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R. DICIOTTI, J. CULURGIONI, S. SERRA, M. TRENTADUE, G. CHESSA, C. T. SATTA, T. CADDEO, A. LUGLIE, N. SECHI, N. FOIS

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First record of *Mnemiopsis leidyi* (Ctenophora, Bolinopsidae) in Sardinia (S'Ena Arrubia Lagoon, Western Mediterranean): a threat to local fishery?

R. DICIOTTI[1], J. CULURGIONI[1], S. SERRA[1], M. TRENTADUE[1], G. CHESSA[1], C.T. SATTA[2], T. CADDEO[2], A. LUGLIÈ[2], N. SECHI[2] and N. FOIS[1]

[1] Agricultural Research Agency of Sardinia. Ichthyc Products Research Service
S.S. Sassari-Fertilia Km 18,600, Bonassai, Olmedo, Italy
[2] Department of Architecture, Design and Urban Planning, University of Sassari, Via Piandanna 4, 07100 Sassari, Italy

Corresponding author: jculurgioni@agrisricerca.it

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Abstract

The invasive comb jelly *Mnemiopsis leidyi* is a lobate ctenophore native of coastal and estuarine waters in the temperate western Atlantic Ocean. Over the last decades, this species has expanded its range of distribution, colonizing marine and transitional environments in Europe. In October 2015, during a fishing survey concerning the European eel *Anguilla anguilla* in Sardinia (western Mediterranean), a massive bloom of this species was observed in the eutrophic S'Ena Arrubia Lagoon for the first time. In November 2015, two sampling tows of an Apstein net were conducted in the lagoon and the abundance of ctenophores was 2.83 ind. m^{-3}. All collected specimens were adults, measuring 18 - 62 mm total length. In addition, a fyke net was deployed for 12h in order to roughly estimate the impact of the ctenophores on the fishing activity. The high number of ctenophores (6837 individuals *per* fyke net) in the net suggests a negative effect on fishing operations by reducing catches and affecting the performance of fishing gears. Since there is no effective mechanical, chemical or biological method to counter *M. leidyi*, the short-term economic damage may be addressed by new fisheries management measures, as well as by reducing the eutrophication of the lagoon.

Keywords: Introduced species, comb jelly, eutrophic waters.

Introduction

The ctenophore *Mnemiopsis leidyi* Agassiz, 1865 is a macroplanktonic comb jelly, native of the temperate to subtropical estuaries of the western Atlantic Ocean, from Massachusetts, USA, to Argentina (Purcell *et al*., 2001; Ghabooli *et al*., 2013). Since the 1980s, this species has extended its distribution through coastal and estuarine environments of Europe, showing a high invasive behaviour with continuous reports in new localities (Costello *et al.,* 2012; Mianzan *et al.,* 2012). At present, *M. leidyi* is included in the International Union for Conservation of Nature (IUCN) list of the 100 most damaging invasive species in the world (Lowe *et al.,* 2000). It is also included in the marine Mediterranean list of alien species (Zenetos *et al.,* 2010) and in the "Black List" of 47 marine invasive species in the Mediterranean Sea (Otero *et al.,* 2013).

Costello *et al.,* (2012) reviewed the spreading pattern of *M. leidyi* in the Mediterranean basin: starting from the first reports in the Black Sea (1982), Azov and Marmara seas (1988). From there, the species reached the Eastern Mediterranean Sea, being reported in the Aegean Sea (1990), in coasts of Turkey (Mersin Bay, 1992), and in Syria (1993). Since the 2000s, the species was detected in the central and western Mediterranean: Adriatic Sea (2005), Gulf of Marseille (2006 and 2010), Catalunya (2009) and Mar Menor (Marambio *et al.,* 2013). In Italy, the species was recorded in Ligurian, Tyrrhenian and Ionian seas (Boero *et al.,* 2009). According to Ghabooli *et al.,* (2013), genetic divergence suggests a further colonization route from the Black Sea to the Caspian Sea (Ivanov *et al.,* 2000), as well as a third direct pattern, started in 2006, from north western Atlantic to the Baltic Sea (Reusch *et al*., 2010; Ghabooli *et al*., 2011).

