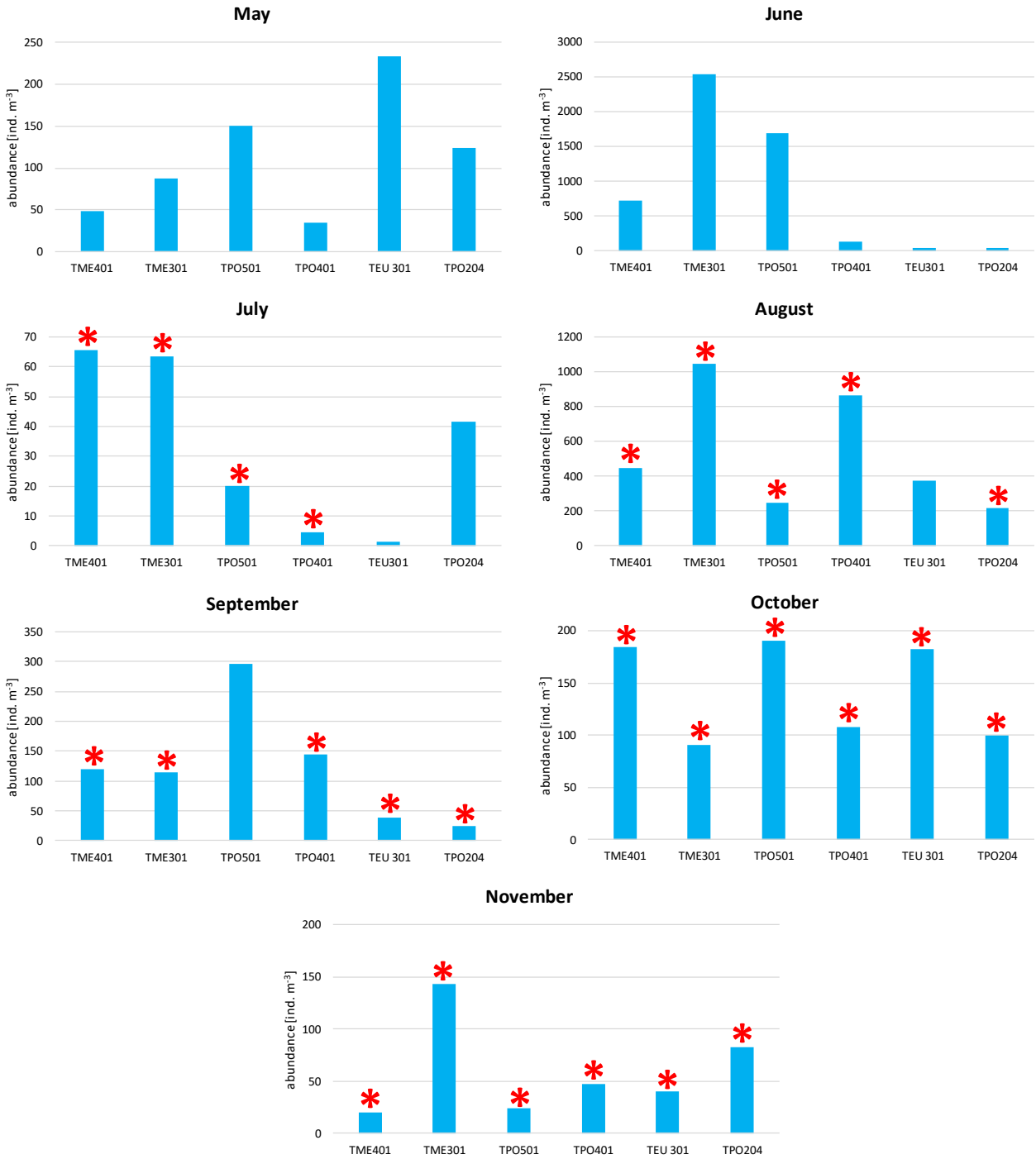


Fig. 11 Total abundance of zooplankton observed at each station in each month in the Marano and Grado Lagoon during May-November 2021. "*" indicates samples in which ctenophores of *Mnemiopsis leidyi* were caught.

During the studied period, the average abundance of taxa observed in each month, expressed as a percentage (Fig. 12), was generally characterized by a marked preponderance of copepods of the genus *Acartia* (*A. clausi* and *A. tonsa* mainly present in spring/fall and summer seasons, respectively), while cladocerans (*P. avirostris* and *P. polyphemoides*) were found primarily in the late summer/early fall months. It is noteworthy the important presence of Brachyura larvae (crab larvae) from spring to early summer, which accounted for more than 90% of the total community in June. In particular the crab *Rhithropanopeus harrisii,* a non-indigenous species identified by molecular approach, was present in almost all sampled stations with extremely high abundances, reaching the maximum value of 2529 ind. m^{-3} (99% of the community) in the Marano basin. Ctenophores of the invasive species *Mnemiopsis leidyi* were present in limited abundance (< 3% of the community), except in November 2021 when *Mnemiopsis* bloomed, as proofed by the high number of small individuals (< 1cm) found representing more than 45% of the community.

