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Review Article

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Alien species in the Mediterranean Sea by 2012. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD). Part 2. Introduction trends and pathways

A. ZENETOS[1], S. GOFAS[2], C. MORRI[3], A. ROSSO[4], D. VIOLANTI[5], J.E. GARCÍA RASO[2], M.E. ÇINAR[6], A. ALMOGI-LABIN[7], A.S. ATES[8], E. AZZURRO[9], E. BALLESTEROS[10], C.N. BIANCHI[3], M. BILECENOGLU[11], M.C. GAMBI[12], A. GIANGRANDE[13], C. GRAVILI[13], O. HYAMS-KAPHZAN[7], P.K. KARACHLE[14], S. KATSANEVAKIS[15], L. LIPEJ[16], F. MASTROTOTARO[17], F. MINEUR[18], M.A. PANCUCCI-PAPADOPOULOU[1], A. RAMOS ESPLÁ[19], C. SALAS[2], G. SAN MARTÍN[20], A. SFRISO[21], N. STREFTARIS[1] and M. VERLAQUE[18]

[1] Hellenic Centre for Marine Research, P.O. Box 712, 19013, Anavissos, Greece
[2] Departamento de Biologia Animal, Facultad de Ciencias, Universidad de Málaga, E-29071 Málaga, Spain
[3] DiSTAV (Dipartimento di Scienze della Terra, dell'Ambiente e della Vita), University of Genoa, Corso Europa 26, I-16132 Genova, Italy
[4] Dipartimento di Scienze Biologiche Geologiche e Ambientali, Sezione Scienze della Terra, Laboratorio Paleoecologia, Università di Catania, Corso Italia, 55- 95129 Catania, Italy
[5] Dipartimento di Scienze della Terra, Università diTorino, via Valperga Caluso 35, 10125 Torino, Italy
[6] Ege University, Faculty of Fisheries, Department of Hydrobiology, 35100 Bornova, Izmir, Turkey
[7] Geological Survey of Israel, 30 Malchei Israel St., Jerusalem 95501, Israel
[8] Department of Hydrobiology, Faculty of Marine Sciences and Technology Çanakkale Onsekiz Mart University, 17100, Çanakkale, Turkey
[9] ISPRA, National Institute for Environmental Protection and Research, Piazzale dei Marmi 2, 57123 Livorno, Italy
[10] Centre d'Estudis Avanç ats de Blanes (CSIC), Acc. Cala, Sant Francesc 14, 17300, Blanes, Girona, Spain
[11] Adnan Menderes University, Faculty of Arts & Sciences, Department of Biology, 09010 Aydin, Turkey
[12] Stazione Zoologica Anton Dohrn, Villa Comunale, 80121 Naples, Italy
[13] Dipartimento di Scienze e Tecnologie Biologiche ed Ambientali, Università del Salento, CONISMA, Via Prov.le Lecce Monteroni, 73100 Lecce, Italy
[14] Department of Zoology, School of Biology, Aristotle University, Box 134, GR-54124, Thessaloniki, Greece
[15] European Commission, Joint Research Centre, Institute for Environment and Sustainability, Water Resources Unit, Ispra, Italy
[16] Marine Biology Station, National Institute of Biology, Fornace 41, 6330 Piran, Slovenia
[17] Department of Biology, University of Bari, Via Orabona 4, 70125 Bari, Italy
[18] Aix-Marseille University, UMR7294 MIO Mediterranean Institute of Oceanography, 13288 cedex 9, Marseille, France
[19] Centro de Investigación Marina de Santa Pola, Universidad de Alicante, 03080 Alicante, Spain
[20] Departamento de Biología (Zoología), Facultad de Ciencias, Universidad Autónoma de Madrid, Canto Blanco, Calle Darwin, 2, E-28049, Spain
[21] Department of Environmental Sciences, Informatics and Statistics, University of Venice, Calle Larga 2137, 30123 Venice, Italy

Abstract

More than 60 marine non-indigenous species (NIS) have been removed from previous lists and 84 species have been added, bringing the total to 986 alien species in the Mediterranean [775 in the eastern Mediterranean (EMED), 249 in the central Mediterranean (CMED), 190 in the Adriatic Sea (ADRIA) and 308 in the western Mediterranean (WMED)]. There were 48 new entries since 2011 which can be interpreted as approximately one new entry every two weeks. The number of alien species continues to increase, by 2-3 species per year for macrophytes, molluscs and polychaetes, 3-4 species per year for crustaceans, and 6 species per year for fish. The dominant group among alien species is molluscs (with 215 species), followed by crustaceans (159) and polychaetes (132). Macrophytes are the leading group of NIS in the ADRIA and the WMED, reaching 26-30% of all aliens, whereas in the EMED they barely constitute 10% of the introductions. In the EMED, molluscs are the most species-rich group, followed by crustaceans, fish and polychaetes. More than half (54%) of the marine alien species in the Mediterranean were probably introduced by corridors (mainly Suez). Shipping is blamed directly for the introduction of only 12 species, whereas it is assumed to be the most likely pathway of introduction (via ballasts or fouling) of another 300 species. For approximately 100 species shipping is a probable pathway along with the Suez Canal and/or aquaculture. Approximately 20 species have been introduced with certainty via aquaculture, while >50 species (mostly macroalgae), occurring in the vicinity of oyster farms, are assumed to be introduced accidentally as contaminants of imported species. A total of 18 species are assumed to have been introduced by the aquarium trade. Lessepsian species decline westwards, while the reverse pattern is evident for ship-mediated species and for those introduced with aquaculture. There is an increasing trend in new introductions via the Suez Canal and via shipping.

Keywords: Biological invasions, marine aliens, biogeography, trends, pathways, Mediterranean Sea.

Introduction

An up-to-date inventory of the alien species in the Mediterranean apart, from its scientific merits can fulfil the needs of the regulatory requirements and environmental management options. This is of particular importance considering the current emergence of the new generation of EU political actions covering major maritime strategic objectives, such as the newest EU Biodiversity Strategy (EU, 2011), the Marine Strategy Framework Directive (MSFD) (EU, 2008a), and the European Strategy for Marine and Maritime Research (EU, 2008b). Alien species are of major importance in those policies. In the MSFD the descriptor D2: "Non-indigenous species introduced by human activities are at levels that do not adversely alter the ecosystems" is actually one of the eleven qualitative descriptors for determining Good Environmental Status (GES).

The two criteria for assessing GES in relation to D2 are: a) abundance and state characterisation of non-indigenous species, in particular invasive species [criterion 2.1], and b) Environmental impact of invasive non-indigenous species [criterion 2.2] (EU, 2010).

With regards to the criterion 2.1, proposed indicators include *Trends in abundance, temporal occurrence and spatial distribution in the wild of non-indigenous species, particularly invasive non-indigenous species, notably in risk areas, in relation to the main vectors and pathways of spreading of such species.*

With regards to criterion 2.2 it must be pointed out that the ecological impacts of invasions are often inferred from distribution data under the assumption that the more abundant the alien species, the more severe the impact (Vilà *et al.*, 2010). There have been more than 1300 marine species introduced in European Seas (Katsanevakis *et al.*, in press) but the impact on local ecosystems has been studied for fewer than 100 species.

The Convention on Biological Diversity (CBD) has recognized the urgent need to address the impact of Invasive Alien Species (IAS) and has included 'Trends in invasive alien species' in the trial indicators to be developed and used for assessing global progress towards the 2010 target. However, due to lack of data on invasive species, the cumulative number of all aliens is used as an alternative. An indicator based on the cumulative number of alien species was used to identify progress toward the 2010 targets (Butchart *et al.*, 2010), and was included in the third edition of the CBD's *Global Biodiversity Outlook* (Secretariat of the CBD, 2010).

At the 2011 CBD meeting operational IAS indicators and their associations within the indicator framework, for assessing progress towards the implementation of the Strategic Plan for Biodiversity 2011–2020 and achievement of the Aichi biodiversity target 9 were discussed (CBD, 2011). 'Trends in number of invasive alien species' (decisions VII/30 and VIII/15) was proposed as a priority tool to be developed at global level, and 'Trends in invasive alien species pathways management' was proposed for consideration at sub-global level respectively (EEA, 2012).

The present work addresses marine Non-Indigenous Species (NIS) in the Mediterranean Sea (non EU countries included) and attempts to assess trends in: 1) temporal occurrence per MSFD area/and introduction rate per major group; and 2) pathways of spreading per MSFD area. Trends in pathways of introduction in the Mediterranean per decade should tell us a story when combined with management policies implemented over the last decades. This work also serves as an updated list of alien species in the Mediterranean accommodating recent findings and latest nomenclatural changes since Zenetos *et al.* (2010; 2011).

Materials and Methods

The study area

In this work the Mediterranean is divided into the four subregions described under the MSFD, namely: (i) the Western Mediterranean Sea (WMED); (ii) the Central Mediterranean Sea (CMED); (iii) the Adriatic Sea (ADRIA); and (iv) the Eastern Mediterranean Sea (EMED). With WMED we include the whole basin lying between Gibraltar and the Straits of Sicily. The borders of CMED are hereby defined as the Kythira-Anti-Kythira Straits (Greece) and Libya-Egypt borders to the east, Otranto Straits (Italy, Albania) to the north, Cap Bon (Tunisia) and Cape Libeleo (south-west Sicily) to the west. ADRIA goes from the Gulf of Trieste to the north to Otranto Straits to the south. The EMED is also commonly referred to as the Aegean-Levantine basin, but in this work the Marmara Sea, bearing more similarities to the Aegean than to the neighbouring Black Sea, is also included. The coastal areas of the countries included in these subregions are listed in Table 1. This division imposes some difficulties in the case of countries whose waters are included in more than one of these subregions such as Albania (CMED + ADRIA), Greece (EMED+CMED), Tunisia (WMED + CMED) and Italy (WMED + CMED + ADRIA).

The data set used in the following analyses was based on the Zenetos *et al.* (2010) inventory, updated and checked to October 2012. Scientific literature (2010-2012) was also taken into account for taxonomic issues and revised distribution ranges. Following criticism that questions their status as aliens (Galil, 2012), we excluded species of tropical Atlantic origin that have expanded their distribution range and vagrant species. In the Mediterranean literature (e.g. Golani, 2010; Orsi-Relini, 2010) the term 'vagrant' has been used for large species be-

Table 1. Countries and coastal sectors included in the four subregions of the Mediterranean studied in this work.

Western Mediterranean (WMED)	**Central Mediterranean** (CMED)	**Adriatic Sea** (ADRIA)	**Eastern Mediterranean** (EMED)
Ligurian Sea	Greek Ionian Sea	Italian Adriatic Sea	Greek Aegean Sea
Monaco	Italian Ionian Sea	Slovenia	Turkish Aegean Sea
France	Albanian Ionian Sea	Croatia	Sea of Marmara
Corsica	South-east Sicily	Montenegro	South Turkey
Sardinia	Malta	Albanian Adriatic Sea	Cyprus
Tyrrhenian Sea	Libya		Syria
Balearic Islands	South Tunisia		Lebanon
Spain			Palestine Authority
Gibraltar			Israel
Morocco			Egypt
Algeria			
North Tunisia			
West Sicily			

longing to the offshore nekton (mainly perciform fishes, sharks, large cephalopods and marine mammals) recorded occasionally as isolated animals. Both established and non established species were considered herein. Very old records reported as non-persisting in the literature were excluded. Cryptogenic species were excluded from the trends analyses.