The occurrence of *M. leidyi* in the Black and Caspian seas resulted in massive blooms, which resulted in dramatic consequences for the balance of the ecosystems and, particularly, for the fish stocks (GESAMP 1997; Ivanov *et al*., 2000; Dumont *et al*., 2004). Thus, all the reports of this species in marine or transitional systems such as ponds, lagoons, estuaries and gulfs, have to be taken into account for the related ecological implications and the potential effects on fisheries and tourism (Marambio *et al.,* 2013, 2014; Thibault *et al.,* 2014).

The most important vector favouring the spread of this invasive species seems to be the transport of specimens in the ballast waters of commercial ships (GESAMP 1997). The species finds suitable conditions in eutrophic waters, and it tolerates wide ranges of salinity, temperature and dissolved oxygen (Purcell *et al.,* 2001; Marambio *et al.,* 2013). Additionally, it actively preys on zooplankton, including eggs and larvae of various fish species (Purcell *et al.,* 1991, 2001). The wide environmental ranges of tolerance, the high abundance of preys in colonised waters, and the paucity of natural predators and competitors are deemed the most important factors favouring fast growth rates of *M. leidyi* populations (GESAMP, 1997; Purcell *et al.,* 2001; Oliveira, 2007). Furthermore, the severe decline of fish stocks, as a consequence of overfishing, may also led to the outburst of *M. leidyi* (Gucu, 2002; CIESM, 2014).

In the present paper, we report the first observation of *M. leidyi* in Sardinia (Italy, western Mediterranean Sea). The species gave rise to a massive bloom in S'Ena Arrubia Lagoon, located on the western coast of the island, during autumn 2015. Data on the abundance of the species were reported, and a rough estimate of the impact on fishing activities was conducted. In addition, environmental conditions, nutrients, chlorophyll *a* and phytoplankton data were acquired.

Materials and Methods

Study area

The S'Ena Arrubia Lagoon is located on the western coast of Sardinia, in the middle of the Oristano Gulf (Fig. 1). It extends within an area rich in islets and reed thicket, with high ecological, faunal and floristic value. S'Ena Arrubia Lagoon is included in the Ramsar list (code: IT016), it is a Special Protection Area for birds (SPA, code: ITB034001), a Site of Community Importance (SCI, code: ITB030016), and a 'Permanent oasis of wildlife protection and capture' according to the Sardinian regional law (LR 29/1998).

The lagoon represents the residual portion of a larger original brackish area (Sassu pond), which was dried up in the 1920s. At present, S'Ena Arrubia comprises a water body of about 1.50 km^2 and has an average depth of 70 cm (maximum 1.0 m). A single canal, protected by two breakwaters, connects the lagoon to the sea. The catchment area covers about 192 km^2 and is mainly used for intensive arable farming and cattle breeding. The St. Anna diversion canal drains most of the waters from the catchment area. In addition, another canal (named 'canal of shallow waters') collects the waters of the old Sassu pond and related hydrographic areas. These drained waters are pumped through a dewatering into the lagoon. S'Ena Arrubia Lagoon is classified as eutrophic and hypereutrophic and occasionally presents dystrophic crises (Sechi, 1982; Trebini *et al.,* 2005; Cannas *et al.,* 2008). The lagoon is exploited for fishing activities, including clam harvesting.