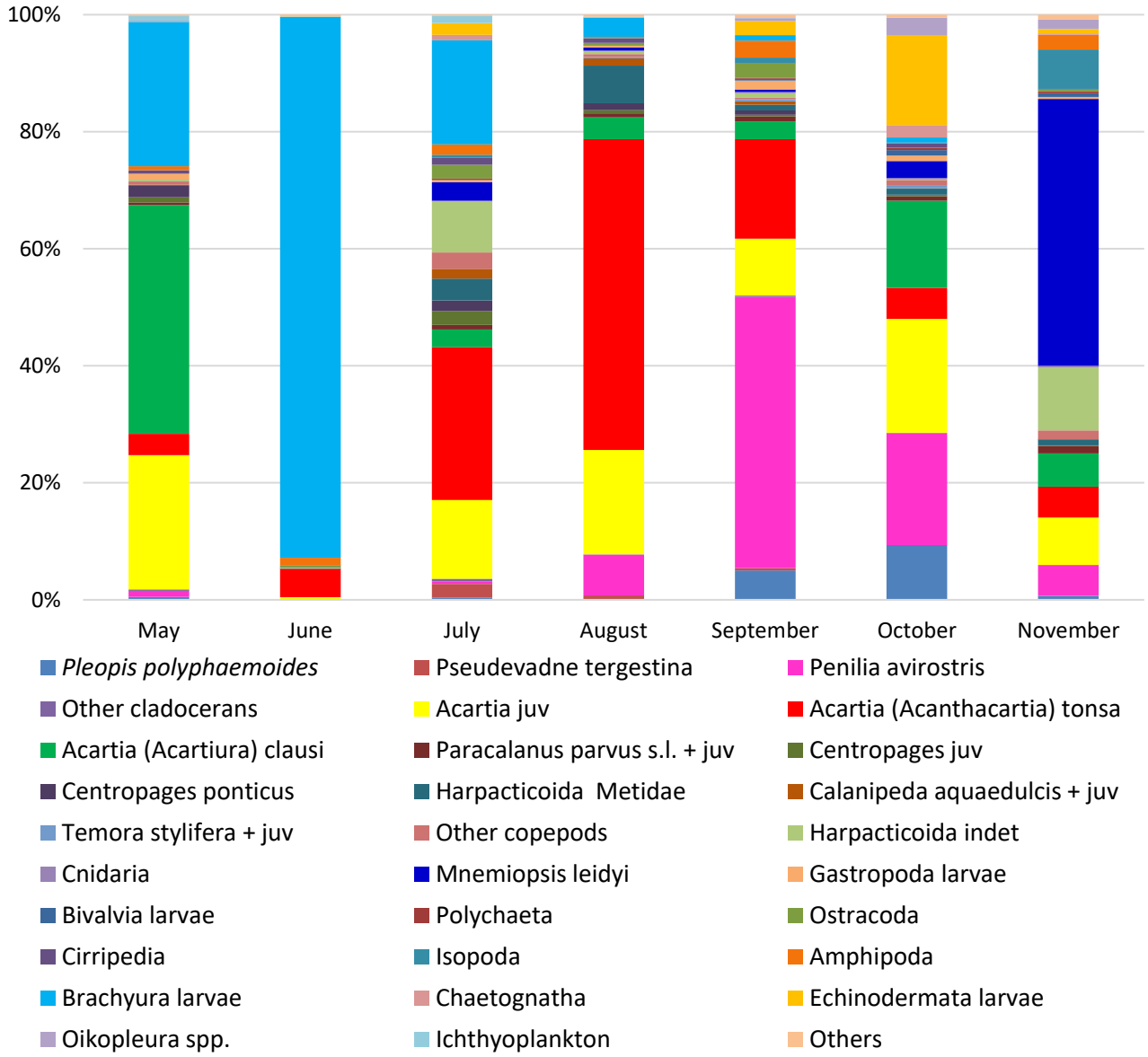


Fig. 12 Average abundance of taxa of the community observed in each month, expressed as a percentage. Taxa with very low abundance are grouped as "Others"; “juv.”: juveniles.

As for the composition of trophic groups (Fig. 13), the community in the lagoon was dominated mainly by omnivores-herbivores and herbivores. These two trophic groups accounted for between 55 and over 90% of the community in the lagoon, in almost every month and were mainly represented by *Acartia* copepods and Cladocera. Omnivore-carnivores (crab larvae) and carnivore taxa (*M. leidyi*) dominated the community in June and November, respectively. The remaining trophic groups - omnivore-detritivores (mostly represented by harpacticoid copepods and ostracods),

omnivores (such as amphipods, isopods and some species of copepods), carnivore-detritivores (pycnogonids) - constituted only a small part of the studied communities.

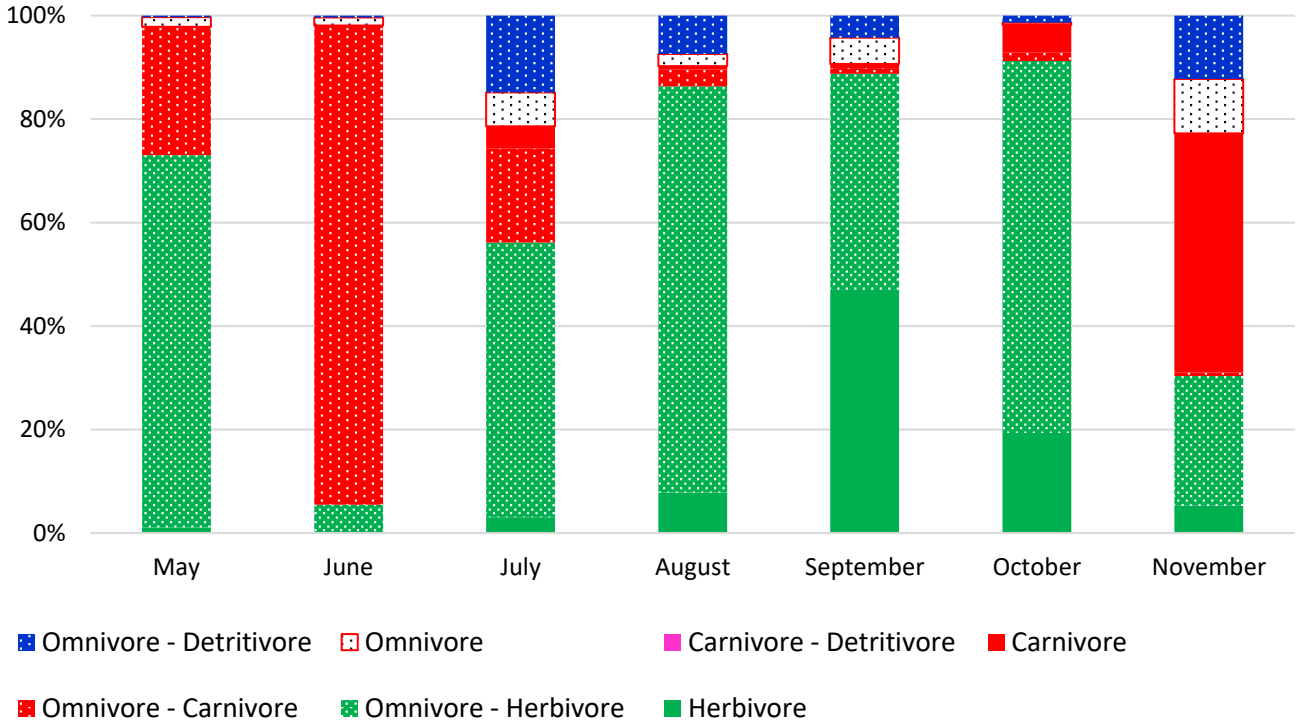


Fig. 13 Average abundance of trophic groups of the communities observed in each month, expressed as relative abundance (%).

ENVIRONMENTAL DNA (eDNA) ANALYSIS

Study area and sampling

The study of environmental DNA (eDNA) was conducted in 17 sites of the Marano and Grado Lagoon (Fig. 14) in spring and autumn 2021 (Tab. 12), contextually with the Water Framework Directive (WFD/2000/60/EC) survey conducted by the Regional Agency for the protection of the environment of the Friuli Venezia Giulia region (ARPA FVG).

At each station, 5 L of water were collected. Water samples were prefiltered through 50 µm mesh, filtered through 1.2 µm PES membrane filters (PALL Laboratory) and stored at −80 °C until further processing. Filtration was performed until clogging of membrane pores, for at least 2 filters per sample. Filtered volumes were in the range of 1–1.5 L (Tab. 13).