Freshwater species reported in estuarine environments, such as the Louisiana crayfish *Procambarus clarkii,* the Nile tilapia *Oreochromis niloticus niloticus,* the mosquito fish *Gambusia hoolbroki,* and the zebra mussel *Dreissena polymorpha,* were not encountered as marine NIS.

For all reported marine NIS in the Mediterranean, we investigated the year of first record and the potential pathway of introduction in all four Mediterranean subregions. The major groups analysed by order of decreasing contribution were: 1) Mollusca; 2) Crustacea; 3) Polychaeta; 4) Macrophyta; 5) Fish; 6) Foraminifera; 7) Cnidaria; 8) Bryozoa; 9) Ascidiacea; and 10) Miscellanea (i.e. Chaetognatha, Ctenophora, Echinodermata, Nematoda, Platyhelminthes, Porifera, Pycnogonida, Sipuncula). Unicellular organisms were not considered except for Foraminifera. Species names have been extracted from WoRMS, the World Register of Marine Species (Appeltans *et al.*, 2012). Against all rules, the authorities are not listed for species due to confusion with the NIS record or reference but these authorities are included in the species tables.

C. Trends in introduction rates

The rate of introduction is represented as the number of marine and brackish waters alien species introduced per decade since 1950. Results are given for the entire Mediterranean Sea and per each MSFD subregion (including non EU member state waters).

Year of introduction is based on reported first collection dates but does not necessarily imply true year of introduction, which may have occurred years earlier. When the exact date of the alien record is not reported, the year of the first relevant publication has been used.

D. Trends in pathways

This trend is represented by the number of new alien species per decade and per pathway of first introduction to the Mediterranean Sea since 1950. The classification of introduction pathways was based on the frameworks proposed by Hulme *et al.* (2008) and Molnar *et al.,* (2008), as adopted by the European Alien Species Information Network (EASIN; 2013; Katsanevakis *et al.,* 2012). We focus on the pathways of the first introduction in the Mediterranean and have not considered pathways of subsequent transfers to other areas. In this analysis we have used the following categorization of pathways: '<u>aquaculture'</u> (including both target species and others occurring as contaminants); '<u>shipping'</u> (including ballasts and fouling); '<u>corridors'</u>: (Suez and inland canals); '<u>aquarium trade'</u> (releases and escapes), and '<u>other'</u> (including live food/ bait trade; floating manufactured objects).

The link between species and pathways was based on scientific literature, i.e. on published justification by experts. In cases of uncertainty or diverging opinions among experts, literature was critically evaluated by the authors to reach a decision. In some cases, the pathway was defined by expert judgment as the best plausible alternative. A modification of the approach proposed by Minchin (2007) was applied, according to which for each species one of the following is true:

1) There is direct information of a pathway (uncertainty category 1). The species was clearly associated to a specific vector(s) of a pathway at the time of introduction to a particular locality. This is the case in intentional introductions (i.e. aquaculture/target species, wholesale

importation of shellfish products) and in many examples of Lessepsian immigrants, when there was direct evidence of a gradual expansion along the Suez Canal and then in the localities around the exit of the Canal in the Mediterranean.

2) One most likely pathway can be inferred (uncertainty category 2). The species appeared for the first time in a locality where a single pathway is known to operate and there is no other rational explanation for its occurrence. In many cases, inference is based on known examples of introductions elsewhere for the same or similar species, the biology and ecology of the species, the habitats and locales it occupies in both the native and introduced range, and its pattern of dispersal (when known). For example, for a fouling species frequently recorded in ports, shipping has been assumed to be the most probable pathway/vector.

3) One or more possible pathways can be inferred (uncertainty category 3). The species cannot be convincingly ascribed to a single pathway. Inference is based on the activities in the locality where the species was found and may include evidence about similarly occurring species reported elsewhere. Species of Indo-Pacific origin found in the EMED, but not yet reported from the Red Sea are assumed to have been introduced via Suez either through the canal or by shipping with uncertainty category 3.

4) Unknown: There is doubt as to any specific pathway explaining the arrival of the species.

More than one pathway were assigned to a species when: (a) different introduction events by different pathways occurred within the Mediterranean; and (b) species been classified in the literature as 'Lessepsian', yet they are suspected to have been (also) transferred with ships (for a few species of Indo-Pacific origin, mainly sessile polychaetes, molluscs and macroalgae). Of the 986 assessed species, 799 have been assigned to a single pathway and 114 have been assigned to two or more possible pathways. The remaining 73 species have been classified as 'unknown'.

Results and Discussion

A. Updates in species records

A total of 986 marine species are reported as NIS in this work. The full list is accessible in the Marine Mediterranean Invasive Alien Species (MAMIAS) database (UNEP RAC/SPA, 2012).

In comparison to previous inventories (Zenetos *et al.,* 2010; 2011), and in the light of new literature and reconsideration of introduction pathways, 35 taxa have been excluded as natural range expansion, six as vagrant and 21 for other reasons (Table 2). Eighty-six species (among which 48 new records since January 2011) have been added (Table 3). The dominant group among alien species is Mollusca (with 215 species), followed by Crustacea (159) and Polychaeta (132) (Fig. 1).

Of the 215 alien molluscs, 76 of which are new additions, 120 form established populations, 14 are questionable, 4 are cryptogenic and the remaining 77 have been recorded only once or twice. Molluscs remain the most species-rich group, in term of NIS, also when ignoring many records based on single shells collected long ago and not qualified as aliens.

Crustaceans increased by eight species. Decapods are the prevalent group, followed by copepods. The latest records are the shrimp *Palaemon macrodactylus* (Torres *et al.,* 2012) in the WMED, and two parasitic copepods, *Caligus fugu* and *Taeniacanthus lagocephali*, caught on a Lessepsian puffer fish in the EMED (Özak *et al.,* 2012). We have added the unusual finding of the brine shrimp *Artemia franciscana* in the saltworks of Margherita di Savoia, Apulia (Mura *et al.,* 2006).

Only 52 polychaete NIS (out of the 132 recorded up to now) have formed established populations in at least one of the subregions. The NIS established in the entire basin include species such as *Ficopomatus enigmaticus*, *Hydroides dianthus* and *H. elegans*, which represent long dated established introductions and are sometimes considered as natural component of Mediterranean habitats: see for instance *Ficopomatus* reefs in many Italian lagoons (Bianchi & Morri, 1996). *Phyllodoce longifrons* has been known from the EMED since 1976 (Ben Eliahu, 1976), but has been just recognised as an alien by Çinar & Dagli (2012). As *Serpula hartmanae* is morphologically similar to the native species *S. concharum* (the main difference is the presence of an asymmetric proximal boss (swelling) in the operculum of *S. hartmanae*), the previous records of *S. concharum* from the region should be re-examined. *Nainereis setosa, Parapionosyllis* cf. *macaronesiensis*, and *Syllis* cf. *mauretanica*, which were previously thought to be probable alien species, have not been included in the present list. *N. setosa* was reported from an aquaculture facility in 2003 near Brindisi (Adriatic Sea), where it was one of the most abundant polychaetes, attaining a population density of 500 individuals m^{-2} (Blake & Giangrande, 2011); at present, it has been eliminated naturally from the area. Del Pilar-Ruso & San Martín (2012) recently reported the syllid species *P.* cf. *macaronesiensis* and *S.* cf. *mauretanica* from the coast of Alicante (Spain), close to Gibraltar: as there is doubt about their taxonomic status, a possible natural range expansion from the nearest Atlantic cannot be excluded.

With 128 NIS, macrophytes rank fourth. Newcomers are comparatively few, the latest being *Solieria* sp. (Mineur *et al.*, 2012), *Ascophyllum nodosum* (Petrocelli *et al.*, 2012), and *Uronema marinum* (Sfriso *et al.*, 2012a). Many macrophytes are especially worrying because they may alter ecosystem structure and functioning by monopolizing space and acting as ecosystem engineers

Table 2. Species from previous inventories (Zenetos *et al.*, 2010; 2011), here removed. Abbreviation for groups: Fi=Fish, Mol=Mollusca, Cru=Crustacea, For=Foraminifera, Misc=Miscellanea, Mac=Macrophytes.

group	species	reasoning	group	species	reasoning
Fi	*Acanthurus monroviae*	range expansion	For	*Amphistegina lessonii*	native
Fi	*Aluterus monoceros*	range expansion	For	*Agglutinella arenata*	fossil record
Fi	*Anarhichas lupus*	range expansion	For	*Agglutinella soriformis*	fossil record
Fi	*Beryx splendens*	range expansion	For	*Amphistegina madagas-cariensis*	synonym
Fi	*Dicologlossa hexophthalma*	range expansion	For	*Miliolinella* cf. *M. hybrida*	fossil record
Fi	*Diplodus bellottii*	range expansion	For	*Operculina ammoniodes*	unsupported record
Fi	*Enchelycore anatina*	range expansion	For	*Planorbulinella larvata*	unsupported record
Fi	*Fistularia petimba*	range expansion	For	*Pyramidulina catesbyi*	fossil record
Fi	*Gephyroberyx darwini*	range expansion	For	*Pulleniatina obliquiloculata*	fossil record
Fi	*Gymnammodytes semisquamatus*	range expansion	For	*Schackoinella imperatoria*	fossil record
Fi	*Halosaurus ovenii*	range expansion	For	*Sorites orbiculus*	fossil record
Fi	*Kyphosus incisor*	range expansion			
Fi	*Microchirus boscanion*	range expansion	Mol	*Echinolittorina punctata*	range expansion
Fi	*Pagellus bellottii*	range expansion	Mol	*Spondylus multisetosus*	unsupported record
Fi	*Pisodonophis semicinctus*	range expansion	Mol	*Siphonaria belcheri*	unsupported record
Fi	*Scorpaena stephanica*	range expansion	Mol	*Septifer bilocularis*	unsupported record
Fi	*Seriola fasciata*	range expansion			
Fi	*Solea senegalensis*	range expansion	Cru	*Pseudocalanus elongatus*	native?
Fi	*Sphoeroides marmoratus*	range expansion	Cru	*Scaphocalanus amplius*	range expansion
Fi	*Sphoeroides pachygaster*	range expansion	Cru	*Scaphocalanus brevirostris*	range expansion
Fi	*Synaptura lusitanica*	range expansion	Cru	*Scolecithrix valens*	range expansion
Fi	*Syngnathus rostellatus*	range expansion	Cru	*Sphaeroma venustissimum*	range expansion
Fi	*Trachyscorpia cristulata echinata*	range expansion	Cru	*Synalpheus tumidomanus africanus*	range expansion
Fi	*Cephalopholis taeniops*	range expansion			
Fi	*Diodon hystrix*	range expansion	Pol	*Pherusa saldanha*	Re-identified as *Stylarioides grubei*
Fi	*Seriola carpenteri*	native?/range expansion	Pol	*Pherusa parmata*	Re-identified as *Se-miodera cinari*
Fi	*Seriola rivoliana*	native?/range ex-pansion			
			Misc	*Neothoracocotyle acantho-cybii*	parasite on Atlantic fish
Fi	*Carcharhinus altimus*	vagrant	Misc	*Hirudinella ventricosa*	parasite on Atlantic fish
Fi	*Carcharhinus falciformis*	vagrant	Misc	*Clytia mccradyi*	range expansion
Fi	*Galeocerdo cuvier*	vagrant	Misc	*Eirene viridula*	range expansion
Fi	*Isurus paucus*	vagrant			
Fi	*Rhizoprionodon acutus*	vagrant	Mac	*Polysiphonia stricta*	misidentification
Fi	*Sphyrna mokarran*	vagrant	Mac	*Osmundea oederi*	range expansion

(Thresher, 2000): major examples include *Stypopodium schimperi*, *Caulerpa taxifolia*, and *C. racemosa* (Bianchi, 2007 and references therein).

