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Toxic invasive pufferfish (Tetraodontidae family) ~~in the Mediterranean Sea and~~ along Italian coasts: ~~data collection and analysis for the~~ assessment of an emerging public health risk

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Abstract

Several pufferfish species belonging to the Tetraodontidae family are currently present in the Mediterranean Sea. Since 2013 *Lagocephalus sceleratus* is increasingly reported also along Italian coasts, where other two species of less concern, *Lagocephalus lagocephalus* and *Sphoeroides pachygaster*, also occur since a long time. Thise ~~present~~ study represents one of the first attempts to describe the occurrence in the Mediterranean, and in particular along Italian coasts, of the three aforesaid species ~~collect and integrate all the available data on the occurrence of these three species in Mediterranean and Italian waters~~, in order to characterize ~~assess~~ an emerging public health hazard ~~and characterize its implications for public health~~. Reports were searched in scientific articles and dedicated online databases. The following data were collected: number of specimens and type of record, geographical location and date of report, fish size, depth and type of seabed. Overall, at least 111079 individuals of the three species were found in the Mediterranean Sea, including 110237 specimens of *L. sceleratus* (since 2003), 126 of *L. lagocephalus* (1878-2017) and 716 of *S. pachygaster* (1979-2017). The evident differences confirm the invasive character of the toxic Lessepsian immigrant *L. sceleratus*, the species of main public health concern. Despite this species was recorded along Italian coasts in low numbers (0.08% of the total individuals of *L. sceleratus*) and its distribution is limited to southern regions, the picture could change rapidly. Moreover, the collected data show that most of *L. sceleratus* specimens have a large size and are mainly caught by commercial fishing gears. These factors may increase the risk of this species entering the seafood chain with serious consequences for consumers' health. Results suggest that the presence of *L. sceleratus* should be strictly monitored. Institutional measures should be implemented to inform people as regards a new hazard that has the potential to affect the Italian seafood chain.

Keywords

Lagocephalus sceleratus, *Lagocephalus lagocephalus*, *Sphoeroides pachygaster*, emerging risk, food safety

1. Introduction

Biological invasions of exotic species represent an issue of increasing concern worldwide. Although natural migrations do occur, biological invasions result from human activities (Katsanevakis et al., 2014). Anthropogenic alterations, such as the break-down of geographical barriers, together with the consequences of climate change, have boosted the spreading of animal species and pathogens in new environments (Kalogirou, 2013). The scale of this phenomenon is well represented in the Mediterranean Sea , one of the regions most severely affected by alien marine invasions (Zenetos et al., 2012), where. In this area the diffusion of Indo-Pacific fish species entering through the Suez Canal ("Lessepsian" migration) is favoured by the consequences of global warming (Lejeusne, Chevaldonné, Pergent-Martini, Boudouresque, & Pérez, 2010), leading to a process of tropicalization (http://www.fpa2.org/downloads/GB_232.pdf).

This changing ecosystem has received attracteda strong interest from the scientific community and international organisations in monitoring and assessing the impact of alien species on local the native ecosystems and on local economies, investigating the causes and patterns of alien introductions, and proposing management measures (Andaloro et al., 2016; Beköz, Beköz, Yilmaz, Tüzün, & Beköz, 2013; Katsanevakis, Tsiamis, Ioannou, Michailidis, & Zenetos, 2009; Ben Souissi et al., 2014). Efforts have been made to assemble fragmentary information on the distribution of alien species in the Mediterranean Sea (Bazairi et al., 2013; Enajjar, Ben Hadj Hamida1, Saadaoui, & Bradaï, 2015; Katsanevakis et al. 2009; Peristeraki, Lazarakis, Skarvelis, Georgiadis, & Tserpes, 2006). In this regard the Reg. (EU) No 1143/2014 on the prevention and management of the introduction and spread of invasive alien species has also been recently issued.

Besides threatening biodiversity, these modifications may impact on food safety in different ways (Marques, Nunes, Moore, & Strom, 2010), such as in the case of the diffusion of pufferfish species belonging to the (Tetraodontidae family ("pufferfish") (Nader, Indary, & Boustany, 2012). In fact, Tetraodontidae are in fact able to accumulate tetrodotoxin (TTX) in their tissues, a potentially lethal thermostable neurotoxin of exogenous origin, in their tissues (EFSA CONTAM

Panel 2017). Despite this, some pufferfish species are traditionally consumed in Asia ~~as a delicacy~~. As a consequence, ~~the~~ TTX intoxications ~~with TTX is~~are mainly diffuse in Asian coastal areas (Islam et al., 2011), while cases ~~of intoxication~~ outside these areas are much rarer and generally due to imported pufferfish species fraudulently commercialized, as reported in California and Hawaii (Coehn et al., 2009). ~~In the past,~~ ~~S~~similar episodes have also been described in Italy in the past, when 10 people were intoxicated in Rome and Jesolo (Venice) in 1977, after the consumption of toxic Tetraodontidae from Taiwan, ~~that had been~~ mixed with batches of monkfish (*Lophius piscatorium*) (Pocchiari, 1977), and in 1978 in Pavia with 3 intoxicated people (Viviani, D'Alessandro, Manzo, & Crema, 1978).

The recent spreading of pufferfish species in the Mediterranean region has changed the picture (Kosker et al., 2016; Rambla-Alegre et al., 2017), introducing a new public health issue ~~for public health~~. At least eleven species of the family Tetraodontidae are reported in the Mediterranean Sea: *Lagocephalus guentheri, L. spadiceus, L. lagocephalus, L. suezensis, L. sceleratus, Torquigener flavimaculosus, Tylerius spinosissimus, Sphoeroides spengleri, Sphoeroides pachygaster, Sphoeroides marmoratus* and *Ephippion guttiferum* (Farrag, El-Haweet & Moustafa, 2016; Irmak et al., 2015). Only ~~T~~three of these pufferfish are currently present in waters surrounding Italy~~:, namely~~ *L. sceleratus* (Silver pufferfish or Silver-cheeked toadfish or Silverstripe blaasop), *L. lagocephalus* (Oceanic pufferfish) and *S. pachygaster* (Blunthead pufferfish). A report of a fourth species, *S. marmoratus* (Guinean puffer), is also present, but it was only found once, in 1977 in Gallipoli, Lecce (Apulia), when it was erroneously identified as *L. lagocephalus* (Vacchi, Bussotti, Miglietta, & Guidetti, 2007). Among them, the species of main concern is *L. sceleratus*, a highly invasive and toxic Lessepsian migrant arrived in the Mediterranean basin in 2003 from the Red Sea (Akyol, Ünal, Ceyhan, & Bilecenoglu, 2005). ~~Cases of I~~intoxications due to the consumption of this species ~~have~~ occurred in countries of the Eastern Mediterranean (Beköz et al., 2013; Bentur et al., 2008; Chamandi, Kallab, Mattar, & Nader, 2009; Eisenman, Rusetski, Sharivker, Yona, & Golani, 2008; Kheifets, Rozhavsky, Girsh Solomonovich, Marianna, & Soroksky, 2012). As a consequence,

interventions aimed at preventing the consumption of pufferfish species as those already organized in other countries bordering the Mediterranean Sea (Andaloro et al., 2016; Kalogirou, 2013; Ben Souissi et al., 2014) ~~have~~ are assuming~~ed~~ increasing relevance also in ~~in the Mediterranean region~~Sea Italy.~~. Informative initiatives have recently been organized in various countries to raise awareness on this issue (Andaloro et al., 2016; Kalogirou, 2013; Ben~~ Souissi et al., 2014).