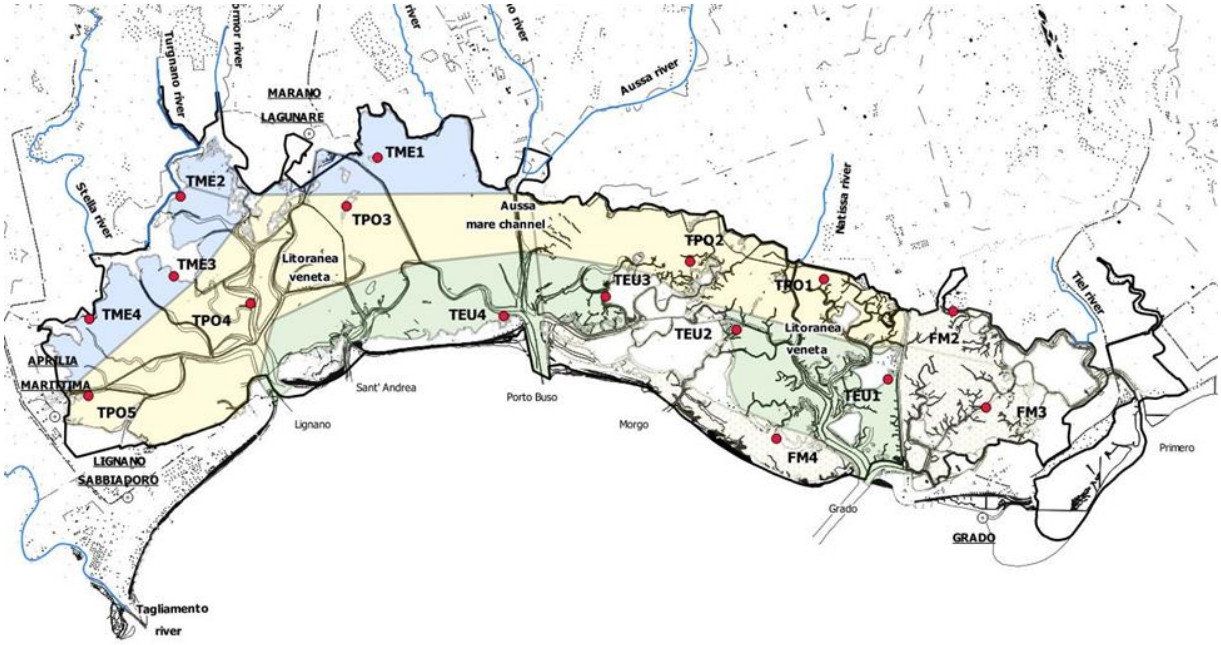


Fig. 14 Map of the eDNA sampling sites in the Marano and Grado Lagoon.

Tab. 12 Sampling sites, coordinates, and sampling data of the study in the Marano and Grado Lagoon.

Site	Latitude	Longitude	Spring 2021	Autumn 2021
FM2	45,724717	13,402167	14/05/2021	24/09/2021
FM3	45,7002	13,415117	14/05/2021	24/09/2021
FM4	45,691133	13,338017	10/06/2021	26/10/2021
TEU1	45,70705	13,3786	10/06/2021	28/09/2021
TEU2	45,719017	13,3226	28/05/2021	29/09/2021
TEU3	45,72675	13,274133	10/06/2021	28/09/2021
TEU4	45,721333	13,236567	11/06/2021	28/09/2021
TME1	45,761217	13,189	11/06/2021	28/09/2021
TME2	45,757983	13,13455	03/06/2021	27/09/2021
TME3	45,74575	13,134333	28/04/2021	27/09/2021
TME4	45,71815	13,08595	28/04/2021	29/10/2021
TPO1	45,7323	13,354433	28/05/2021	01/10/2021
TPO2	45,736283	13,304933	28/05/2021	28/09/2021
TPO3	45,7485	13,178	11/06/2021	26/10/2021
TPO4	45,72285	13,1434	03/06/2021	27/09/2021
TPO5	45,6964	13,10275	28/04/2021	29/10/2021

Tab. 13 Volume of water (L) filtered per each site to collect eDNA.

	Spring		**Autumn**	
	Filter #1	#2	Filter #1	#2
FM2	1.2	1	1	1
FM3	1	1.1	1	1
FM4	1.5	1.5	1.5	1.5
TEU1	1.5	1.5	1.6	1.6
TEU2	1.35	1.45	1.5	1.45
TEU3	1.48	1.45	1.5	1.5
TEU4	1.5	1.5	1.5	2
TME1	1	1.2	1.25	1.25
TME2	1.5	1.5	1	1
TME3	1	1	1	1.2
TME4	1	1.5	1.25	1.25
TPO1	1.38	1	1.5	1.5
TPO2	1	1	1.1	1.1
TPO3	1.6	1.55	1.5	1.5
TPO4	1.5	1.5	1.5	1.5

TPO5	1	1	1.25	1.25

DNA extraction and amplicon sequencing

DNA was extracted from membrane filters using the DNeasy PowerWater Kit (Qiagen). Two filters were extracted for each sample, and then the eluted DNA were pooled together. The DNA extraction was also performed on 2 filters treated only with distilled water (1L each) to assess possible contaminations due to samples processing. These blank extractions were used as additional negative controls during the subsequent PCR amplifications.

For the Fish DNA metabarcoding, the mitochondrial 12S rRNA gene was amplified using Teleo_f/L1848 (5′- ACACCGCCCGTCACTCT-3′) and Teleo_r/H1913 (5′- CGYCAATTYMTTTRAGTTT-3′) primers (Valentini et al. 2016) combined with teleo_blk (5’- ACCCTCCTCAAGTATACTTCAAAGGAC-SPC3I-3’) primer to prevent human DNA amplification (Valentini et al. 2016). PCR amplifications were performed in duplicates for each sample, in a total volume of 50 µl with 1 U of HiProof HF Master Mix (Bio-Rad), 0.5 µM of F and R primers, 10 µM of blocking primer, and 5 µl of DNA. The thermal cycling profiles started with 98 °C for 30 s, followed by 35 cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 30 s and extension at 72 °C for 30 s, with a final extension at 72 °C for 7 min.

For the Metazoa DNA metabarcoding, the mitochondrial mitochondrial Cytochrome-c-Oxidase I gene (COI) was amplified using mlCOIintF (5′-GGWACWGGWTGAACWGTWTAYCCYCC-3′) and jgHCO2198 (5′-TAIACYTCIGGRTGICCRAARAAYCA-3′) primers (Leray et al. 2013; Geller et al. 2013). PCR amplifications were performed in duplicates for each sample, in a total volume of 25 µl with 1 × AccuStart™ II PCR ToughMix (QuantaBio), 0.2 µM of each primer, and 5 µl of DNA. The thermal cycling profiles started with 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 20 s, annealing at 55 °C for 20 s and extension at 72 °C for 1 min, with a final extension at 72 °C for 1 min.

Library preparation and Illumina MiSeq System runs for a read length of 2 × 150 bp and 2 × 300 (for 12S and COI respectively) were performed at BMR Genomics S.r.l., Padua, Italy (www.bmr-genomics.it).