Fish rank fifth, although close to macrophytes. Of the 126 fish NIS, 124 are actinopterygians, whereas only two (*Himantura uarnak* and *Torpedo sinuspersici*) belong to the elasmobranchs. Twenty-seven species of tropical Atlantic origin have been removed from our previous list because their presence can be explained by a natural range expansion via Gibraltar rather than as human mediated introductions (Table 2). The Guinean amberjack *Seriola carpenteri* and the Almaco jack *Seriola rivoliana* have been eliminated because they were probably present in the Mediterranean but overlooked in the past (D. Golani, pers. comm.). Six additional species of sharks (*Carcharhinus altimus, C. falciformis, Galeocerdo cuvier, Isurus paucus, Rhizoprionodon acutus* and *Sphyrna mokarran*) have been excluded because the Mediterranean records are based on vagrant individuals. Invasive fish can have significant ecological and economical impacts and cause profound damage to natural habitats, as recently demonstrated for Lessepsian siganids in the EMED (Sala *et al.*, 2011).

Table 3. New Additions to the alien species inventory by Zenetos *et al.,* (2010; 2011). Grey shaded are species reported <2010.

Taxon	*Species/Author*	Location	source
Mollusca/Bivalvia	*Mimachlamys sanguinea* (Linnaeus, 1758)	Israel	Shefer *et al.,* 2012
Mollusca/Bivalvia	*Teredothyra dominicensis* (Bartsch, 1921)	Turkey	Müller, 2011
Mollusca/Gastropoda	*Marginella glabella* (Linnaeus, 1758)	Spain	Luque *et al.,* 2012
Mollusca/Gastropoda	*Pseudorhaphitoma iodolabiata* (Hornung & Mermod, 1928)	Turkey	Öztürk, 2012
Mollusca/Gastropoda	*Spurilla major* (Eliot, 1903)	Turkey	Turk & Furlan, 2011
Crustacea/Anostraca	*Artemia franciscana* (Kellogg, 1906)	Italy	Mura *et al.,* 2006
Crustacea/Copepoda	*Lernanthropus callionymicola* El-Rashidy & Boxshall, 2012	Israel, Egypt	El Rashidy & Boxshall, 2012
Crustacea/Copepoda	*Caligus fugu* Yamaguti & Yamasu, 1959	Turkey	Özak *et al.,* 2012
Crustacea/Copepoda	*Taeniacanthus lagocephali* Pearse, 1952	Turkey	Özak *et al.,* 2012
Crustacea/Copepoda	*Pseudodiaptomus marinus* Sato, 1913	Italy	De Olazabal & Tirelli, 2011
Crustacea/Decapoda	*Elamena mathoei* (Desmarest, 1823)	Tunisia	Zaouali *et al.,* 2011
Crustacea/Decapoda	*Lysmata kempi* Chace, 1997	Italy	Froglia & Deval, 2012
Crustacea/Decapoda	*Palaemon macrodactylus* Rathbun, 1902	Spain	Torres *et al.,* 2012
Annelida/Polychaeta	*Laonice norgensis* Sikorski, 2003	Turkey	Dagli *et al.,* 2011
Annelida/Polychaeta	*Phyllodoce longifrons* Ben Eliahu, 1976	Israel, Turkey	Ben Eliahu, 1976; Çinar & Dagli, 2012
Annelida/Polychaeta	*Perkinsyllis augeneri* (Hartmann-Schröder, 1979)	Israel	Faulwetter *et al.,* 2011
Annelida/Polychaeta	*Serpula hartmanae* Reish, 1968	Lebanon	Ben Eliahu & Ten Hove, 2011
Annelida/Polychaeta	*Spiophanes algidus* Meißner, 2005	Turkey	Dagli *et al.,* 2011
Annelida/Polychaeta	*Stylarioides grubei* Salazar-Vallejo, 2011	Turkey	Salazar-Vallejo, 2011
Chlorophyta	*Codium arabicum* Kützing, 1856	Israel	Hoffman *et al.,* 2011
Chlorophyta	*Uronema marinum* Womersley, 1984	Italy	Sfriso *et al.,* 2012a
Ochrophyta	*Microspongium globosum* Reinke, 1888	Turkey	Taşkin *et al.,* 2006
Ochrophyta	*Ascophyllum nodosum* (Linnaeus) Le Jolis	Italy	Petrocelli *et al.,* 2012
Rhodophyta	*Palisada maris-rubri* (K.W.Nam & Saito) K.W. Nam	Italy	Serio *et al.,* 2010
Rhodophyta	*Solieria* sp.	France	Mineur *et al.,* 2012
Fish/Actinopterygii	*Champsodon capensis* Regan, 1908	Turkey	Dalyan *et al.,* 2012
Fish/Actinopterygii	*Stolephorus insularis* Hardenberg, 1933	Israel	Fricke *et al.,* 2012

(continued)

Table 3. (continued). New Additions to the alien species inventory by Zenetos *et al.* (2010, 2011). Grey shaded are species reported <2010.

Taxon	*Species/Author*	Location	source
Fish/Actinopterygii	*Chaetodon austriacus* Rüppell, 1836	Israel	Goren *et al.,* 2011
Fish/Actinopterygii	*Chaetodon larvatus* Cuvier, 1831	Israel	Salameh *et al.,* 2011
Fish/Actinopterygii	*Chanos chanos* (Forsskål, 1775)	Turkey	Özvarol & Gökoğlu, 2012
Fish/Actinopterygii	*Epinephelus fasciatus* (Forsskål, 1775)	Lebanon	Bariche & Heemstra, 2012
Fish/Actinopterygii	*Epinephelus merra* Bloch, 1793	France	Lelong, 2005
Fish/Actinopterygii	*Equulites elongatus* (Günther, 1874)	Israel	Golani *et al.,* 2011a
Fish/Actinopterygii	*Holacanthus ciliaris* (Linnaeus, 1758)	Croatia	Dulčić & Dragičević, 2012b
Fish/Actinopterygii	*Ostracion cubicus* Linnaeus, 1758	Lebanon	Bariche, 2011
Fish/Actinopterygii	*Paranthias furcifer* (Valenciennes, 1828)	Croatia	Dulčić & Dragičević, 2012a
Fish/Actinopterygii	*Scatophagus argus* (Linnaeus, 1766)	Malta	Zammit & Schembri, 2011
Fish/Actinopterygii	*Synanceia verrucosa* Bloch & Schneider, 1801	Israel	Edelist *et al.,* 2011
Foraminifera	*Articulina mayori* Cushman, 1922	Turkey	Oflaz, 2006
Foraminifera	*Articulina pacifica* Cushman, 1944	Israel	Hyams-Kaphzan *et al.,* 2008
Foraminifera	*Cibicides mabahethi* Said, 1949	Israel	Hyams-Kaphzan *et al.,* 2008
Foraminifera	*Cycloforina quinquecarinata* (Collins, 1958)	Israel	Hyams-Kaphzan *et al.,* 2008
Foraminifera	*Epistomaroides punctatus* (Said, 1949)	Israel	Almogi-Labin & Hyams-Kaphzan, 2012
Foraminifera	*Lagena oceanica* Albani, 1974	Israel	Hyams, 2006
Foraminifera	*Loxostomina* cf. *L. africana* (Smitter, 1955)	Israel	Hyams-Kaphzan *et al.,* 2008
Foraminifera	*Loxostomina costulata* (Cushman, 1922)	Israel	Hyams-Kaphzan *et al.,* 2008
Foraminifera	*Pararotalia* cf. P. *socorroensis* McCulloch, 1977	Israel	Hyams-Kaphzan *et al.,* 2008
Foraminifera	*Pararotalia spinigera* (Le Calvez)	Israel	Bresler & Yanko, 1995
Foraminifera	*Paratrochammina madeirae* Bronniman, 1979	Israel	Hyams-Kaphzan *et al.,* 2008
Foraminifera	*Planispirinella exigua* (Brady, 1879)	Croatia	Wiesner, 1911
Foraminifera	*Pseudohauerinella dissidens* McCulloch, 1977	Israel	Hyams-Kaphzan *et al.,* 2008
Foraminifera	*Pseudomassilina australis* (Cushman, 1932)	Israel	Hyams, 2000
Foraminifera	*Pseudotriloculina subgranulata* (Cushman, 1918)	Israel	Hyams-Kaphzan *et al.,* 2008
Foraminifera	*Quinqueloculina* cf. Q. *multimarginata* Said, 1949	Israel	Hyams-Kaphzan *et al.,* 2008

(continued)

Table 3. (continued). New Additions to the alien species inventory by Zenetos *et al.* (2010, 2011). Grey shaded are species reported <2010.

Taxon	*Species/Author*	Location	source
Foraminifera	*Quinqueloculina milletti* (Wiesner, 1923)	Turkey	Oflaz, 2006
Foraminifera	*Septloculina rotunda* El-Nakhal, 1990	Turkey	Oflaz, 2006
Foraminifera	*Septloculina tortuosa* El-Nakhal, 1990	Turkey	Oflaz, 2006
Foraminifera	*Sigmoihauerina bradyi* (Cushman, 1917)	Turkey	Oflaz, 2006
Foraminifera	*Siphonaperta distorqueata* (Cushman, 1954)	Israel	Hyams-Kaphzan *et al.,* 2008
Foraminifera	*Siphonaperta pittensis* (Albani, 1974)	Israel	Hyams-Kaphzan *et al.,* 2008
Foraminifera	*Spirolina acicularis* (Batsch, 1791)	Turkey	Meriç *et al.,* 2011
Foraminifera	*Spiroloculina* aff. *S. communis* Cushman & Todd, 1944	Israel	Hyams-Kaphzan *et al.,* 2008
Foraminifera	*Spiroloculina attenuata* Cushman & Todd, 1944	Israel	Hyams-Kaphzan *et al.,* 2008
Foraminifera	*Spiroloculina nummiformis* Said, 1949	Israel	Hyams-Kaphzan *et al.,* 2008
Foraminifera	*Tretomphaloides clara* (Cushman, 1934)	Israel	Hyams-Kaphzan *et al.,* 2008
Foraminifera	*Triloculina asymmetrica* Said, 1949	Turkey	Oflaz, 2006
Foraminifera	*Varidentella* cf. *V. neostriatula* (Thalmann, 1950)	Israel	Hyams-Kaphzan *et al.,* 2008
Ascidiacea	*Didemnum vexillum* Kott, 2002	Italy	Tagliapietra *et al.,* 2012
Bryozoa	*Catenicella paradoxa* Rosso, 2009	Italy	Rosso, 2009
Bryozoa	*Celleporaria fusca* (Busk, 1854)	Israel	d' Hondt, 1988
Bryozoa	*Hippopodina iririkiensis* Tilbrook, 1999	Israel	Eitan, 1972
Bryozoa	*Microporella browni* Harmelin *et al.,* 2011	Lebanon	Harmelin *et al.,* 2011
Bryozoa	*Microporella genisii* Harmelin *et al.,* 2011	Lebanon	Harmelin *et al.,* 2011
Bryozoa	*Microporella harmeri* Hayward, 1988	Lebanon	Harmelin *et al.,* 2011
Bryozoa	*Parasmittina protecta* (Thornely, 1905)	Israel	d'Hondt,1988
Bryozoa	*Scorpiodinipora costulata* (Canu & Bassler, 1929)	Lebanon	Harmelin *et al.,* 2012
Cnidaria/Hydrozoa	*Aequorea globosa* Eschscholtz, 1829	Turkey	Turan *et al.,* 2011
Cnidaria/Hydrozoa	*Campanularia morgansi* Millard, 1957	Israel	Piraino *et al.,* 2010
Cnidaria/Hydrozoa	*Eucheilota ventricularis* McCrady, 1859	Lebanon	Lakkis & Zeidane, 1985
Cnidaria/Hydrozoa	*Fabienna oligonema* (Kramp, 1953)	Lebanon	Goy *et al.,* 1988
Cnidaria/Hydrozoa	*Haliscera bigelowi* Kramp, 1947	Croatia	Schmidt & Benovic, 1977

(continued)

Table 3. (continued). New Additions to the alien species inventory by Zenetos *et al.* (2010, 2011). Grey shaded are species reported <2010.