The aim of this work, developed in the framework of a project funded by the Italian Ministry of Health, was to ~~collect data~~ describe ~~on~~ the occurrence in Mediterranean, and in particular along Italian coasts ~~Italian waters~~, of the three pufferfish species (*L. sceleratus*, *L. lagocephalus* and *S. pachygaster*) ~~currently reported as present along Italian coasts (*L. sceleratus*, *L. lagocephalus* and *S. pachygaster*)~~ in order to characterize their different geographical distribution and frequency. This study represents one of the first attempts to characterize an emerging and less-known hazard still marginally affecting Italy and assess its implications for public health. Finally, it lays down the basis for a proper communication to all the actors involved in the seafood chain.

2. Materials and methods

2.1 Collection of reports in Mediterranean and Italian waters

~~In order to investigate the risk associated to the presence of~~ In this study, focused on the presence of the three-pufferfish species along Italian coasts, their reports in other areas of the Mediterranean Sea were also collected to have a more precise picture of their distribution pattern. Reports of *L. sceleratus*, *L. lagocephalus* and *S. pachygaster* ~~, reports of these three species~~ in Mediterranean and Italian waters were searched ~~by analysing data from~~in scientific articles ~~which were~~ retrieved from Web of Science, Pubmed and Google Scholar using different combinations of the following keywords: Mediterranean Sea, Italy, pufferfish, Tetraodontidae, invasive alien species (IAS), toxic fish species, *Lagocephalus*, *Sphoeroides*, *Lagocephalus lagocephalus*, *Lagocephalus sceleratus*, *Sphoeroides pachygaster*, Lessepsian species, tropicalization, tetrodotoxin intoxication. Only papers describing the report in the Mediterranean Sea ~~of one of the three above mentioned species~~, indicating ~~ed by its~~ the scientific name of one of the three above mentioned species, ~~with~~ a

date of report (at least the year) and a place of report (at least the country), published in English, French, Italian ~~and~~ or Spanish, were included. The reference list of the selected articles was also screened for other eligible records.

A number of online databases was also analysed: Seawatchers (http://www.observadoresdelmar.es/index.php), AquaNIS (http://www.corpi.ku.lt/databases/index.php/aquanis/search/search_result/for/ie/sid/7679), Marine Mediterranean Invasive Alien Species (http://www.mamias.org), EASIN - European Alien Species Information Network (https://easin.jrc.ec.europa.eu/Services/SpeciesSearch). In addition, annual reports of the ICES Working Group from 2003 to 2017 (available online) were consulted.

The following data were collected for each record: number of specimens and type of record (caught, observed or found stranded), geographical location and date of report, fishing method (commercial/recreational), fish size (length and/or weight), depth and type of seabed. The highest available level of detail was registered while collecting these ~~above listed~~ data. However, not all of them ~~data~~ were present for all the records. In particular, a precautionary approach was followed when the specimens' number was not mentioned, and a single specimen was assigned to the record. In a few cases, when the available data suggested the record of more than 1 specimen (e.g. if two fishing gears or a total length range were indicated) 2 specimens were counted. In other cases, when the number of specimens was expressed as a range, the average was used in the calculation.

2.2 Data analysis

The collected data were used to calculate the overall number of specimens recorded, the range of specimens per record, the type of record and the fishing gear used, the ratio between the n of specimens recorded in Italian regions and the overall number in the Mediterranean, the mean total length (TL) and the TL range, the mean weight and weight range, the mean depth of catch or observation, the depth range and the most commonly reported seabed. On the basis of these data, the geographical distribution and the annual and monthly distribution were also described. Due to the high variability in data presentation and aggregation found in the analysed sources and to the

different level of given details, the number of specimens (n) used for the calculation of each of the aforesaid parameters was different. Records from waters around Italy were used for producing an updated map of their distribution.

3. Results

The main limitations of our study regards the nature of observations, which are generally occasional and not deriving from systematic searches. Overall, 111079 records of ~~*L. sceleratus, L. lagocephalus* and *S.* pachygaster~~the three species of interest were found in different areas of the Mediterranean Sea, reporting~~. In particular,~~ at least 110237 specimens of *L. sceleratus* (since 2003), 126 of *L. lagocephalus* (since 1878) and 716 of *S. pachygaster* (since 1979)~~ in different areas of the Mediterranean Sea were registered~~. In Italy, the overall number was 687 (92 *L. sceleratus*, 30 *L. lagocephalus*, 565 *S. pachygaster*) (Table 1).

~~The main re are some limitations to our data and approach are, mainly due to the nature of observations, which are generally occasional and not deriving from systematic searches. However,~~ ~~b~~By comparing the number of specimens of the three species ~~found~~ reported from 2003 to date, an evident difference was observed between the frequency of *L. sceleratus* (n=110237, 99.2% of the total number of records) and those of *L. lagocephalus* (n=111, 0.1%) and of *S. pachygaster* (n=104, 0.1%). A summary of ~~all the data collected~~the results obtained after the analysis of the data reported for the three species is presented~~reported~~ in Table 1.

3.1. Lagocephalus sceleratus

Of the total 110237 specimens reported from 2003 ~~to date~~ in the Mediterranean Sea, 110214 specimens were retrieved from reports indicating the exact number of specimens caught or observed, while in 21 reports the precise number was not available. In these cases, 1 or 2 specimens were assigned to each record depending on the available details (as described in section 2.1), for a total of at least 23 individuals. The number of individuals observed or caught per single event varied from 1 to more than 50 (in 7 cases). However, in the case of articles reporting the catch or observation of a higher number of specimens over several months or years, it was not possible to

assess the exact number of individuals per event (Table S1). Data on the type of record~~, total length, weight~~fish size, ~~and~~ depth of capture/observation and type of seabed are reported in Table 1. ~~was available for 110214 individuals (99.9%): most of the specimens had been caught (N=109594, 99.4%), while only a minority of reports described observed specimens (N=620, 0.6%). As regards the caught specimens, more than one fishing gear was often reported, so precise data could be collected only for 1260 specimens (1.1%): of these, the large majority (99.6%) was caught with professional commercial fishing instruments, while only the few remaining specimens were caught with amatorial gears such as fishing rods and spear guns (Table 1).~~