Fish taxonomic assignment

Bioinformatic analyses were performed with QIIME2 2022.2 (Bolyen et al. 2019). Raw sequences were quality filtered, trimmed, and denoised with DADA2 (Callahan et al. 2016).
For taxonomic assignment, the list of Mediterranean species was retrieved from Fishbase (https://www.fishbase.se/trophiceco/FishEcoList.php?ve_code=13). The 758 species, plus *Knipowitschia panizzae* and *Pomatoschistus canestrinii* that were recovered by WFD ARPA survey but not included in Fishbase were used as Entrez query from NCBI on 13 April 2022. The query was
12S[All Fields] OR mitochondrion[All Fields] OR mithocondria[All Fields]) AND (("*Species*"[Organism] OR *Species*[All Fields]) NOT ("predicted" [All Fields]) NOT ("unverified"[All Fields])) AND ("80"[SLEN] : "25000"[SLEN]).
The query resulted in 6334 sequences, representing 737 species. All the species of the fish recovered in the lagoon by WFD ARPA survey were present.
RESCRIPt v.2021.11.0 (Robeson et al. 2021) was used to construct the QIIME2-formatted database. ASVs taxonomic assignment was performed using classify-consensus-blast in QIIME2 (Bolyen et al. 2019) set with decreasing identity percentages (1, 0.99, and 0.97), and the assignment was manually inspected, comparing the different results. ASVs were also aligned against the NCBI nucleotide collection using BLASTN 2.12.0+ (Altschul et al. 1997) as an additional identify check.

Metazoa taxanomic assignment

Bioinformatic analyses were performed with QIIME2 2022.2 (Bolyen et al. 2019). Raw sequences were quality filtered, trimmed, and denoised with DADA2 (Callahan et al. 2016).
ASVs taxonomic assignment was performed with the GenBank249 Unique COI MIDORI Reference 2 Database (Leray et al. 2022) using RDP Classifier (Wang et al. 2007) set with 0.97 confidence cutoff.
ASVs were also aligned against the NCBI nucleotide collection using BLASTN 2.12.0+ (Altschul et al. 1997) as an additional identify check.

Results

Fishes

A total of 2,628,001 raw sequences were generated. After the denoising procedure, 2,149,118 were retained with an average of 67,160 ± 16,273 per sample. The average number of ASVs were 105 ± 60 per sample. The reads belonging to Actinopterygii and Chondrichthyes were 1,446,482, representing the 67% of the clean dataset, with an average proportion of 67 ± 19 % per sample.

The total number of species detected was 31 (Tab. 14). Overall, a core group of species were detected in all sites (*Sparus aurata, Atherina boyeri, Chelon auratus, Dicentrarchus labrax*), and correspond also the highest number of reads. About one third of the species were detected only in Autumn (*Alosa fallax, Mustelus mustelus, Trachurus trachurus, Symphodus melops, Pomatomus saltatrix, Salmo salar, Scomber scombrus, Sprattus sprattus, Pomatoschistus knerii, Hippocampus hippocampus, Torpedo marmorata, Arnoglossus laterna*).

Tab. 14 Fish species detected by eDNA metabarcoding in the sampling sites for the two seasons. Green: detected; yellow: not detected. √: detected by WFD ARPA survey.

SPRING	FM			TEU				TME				TPO				
	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	5
Sparus aurata	√	√								√	√		√			
Atherina boyeri			√	√	√	√	√	√		√		√	√	√	√	
Chelon auratus				√	√							√				
Dicentrarchus labrax	√	√											√			
Oncorhynchus mykiss																
Chelon ramada	√									√	√	√	√			√
Aphanius fasciatus	√			√	√	√		√				√	√	√		
Syngnathus typhle							√									
Zosterisessor ophiocephalus																
Mugil cephalus																
Sardina pilchardus																√
Solea solea																
Gobius niger																
Salmo trutta																
Pomatoschistus minutus																
Engraulis encrasicolus																
Squalus acanthias																
Chelon labrosus																
Anguilla anguilla																
Alosa fallax																
Mustelus mustelus																
Trachurus trachurus																
Symphodus melops																
Pomatomus saltatrix																
Salmo salar																
Scomber scombrus																
Sprattus sprattus																
Pomatoschistus knerii																
Hippocampus hippocampus																
Torpedo marmorata																
Arnoglossus laterna																

AUTUMN	FM			TEU				TME				TPO				
	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	5
Sparus aurata																
Atherina boyeri	√	√	√		√	√	√	√	√	√	√	√	√	√	√	√
Chelon auratus																
Dicentrarchus labrax																
Oncorhynchus mykiss																
Chelon ramada	√															
Aphanius fasciatus	√	√	√	√	√	√		√	√	√	√	√	√	√		√
Syngnathus typhle															√	
Zosterisessor ophiocephalus							√									
Mugil cephalus																
Sardina pilchardus																
Solea solea																
Gobius niger																
Salmo trutta																
Pomatoschistus minutus																
Engraulis encrasicolus																
Squalus acanthias																
Chelon labrosus																
Anguilla anguilla																
Alosa fallax																
Mustelus mustelus																
Trachurus trachurus																
Symphodus melops																
Pomatomus saltatrix																
Salmo salar																
Scomber scombrus																
Sprattus sprattus																
Pomatoschistus knerii																
Hippocampus hippocampus																
Torpedo marmorata																
Arnoglossus laterna																

Comparison with WFD ARPA survey

The WFD monitoring carried out by ARPA FVG detected 18 Teleostei species (Tab. 14, Fig. 15). Of the 18 species, 11 were detected also with eDNA (Tab. 14, Figs. 15, 16). The other seven, beside present in the database, were not detected or because the 12S target region present in NCBI was not present in the full length preventing the assignment (*Chelon saliens*, *Knipowitschia panizzae*, *Nerophis ophidion*, *Platichthys flesus*, *Pomatoschistus canestrinii*, *Salaria pavo*) or because the primer site contains a mismatch, thus preventing amplification (*Syngnathus abaster*).

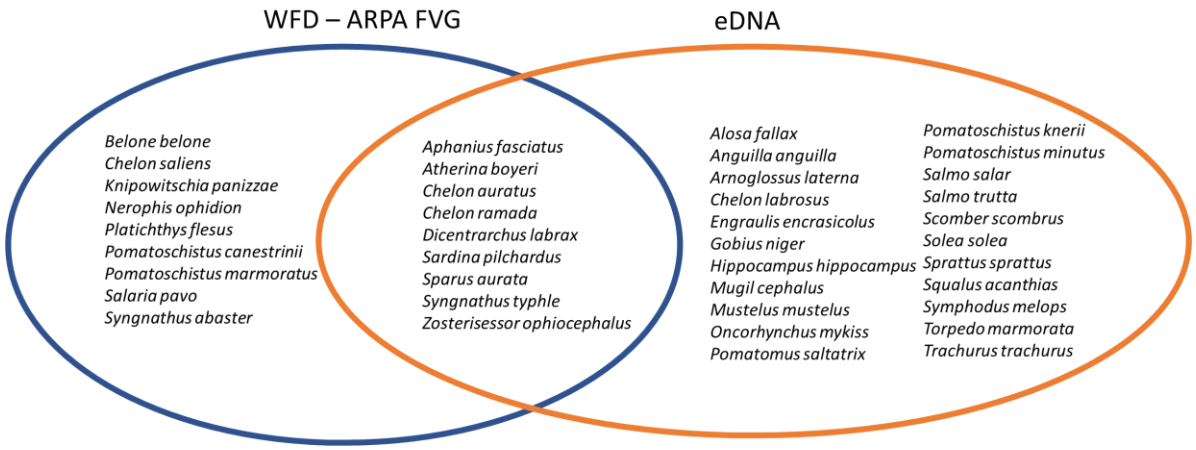


Fig. 15 Venn diagram showing the species detected by the WFD ARPA survey and with eDNA analysis.