Taxon	*Species/Author*	Location	source
Cnidaria/Hydrozoa	*Halitiara inflexa* Bouillon, 1980	Lebanon	Goy *et al.*, 1991
Cnidaria/Hydrozoa	*Sphaerocoryne bedoti* Pictet, 1893	Lebanon	Goy *et al.*, 1991
Echiura	*Arhynchite arhynchite* (Ikeda, 1924)	France	Saiz Salinas & Amouroux, 2010
Echiura	*Ochetostoma erythrogrammon* Leuckart & Ruppell, 1828	Croatia	Saiz Salinas & Amouroux, 2010
Sipuncula	*Nephasoma eremita* (Sars, 1851)	Turkey	Açik, 2011

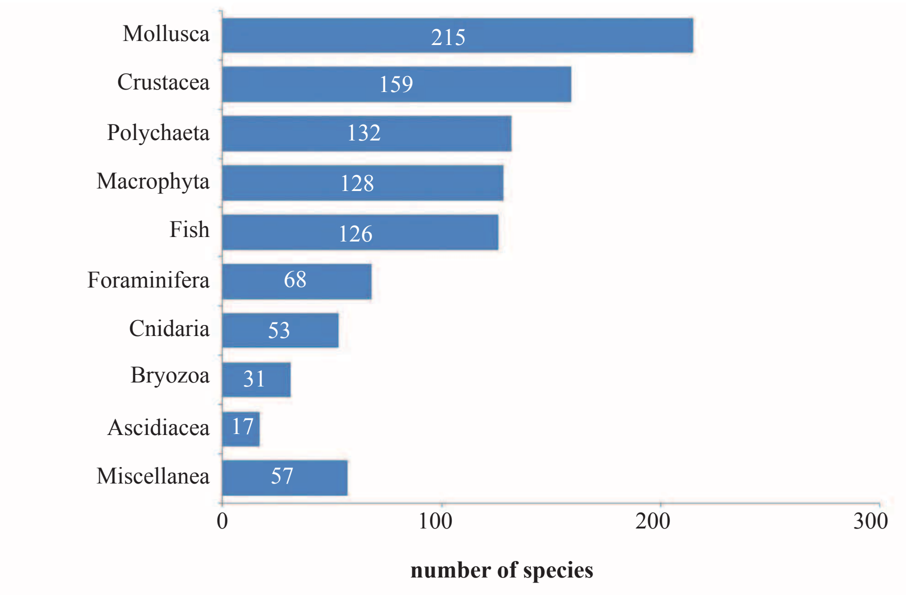

Fig. 1: Contribution of marine alien taxa in the Mediterranean Sea.

Foraminifera include 68 alien species, thus ranking sixth. Eleven out of the 50 foraminiferan NIS listed by Zenetos *et al*. (2010; 2011) were based on fossil records (Tapiero, 2002; Milker & Schmiedl, 2012) and were thus excluded (Table 2). On the contrary, 29 more foraminifers were included in our inventory (Table 3) based on new reports (Bresler & Yanko, 1995; Oflaz, 2006; Hyams-Kaphzan *et al.*, 2008).

Cnidarians, represented by 53 NIS, rank seventh. They show an increase of seven species (13.2 %) compared to the inventory by Zenetos *et al.* (2010). All these seven species belong to the Hydrozoa (Table 3). The recent record of the jellyfish *Aequorea globosa* is considered dubious by S. Piraino (pers. comm.).

With a total of 31 aliens, bryozoans rank eighth. Eight species (more than 25%) represent new additions to the previous list (Zenetos *et al.*, 2010). Nevertheless, none of these introductions actually took place in the last few years as all records relate to a critical re-examination of past literature. *Celleporaria fusca* and *Parasmittina protecta* were reported by d'Hondt (1988) for the Israeli coast, whereas *Hippopodina iririkiensis* was added according to Tilbrook (1999; and pers. comm.). Three species, all belonging to the genus *Microporella*, were recorded by Harmelin *et al.* (2011), two of them described as new taxa.

Ascidians include 17 aliens, and rank therefore ninth. Except for *Distaplia bermudensis,* they are mostly established or even invasive species such as *Microcosmus squamiger.* What will happen to the NIS ascidian recently recorded, such as the cryptogenic *Ecteinascidia thurstoni* in Israel (Shenkar & Loya, 2009) and the invasive *Didemnum vexillum* in the Venice Lagoon (Tagliapietra *et al.,* 2012) cannot be foreseen.

B. Distribution of aliens across the Mediterranean

The number of aliens across the four Mediterranean MSFD subregions (including non EU state waters) is illustrated in Figure 2 and the detailed contribution of major groups in Figure 3. The vast majority of aliens occur in the EMED (775), whereas a lower number of species is

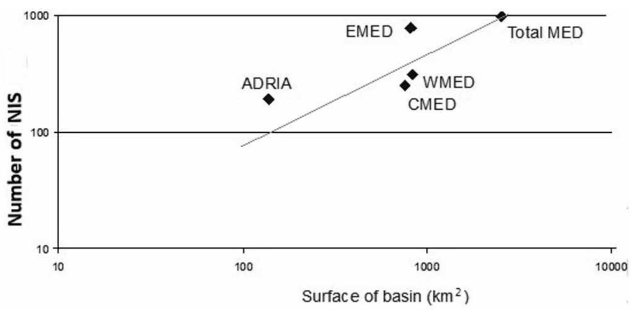

Fig. 2: Species-area plot of the number of NIS in the 4 MSFD subregions of the Mediterranean Sea as compared with the whole basin.

known for the WMED (308) and CMED (249), and even lowest for the ADRIA (190). Since the areas of WMED, CMED and EMED (760,000 to 860,000 km^2) are roughly comparable, the differences in numbers are meaningful. The ADRIA is much smaller (138,000 km^2) and appears heavily impacted by aliens if numbers are scaled (Fig. 2).

The decreased number of NIS in the WMED and CMED in relation to the 2010 figures is due to the removal from previous lists of species (mostly fishes) originating from the tropical Atlantic and of those classified as vagrant. On the other hand, in the EMED the NIS number has climbed to 775 (from 718 in 2010), as a result of the continuous influx of Indo-Pacific species found mainly along the Turkish and Israeli coasts (Table 3).

The number of alien molluscs is one order of magnitude higher in the EMED than in the remaining subregions. Several of the 76 newly reported species are already well established and spreading. Such is the case for the invasive opistobranch *Aplysia dactylomela* currently established in the EMED, CMED and ADRIA (Zenetos *et al.*, 2010); its latest record is from Montenegro (Kljajić & Mačić in Thessalou-Legaki *et al.*, 2012). Other introduced opistobranchs are also expanding their geographical range. *Polycerella emertoni* is now established in the WMED (Tunisia: Antit *et al.*, 2011); *Polycera hedgpethi* is well established in the ADRIA (Lagoon of Venice: Keppel *et al.*, 2012); *Bursatella leachii* in the WMED (Izquierdo-Muñoz *et al.* in Nicolaidou *et al.*, 2012; Ibañez-Yuste *et al.*, 2012).

The highest proportion of crustacean NIS occurs in the EMED. The highest proportion (78%) of crustacean NIS occurs in the EMED, being 28% in the WMED, 23% in the CMED, and 14% in the ADRIA. The mud crab *Dyspanopeus sayi*, hitherto known only from the ADRIA, has been found in the WMED (Alfacs Bay, Ebro River Delta, Spain) (Schubart *et al.*, 2012).

Also in the case of polychaetes, most NIS occur in the EMED, as is especially obvious in the case of serpulids. Numbers in the WMED, CMED and ADRIA are comparable.

It is worth noting the dominance of macrophytes among NIS in the ADRIA and the WMED, where they reach 26-30% of all aliens, whereas in the EMED they hardly add up to 10% of the introductions. This pattern is related to the introduction of macrophytes in coastal lagoons (Boudouresque *et al.*, 2011). Many macroalgae have expanded considerably their distribution range within the whole Mediterranean Sea. *Scytosiphon dotyi*, previously reported from the ADRIA and the WMED only, is now established in the Marmara Sea (EMED) (Taşkin, 2012). *Botrytella parva*, hitherto restricted to the ADRIA, was recently found on the shore of the Dardanelles Straits (EMED) (Taşkin & Pedersen, 2012). *Polysiphonia morrowii,* already established in the lagoon of Thau (WMED) and in the Venice Lagoon (ADRIA), has also colonized the Mar Piccolo of Taranto (CMED) (Petrocelli *et al.*, 2012). Although seaweeds are particularly prone to becoming invasive in the shallow areas they colonise, this does not seem to be the case throughout the Mediterranean. Up to 14 species have so been reported in the Medi-

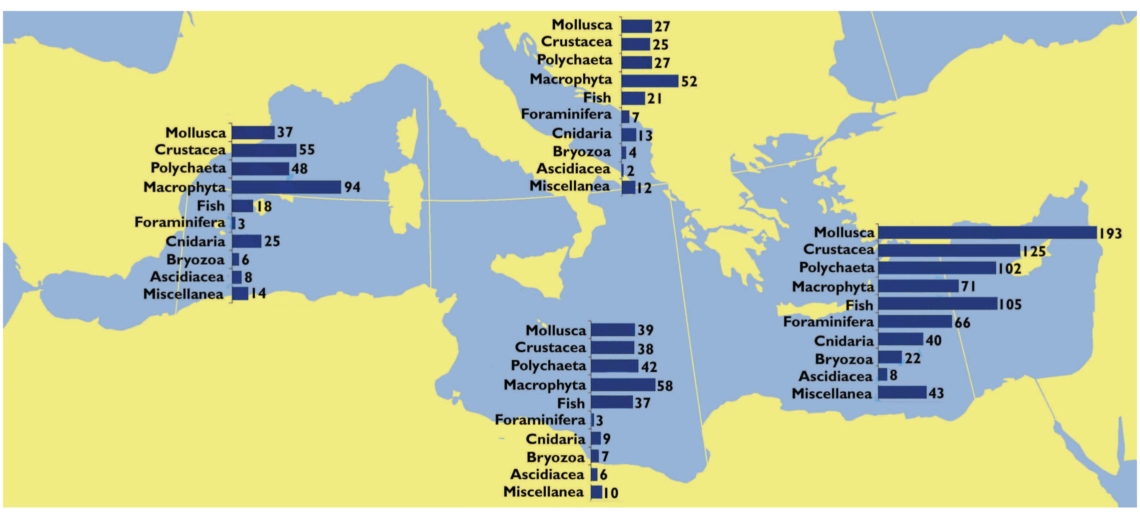

Fig. 3: Number of marine alien species per major groups in the MSFD subregions of the Mediterranean Sea.

terranean but only one (*Caulerpa racemosa* var. *cylindracea*) displays an invasive trait in all the subregions. Other species, such as the Rhodophyta *Womersleyella setacea*, *Lophocladia lallemandii*, *Asparagopsis taxiformis*, and the Chlorophyta *Codium fragile*, and *Caulerpa taxifolia*, occur in all the subregions but are not invasive everywhere. Such differences between subregions may have multiple explanations (e.g. time between introduction and observation, differences in climate conditions or competitive ability of native assemblages, etc.).