As concerns the geographical distribution (reported in detail in Table S1), the first records in the Mediterranean Sea date back to 2003 and occurred in the Southern Aegean Sea in front of Turkey (Akyol et al., 2005; Filiz and Er, 2004). The first confirmed Italian finding took place in Lampedusa in 2013 (Azzurro, Castriota, Falautano, Giardina, & Andaloro, 2014). Subsequently, other specimens were fished in 2013 and 2014 along the Apulian coasts in Bari and then again in Sicily (Andaloro et al., 2016). In 2014 a specimen was caught in front of Trani (Apulia) (Carbonara et al., 2017) and another one was found for the first time in the Ionian Sea (Tiralongo & Tibullo, 2014). Other 2 specimens were found in Calabria in 2016 (http://www.observadoresdelmar.es/observacio-detall.php?projecte_id=9&id=5373; http://www.isprambiente.gov.it/it/news/primo-esemplare-di-pesce-palla-maculato-nel-mar-ionio). The last Italian record occurred in Molfetta (Bari) on the 24th of October 2017 (http://www.ansa.it/canale_ambiente/notizie/animali/2017/10/24/pescato-un-pesce-palla-maculato-ispra-avverte-su-tossicita_c651b82d-1425-4823-ba54-1ae100f3f540.html). Thus, currently, *L. sceleratus* is known to be present along the coasts of Sicily, Apulia and Calabria. The geographical distribution of Italian records is visible in Fig. 1. ~~Overall, 12 specimens have been caught and 80 observed in Italian waters since 2013, representing around 0.08% of the total specimens of *L. sceleratus* included in this study. Currently, *L. sceleratus* is known to be present along the coasts of the following regions: Sicily, Apulia and Calabria. The geographical distribution of Italian records is visible in Fig. 1.~~

~~As regards the annual distribution in the Mediterranean, reports were present for every year since 2003. A peak of reports was observed in 2009-2010 with a total of 105264 individuals reported by Michailidis, (2010) and Rousou, Ganias, Kletou, Loucaides, & Tsinganis, (2014) around Cyprus. A second peak was observed in 2012, when 1832 specimens were reported. Most of them (1736) were caught along the Egyptian coast and reported by Farrag, El-Haweet, & Moustafa, (2016). A large number of individuals was also reported in 2015 and 2016 (450 and 206 respectively), mainly from Greece, Tunisia and Lybia (Table S1 SM).~~ The monthly distribution~~,~~ ~~only evaluated for~~of records along Italian coasts~~,~~ showed that most of the records occurred in Autumn and Winter (Table S1).

~~Data on the total length (TL) was available for 55042 specimens (49.9% of the total specimens recorded in this study), but for the most part of them only a TL range was indicated. The overall TL range was 50-830 mm. The average length, calculated only on the subjects for which single TL measurements were available (n=70, 0.06%), was 442.67 mm (SD 115.03 mm). Similarly, data on the weight was reported for 51963 specimens (47.1%), but for many them only a range was indicated. The overall weight range was 5-5600 g (n=51963), while the average weight was 1821.9 g (SD 1268.3) (n=59, 0.05%) (Table 1).~~

~~The overall depth range varied from 2 to 400 m (n=64903) and the mean depth was 23.9 ± 18.1 m (n=59) (Table 1). Specific information on the seabed type, available for 61874 individuals (56.1%), are detailed in Table 1.~~

3.2 Lagocephalus lagocephalus

Totally, at least 126~~7~~ specimens were reported from 1878 to date in the Mediterranean Sea. Of these, 122~~3~~ were retrieved from reports indicating the exact number of specimens, while for 4 reports the precise number of specimens was not available (1 specimen was assigned to each record). The number of individuals per single catch or observation varied from 1 to 3 (which were found only in 3 cases). Data on the type of record, ~~total length, weight~~fish size, ~~and~~ depth of capture/observation and type of seabed are reported in Table 1. ~~Data on the type of record was~~

~~available for 123 specimens: the great majority of the individuals were caught (n=116, 94.3%), while the remaining were found stranded (n=7, 5.7%). Commercial fishing gears were mainly indicated (86.1%). Less common fishing gears were: fishing rod (7.6%), *charfia* (a traditional Tunisian fishing method, 5.1%) and small hand net (1.3%) (Table 1).~~

The species has long been known from around Sicily (Doderlein, 1878-1879) and along the Algerian coasts (Dieuzeide, Novella, & Roland, 1955). A complete list of the records of this species in the Mediterranean Sea is presented in Table S2. ~~Overall, 30 of the 1276~~ specimens ~~(23.86%) were reported from waters surrounding Italy. Of these~~As concerns the 30 Italian records~~,~~ 23 specimens (20 caught and 3 found on the beach) were found between 1999 and 2004. All except one (reported from Calabria) were found in Sicilian waters (Zava et al., 2005). Other 5 specimens were found in Calabria between 2007 and 2012, between October and March (Sperone, Paolillo, Circosta, & Tripepi, 2012). The most recent Italian reports occurred in February 2014, when a specimen was found in the waters surrounding Elba Island (Tuscany, Italy) (http://www.aiam.info/index.php/component/content/?view=featured&start=15) and in February 2015 when another specimen was found in the Gulf of Orosei, Sardinia (http://lanuovasardegna.gelocal.it/regione/2015/02/03/news/pesca-a-sorpresa-uncapolepre-1.10794873). ~~Italian records represent 24.4% of the total Mediterranean records included in the study.~~ Currently, the coasts of Sicily, Calabria, Tuscany and Sardinia~~the following regions~~ are interested by the presence of *L. lagocephalus*~~: Sicily, Calabria, Tuscany and Sardinia~~ (Fig. 1). Italian records represent 24.4% of the total Mediterranean records included in the study.

~~The annual distribution of reported specimens is quite variable: in most of the years 1 to 6 specimens were reported. The highest number of specimens was found in 2004 (n=31) followed by 2012 (n=21), 2014 (n=21) and 2003 (n=16).~~ The monthly distribution~~, only evaluated for~~ of records along Italian coasts~~,~~ showed that most of the records occurred in Autumn and Winter, as ~~shown~~ for *L. sceleratus*.

~~The overall TL range was 50-765 mm (n=97), the average length was 536.5 mm (SD 83.0 mm) (n=77, 60.6%). The overall weight range was 100.5-3783 g (n=72, 57.6%), while the average weight was 1618.23 g (SD 687.93 g) (n=72) (Table 1). The depth of capture or observation ranged from 1 to 200 m. The average depth was 25.12 m (SD 33.5 m, n=8, 6.3%). The type of seabed, described as sandy, was mentioned only in the report of 2 specimens from Libya (Table 1).~~

3.3 Sphoeroides pachygaster

Totally, at least 716 individuals were recorded from 1979 to date in the Mediterranean Sea. For 712 of them (99.4%) the exact number was indicated in the report, while for 4 reports (0.6%) the precise number was not available. The number of individuals per each catch or observation generally varied from 1 to 3. However, as already described for *L. sceleratus*, in some cases it was not possible to assess the exact number of individuals per event (Table S3). Most of the fishes had been caught (n=694, 99.7%), only 1 was reported as observed in front of Reggio Calabria, Italy (Costa 2004) while another one was found on the beach of Torremezzo di Falconara, Calabria, Italy (Visentin & Borg, 2014). Data on the type of record, fish size, depth of capture/observation and type of seabed are reported in Table 1.