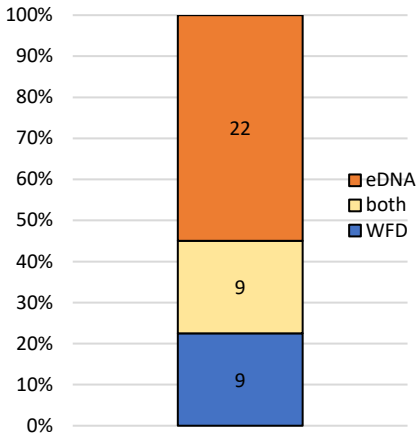


Fig. 16 Bar chart showing the species detected only by the WFD ARPA survey, only with eDNA analysis, and with both.

For this reason, in the perspective to implement a site-specific eDNA monitoring of the lagoon, these biases can be at least partially prevented by a combined strategy. For the database issue, the DNA of these species can be directly extracted form lagoon specimens, the targeted barcode region sequenced by Sanger approach, and those sequences added at the reference database to increase its resolution.
For the primer mismatch issue, primers with degenerated nucleotides or a mix with different primer presenting specific polymorphisms could be tested in order to increase the number of species detected in a Marano and Grado Lagoon-tailored study. Moreover, other freshwater and marine fish-specific systems could be applied alone or together with the one by Valentini et al. (2016) that we used, such as the one on 16S rRNA gene (16SF/D/16S2R-degenerate; Berry et al. 2017; Deagle et al. 2007) or 12S rDNA (MiFish-U-F/MiFish-U-r; Miya et al. 2015). These approaches, however, target longer DNA regions (160-400 bp and ~170 bp respectively) in respect to the Valentini et al. (2016), which is 70-80 bp. In eDNA surveys, due to the possible degradation of DNA, the use of the so-called “minibarcodes” facilitates the detection of such genetic materials (Meusnier et al. 2008).

Metazoa

A total of 2,654,174 raw sequences were generated. After the denoising procedure, 1,940,607 were retained with an average of 60,644 ± 23,751 per sample. The average number of ASVs were 393 ± 182 per sample. Overall, 11 phyla for a total of 56 species were detected by the molecular approach. A core group of species (present in both Spring and Autumn) were represented by Anellida, Arthropoda, Bryozoa, Chordata, Cnidaria and Mollusca (Tab. 15). These data were compared with data obtained by traditional microscopic analyses of zooplankton samples collected in the 6 stations of the Marano and Grado Lagoon during the same seasons as the eDNA sampling (in Spring: May and June, in Autumn: September and October) (see section "*Selected specimens Sanger sequencing*" in the chapter "*Zooplankton community*"). Using the traditional approach, over 31,000 organisms were examined and 69 taxa were detected. Comparison showed that the two methods revealed almost the same number of phyla (11 with the traditional approach), while microscopic analysis revealed a lower number of species (29). To evaluate the detection performance of the molecular approach versus the traditional morphological analysis, the datasets were classified into 12 main taxonomic groups applicable to both approaches (Fig. 17 A).

Overall, the performance of the molecular and microscopic analyses in finding taxonomic units differed among the taxonomic groups: the molecular approach detected a greater number of meroplanktonic organisms (Teleostea, Mollusca, and Polychaeta) that are normally only identified at a higher taxonomic level by the microscope, due to the lack of specific morphological features at the larval stage; in contrast, a fivefold greater number of copepod species were observed with the microscope, and the presence of taxa belonging to Cladocera, Chaetognatha, and Echinodermata has been almost exclusively detected with this approach. As for the copepod community, 22 species were found by microscopic analysis, while the molecular approach yielded only 6 species, probably due to the paucity of copepods' sequences in reference databases (Fig. 17 B). Only 5 species were detected with both approaches: *A. clausi*, *A. tonsa*, *Euterpina acutifrons* and the non-native species *Pseudodiaptomus marinus* and copepod *Oithona davisae*.

Tab. 15 Metazoan species detected by eDNA metabarcoding at the sampling sites in the two seasons. Green: detected; yellow: not detected; "√": species detected by morphological approach.