Sampling and processing

The first sampling in the S'Ena Arrubia Lagoon was conducted on October 21 2015, during the monitoring carried out in the Sardinian lagoons by the Agricultural Research Agency of Sardinia (AGRIS). The aim of this monitoring was to assess the local stock of European eel (*Anguilla anguilla* Linnaeus, 1758), and to evaluate the effects of the regional management plan of the species requested by the Council Regulation (European Community) No 1100/2007. As pointed out by the local fishermen (St. Andrea Cooperative), a large number of ctenophores were observed in the water and, in particular, inside the fyke nets. These gears consist in cylindrical netting bags mounted on rings. They have wings or leaders guiding

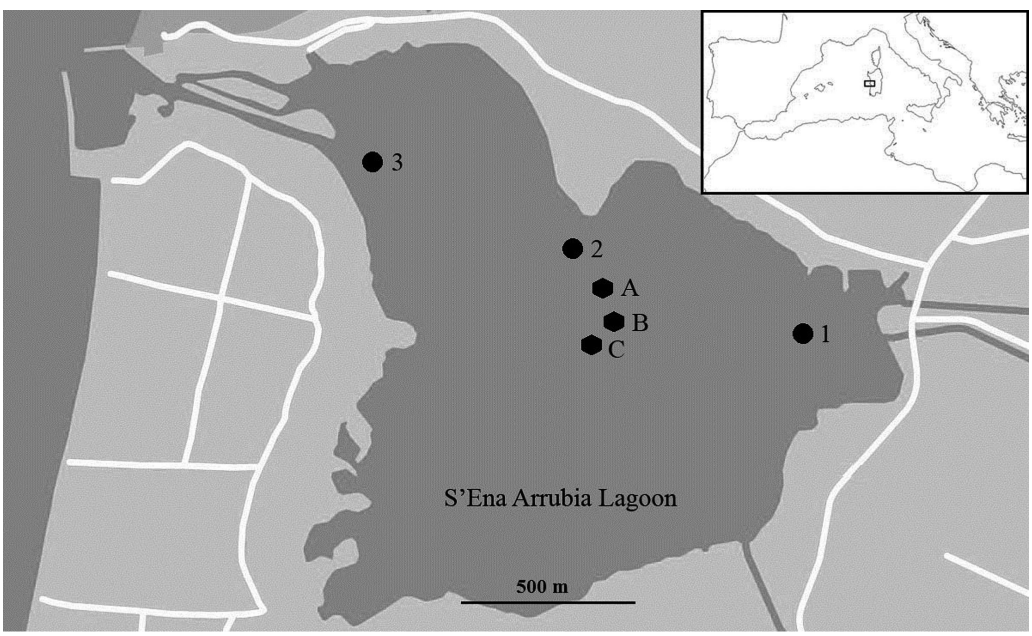

Fig. 1: S'Ena Arrubia Lagoon and localization of sampling stations. Letters, sampling points of net samples; numbers, sampling stations of environmental variables.

the fish towards the entrance of the bags. The fyke nets are fixed onto the bottom by stakes (FAO, 2016) (Supp. data Fig. S1). During the first sampling, 10 specimens were collected in plastic bottles and transported to the laboratory for the taxonomic identification, according to morphological descriptions available in the literature (Greve, 1975; Shiganova & Panov 2006; Oliveira, 2007).

The second sampling was performed on November 3 2015. Three sampling points were fixed by a Garmin eTrex GPS and named A (39.824200° N; 8.567533 E°), B (39.823133° N; 8.567350° E), and C (39.823633° N; 8.567750° E) (Fig. 1). Two samples were collected towing an Apstein conic net (mesh size 200 µm), equipped with a flowmeter, between points A and B (route distance: 90 m), and points B and C (route distance: 80 m), at a depth of 0.50 m. The ctenophores collected in the two tows were immediately counted to estimate the abundance according to the filtered volumes of water. The total length (TL: oral-aboral length including lobes) of the ctenophores was measured *in vivo* with a digital calliper, and the weight was obtained through a Radwag WTB2000 scale. The content of one fyke net, with a capacity of about 0.2 m^{-3} (placed near the sampling point B), was examined after 12 hours of fishing in order to roughly evaluate the impact of ctenophores on fishing activities. Firstly, the net was emptied into an appropriate tank (Supp. data Fig. S2) and the total volume and weight of the bulk of specimens were determined. Subsequently, the number of individuals present in the fyke net was obtained by dividing the total weight and the average weight *per* individual. The average weight *per* individual was obtained by measuring 30 specimens. The number of ctenophores in the fyke net was then calculated. Fifty specimens were transported to the laboratory for further taxonomic evaluations. The ctenophores were put in tanks with aerated sea water, and fed with *Artemia salina* (Linnaeus, 1758) nauplii.