~~As concerns the catch method, the most frequently indicated fishing gear was trawl (99.0%), only in a few cases gill nets (0.8%) or bottom line (0.2%) were used (Table 1).~~

The first record of this species, initially erroneously identified as *Sphoeroides cutaneus*, occurred in Cala Ratjada, Maiorca, Western Mediterranean, in 1979 (Oliver, 1981; Visentin & Borg, 2014). A complete list of records of *S. pachygaster* in the Mediterranean Sea is reported in Table S3. As specifically regards Italy, specimens were found in the Ligurian sea (Barletta & Torchio, 1986; Fiorentino & Zamboni, 1990; Ragonese, Jereb, & Morara, 1992), in the south of Sardinia (Vacchi & Cau, 1986) and around Sicily (Arculeo, Riggio, & D'Anna, 1994; Ragonese, Jereb, & Morara, 1997; Vacchi & Cau, 1986). Other records occurred from the southern Adriatic (Bello, 1993), the Ionian Sea (Tursi, D'Onghia, & Matarrese, 1992;), the Elba Island (Bedini, 1998; Ligas, Sirna, & Sartor 2006) and Anzio, Latium (Psodomadakis, Ceddia, & Vacchi, 2008). More

recently, a specimen was caught in the Strait of Messina (Giordano et al., 2012) and another was found in Calabria on Falconara Albanese (CS) beach at Torre Mezzo (Visentin & Borg, 2014). ~~Italian records (n=565) represent 79.2% of the total Mediterranean records of the species.~~ Currently, the following regions are interested by the presence of *S. pachygaster*: Sicily, Sardinia, Apulia, Calabria, Liguria, Latium and Tuscany (Fig. 1). ~~As regards the annual distribution a peak of reports was found in 1992 and 1993: in addition to the 25 specimens found in 1992, and to other 14 in 1993, Bello (1993) reported 120 individuals between 1992 and 1993 (which could not be assigned to a specific year). Moreover, 403 specimens were reported between 1990 and 1994 (Ragonese et al., 1997).~~ The monthly distribution, available for only 13 of the 550 records along Italian coasts (2.4%) did not show a seasonal pattern.

~~The overall TL range was 85-765 mm (n=493), the average length was 283.4 mm (SD 127.6 mm) (n=76, 10.6%). The overall weight range was 41.9-2640 g (n=54, 7.6%), while the average weight was 1159.4 g (SD 708.1 g) (n=37, 5.2%). As concerns the depth of capture, for most of the records a range was indicated (30-130 m for approximately 120 individuals reported by Bello, 1993; 50-250 m for 403 individuals reported by Ragonese et al., 1997). The depth range, calculated on all the data (n=621), varied from 22 to 400 m (n=621), while the mean depth, calculated on 42 precise measures was 151.2 m (± 93.8 SD). (Table 1). Details on the type of seabed, available for 21 specimens, are in Table 1. In addition, for the 120 specimens reported by Bello, (1993) the type of seabed was indicated as muddy, sandy/muddy, sandy and seagrass.~~

4. Discussion

4.1 Collection of reports in Mediterranean and Italian waters

A large number of networks and online databases have been created to provide information on biological invasions (Katsanevakis, Bogucarskis, Gatto, Vandekerkhove, Deriu, & Cardoso, 2012). ~~Although first records of invasive fish species are of high interest, subsequent records are important as well, as they confirm previous records and document the rate of establishment of migrant species (Golani & Levy, 2005).~~ An enormous amount of information scattered in various databases,

institutional repositories and scientific and technical papers is available for ~~alien species in~~ the Mediterranean Sea (Katsanevakis et al., 2014). Often, such information remains in the grey literature and is thus largely unavailable to the scientific community (Katsanevakis et al., 2014).

The EASIN, an initiative of the Joint Research Centre of the European Commission, was released and further upgraded by creating the EASIN-Lit geo-database, with the aim to gather data in distributed sources (Katsanevakis et al., 2012; Trombetti, Katsanevakis, Deriu, & Cardoso, 2013). However, data concerning vagrant marine species that have entered the Mediterranean via Gibraltar such as *L. lagocephalus* and *S. pachygaster* are excluded from the database (Katsanevakis, Gatto, Zenetos, & Cardoso, 2013) and even records on *L. sceleratus*~~, the most relevant from a public health perspective,~~ are very limited (https://easin.jrc.ec.europa.eu/EASINLits).

4.1.1 Number of specimens, and geographical location. ~~type of record.~~

Only few decades ago Lessepsian immigrants were restricted to the Eastern Mediterranean, with the Sicily Strait as their western barrier (Quignard & Tomasini, 2000). However, combined effects of the present warming trend of Mediterranean waters and other factors, such as the deepened and widened Suez Canal, damming of the Nile River, gradual extinction of physical barriers through the canal (i.e. salinity), faunal impoverishment of the Levant Basin and overfishing along the eastern Mediterranean coasts are now favouring their distribution (Bilecenoglu, 2016). ~~The evident difference was observed in the number of Mediterranean records for the three species considered in this study~~ Our results~~,~~ confirm~~ed~~ing that *L. sceleratus* is by far the most invasive species, especially considering its relatively recent introduction. On the contrary, *L. lagocephalus* and *S. pachygaster* do not show an invasive pattern. (Table 1). ~~This species~~*L. sceleratus* shows an unprecedented invasive character in terms of both abundances and geographical range (Nader et al., 2012) so to be considered among the fastest expanding Lessepsian immigrants (Torcu Koç, Erdoğan, & Üstün, 2011). Furthermore, when established, *L. sceleratus* contributes to a large part of the biomass, such as observed in Rhodes, Cyprus Greece, Egypt, Lebanon and Turkey (Kalogirou, 2013; Rousou et al., 2014). This species is reported as a common catch also by Lybian fishermen (Milazzo, Azzurro,

& Badalamenti, 2012). In Italy, the first confirmed record dates back to October 2013 from the Island of Lampedusa (Azzurro et al., 2014). Interestingly, during the present project an earlier record was reported to us by a fisherman from Reggio Calabria who caught a specimen of *L. sceleratus* with a long line in Gallico Marina (RC) in July 2010, at a depth of 80 m. Unfortunately, since pictures of the specimens were not available, the record could not be confirmed. Analogously, the occurrence of anecdotal evidences (via videos, articles and social networks posts) was observed in Tunisia and considered as unverified warnings about the spread of this fish (Ben Souissi et al., 2014). After reaching Italian coasts in 2013, Spanish coasts in 2014 and Algerian coasts in 2015, thus demonstrating its propensity in spreading towards the Northern and Western Mediterranean, it was also found in the North of the Adriatic Sea and in the Sea of Marmara (Table S1). The rapid spread and successful establishment of *L. sceleratus* in the Mediterranean Sea could be due to its broad thermal tolerance and to its adaptability to many habitats (Michailidis, 2010). In fact, *L. sceleratus* is reported to inhabit different kind of seabed types, such as sandy, muddy, rocky and seagrass substrates (Michailidis, 2010) (Table 1) and to be able to exploit resources competing with the native carnivore species (Nader et al., 2012).