Phylum	Class	Order	Family	Genus	Species	Spring	Autumn
Annelida	Polychaeta	Phyllodocida	Nereididae	*Hediste*	*Hediste diversicolor*		
Annelida	Polychaeta	Spionida	Spionidae	*Streblospio*	*Streblospio shrubsolii*		
Annelida	Polychaeta	Terebellida	Pectinariidae	*Pectinaria*	*Pectinaria koreni*		
Annelida	Polychaeta	Capitellida	Capitellidae	*Capitella*	*Capitella teleta*		
Annelida	Polychaeta	Eunicida	Eunicidae	*Marphysa*	*Marphysa sanguinea*		
Annelida	Polychaeta	Phyllodocida	Glyceridae	*Glycera*	*Glycera sp.*		
Annelida	Polychaeta	Phyllodocida	Hesionidae	*Syllidia*	*Syllidia armata*		
Annelida	Polychaeta	Sabellida	Sabellariidae	*Sabellaria*	*Sabellaria spinulosa*		
Annelida	Polychaeta	Sabellida	Serpulidae	*Ficopomatus*	*Ficopomatus enigmaticus*		
Annelida	Polychaeta	Sabellida	Serpulidae	*Hydroides*	*Hydroides dianthus*		
Annelida	Polychaeta	Sabellida	Serpulidae	*Pomatoceros*	*Pomatoceros triqueter*		
Annelida	unclassified_Anellida	Haplotaxida	Naididae	*Nais*	*Nais elinguis*		
Annelida	Polychaeta	Capitellida	Capitellidae	*Heteromastus*	*Heteromastus filiformis*		
Annelida	Polychaeta	Capitellida	Maldanidae	*Clymenura*	*Clymenura sp.*		
Annelida	Polychaeta	Sabellida	Serpulidae	*Hydroides*	*Hydroides elegans*		
Annelida	Polychaeta	Spionida	Spionidae	*Polydora*	*Polydora cornuta*		
Arthropoda	Malacostraca	Amphipoda	Corophiidae	*Grandidierella*	*Grandidierella japonica*		
Arthropoda	Maxillopoda	Calanoida	Acartiidae	*Acartia*	*Acartia (Acartiura) clausi*	√	√
Arthropoda	Maxillopoda	Calanoida	Acartiidae	*Acartia*	*Acartia (Acanthacartia) tonsa*	√	√
Arthropoda	Maxillopoda	Cyclopoida	Oithonidae	*Oithona*	*Oithona davisae*	√	
Arthropoda	Branchiopoda	Diplostraca	Macrothrichidae	*Macrothrix*	*Macrothrix sp.*		
Arthropoda	Malacostraca	Amphipoda	Corophiidae	*Monocorophium*	*Monocorophium insidiosum*		
Arthropoda	Malacostraca	Amphipoda	Gammaridae	*Echinogammarus*	*Echinogammarus sp.*		
Arthropoda	Malacostraca	Amphipoda	Gammaridae	*Gammarus*	*Gammarus sp.*		
Arthropoda	Malacostraca	Decapoda	Carcinidae	*Carcinus*	*Carcinus aestuarii*		
Arthropoda	Maxillopoda	Cyclopoida	Cyclopidae	*Acanthocyclops*	*Acanthocyclops americanus*		
Arthropoda	Maxillopoda	Sessilia	Balanidae	*Amphibalanus*	*Amphibalanus amphitrite*		
Arthropoda	Malacostraca	Decapoda	Panopeidae	*Dyspanopeus*	*Dyspanopeus sayi*		
Arthropoda	Maxillopoda	Calanoida	Pseudodiaptomidae	*Pseudodiaptomus*	*Pseudodiaptomus marinus*		√
Arthropoda	Maxillopoda	Harpacticoida	Euterpinidae	*Euterpina*	*Euterpina acutifrons*	√	√
Bryozoa	Gymnolaemata	Cheilostomatida	Bugulidae	*Bugula*	*Bugula neritina*		
Bryozoa	Gymnolaemata	Cheilostomatida	Candidae	*Tricellaria*	*Tricellaria occidentalis*		
Bryozoa	Gymnolaemata	Cheilostomatida	Candidae	*Tricellaria*	*Tricellaria sp.*		
Bryozoa	Gymnolaemata	Ctenostomatida	Vesiculariidae	*Amathia*	*Amathia verticillata*		
Chordata	Actinopteri	Actinopteri	Moronidae	*Dicentrarchus*	*Dicentrarchus labrax*		
Chordata	Actinopteri	Cypriniformes	Cyprinidae	*Carassius*	*Carassius sp.*		
Chordata	Actinopteri	Gobiiformes	Gobiidae	*Ninnigobius*	*Ninnigobius sp.*		
Chordata	Actinopteri	Mugiliformes	Mugilidae	*Liza*	*Liza aurata*		
Chordata	Actinopteri	Mugiliformes	Mugilidae	*Liza*	*Liza saliens*		
Chordata	Actinopteri	Spariformes	Sparidae	*Sparus*	*Sparus aurata*		
Chordata	Ascidiacea	Enterogona	Didemnidae	*Diplosoma*	*Diplosoma sp.*		
Chordata	Actinopteri	Mugiliformes	Mugilidae	*Mugil*	*Mugil cephalus*		
Chordata	Actinopteri	Salmoniformes	Salmonidae	*Oncorhynchus*	*Oncorhynchus mykiss*		
Chordata	Ascidiacea	Enterogona	Ascidiidae	*Ascidia*	*Ascidia ahodori*		
Chordata	Chondrichthyes	Squaliformes	Squalidae	*Squalus*	*Squalus acanthias*		
Cnidaria	Hydrozoa	Leptothecata	Campanulariidae	*Laomedea*	*Laomedea angulata*		
Cnidaria	Scyphozoa	Rhizostomeae	Rhizostomatidae	*Rhizostoma*	*Rhizostoma pulmo*		
Cnidaria	Anthozoa	Actiniaria	Actiniidae	*Anthopleura*	*Anthopleura elegantissima*		
Cnidaria	Anthozoa	Actiniaria	Diadumenidae	*Diadumene*	*Diadumene lineata*		
Cnidaria	Anthozoa	Actiniaria	Sagartiidae	*Sagartiidae sp.*	*Sagartiidae sp.*		
Cnidaria	Hydrozoa	Anthoathecata	Bougainvilliidae	*Bougainvillia*	*Bougainvillia sp.*		
Cnidaria	Hydrozoa	Leptothecata	Campanulariidae	*Obelia*	*Obelia bidentata*		
Ctenophora	Tentaculata	Lobata	Bolinopsidae	*Mnemiopsis*	*Mnemiopsis leidyi*		√
Gastrotricha	Gastrotricha	Chaetonotida	Chaetonotidae	*Chaetonotus*	*Chaetonotus sp.*		
Gastrotricha	unclassified_Gastrotricha	Chaetonotida	Chaetonotidae	*Chaetonotidae sp.*	*Chaetonotidae sp.*		
Mollusca	Bivalvia	Myoida	Corbulidae	*Corbula*	*Corbula gibba*		
Mollusca	Bivalvia	Ostreoida	Ostreidae	*Crassostrea*	*Crassostrea gigas*		
Mollusca	Bivalvia	Veneroida	Cardiidae	*Cerastoderma*	*Cerastoderma glaucum*		
Mollusca	Bivalvia	Veneroida	Cardiidae	*Parvicardium*	*Parvicardium sp.*		
Mollusca	Bivalvia	Veneroida	Veneridae	*Ruditapes*	*Ruditapes philippinarum*		
Mollusca	Gastropoda	unclassified_Gastropoda	Trochidae	*Gibbula*	*Gibbula sp.*		
Mollusca	Bivalvia	Myoida	Hiatellidae	*Hiatella*	*Hiatella arctica*		
Mollusca	Bivalvia	Myoida	Pholadidae	*Pholas*	*Pholas dactylus*		
Mollusca	Bivalvia	Mytiloida	Mytilidae	*Modiolus*	*Modiolus barbatus*		
Mollusca	Bivalvia	Veneroida	Veneridae	*Polititapes*	*Polititapes aureus*		
Mollusca	Bivalvia	Mytiloida	Mytilidae	*Xenostrobus*	*Xenostrobus securis*		
Mollusca	Bivalvia	Veneroida	Veneridae	*Callista*	*Callista chione*		
Mollusca	Gastropoda	Cephalaspidea	Haminoeidae	*Haminoea*	*Haminoea orteai*		
Mollusca	Gastropoda	unclassified_Gastropoda	Haminoeidae	*Haminoea*	*Haminoea japonica*		
Nemertea	Palaeonemertea	Cephalotrichidae	Cephalotrichidae	*Cephalothrix*	*Cephalothrix sp.*		
Nemertea	Anopla	Heteronemertea	Lineidae	*Riseriellus*	*Riseriellus occultus*		
Porifera	Demospongiae	Halichondrida	Hymeniacidonidae	*Hymeniacidon*	*Hymeniacidon sp.*		
Porifera	Demospongiae	Halichondrida	Halichondriidae	*Halichondria*	*Halichondria panicea*		
Rotifera	Eurotatoria	Ploima	Synchaetidae	*Synchaeta*	*Synchaeta sp.*		