Environmental conditions, chlorophyll a, nutrients and phytoplankton

Temperature (T), conductivity (Cond), salinity (Sal), dissolved oxygen (DO), pH, and redox potential (Eh) values were obtained *in situ* using a multiparameter probe (Ocean Seven 316CTD Idronaut) from three sampling stations located along an axis from the outlet of the 'shallow water canal' to the sea mouth (Fig. 1): station 1 (39.823033 N°; 8.573217° E), near the outlet; station 2 (39.824833 N°; 8.566300 E°) in the central part of the lagoon, in proximity of the abovementioned sampling points; station 3 (39.827083 N°; 8.559933 E°), near the inlet of the lagoon. Samples for chlorophyll *a* estimation, nutrients and phytoplankton analyses were collected from the same three sampling stations. The concentrations of nitrate ($N–NO_3$), ammonium ($N–NH_4$), and nitrite ($N–NO_2$), reactive ($P–PO_4$) and total phosphorous (P–Ptot), total nitrogen (N–Ntot), and reactive silica ($Si-SiO_4$) were determined according to Strickland & Parsons (1972), using a 200 DMS (Varian) spectrophotometer. The sum of nitrate, ammonium, and nitrite was reported as dissolved inorganic nitrogen (DIN). Chlorophyll *a* was determined by spectrophotometer analysis following the UNESCO protocol (1997). Phytoplankton samples were fixed in Lugol's solution and analysed with the Utermöhl's technique (1958), using an inverted microscope (Zeiss, Axiovert 10), after the sedimentation of variable volumes of water (5–10 mL), depending on phytoplankton density. Cell counts were made at a magnification of 100X on the entire bottom of the sedimentation chamber for the larger and more easily identifiable species, and at magnifications of 200X, and 400X in an adequate number of fields for the smaller cells. The species were determined according to the literature listed in Pulina *et al.* (2012).

Results

Morphology and abundance

The body of *M. leidyi* individuals from the S'Ena Arrubia Lagoon was oval in shape, transparent and laterally compressed (Supp. data Fig. S3). Papillae were absent, and the four auricles and two oral lobes originated at the level of the statocyst.

Three *M. leidyi* specimens were collected with the Apstein net in the first transect (from point A to B), and 28 in the second (from point B to C), resulting in an overall abundance of 2.8 ctenophores m^{-3} in the lagoon. The mean body mass was 4.71 g (minimum-maximum range of 2.05-7.31 g, n=30), the mean total length was 45 mm (minimum-maximum range of 18-62 mm, n=30) (Supp. data Table 1). The fyke nets were completely filled by thousands of ctenophores; the estimated total number of ctenophores in the fyke net (whose capacity was 0.2 m^{-3}) was 6837, and the total gelatinous mass filling the fyke net was 32.2 kg.

Environmental conditions, nutrients and phytoplankton

Temperature and salinity values during the sampling ranged from 17.84 °C (Station 2) to 18.32 °C (Station 3) and from 23.39 (Station 1) to 33.44 (Station 3), respectively. The water saturation in dissolved oxygen ranged from 86.57% (Station 3) to 90.83% (Station 2) (Table 1).

DIN values ranged from 10.8 µM (Station 3) to 52.5 µM (Station 1). The most important nitrogen form was $N-NH_4$ (Table 1). P-PO4 and P-Ptot ranged respectively from 0.1 µM (Station 3) to 2.8 µM (Station 1) and from 3.4 µM (Station 3) to 5.8 µM (Station 1).