L. lagocephalus was found to be the less frequent species among the three, confirming its rarity (Zava et al., 2005). Contrarily to *L. sceleratus*, this species does not appear to be invasive. In fact, although ~~, especially considering that its presence has been known in the Mediterranean Sea for at least a century (Table S2).~~ the presence of *L. lagocephalus* in the Mediterranean Sea has long been known (Dulčić & Pallaoro, 2006), reports are rare and biological information rather scarce (Zava et al., 2005). It is believed that the species entered the Mediterranean through the Strait of Gibraltar (Zava et al., 2005) as most of the reports occurred in the Central and Western Mediterranean Sea, while reports from the Eastern basin are scarce (Table S2).

The occurrence of *S. pachygaster* has changed over the years. After its first record in Mallorca in 1979 (Oliver, 1981), evidence of its spreading throughout the whole Mediterranean has been reported (Ragonese & Morara, 2012), especially in the Southern Tyrrhenian and Ionian Sea

including the Strait of Sicily (Bello, 1993; Ragonese et al., 1997; Bianchini & Ragonese, 2007). Although it is widely accepted that *S. pachygaster* is an Atlantic immigrant, a possible ancient Lessepsian immigration has also been hypothesized (Ragonese & Morara, 2012). Since the 1990s the area of the Sicilian Strait and the Southern Adriatic Sea hosted an established population (Bello, 1993; Ragonese et al., 1997; Ragonese & Morara, 2012). It has even been suggested that this species arrived in the Sicily Strait prior to the date of the first Mediterranean record (Ragonese et al., 1992) and that the area could represent a centre from which the population has spread in different directions. However, a reduction of the population in the waters of southern Sicily was recently observed by Ragonese & Morara, (2012). Our data seem to confirm the contraction of the population of *S. pachygaster*, as only 14% of the total specimens of this species recorded so far in the Mediterranean was found after 2003 (Table 1 and Table S3). The current low standing stock off the Southern Sicilian coast of *S. pachygaster* might be due to the fact that this species, considered as a bycatch and always discarded at sea, suffers 100% discard mortality~~. In fact, pufferfish are mortality prone~~ due to the phenomenon of body inflation that occurs after the catch (Ragonese & Morara, 2012). Interestingly, most of the records of *S. pachygaster* in the references included in this study occurred from waters surrounding Italy, although the large majority of them dates back to more than 10 years ago.

~~Almost all the recorded individuals of *L. sceleratus*, *L. lagocephalus* and *S. pachygaster* were caught (99.4%, 94.3% and 99.7%, respectively). Only in the case of *L. lagocephalus* 5.7% of specimens were found stranded on the beach. *L. sceleratus* and *S. pachygaster* were always caught by commercial fishing gears (99.6-100%), while 13.9% of the catches of *L. lagocephalus* were performed with recreational fishing gears (Table 1). These~~

4.1.2 Type and date of record. Our results as regards the type of records (Table 1) are in accordance with the observations of other authors. Carpentieri et al., (2009) reported that *L. suezensis*, *L. sceleratus* and *L. spadiceus* represent a common by-catch of trammel net and small mesh-size gillnet. According to El- Haweet, Fishar, Geneid, & Abdel-Moula, (2016) pufferfish

were mainly caught by bottom long-line followed by bottom otter trawl, only accidentally they appeared in the catch of purse seine, trammel and gill nets. In the same way *S. pachygaster* ~~are~~ is a by catch of commercial trawlers operating in the Sicily strait (Ragonese & Morara, 2012).

~~*4.1.2 Geographical location and date of record.* Only few decades ago Lessepsian immigrants were restricted to the Eastern Mediterranean, withand the Sicily Strait has long been considered as their ultimate western barrier to their dispersal (Quignard & Tomasini, 2000). Water temperature is often considered the most important abiotic factor affecting dispersal of Lessepsian species, and the present warming trend of the Mediterranean waters facilitates their spread. However, Ccombined effects of the present warming trend of Mediterranean waters and other factorsdrivers of the invasion, such as the deepened and widened Suez Canal, damming of the Nile River, gradual extinction of physical barriers through the canal (i.e. salinity), faunal impoverishment of the Levant Basin and overfishing along the eastern Mediterranean coasts are now favouring their distribution, should also not be neglected (Bilecenoglu, 2016). Among other Lessepsian species, Tthe rapid spread and successful establishment of *L. scleratus* in the Mediterranean Sea could be due to its somewhat broad thermal tolerance and to its adaptability to many habitats (Michailidis, 2010). In fact, *L. scleratus* is reported to have a great tolerance to environmental variations, inhabiting different kind of seabed types, such as sandy, muddy, rocky and seagrass substrates (Michailidis, 2010) (Table 1) and to have the ability to exploit resources competing with the native carnivore species (Nader et al., 2012). After colonizing the eastern basin *L. scleratus* has already colonized the eastern basin and it has reached Italian coasts in 2013, Spanish coasts in 2014 and Algerian coasts in 2015, thus demonstrating its propensity in spreading towards the Northern and Western Mediterranean. In addition, it was also found in the North of the Adriatic Sea and in the Sea of Marmara (Table S1).~~

~~In Italy, the first confirmed record dates back to October 2013 from the Island of Lampedusa (Azzurro et al., 2014). Interestingly, during the present project an earlier record was reported to us by a fisherman from Reggio Calabria who caught a specimen of *L. scleratus* with a long line in~~

Gallico Marina (RC) in July 2010, at a depth of 80 m. Unfortunately, since pictures of the specimens were not available, the record could not be confirmed. Analogously, the occurrence of anecdotal evidences (via videos, articles and social networks posts) was observed in Tunisia and considered as unofficial and unverified warnings about the spread of this fish (Ben Souissi et al., 2014).

The presence of *L. lagocephalus* in the Mediterranean Sea has long been known (Dulčić & Pallaoro, 2006), although it is rarely reported and also biological information is rather scarce (Zava et al., 2005). It is believed that the species has entered the Mediterranean through the Strait of Gibraltar (Zava et al., 2005) and as most of the reports occurred in the Central and Western Mediterranean Sea, while reports from the Eastern basin are scarce (Table S2).

After the first record of *S. pachygaster* in Mallorca in 1979 (Oliver, 1981), evidence of its spreading throughout the whole Mediterranean has been reported (Ragonese & Morara, 2012), especially in the Southern Tyrrhenian and Ionian Sea including the Strait of Sicily (Bello, 1993; Ragonese et al., 1997; Bianchini & Ragonese, 2007). Although it is widely accepedt that *S. pachygaster* is an Atlantic immigrant, a possible ancient Lessepsian immigration has also been hypothesized (Ragonese & Morara, 2012). Interestingly, most of the records of *S. pachygaster* in the references included in this study occurred from waters surrounding Italy, although the large majority of them goes dates back to more than 10 years ago.