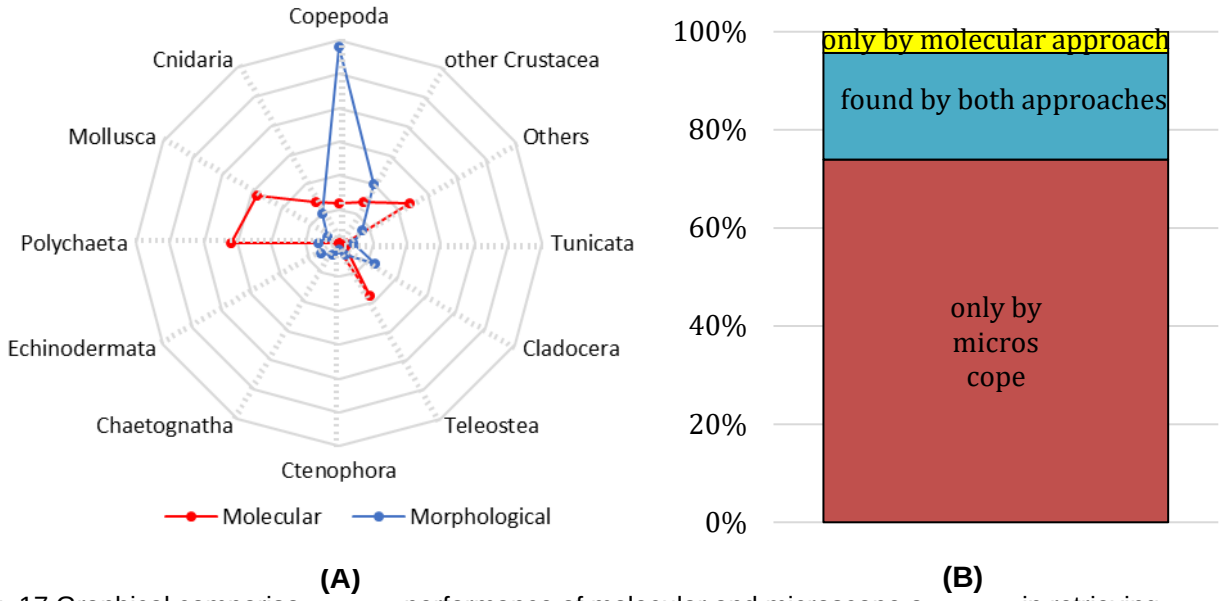


Fig. 17 Graphical comparison of the performance of molecular and microscope analyses in retrieving taxonomical units. (A) Overall performance by the two approaches by major groups and (B) proportion of copepod species identified by the two approaches.

Mnemiopsis leidyi detection in eDNA

The detection of invasive alien species through eDNA is of extreme interest and as it allows early warning is fundamental for the environmental protection. In this case, we have set up an assay to detect the ctenophore *Mnemiopsis leidyi* from the eDNA extracts.

A positive control formed by *M. leidyi* specimens was used, extracted, and amplified as described in the previous section but using the species-specific Ml-COIF (5′-TGTCGCCCAAATTACTGTTTC -3′) Ml-COIR primers (5′-TGACGGGGTAAACCTCATAAA -3′) (Ghabooli et al. 2013), that target a 656 bp COI fragment.

The amplicon was Sanger sequenced and aligned against the NCBI nucleotide collection using BLASTN 2.12.0+ (Altschul et al. 1997) to check its identity.

PCR were performed in a mix containing 5 µL eDNA, AccuStart™ II PCR ToughMix (QuantaBio), 1× EvaGreen™ (Biotium), and 200 nM of each primer.

The thermal cycling profiles started with 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 20 s, annealing at 55 °C for 20 s and extension at 72 °C for 1 min, with a final extension at 72 °C for 1 min, run on a CFX96 Touch Real-Time PCR Detection System (Bio-Rad). A melting curve analysis (65 °C - 95 °C increment 0.5 °C

for 5 s) was performed to verify the absence of non-specific amplification products. Amplicons were run on 1.5% agarose gel to check for the correct length, purified with QIAquick PCR Purification Kit (Qiagen) and Sanger sequenced as described above. Sequences were aligned against the NCBI nucleotide collection using BLASTN 2.12.0+ (Altschul et al. 1997) to verify the correct species identity (Fig. 18).
In all Autumn samples it was possible to detect *M. leidyi* DNA, while no detection was found in Spring samples (Fig. 19). This result is in agreement with zooplankton sampling data.

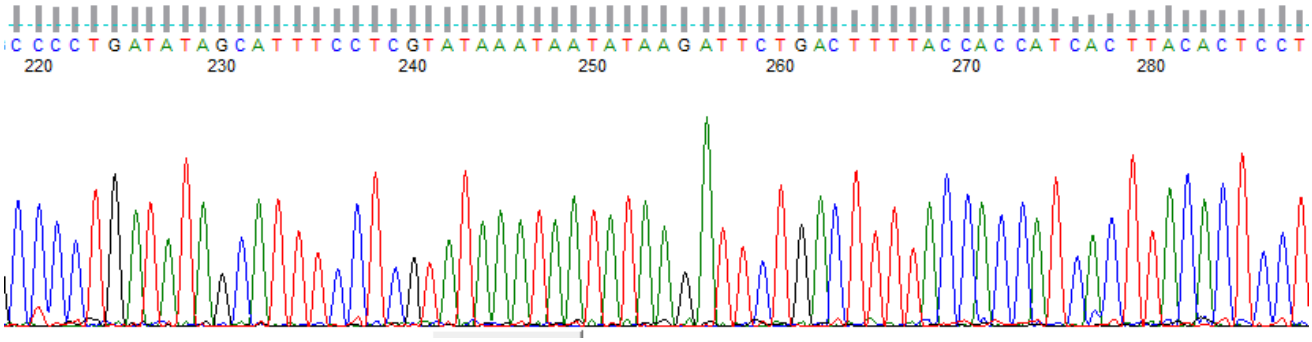


Fig. 18 Portion of an electropherogram of *M. leidyi* sequenced with Sanger method.

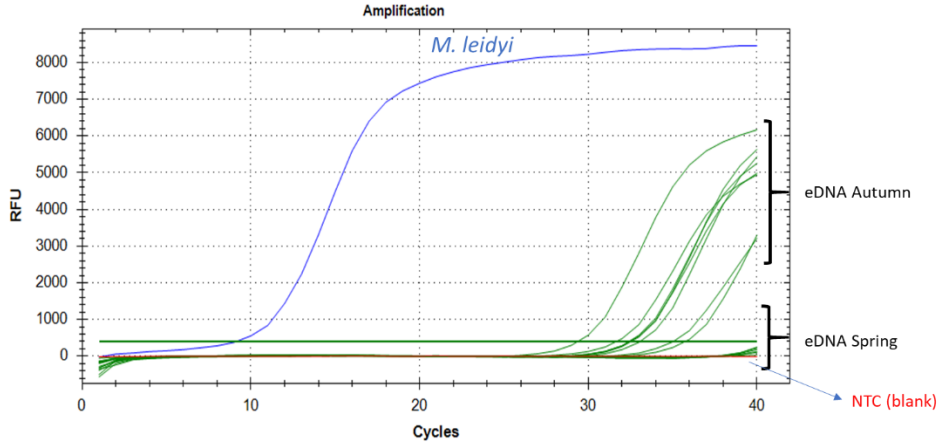


Fig. 19 Example of real-time amplification of eDNA samples. Blue line: *M. leidyi* DNA; Green lines: Spring and Autumn eDNA samples; Red line: No template control (NTC).

TRENDS OF SMALL-SCALE FISHERIES CATCHES in the MARANO and GRADO LAGOON

The data we collected at the Marano Lagunare fish market for the period 2010-2021, provided by the San Vito Fisheries Cooperative, show an overall increase in annual catches (Fig. 20). However, looking in detail at the trend of the target species of fyke nets (the big-scale sand smelt (*Atherina boyeri*), the grey shrimp (*Crangon crangon*) and the Baltic prawn (*Palaemon adspersus*)) a slight but steady increase can be seen from 2010 to 2014, followed by a slight decrease in 2015 and a sharp decline in these resources from 2016 (Fig. 20), the year of the first massive occurrence of the ctenophore *Mnemiopsis leidyi* in the Marano and Grado Lagoon (Malej et al., 2017).