The vast majority of fish (106 species - 84.1%) has been reported from the EMED. Of these, 70 species have not been reported, to date, from any other subregion of the basin. The CMED is second in number of recorded NIS fish (34 species - 27.0%), with five exclusive records, whereas the WMED and the ADRIA have almost the same number of total records (18 species -14.3% and 19 species -15.1%, respectively) and of exclusive records (6 and 5, respectively). In all subregions the majority of NIS consists of casual records (WMED: 13 - 72.2%; CMED: 12 species - 35.3%; ADRIA: 15 species - 78.9%; EMED: 42 species - 39.6%), followed by the NIS that have been successfully established in their new environments (WMED: 4 species - 22.2%; CMED: 10 species - 29.4%; ADRIA: 4 species - 21.1%; EMED: 40 species - 37.7%).

Foraminifera play an important role among newcomers in the EMED. Warm tropical to subtropical amphisteginids (e.g. *Amphistegina lobifera*) have become very abundant locally in the Levantine Basin and along the northern coasts of Africa and are expanding their range northwestward as a consequence of the recent warming (Langer *et al.*, 2012). In the ADRIA the number of aliens is also increasing due to the spreading of species introduced and already established in the EMED and CMED, as predicted by Katsanevakis *et al.* (2011).

As regards cnidarians, Gravili *et al.* (in press) have recently reviewed the biodiversity of Mediterranean hydrozoans, alien species included. Most NIS were found exclusively in the EMED, but the number is comparatively high also in the WMED. In the ADRIA and the CMED alien cnidarians are less species-rich.

NIS bryozoans follow the general distribution trend, with the highest numbers reached in the EMED (22 species - 71%). Seven species have been found in both the WMED and the CMED. The EMED and the WMED were the only subregions from where alien bryozoans had been reported before 1950, often already recognised as such (Hastings, 1927). Only three bryozoans NIS are known from the ADRIA subregion. Several records in time exist for different aliens in the EMED, attesting of their establishment. In contrast, this does not occur usually in other subregions, except for *Tricellaria inopinata*, a species repeatedly recorded from the Venice Lagoon, whose spread in CMED and WMED has been well documented in recent times (Occhipinti Ambrogi & d'Hondt, 1994; Corriero *et al.*, 2007). A further species, *Electra tenella*, first recorded from the CMED, seems to be currently established, though not invasive, and also spreading in the WMED (Rosso in Thessalou-Legaki *et al.*, 2012).

Ascidians are similarly represented in the EMED, the CMED and the WMED, whereas their number is distinctly lower in the ADRIA. Five species seem to have spread to other areas from the original settlement: *Microcosmus squamiger* spread from the WMED to the CMED (Malta) (Izquierdo *et al.*, 2009); *Phallusia nigra* spread from the Levantine basin to the North Aegean Sea within the EMED (Koutsogiannopoulos *et al.* in Thessalou-Legaki *et al.,* 2012); *Herdmania momus* and *Symplegma brakenhielmi* from the south to the north Levantine basin (Çinar *et al.,* 2006); and *Microcosmus exasperatus* from the Gulf of Gabes in the CMED to the EMED (Izquierdo *et al.*, 2009; Ramos-Esplá *et al.,* in press).

C. Trends in introduction rates

The introduction rate per major group and per decade is depicted in Fig. 4. By 1950 there were already 176 introduced species, limited mostly to the Levantine basin. An overall increasing rate is evident since the 1970s, which however is variable among major groups. In the period 2001-2010 the introduction of molluscs, crustacean and fish is prominent. Many foraminiferal species have been added to the list since the 1980s, but it is unclear whether this reflects a real process, or an artefact due to an increase in taxonomic studies. Furthermore, this group does not have a pre-1950 baseline as detailed as for groups such as molluscs, fish or crustaceans decapods. On average, the number of macrophytes, molluscs and polychaetes increases by 2-3 new species per year, that of crustaceans by 3-4 species per year, while 6 fish species are introduced every year. The rate of introductions which was calculated to be *ca*. 1 species per 1.5 weeks between 2005 and 2010 (Zenetos, 2010), appears to slow down more recently. In the period January 2011 to October 2012, 48 new species have been reported (Table 2), which corresponds approximately to one new introduction every 2 weeks.

Overall, 211 new alien species have been collected during the last decade in the Mediterranean Sea, the majority of them in the EMED. Many more have been reported but some of them refer to earlier collections. In addition to the newcomers, established NIS appear to have expanded their distribution range considerably from one MSFD area to another. Thus there are 63 new findings in the WMED, 90 in the CMED, 52 in the ADRIA and 182 in the EMED. The temporal and spatial distribution of the main taxa is shown in Figure 5.

The number of alien molluscs has increased by 54.7% in the last decade; 215 species at present *vs* 139 reported in Gofas & Zenetos (2003). Of the 76 newly reported species, 46 were collected for the first time after 2001 and

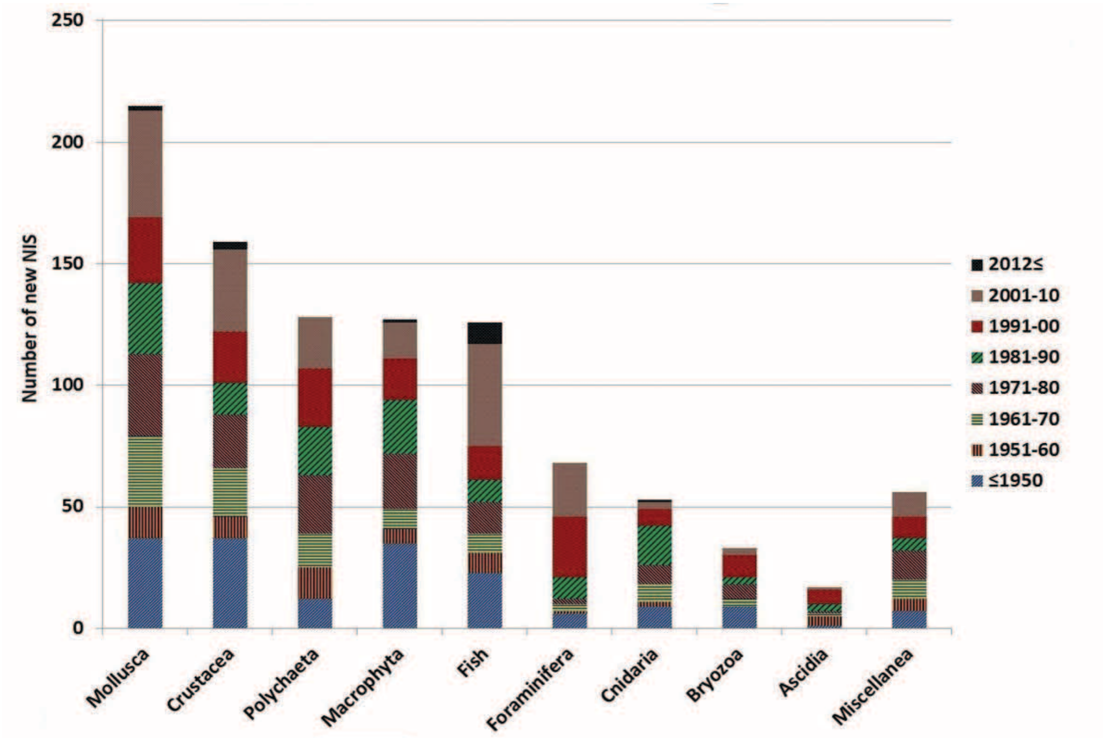

Fig. 4: Number of NIS introduced per decade, according to major groups, in the Mediterranean Sea.

some of them are already well established and spreading.

Only 37 alien crustaceans were known before 1950s in the Mediterranean. Between the 1950s and the-2000s, further 122 species were recorded, which represents an increase of 327%. The highest increase in decapod NIS occurs in the EMED. The analysis of the temporal trend of NIS belonging to small crustaceans (e.g. copepods, mysids, cumaceans and others) is for the moment unreliable, because information is scarce, and sometime too recent. For instance, the copepods are the most abundant group in ballast waters (adult specimens very abundant and common), but only 12 NIS have been reported in the WMED, and 10 of these since the 1970s. This is a low value when compared with other groups of larger sizes (e.g. decapods, with 22 species in the WMED).

Among the recent introductions of polychaetes, *Branchiomma luctuosum* became established in most of the Mediterranean areas in a relatively short period of time. It was introduced into the Mediterranean Sea, probably from the Red Sea, in 1983 and is at present observed in dense populations in many sheltered areas. Another *Branchiomma* species, *B. bairdi*, recently intro-

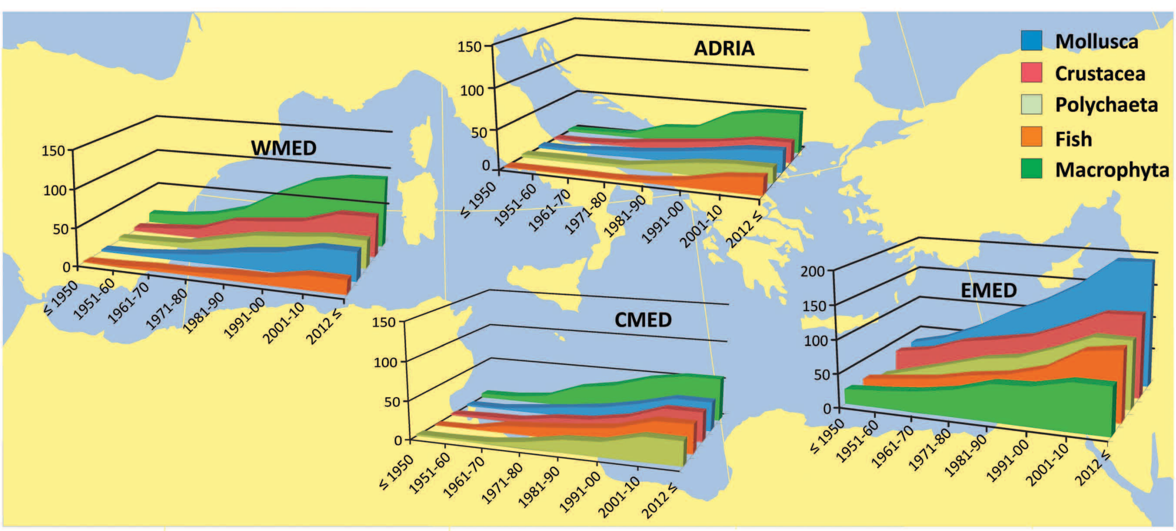

Fig. 5: Cumulative number of NIS of the main taxa by MSFD subregion.

duced from the Caribbean Sea (Çinar, 2009; Arias *et al.*, pers. commun.), also forms dense populations on many submerged structures, appearing even more invasive than the co-generic *B. luctuosum*. Mediterranean polluted sediments are densely colonized by NIS spionid polychaetes (i.e. *Polydora cornuta*, *Prionospio pulchra* and *Streblospio gynobranchiata*) that can now be accepted as new pollution indicators (Çinar *et al.*, 2012).