As concerns the month distribution of Italian records, most of the reports for *L. sceleratus* and *L. lagocephalus* occurred in Autumn and Winter. The seasonal trend observed for *L. lagocephalus* is in agreement with previous observations in Sicily and Calabria, suggesting that adults live closer to the coast in autumn and winter, while they are pelagic the rest of the year (Sperone et al., 2012; Zava et al., 2005). This seasonality may be relevant in terms of toxicity, as TTX level of *L. sceleratus* caught from the Mediterranean Sea was found to be higher in autumn and winter (Katikou, Georgantelis, Sinouris, Petsi, & Fotaras, 2009; Rodríguez et al., 2012). It is thought that

this situation may be specific in the Mediterranean and Aegean Sea (Kosker et al., 2016). On the contrary a seasonal pattern was not observed for *S. pachygaster*, as already reported by Bello, 1993.

4.1.3 Fish size. Most of the specimens of *L. sceleratus* included in this study were of big size (46.5 cm, SD 15.9 cm). Studies from the Mediterranean Sea show that the size of *L. sceleratus* may influence toxicity levels (Katikou et al., 2009; Rodriguez et al., 2012). ~~In particular, a~~A significant positive correlation between toxicity ~~levels~~ and ~~the~~ size ~~has been~~was reported by Katikou et al., (2009). In all the toxic specimens analysed in this latter study, gonads, gastrointestinal tract and liver ~~were the tissues possessing~~showed the highest toxicities. Even though muscle toxicity was lower it was pointed out that the consumption of approximately 200 g of flesh from the largest specimen of the study could be lethal. Extracts from the positive samples also contained PSP (Paralytic Shellfish Poisoning) toxins. On the contrary, toxicity was not detected in the smallest individuals analysed (Katikou et al., 2009). However, in the study of Acar, Ishizaki, & Nagashima, (2017) no relation between TTX and body size was found. Another interesting aspect is that *L. sceleratus* toxicity increases as the spawning season starts (Ali, Gomaa, & Othman, 2011) ~~and that a positive correlation between fish size and sexual maturity exists~~. In the study of Rousou et al., (2014) the minimum body size for sexual maturity was shown to be 30 cm and in the study of Boustany, Indary, & Nader, (2015) all the individuals with a length of 48 cm were sexually mature. This is particularly interesting from a public health perspective, considering that the consumption of large specimens is reported (see section 4.2).

As concerns *L. lagocephalus*, a study in Tunisia showed that the flesh and the liver of this puffer fish contains toxic compounds able to induce gastrointestinal disorders and oxidative stress, while TTX was not found (Saoudi et al., 2008). No proof of poisonous flesh has been reported for the Mediterranean specimens of *S. pachygaster* (Ragonese & Morara, 2012). A recent study confirmed the absence of TTX in both species (Rambla-Alegre et al., 2017).

4.2 The spreading of *L. sceleratus* throughout ~~the Mediterranean Sea~~Italian waters: implications for public health

Despite *L. sceleratus* has relevant ~~is causing important~~ ecological and economic impacts (Nader et al., 2012) the discussion of these ~~relevant~~ aspects is not the aim of this study~~, which focuses on public health issues~~.

The rapid expansion and establishment in the Eastern basin of *L. sceleratus* has raised health concerns in many countries such as Turkey, Israel, Greece, Cyprus, Egypt, Lebanon (Tunçer & Önal, 2014) and Libya (Milazzo et al., 2012). ~~Several cases of hospitalization (Bentur et al., 2008; Chamandi et al., 2009; Eisenman et al., 2008; Kasapidis, Peristeraki, Tserpes, & Magoulas, 2007), a mass poisoning of the crewmembers of a tanker (Ben Souissi et al., 2014) as well as some fatal intoxications (Zaki, 2004; Beköz et al., 2013) have been reported.~~Due to this risk, fishing, marketing and consumption of *L. sceleratus* have recently been banned in Egypt, Turkey, Lebanon (Nader et al., 2012) and Cyprus (EastMed, 2010). Despite this, the fish is generally consumed by local fishermen ~~due to its cheap prize, nice taste and large size~~ (Aydin, 2011) and reports o~~n~~ illegal sales ~~of *L. sceleratus*~~ on local fish markets in the Eastern Mediterranean exist (Farrag et al., 2016; Tunçer & Önal, 2014)~~. Researchers observed individuals of *L. sceleratus* being packed to be sold in local fish markets and along the Libyan coast (Milazzo et al., 2012)~~exist. ~~*L. sceleratus* is considered as a delicacy in Suez City, Egypt (Jribi & Bradai, 2012) and it is illegally landed and consumed also on the Mediterranean coasts of Turkey. Cases of fishermen attempting to market large *L. sceleratus* specimens in Turkish fishing ports are known (L. Konuk, pers. comm. in Bilecenoglu, Kaya, & Akalin, 2006)~~. The risk of consumption ~~of silver cheeked toadfish~~ is particularly high in north-western Turkey due to low levels of awareness of both fishermen and consumers and to the weak control of fish markets (Tunçer & Önal, 2014). However, no cases of poisoning have been reported in Turkey so far, probably because the parts generally containing tetrodotoxin are normally not consumed in this country (Aydin, 2011). On the contrary, consumption of fish liver and gonads is common in other Eastern Mediterranean countries where cases of poisoning have recently been recorded (Bentur et al., 2008).

To manage the health risk related to the presence of *L. sceleratus* effectively, risk assessment and communication should be implemented. Informative campaigns have been conducted in Greece (Kalogirou, 2013; Zenetos, Koutsogiannopoulos, Ovalis, & Poursanidis, 2013), Malta (Andaloro et al., 2016), Turkey (Beköz et al., 2013) and Tunisia (Enajiar et al., 2015; Ben Souissi et al., 2014). Despite this, a serious case of intoxication was registered in August 2013 in the internal areas of the country, where no specific actions were carried out to inform people about thise risks ~~posed by this species~~ (Ben Souissi et al., 2014). An analogous case was described in 2008 in Bangladesh, where a mass intoxication occurred in an inland village ~~, where people are not used to recognise pufferfish~~ (Islam et al., 2008). However, other authors reported that even when risk communication had been performed, sometimes fishermen continued to sell~~old~~ pufferfish ~~on the market~~ (Beköz et al., 2013). On the other side, communication resulted in familiarization of ~~the~~ Greek fishermen with this species and even in over-reporting (Katikou et al., 2009).

As regards Italy, following the first report of *L. sceleratus* (Azzurro et al., 2014) a national informative campaign by ISPRA (*Istituto Superiore per la Protezione e la Ricerca Ambientale*), the agency of the Italian Ministry of Environment, was issued (Andaloro et al., 2016). An informative campaign was launched also in Spain soon after the first occurrences of *L. sceleratus* in this country (Izquierdo-Muñoz & Izquierdo-Gomez, 2014) and in 2015 these two separate actions were ~~extended and~~ interconnected (Azzurro et al., 2016).