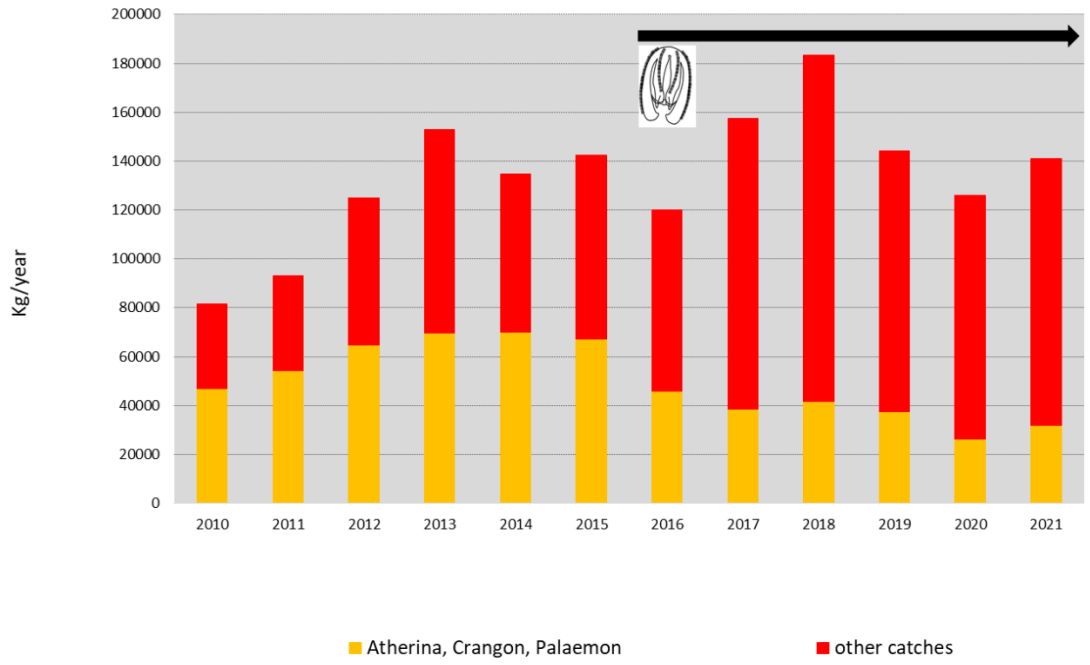


Fig. 20 Annual catches recorded at the Marano Lagunare Market from 2010 to 2021.

Based on catch data from the Marano Lagunare market, big-scale sand smelt and shrimp fisheries accounted for about 80% of the catch in the Marano and Grado lagoon

from 2000 to 2010 (Bettoso et al., 2013). As recently as 2010, the three target species accounted for less than 60% of the total catch of Marano fishermen, but this share gradually declined to just over 20% in 2017 and has not recovered to date (Fig. 21).

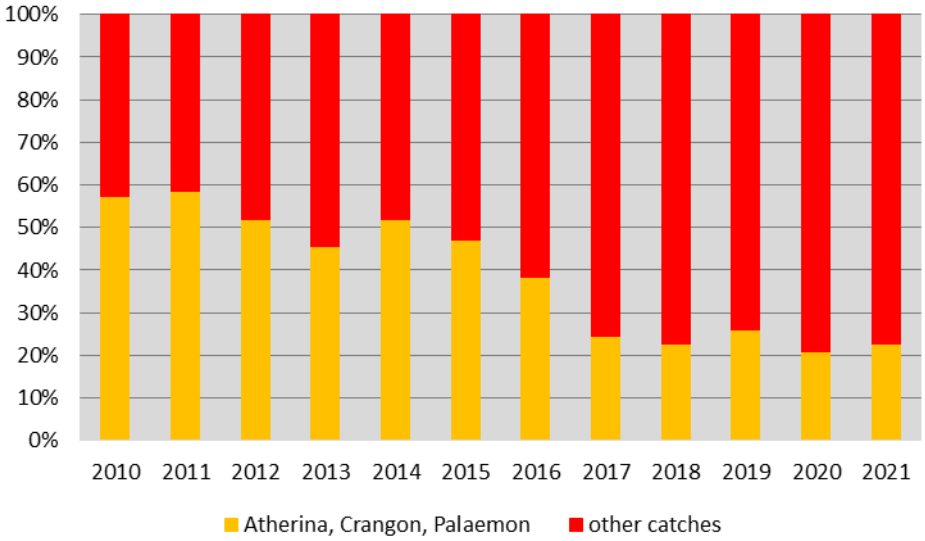


Fig. 21 Relative importance of the target species of fyke nets: data from Marano Lagunare market.

CONCLUSIONS

- Argos provided an important contribution to the knowledge of the food web in the Marano and Grado Lagoon: this study and the NOCE di MARE projects (Italian national project funded by the Regione Autonoma Friuli Venezia Giulia, l.r.n. 14/2018, art2., commi 51-55 and l.r.n. 26/2020, art. 4, commi 33-34) are the **first zooplankton surveys carried out in this area**

- herbivore and herbivore-omnivore taxa dominated the zooplankton community, confirming **the important role of zooplankton in transferring energy from the primary producers (phytoplankton) to higher trophic levels (fish) in the lagoon**

- 6 non-indigenous species (NIS) were identified in zooplankton samples (3 of which were first records in the study area): in particular the invasive alien species *Mnemiopsis leidyi* was observed in almost all the monitored sites in the Marano and Grado Lagoon from July to November 2021

- data collected at the Marano Lagunare fish market for the period 2010-2021, indicated a sharp decline of small-scale fishery catches (target fish species of fyke nets: Atheryna boyeri, Palaemon adsperus and Crangon crangon) from 2016, the year of the first massive occurrence of the ctenophore *Mnemiopsis leidyi* in the Marano and Grado Lagoon. The results of Argos confirmed the presence of this species and the great threat that this ctenophore poses to the ecosystem of the lagoon (as voracious predator of zooplankton and therefore competitor with planktivorous fish species) and, in particular, to the fishery with fyke nets (*cogolli*) (due to the mechanical occlusion of the nets)

- the molecular approach was crucial to identify meroplanktonic species (e.g. crab and fish larvae)

- **eDNA approach was applied for the first time to identify fish and metazoa of the Marano and Grado Lagoon**

- eDNA analysis was successfully applied to the Marano and Grado Lagoon: more than 30 fish species and *Mnemiopsis leidyi* were detected

- some fish species (e.g., *Allosa fallax*, *Anguilla anguilla*) have been detected only by metabarcoding

- in the perspective to implement a site-specific eDNA program in the Marano and Grado Lagoon, the DNA of missing species should be directly extracted and sequenced from lagoon specimens and added at the reference databases to increase its resolution

- the combination of molecular techniques with more traditional approaches can significantly **improve the assessment and the monitoring of the lagoon biodiversity and can be used for invasive species early warning**.

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