The number of introduced macrophytes has steadily increased over time. Their pattern of introduction is different in the four MSFD subregions. Among NIS, they dominate the benthic ecosystems in the WMED reaching more than 58 species in the French lagoon of Thau (a Japanese biological enclave in the Mediterranean Sea), which represent 32% of the species diversity and 48–99% of the macrophyte biomass on hard substrates (Boudouresque *et al.*, 2011). The situation in the ADRIA is similar: the increasing naval exchanges with extra-Mediterranean countries, for the import of fish products or aquaculture activities, introduce new species every year, mainly macroalgae (Sfriso & Curiel, 2007; Cecere *et al.*, 2009). In particular, the Venice Lagoon with 35 NIS macrophytes (*ca.* 70% of the algal flora, Sfriso *et al.*, 2012b), out of the 52 reported in the ADRIA, confirms to be the major NIS hot spot of the Adriatic Sea.

Fish invasions have dramatically increased in the last decade: 51 fish NIS (40.5%) were recorded for the first time in the Mediterranean Sea after 2001 i.e. during a period of 11 years, whereas the remaining 75 species (59.5%) were recorded in a span of 118 years (1882 to 2000). The early settlers have expanded their first ranges, and both the number of species and the affected areas have been increasing since 1950. Some recent immigrants, such as *Lagocephalus sceleratus* and *Fistularia commersonii*, are showing an unprecedented invasive character in terms of both abundances and geographical expansion, reaching rates of spread of approximately 1,000–1,500 km year^{-1} in the case of *Fistularia commersonii* (Azzurro *et al.*, 2012). The list of NIS fishes continues to increase, especially due to the influx of Lessepsian migration, with the Indian Ocean anchovy *Stolephorus insularis* as the latest published of these records (Fricke *et al.*, 2012).

Fewer than 8% of the alien foraminiferans were reported before 1950s, all from ADRIA. The majority of the species, 42%, was reported in the 1990s, and 33% since 2000. All species reported since the 1980s were from the EMED. The sharp increase in reports on foraminifera is partially related to the increasing awareness of foraminiferal specialists and to the publication of the Atlas on Recent Foraminiferida from the Gulf of Aqaba, Red Sea by Hottinger *et al.* (1993).

The majority of alien cnidarians were recorded in the 1980s, with an apparent decreasing trend in the subsequent decades.

As regards bryozoans, a total of 24 aliens, of the 31 recognised as NIS in the Mediterranean, has been recorded in the last 6 decades. This means a mean number of four new NIS per decade. Two peaks in the introduction rate, involving a new introduction every 2 to 3 years, can be observed in the 1970s and the 1990s, with seven and eight introduced species, respectively. In both periods, and especially in the 1990s, most bryozoans NIS were found in the EMED.

The majority of ascidian NIS has been recorded in the 1990s. However, ascidians are a taxonomically difficult group, which hampers monitoring the introduction of aliens in the Mediterranean Sea, especially in the case the introduced species appears superficially similar to a native one. Ascidians therefore include many cryptogenic species (e.g., *Cystodytes philippiniensis, Trididemnum* cf. *savignyi, Distaplia bermudensis* and *Perophora multiclathrata*). The list could be further enlarged considering newly described or still undescribed species, such as *Botrylloides pizoni*, which is suspected to be an introduced species (Brunetti & Mastrototaro, 2012).

D. Pathways

In addition to the MSFD, the EU Biodiversity Strategy (EU, 2011) specifically stresses the need to assess pathways of biological invasions through its Target 5: ‘By 2020, invasive alien species and their pathways are identified and prioritized, priority species are controlled or eradicated, and pathways are managed to prevent the introduction and establishment of new invasive alien species’. Ideally, a pattern in pathways is related to policy actions and the indicator is sensitive enough to convincingly demonstrate this kind of change. Trends in NIS related to pathways will be a tool towards this, particularly in hot spot areas such as ports, lagoons and aquaculture sites. Efforts to manage pathways should be reflected in the short and medium term in a declining trend of new introductions. A levelling off of the current increase in cumulative numbers of NIS, a reduction in their rate of establishment in new countries and subregions, and/or a shrinking distribution of these within European Seas would be a signal that this target is addressed successfully.

More than half (54%) of the marine NIS in the Mediterranean Sea were probably introduced by corridors (mainly Suez). In addition to the many Indo-Pacific species introduced via the Suez Canal, two crustacean mysids, *Hemimysis anomala* and *Neomysis integer*, were presumably introduced via inland canals (Wittmann & Ariani, 2007). Shipping is the second most common pathway of introduction, followed by aquaculture and aquarium trade (Fig. 6).

The Suez Canal, as a pathway of NIS, is believed to be responsible for the introduction of 493 alien species into the Mediterranean, approximately 11% being invasive (55 species), in good agreement with the “tens

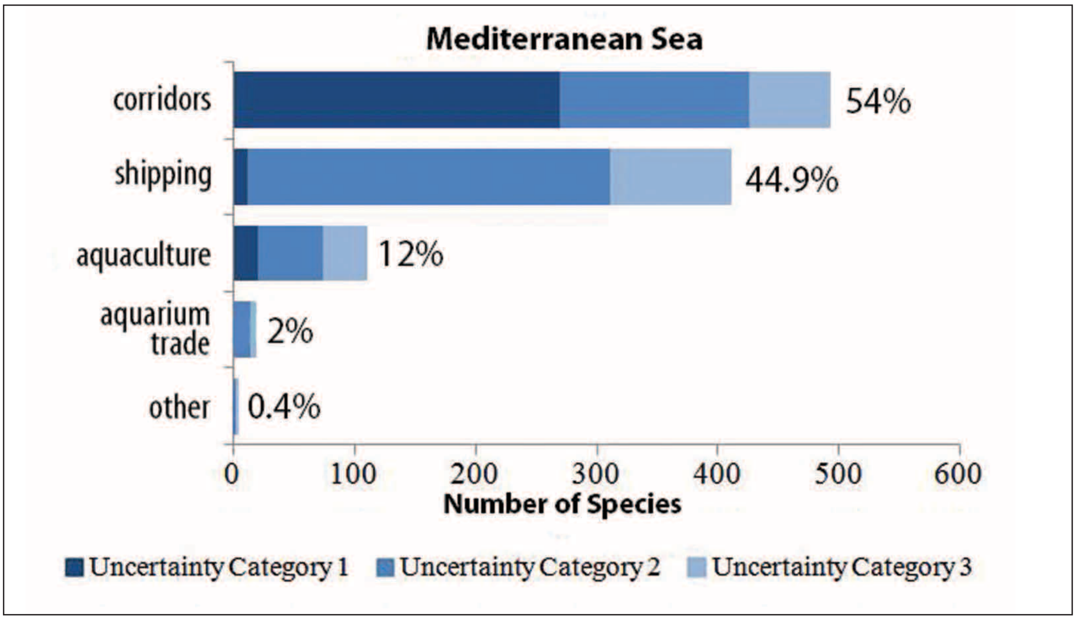

Fig. 6: Number of NIS known to be or likely to be introduced by each of the main pathways. Percentages add to more than 100% (i.e. 113%) as some species are linked to more than one pathway.

rule" of Williamson (1996). However, only 270 of these species are definitely classified as Lessepsian immigrants (uncertainty category 1). Of these 270 Lessepsian immigrants, 71 consist of casual records (based on one or two findings) while 175 are successfully established. 126 out of them (including 17 invasive ones) are limited to the EMED, whereas the others are progressively spreading in the neighbouring MSFD subregions.

Shipping is blamed directly for the introduction of 12 species only, whereas it is assumed to be the only pathway of introduction (via ballasts or fouling) of further 300 species (uncertainty category 2). Finally, for approximately 100 species shipping counts as a parallel possible pathway along with the Suez Canal or aquaculture (uncertainty category 3).

About 20 NIS have been introduced with certainty via aquaculture, either as escapees of imported species, mostly molluscs, or associated as contaminants: parasites such as *Myicola ostreae;* epibionts; endobionts; or in the packing materials (sessile animals, macrophytes). Many macroalgae occurring in the vicinity of oyster farms are assumed to be introduced accidentally via aquaculture activities: 56 NIS were retained as 'likely' or 'very likely' to have been introduced into the Europe from the north western Pacific through oyster trade (F. Mineur *et al.,* unpubl.data).

Aquarium trade, although currently limited to 2%, is gaining points as a pathway of introduction. A total of 18 species are assumed to have been introduced by aquarium trade, the only certain case (uncertainty level 1) is that of *Caulerpa taxifolia*. With the exception of four species, for which aquarium trade is suspected to be a parallel mode of introduction, the remaining 13 species are all tropical fish species kept in marine aquaria. The most plausible explanation for their presence appears to be accidental release (uncertainty category 2), though unaided introduction via the Suez Canal cannot be ruled out for those occurring in the Red Sea.

The contribution of the different pathways, expressed as percentage, is presented in Figure 7. NIS introduced via corridors (essentially the Suez Canal) are the majority in the EMED, and their proportion declines towards the western basin. The reverse pattern is evidenced for ship-mediated species and for those introduced with aquaculture. Here we show separately those species linked to both the Suez Canal and to shipping (uncertainty level 3); some of these Indo-Pacific species might have actually been introduced by shipping and not by natural means via the Suez Canal but there is insufficient information. They constitute a considerable portion ranging from nearly 9% in the EMED to ~6% in the WMED.

E. Pathway per group

Mollusca. Corridors (i.e. the Suez Canal) rank as the first pathway for their introduction. However, this is driven by the situation in the EMED where they achieve the largest number of species and where most of those came across the Canal, aided or not by shipping. In the ADRIA and WMED, shipping and aquaculture are the main vectors: there molluscs lag behind macrophytes and crustaceans. The sharing of different pathways is more balanced in the CMED, where the input from corridors is in the same order of magnitude as that from other sources (Antit *et al.*, 2011). With the natural expansion of many species first arrived in the EMED, the contribution of corridors to the CMED and WMED is expected to increase.

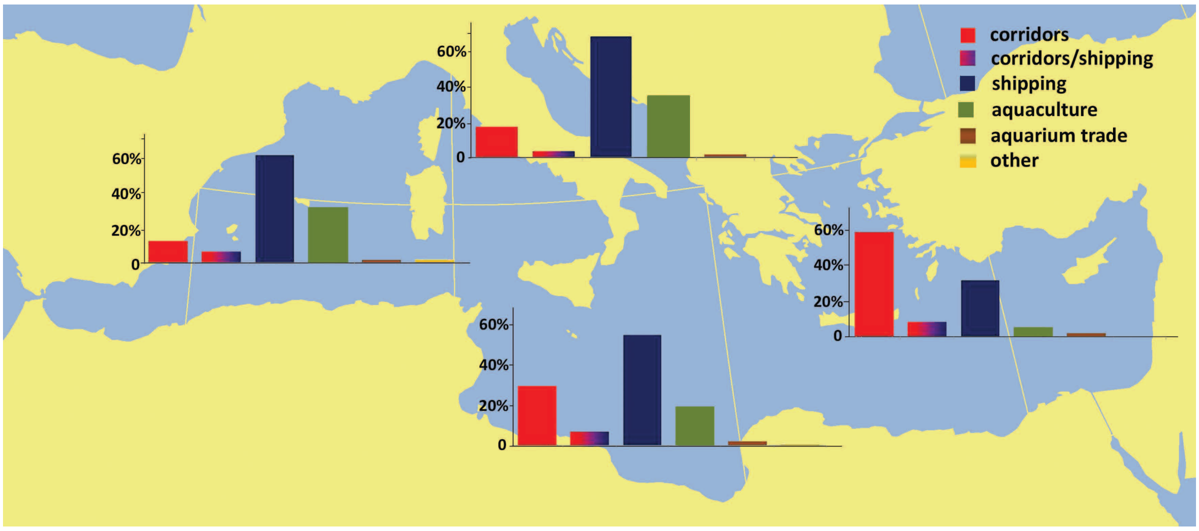

Fig. 7: Percentages of marine NIS known or likely to be introduced by each of the main pathways by MSFD subregion. Percentages add to more than 100% as some species are linked to more than one pathway.