Accordingly, the project "*Climate change and food safety: molecular, microbiological and toxicological analysis on toxic fish species in the Tyrrhenian Sea*" led by the Experimental Zooprophylactic Institute of Lazio and Tuscany in partnership with FishLab, Department of Veterinary Sciences, University of Pisa, and the Veterinary Services and Animal Health, Ministry of Agriculture & Rural Development, Israel, was funded by the Italian Ministry of Health. ~~It aims to monitor the occurrence of toxic fish species along the Tyrrhenian Sea coast and to characterize them under a molecular, microbiological and toxicological profile. In the first part of the project~~ ~~t~~The data presented in this work were collected in order to lay down the basis for the

characterization of this emerging risk in an early stage of the process. These data will contribute to national communication activities to governmental organizations, seafood chain stakeholders and consumers. ~~In fact, a proper communication relies on a correct assessment and the lack of data represents a major limit in assessing public health risk related to biological invasions. In addition, the present study highlighted a high variability of available information stressing the need of harmonizing the presentation and aggregation of data coming from different kind of sources.~~

Evidences from this work show the increasing potential risk for Italian consumers, particularly those inhabiting the southern regions (Fig 1). In fact, even though Italy appears to be still marginally affected by the presence of *L. sceleratus*~~, as only 0.08% of the total 110259 specimens recorded in the Mediterranean Sea so far were found along Italian coasts~~ (Table 1), the phenomenon should be strictly monitored considering the toxicity and adaptability to new areas of this species. In addition, climate change may boost this risk by facilitating its spreading (Aydin, 2011). Recently, Coro et al., (2018) estimated that the increasing sea temperatures will lead to a growth of population of *L. sceleratus* along western Mediterranean coasts, including Italy. ~~The role of climate change on the spreading of *L. sceleratus* has been recently highlighted by the work of Coro et al. (2018in press). Scientists, on the basis of global climate change forecasts up to 2100, has estimated that increasing of sea temperatures will lead to a growth of population along western coasts, including the Italian ones.~~

The main potential risk factors ~~of specimens of~~influencing *L. sceleratus* entering the European seafood chain identified in this study are ~~mainly related to~~ the specimens size ~~of the specimens~~ and ~~to~~ the fishing method. In fact, a positive correlation between size and toxicity level exists ~~The big~~ size of Ccaught specimens of ~~*L. sceleratus* are often of a big size, which might be one of the reasons behind this species being sold. However, this increases the probability they harbour TTX in their organs~~ (see section 4.1.3) and this species is mainly caught by widely used commercial fishing gears~~ that are widely used in the seafood primary production sector~~. Therefore, if fish are not correctly identified in the primary production, ~~starting from this first step~~ single specimens mixed

up with other fish could go unnoticed during self-control checks or inspection activities and ~~they~~ could reach the market. Even though whole specimens present peculiar morphological features, common to the Tetraodontidae family (Nader et al., 2012), the removal of anatomical portions during the preparation (beheading, gutting, skinning) could increase the risk related to their commercialization, unless all the actors of the seafood chain are adequately informed. In the European Union (EU) ~~poisonous~~ fish of the family Tetraodontidae and products derived from them must not be placed on the market at all (Regulation CE n. 854/2004) and the same ban was already present at the European level starting from 1991 (Council Directive 91/493/CEE). However, ~~as the Mediterranean population of the silver-cheeked pufferfish along European coasts grows, so does the health risk they pose, especially when local people are unaware of the danger. In fact,~~ TTX is a new phenomenon for Mediterranean countries and people occupationally involved in seafood harvesting, shipping and processing, as well as officials, seafood consumers, and generic sea users may not be used to recognize these species (http://www.isprambiente.gov.it/it/archivio/notizie-e-novita-normative/notizie-ispra/2016/04/nuova-cattura-di-pesce-palla-maculato-nei-nostri-mari-la-specie-tossica-raggiunge-le-coste-della-calabria). ~~In fact, intoxication cases are mainly due to lack of awareness among consumers, misidentification of species or due to erroneous traditional conception of detoxification methods (Nader et al., 2012).~~ Finally, given the novelty of TTX presence, symptoms may not be immediately recognised in hospitals. Therefore, ~~considering that public health prevention relies on qualified personnel,~~ also health careers must be adequately trained in diagnosis and treatment of TTX human intoxications (Ben Souissi et al., 2014). Even though data on TTX in pufferfish in the Mediterranean Sea are still limited, all the available studies (Acar et al., 2017; Katikou et al., 2009; Kosker et al., 2016; Rambla-Alegre et al., 2017) confirm the toxicity of *L. sceleratus*. ~~Considering that no toxicity was found to be associated with *L. lagocephalus* and *S. pachygaster* (Rambla-Alegre et al., 2017), it is also important that people are able to distinguish *L. sceleratus* from these latter species. In fact, a correct identification would avoid excessive alarms and contribute to a correct mapping of the spread of each species.~~

Finally, it has to be considered that the effects of climate change on these aspects of seafood safety are a relatively new topic. ~~Influences of climate change on the increased production and accumulation of biotoxins by marine organisms have been described in other parts of the world (Center for Disease Control, 2009).~~ Poletti, Milandri, & Pompei, (2003) asserted that TTX had not been found in the Mediterranean, at least until 2003. Probably, the propagation of TTX-producing bacteria in the Mediterranean waters has been favoured by climatic changes and transports through the Suez Canal (Saoudi et al., 2008). Therefore, it cannot be excluded that other Mediterranean species currently considered as non-toxic ~~or weakly toxic~~ may accumulate the TTX through the trophic chain. ~~In this regard r~~Recent reports of the presence of TTXs in gastropods from Portugal and in shellfish from Greece confirm this theory, highlighting that the occurrence of TTX is becoming a matter of concern for the Mediterranean Sea (Katikou et al., 2009; EFSA CONTAMIN Panel, 2017; Rambla-Alegre et al., 2017).

5. Conclusions

This study represents one the first attempt to collect, harmonize and integrate information distributed in various sources, ~~for the assessment~~to depict ~~of~~ an emerging risk related to the spreading of toxic pufferfish, in particular *L. sceleratus*, ~~in the Mediterranean Sea and~~ along Italian ~~waters~~ coasts. Evidence shows the increasing potential risk for Italian consumers, particularly those inhabiting the southern regions. TTX represents in fact a new and less known issue affecting the seafood chain in the Mediterranean. Public health authorities are thus facing new challenges and the current systems need to be adapted in order to guarantee food safety. An interdisciplinary approach involving expertise from food inspection, environmental and climatic sciences, economic science, should be adopted to mitigate the risk (Marques et al., 2010). Governments should implement measures to train people employed in the seafood chain as well as public authorities and the general public on the potential risk for tetrodotoxin poisoning, as in the case of the current project. ~~Despite the current distribution of *L. sceleratus* appears to be limited to the southern Italian regions, a~~ A

rapid dissemination on the risk should be performed throughout the national territory, due to the invasive behaviour of the species observed in the eastern Mediterranean.