Chl *a* minimum value (7.8 mg m^{-3}) was recorded at Station 3, and the maximum value (17.0 mg m^{-3}) at Station 2. Total phytoplankton density values showed the same pattern, with the minimum (29 x 10^6 cells L^{-1}) recorded at Station 3 and the maximum value (47.2 x 10^6

Table 1. Environmental, nutrients, chlorophyll *a* and phytoplankton data, means and standard deviations (SD).

	Station 1	Station 2	Station 3	Mean	±SD
Tem (°C)	18.1	17.8	18.3	18.1	0.2
Cond (mS cm^{-1})	32.0	37.6	44.4	38.0	6.2
Sal	23.4	28.1	33.4	28.3	5.0
DO (%)	90.5	90.8	86.6	89.3	2.4
DO (mg l^{-1})	7.4	7.3	6.7	7.1	0.4
pH	8.6	8.8	8.9	8.8	0.2
Eh (mV)	283.7	156.8	139.8	193.4	78.6
DIN (µM)	52.5	25.1	10.8	29.5	21.2
N-NO_3 (µM)	24.7	9.7	4.0	12.8	10.7
N-NH_4 (µM)	25.4	14.1	6.1	15.2	9.7
N-NO_2 (µM)	2.4	1.4	0.7	1.5	0.9
P-PO_4 (µM)	2.8	0.4	0.1	1.1	1.5
P-Ptot (µM)	5.8	5.6	3.4	5.0	1.3
N-Ntot (µM)	82.7	62.0	41.6	62.1	20.6
Si-SiO_4 (µM)	127.3	72.2	36.0	78.5	46.0
Chl *a* (mg m^{-3})	9.9	17.0	7.8	11.6	4.8
Total density (cells x 10^6 L^{-1})	29.0	47.2	39.2	38.5	29.0
Bacillariophyceae (cells x 10^6 L^{-1})	5.1	2.2	1.1	2.8	5.1
Undetermined rounded cells <5µm (cells x 10^6 L^{-1})	21.8	44.3	36.5	34.2	21.8
Others (cells x 10^6 L^{-1})	2.1	0.8	1.6	1.5	2.1

cells L^{-1}) at Station 2 (Table 1). Phytoplankton was dominated at the three stations, by small (<5 µm) not flagellate rounded cells, which was impossible to determine neither at Class level (Table 1).

Discussion

Mnemiopsis leidyi is reported in the western Mediterranean mainly in coastal waters (Boero *et al.,* 2009; Fuentes *et al.,* 2010), but the most recent observations include transitional environments, such as lagoons (Marambio *et al.,* 2013; Thibault *et al.,* 2014). The presence of *M. leidyi* in the S'Ena Arrubia Lagoon represents the first report in Sardinia and the first report of the species in a transitional ecosystem along the Italian coasts.

Morphology, abundances and environmental conditions

The morphology of *M. leidyi* from S'Ena Arrubia Lagoon matches the one reported in literature (Greve, 1975; Shiganova & Panov 2006; Oliveira, 2007; Otero *et al.,* 2013), showing the two oral lobes originating at the statocyst level. This feature is a characteristic one, since it distinguishes *M. leidyi* from the closest species *Bolinopsis vitrea* (L. Agassiz, 1860), the oral lobes of which originate in the middle of the body (Öztürk *et al.,* 2011). The total length of specimens collected in the S'Ena Arrubia Lagoon in autumn 2015 is in the same range as those from the Mar Menor Lagoon (Marambio *et al.,* 2013), but smaller than those from the Black Sea, which can grow up to 180 mm TL (Shiganova *et al.,* 2004).