Crustacea. Their principal pathway of introduction varies according to the subregion. In the EMED almost 80% are derived from the Indo-Pacific through the Suez Canal, although in some cases these inputs can be dual (corridors and shipping, either in ballast water or among hull fouling) or even caused by aquaculture. In the WMED the situation is different, as a considerable proportion of NIS (between 57% and 71%) has been introduced by shipping, 24% to 33% used corridors as a primary pathway (Suez Canal, and in a few cases inland canals), and only 10% to 14% can be linked to aquaculture. The increase of maritime traffic is an important pathway for introduction and dispersal of alien decapod species, since larvae can survive long periods in ballast water: such is the case for *Dyspanopeus sayi* (Mizzan, 1999; Occhipinti Ambrogi, 2000). The presence of NIS populations in some Mediterranean areas can also be related to their trade: *Necora puber* and *Paralithodes camtschaticus* (Faccia *et al.,* 2009) are quite frequently found alive in the markets. The introduction of *Marsupenaeus japonicus* has been attributed to escapes from aquaculture activities. Parasites such as *Myicola ostreae* are also the result of aquaculture activities.

Polychaeta. A recent analysis of alien polychaete species worldwide indicated that shipping is the major vector for species introduction (Çinar, 2013). Sessile species belonging to the Sabellidae and Serpulidae have been mainly transferred on ship hulls, whereas mud-dwelling species belonging to the Spionidae and Syllidae have become dominant components of the harbour environments due to ballast water discharges (Çinar, 2013). The step by step migration through the Suez Canal is a major pathway for species that have planktotrophic larval stages. In the Mediterranean Sea, the majority of species are of Red Sea or Indo-Pacific origin (almost 80% of alien polychaete species) and the main vector for species introduction largely depends on the sub region. As a whole, almost 50% of the species (including questionable species) have been introduced to the region via the Suez Canal corridor and the rest by ships. Species introduction via the other vectors such as aquaculture or sea-bait trade which are important in the Atlantic coast of Europe and some Indo-Pacific areas (Çinar, 2013) have not been reported in the Mediterranean so far. In general, Lessepsian immigrants mainly dominate the shallow-water habitats in the Levantine Sea, whereas many species carried by ships abundantly occur in harbours and lagoons of the WMED, ADRIA and other EMED basins (Aegean Sea and Sea of Marmara). While the Levantine Sea hosts 51 Lessepsian species (64% of the total number of alien species), the Aegean Sea hosts seven Lessepsian species (17%) and the Sea of Marmara only two (*Nereis persica* and *Metasychis gotoi*). Lessepsian immigrants comprise almost 51% of the alien polychaete species on the Levantine coasts of Turkey (Çinar *et al.,* 2011), but only 6% on the Italian coast (Occhipinti Ambrogi *et al.,* 2011).

Macrophyta. Unlike for most other groups, the main vector of introduction for macrophytes are not corridors or shipping but aquaculture and trade (import of shellfish products). It is not a coincidence that the hot spots of the new findings are not the coastal areas but lagoons such as the lagoon of Thau (WMED), the lagoon of Venice (ADRIA) and the Mar Piccolo of Taranto (CMED) where important oyster, Manila clam and mussel plants are located. Many species of macroalgae are used for keeping the imported fish and molluscs fresh and at the end of the working day they are discharged: in Venice Lagoon the greater part of the NIS have been reported from canals adjacent to fish markets. In this way the majority of large species were introduced and many small epiphytes associated with them. This is also the case of *Ascophyllum nodosum* for the Mar Piccolo of Taranto (Petrocelli *et al.*, 2012). However, *A. nodosum* has been present for many years in the fish markets of Venice and Chioggia to keep fresh *Pecten jacobeus* but has not yet been found attached to the canal banks as was the case for many other species. The lagoon of Thau is the Mediterranean area with the highest macroalgal introductions and this is mainly associated with the oyster farms that cover *ca.* 30% of the lagoon surface. Different pathways are likely for *Uronema marinum* (Sfriso *et al.*, 2012a; b), a very small species that can grow on multiple substrates (macrophytes, shells, hulls ...).

Fish. Corridors are the main pathway for alien fish introduction in the Mediterranean Sea, followed by relatively low percentages of aquarium trade and shipping. In cases of single records, it may be uncertain how the species arrived, and only research may resolve doubts; for example, the pathway of introduction for *Platax teira* has been unclear (Bilecenoglu & Kaya, 2006) until additional individuals were captured from the Levantine Sea indicating Suez Canal as the probable pathway (Golani *et al.,* 2011b). Similar substantiations are required for some recent fish records from EMED, such as *Epinephelus fasciatus* (Bariche & Heemstra, 2012) and a few more NIS including *Ostracion cubicus, Pomacanthus imperator* and *Pomacanthus maculosus*, all species purchased for the aquarium trade. Also the occurrence of *Epinephelus merra* in the WMED and of *Selene dorsalis* and *Scatophagus argus* in the CMED is attributed to aquarium trade. Aquarium-related NIS are not related to geographical regions, since records are sparsely spread within all MSFD subregions, but rather to the increasing exchange of marine species between aquaria. Shipping-mediated fish introductions have quite similar proportions in the four subregions: latest introductions, such as *Paranthias furcifer* (Dulcic & Dragicevic, 2012a) and *Holacanthus ciliaris* (Dulcic & Dragicevic, 2012b), have been reported from ADRIA. Shipping and aquaculture transfers should be of great concern, since they reveal that (a) in the case of shipping, the current regulations regarding ballast water exchange clearly seem to be inadequate to prevent translocation of species and (b) public awareness about the impact on the environment of releasing NIS in the wild is not sufficient.

Foraminifera. The main pathways for the diffusion of alien foraminifera in the Mediterranean are inferred to be the Suez Canal and shipping. Most of them are Indo-Pacific species, common or also originally described in the Red Sea (i.e. *Cibicides mabahethi*). The highest taxa diversity and abundance occur in the EMED, mainly along the Israeli and Turkish coasts, so suggesting that many species act as Lessepsian immigrants. Direct invasion through embryos diffusion is likely for the well established amphisteginids: shipping most probably favoured the introduction of many rare species collected in harbour areas only (*Articulina mayori*, *Septloculina* spp., and others in Iskenderun Gulf; *Planispirinella exigua*, along the Croatian coast).

Cnidaria. Most NIS were found exclusively in the EMED, and are either Indo-Pacific or circumtropical in origin; this distributional pattern may suggest they penetrated through the corridor of the Suez Canal, thanks to their free-swimming medusa stages. However, species having a hydroid stage are often important component of ship hull foul-

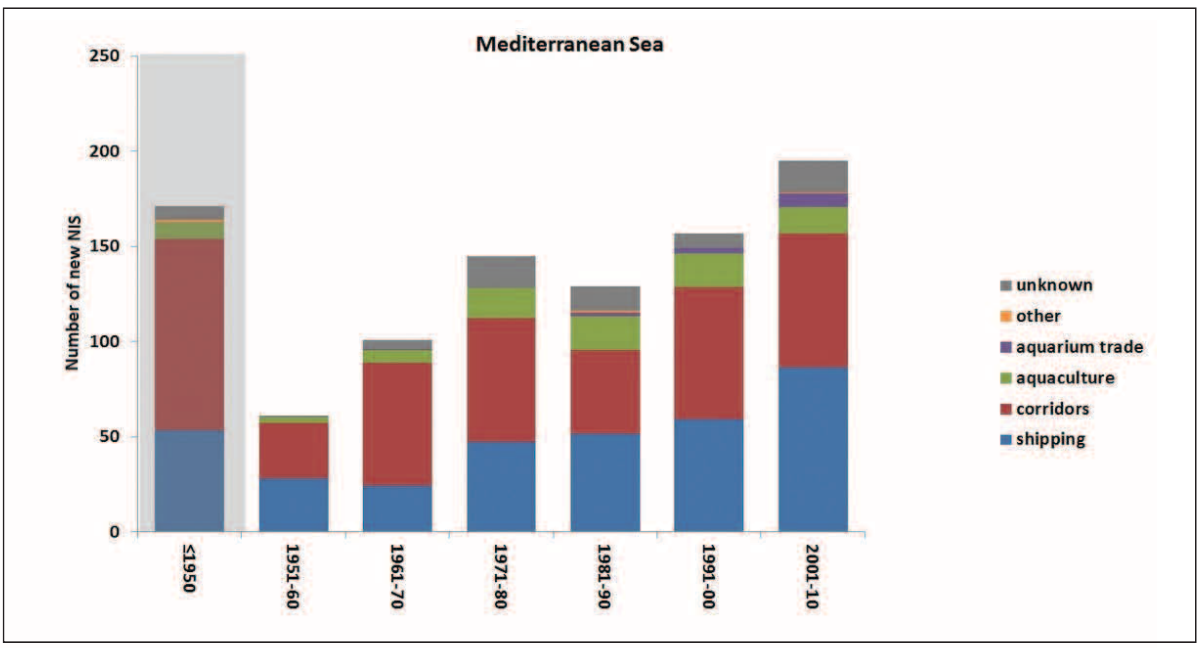

Fig. 8: Temporal trends in the numbers of newly recorded marine NIS in the Mediterranean in relation to the pathway of introduction. Some species that were linked to more than one pathway were given a value of 1/k for each of the k associated pathways so that the overall contribution of each species to the total number of new NIS per decade was always 1.

ing (Morri & Boero, 1986; Shoukr, 1987), so that a corridor pathway for these species cannot be taken for granted, and shipping may be also involved (Morri *et al.*, 2009). Shipping is the second vector of introduction for cnidarians species, whereas aquaculture possibly has a role.

Bryozoa. The main pathways for the introduction of alien bryozoans in the Mediterranean are shipping and corridors. The arrival through the Suez corridor has been documented or at least suggested for aliens occurring in the EMED (Harmelin *et al.*, 2011, for instance) whereas shipping has been considered as the principal vector for CMED and WMED aliens, probably among hull fouling. In contrast, the introduction through aquaculture activities has been suggested only for *Crepidacantha poissoni* in the CMED, but the same vector cannot be excluded for other species. Finally, drifting plastics could represent a further vector for spreading inside the Mediterranean, as suggested for *Electra tenella* (Rosso in Thessalou-Legaki *et al.*, 2012).

Ascidia. Their lecitotrophic larval stages have a relatively short pelagic period (minutes to hours), so that the principal vector for ascidian introduction must be shipping (among hull fouling rather than in ballast waters). These NIS ascidians occur in harbours, and there is some dispersion mainly by colonization of degraded and/or artificial habitats. This notwithstanding, a few species may have been introduced through aquaculture (certainly *Styela clava* and, perhaps, *Botrylloides violaceus, Polyandrocarpa zorritensis* and *Didemnum vexillum*, associated to shellfish culture). *Ecteinascidia thurstoni* probably penetrated the EMED (Shendar & Loya, 2009) through the Suez Canal (Gab-Alla, 2008).