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Declarations of interest

None.

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845 **Table 1** Summary of the results collected for the three investigated species

Collected data	*Lagocephalus sceleratus*	*Lagocephalus lagocephalus*	*Sphoeroides pachygaster*
First report in the Mediterranean sea	2003	1878	1979
Number of individuals recorded since the first report	110237	126	716
Number of individuals recorded since 2003	110237	110	104
- % of the number of records since 2003 over the total number of records since the first Mediterranean report	100.0%	87.3%	14.5%
Number of individuals reported from Italian waters	92	30	565
- % of the Italian records over the total number of records for each species	0.08%	23.8%	78.9%
Number of references:			
- n total	72	31	52
- n scientific articles	51	13	42
- n from databases	19	16	9
- n from others sources	2	2	1
Type of record			
- n caught (%)	109595 (99.4%)	115 (94.3%)	694 (99.7%)
- n observed (%)	620 (0.6%)	0 (0%)	1 (0.1%)
- n stranded (%)	0 (0%)	7 (5.7%)	1 (0.1%)
- n total	110215	122	696
Fishing method			
Commercial fishing gears[a]	99.6%	86.1%	100%
recreational fishing gears	0.4%	13.9%	0%
n	1261	78	617
Total length range	50-830 mm (n=55043)	50-765 mm (n=97)	85-540 mm (n=497)
Average total length	445.0 mm (SD 154.8 mm) (n=71)	536.5 mm (SD 83.0) (n=77)	284.1 mm (SD 125.1 mm) (n=80)
Weight range	5-5600 g (n=51964)	100.5-3783 g (n=72)	41.9-2640 g (n=58)
Average weight	1823.9 g (SD 1270.3 g)(n=60)	1618.2 g (SD 687.9 g) (n=72)	1075.3 g (SD 720 g) (n=41)
Depth range	2-400 m (n=64904)	1-200 m (n=17)	22-400 m (n=625)
Average depth of capture or observation	24.1 m (SD 18.5 m)(n=60)	25.1 m (SD 33.5 m)(n=8)	149.3 m (SD 91.3 M)(n=44)
Type of seabed			
- sandy n (%)	21207 (34.3%)	2	3 (14.3%)
- seagrass n (%)	14999 (24.2%)		
- rocky n (%)	13914 (22.5%)		
- muddy n (%)	11754 (19.0%)		6 (28.6%)
- muddy/sandy n (%)			7 (33.3%)
- seagrass/stones n (%)			4 (19.0%)
- mud/stones n (%)			1 (4.8%)
- n	61874	2	21

846

847 **Figure caption**

848 **Figure 1** Geographical distribution of Italian records of *Lagocephalus sceleratus* (blue dots), *Lagocephalus lagocephalus* (orange dots) and
849 *Sphoeroides pachygaster* (green dots). The interactive version of the map with details of specimens, precise location and reference for each record is
850 available at the following link:
851 https://www.google.com/maps/d/viewer?mid=1njH7upSFkXkLsiS20DHzIkKN2pHCBrxd&ll=39.973042081318034%2C13.53790285625007&z=6
852
853

Figure

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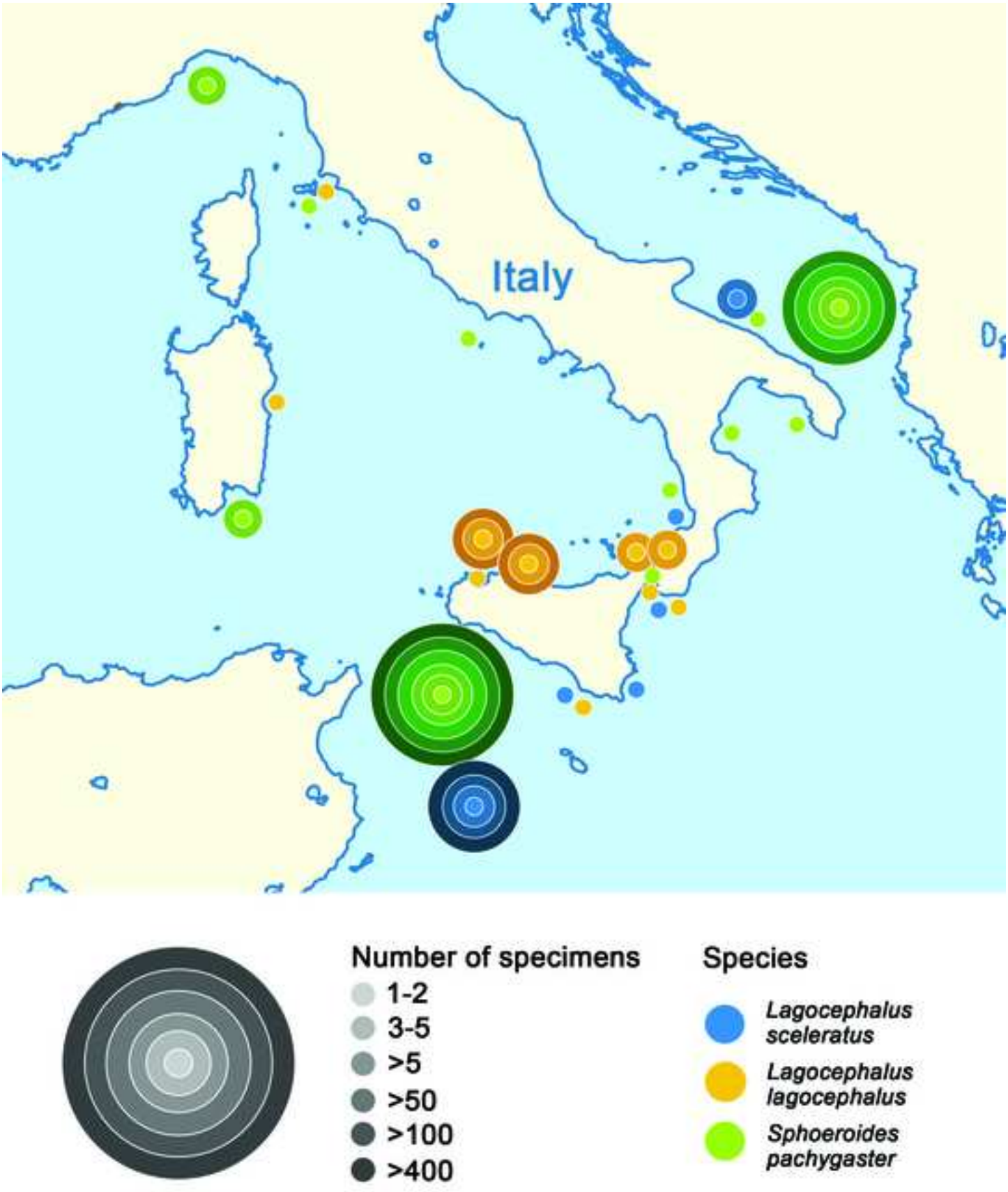

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