The population abundance of 2.8 ctenophores m^{-3} observed in the present study is comparable to the maximum value reported in the Mar Menor Lagoon (1.16 ctenophores m^{-3}; Marambio *et al.,* 2013), and much lower than those reported in the the Berre Lagoon (23 ctenophores m^{-3}; Thibault *et al.,* 2014). Temperature and salinity values detected in the S'Ena Arrubia Lagoon in autumn 2015 are within the range of those reported from invaded Mediterranean and worldwide localities (Costello *et al.,* 2012). Further, the high nutrient availability and the notable chlorophyll *a* concentrations describe an environmental scenario that appears adequate to intensive development of *M. leidyi* (Costello *et al.,* 2012).

Impact on fishery and management and mitigation proposals

Our results highlight the probable impact of *M. leidyi* on fishing activities in the S'Ena Arrubia Lagoon in Autumn 2015. In fact, the presence of thousands of ctenophores in the fyke net blocks the flow of water, making nearly impossible to capture fish (European eel). Moreover, this condition may determine the death of the fish inside the fyke nets (fishermen communication), probably because of the formation of an anoxic microenvironment and the mechanical pressure against the fishing net, both due to the clogging of the gear. Fishermen's activity is also extremely burdened by gelatinous mass, which complicates the operations of emptying the fishing gear. Furthermore, the massive accumulation of the gelatinous mass near the fishing barricades may affect the lagoon-sea exchanges. According to the fishermen's reports, the masses of *M. leidyi* clogging the fyke nets affected the European eel fishing activities in the S'Ena Arrubia Lagoon in autumn 2015. This aspect, accurately discussed by Palmieri *et al*. (2014) concerning the jellyfish blooms in Northern Adriatic, underlines the potential socio-economic harmfulness of this species.

Mnemiopsis leidyi may generate other environmental and economic damages in addition to the negative effects reported by the fishermen in the S'Ena Arrubia Lagoon. In fact, the predatory activity of *M. leidyi* on zooplankton may result in the reduction of veliger larvae of bivalves (see Purcell *et al.,* 1991) and of juvenile fish, as observed during invasions in the Black, Caspian and Baltic seas (Ivanov *et al.,* 2000; GESAMP, 2007; Kube *et al.,* 2008).

Thus, a constant monitoring activity of *M. leidyi* is needed to obtain data about the population dynamics of this species within the S'Ena Arrubia Lagoon and the nearby coastal area. Furthermore, a deeper study of the phenomenon, including the ability of the species to develop its entire life cycle in these western Mediterranean coastal environments, is required (Boero *et al.,* 2009). Indeed, the described outbreak of *M. leidyi* in the S'Ena Arrubia Lagoon during autumn 2015 may be an outstanding event, but it cannot be overlooked in light of the effects attributed to the proliferation of this species in the Caspian and Black seas (GESAMP, 1997; Purcell *et al.,* 2001). It would be appropriate to quantify to what extent such abundance of *M. leidyi* in the S'Ena Arrubia Lagoon might negatively interfere with fishing.

Currently, there are no effective methods to counter *M. leidyi* using mechanical removal and chemical treatments (Shiganova & Panov, 2006) or biological approaches that do not involve other potential risks, like the introduction of alien species such as the ctenophore *Beroe ovata* Bruguière, 1789 (an invasive species), and other predatory comb jellies (GESAMP, 1997; CIESM, 2014). Rather, a reduction of the eutrophic conditions can mitigate the probability of a stable settlement of *M. leidyi* in the S'Ena Arrubia lagoon. In fact, as reported by Shiganova *et al.* (2004), the low prey (zooplankton) density under oligotrophic conditions (i. e. of the Aegean Sea) limits the creation of semi-reproductive populations of *M. leidyi*. The reduction of the eutrophic condition in the S'Ena Arrubia Lagoon, as already suggested by Trebini *et al*. (2005), might reduce the abundance of *M. leidyi* in the lagoon and its potential spread into nearby lagoons (mitigation action).

The assessment of impacts may require management actions, e.g. the adaption of the fishing calendar during the presence of ctenophore. The combination of adaptive and mitigation actions (see Graham *et al*., 2014) will be essential to reduce the potential consequences on fisheries productivity in the short and long-term scales.

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