F. Trends in pathways

The dynamics of the invasion pathways since 1950 (Fig. 8) indicate a steady rise in numbers of NIS introduced via corridors and of those that are ship-transferred. Increasing trends of NIS with time are seen in all MSFD subregions, being more evident in the EMED and CMED (Fig. 9).

The rate of Lessepsian migration has been increasing particularly in the last decade. This is partly attributed to the continued enlargement of the Suez Canal. According to Rilov & Galil (2009) this is the main cause of the apparent acceleration in the rate of Lessepsian invasion over the last five decades. This trend is particularly evident for fish (Golani, 2010).

Increase in trade, tourism and maritime activities have provided new and enhanced pathways for the spread of marine NIS through shipping. Shipping has been reported to be responsible for the introduction (either among hull fouling or in ballast waters) of 54 NIS until 1950. The current rate (based on the last decade) of ship-mediated NIS in the Mediterranean is one new species every ~six weeks. The observed increased trend in new introductions by shipping is not expected to halt unless effective measures are taken. Trends in new introductions of alien species by shipping are expected to decrease only when the ‘International Convention for the Control and Management of Ships’ Ballast Water and Sediments’ (BWM Convention) becomes legally binding, by substantially reducing the transfer of marine species via ballast water. Nevertheless, introductions by hull-fouling will remain.

Introduction of NIS through aquaculture is apparently slowing down. In the last decade, aquaculture has been responsible for 14 new NIS in the Mediterranean *vs* 18 species in the previous two decades 1981-1990 and 1991-2000, but new NIS continue to appear in the vicinity of oyster farms (M. Verlaque & F. Mineur, unpubl. data). The Aquaculture Regulation -Council Regulation (EC) No 708/2007 of 11 June 2007- concerning use of aliens and locally absent species in aquaculture - estab-

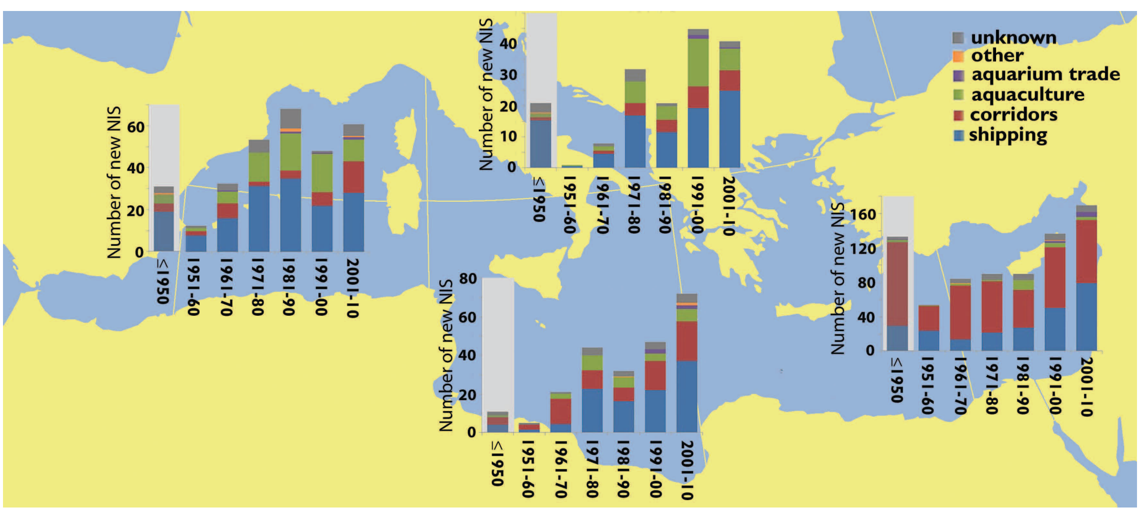

Fig. 9: Trends of NIS introduction per decade by MSFD subregion. subregion. Some species linked to more than one pathway were given a value of 1/k for each of the k associated pathways so that the overall contribution of each species to the total number of new NIS per decade was always 1.

lished a framework with which to assess and minimise the possible impacts of aliens and locally absent species used in aquaculture, including procedures for risk assessment, to ensure adequate protection of aquatic habitats from non-native species. However, since 2008 the whole European shellfish aquaculture is affected by severe and repetitive oyster mortalities and massive imports of non-European livestock are again being considered as a solution for the crisis, despite the risks of accidental NIS introduction associated with these imports.

In the **WMED**, shipping remains the most prominent pathway of introductions and its lower proportion in the two last decades reflects the increase of other sources rather than a genuine decline. Shipping at large may include species introduced with fishing discards, as documented for the gastropods *Bostrycapulus odites* and *Marginella glabella* in the fishing harbours of Alicante and Málaga, respectively. The decades of 1970 to 2000 represent the heyday of introductions through aquaculture, both intentional and accidental. The decline of native or anciently introduced commercial species such as the bivalves *Venerupis decussata* and *Crassostrea gigas* triggered imports of substitute species, or massive imports of spat for restocking (Mineur *et al.*, 2007, and references therein). Nowadays, such imports have been considerably reduced as a consequence of the self-sustaining spat production, and also the stock of alien species most likely to become introduced is now established in the Mediterranean. The rise of corridors as a pathway to the WMED in the last decade is a consequence of the slow but steady progress of species, which first arrived in the EMED through the Suez Canal to successively spread throughout the whole basin. Among the forerunners, the opistobranch *Bursatella leachii* is the first Lessepsian species to have reached the Alboran Sea (Ibañez-Yuste *et al.*, 2012).

The increasing importance of **NIS** is particularly evident for the **CMED**, which separates the western from the eastern sectors of the basin. In this subregion shipping is the main pathway that accounts for the introduction of most species. The incidence of this pathway has increased since the 1971-80 decade, and particularly in the last period from 2001 to 2010, roughly paralleling the general trend in increasing introductions, notwithstanding a light drop in the 1980s. But the corridor pathway seems to feed new aliens irregularly in this subregion, with no apparent correlation with the general trend or the trend of introductions in the nearby EMED. This is probably the result of dispersal mechanisms and pathways between the different areas within the Mediterranean; their timing has not been specifically investigated and still remain poorly known. Aquaculture is a subordinate source of introduction of alien species. However, it shows an increasing pattern, more evident in the last decade. Finally, other pathways are very subordinate. One of the last reported species is *Lagocephalus sceleratus* (Jribi & Bradai, 2012), a highly invasive fish, known for its toxicity and potential danger to humans. Only few decades ago, the CMED was basically not affected by Lessepsian immigrants (Por, 1978) and the Sicily Straits has been long considered as the ultimate western barrier to their dispersal (Quignard & Tomasini, 2000). Yet, the number of Lessepsian immigrants has dramatically increased in the central sectors of the basin, with an increasing number of established invaders such as *Fistularia commersoni* and siganids that have already migrated to the west. The CMED would deserve to be continuously monitored to detect recent colonization events, being a platform of dispersal to the WMED rather than a barrier to that.

The number of NIS currently recorded in the **ADRIA** is considerably lower than that in other subregions, but - as mentioned above - this must be qualified and scaled to the substantially smaller area involved. The majority of species have arrived through shipping, followed by aquaculture. The rate of ship mediated NIS in the ADRIA is constantly increasing as in the EMED and CMED. It has been estimated that 25 NIS have been introduced via shipping in the last decade. Aquaculture associated introductions peaked in the decade 1991-2000 with an average of 15 new NIS (the second highest rate after the WMED). However, this rate has been reduced to half (8 NIS in the period 2001-2010) presumably as a result of the Aquaculture Regulation. Among the NIS introduced through aquaculture, the most successful invaders are the bivalves *Arcuatula senhousia, Crassostrea gigas* and *Ruditapes philippinarum.* The occurrence of *Gracilaria vermiculophylla* (Sfriso *et al.*, 2012b), a species previously introduced in the NE Atlantic, may be attributed to the aquaculture activity or the shellfish trade via importations from the Atlantic. Corridors are also significant as a pathway (spreading of Lessepsian NIS), however, it is less important than in other MSFD subregions. The occurrence of Indo-Pacific fish has been documented mainly after the mid 1980s, when the Adriatic ichthyofauna was well known. To date, at least 16 NIS fish have been recorded in the ADRIA. Almost all of them are considered Lessepsian immigrants. However, very few Lessepsian immigrants can be considered as established, since most of them were, till now recorded only as single or few specimens. The rate of Lessepsian NIS extending their distribution in the ADRIA has doubled the last two decades. It seems that the changes in the patterns of water exchange between the Adriatic Sea and the Mediterranean as well as a rise in the eastern Adriatic Sea surface temperatures in 1985-1987 and 1990-1995 are correlated with the occurrence of Indo-Pacific species, some of them for the first time, others expanding their distribution from the neighbouring subregions where they are already established. The latest finding is that of *Lagocephalus sceleratus* (L. Lipej, 2012 unpubl. data), one of the most invasive species in the EMED. The Gulf of Venice and other North Adriatic coastal lagoons are at the moment the areas the most colonized by NIS, as previously reported by Mizzan (1999) and Occhipinti Ambrogi *et al.* (2011). In the northern Adriatic Sea alien macroalgae, crustaceans and molluscs are more numerous than in the central and southern part. The majority of molluscs and macrophytes were introduced via aquaculture, whereas alien crustacean and polychaeta NIS were mostly related to the introduction by shipping ballast waters or as fouling organisms.

In the **EMED** subregion, the Levantine Sea ranks first in terms of NIS belonging to all groups, followed by the Aegean Sea and the Sea of Marmara. The main pathway of their introduction is the Suez Canal corridor but shipping plays an equally important role. The number of NIS introduced via either the Suez Canal or shipping has tripled since the 1950s (Figure 9), but the increasing pattern was not the same. For Lessepsian immigrants there was an abrupt increase in the 1960s which continued over the next decades and has remained steady during the last two decades, with an average of more than 70 introductions every 10 years. A decline in NIS was observed in the 1980s, which Galil (2009) attributed to the closure of the Suez Canal between 1967 and 1975, during the June 1967 six-day Arab-Israeli war, and the impact of the Arab Oil Embargo on oil shipping and international trade that limited the number of vessels entering in the Mediterranean. On the other hand, the contribution of shipping has steadily increased reaching approximately 78 species in the 2010s. Some international harbours, such as Port Said, Haifa, Iskenderun, Mersin, Alsancak and Peiraias, are the hot spot sites for NIS establishment. In these areas, species introduced by shipping, such as *Polydora cornuta* and *Streblospio gynobranchiata*, dominated the zoobenthic community, more or less removing the long-known pollution indicator species such as *Capitella* spp. and *Scolelepis fuliginosus* from the area (Çinar *et al.,* 2012). In the Sea of Marmara, 69 alien species were reported (Çinar *et al.,* 2011). The main vector is shipping (71% of the total number of species). In addition, 23% of the species were the Lessepsian immigrants, and 4% of the species were transferred to the area via aquaculture.

It is worth mentioning that, since 2001, the number of NIS fish introduced in the Mediterranean via shipping and aquarium trade has increased notably. Unlike other groups, to date, we do not know of any foraminifera that were introduced to the EMED via shipping.

The increase of marine NIS in the Mediterranean is certainly attributed to an increase of human activities but it is also a consequence of climate change. From 1985 to 2006 the temperature in the upper layer of the Mediterranean Sea has been increasing at an average rate of 0.03°C year^{-1} for the WMED and 0.05°C year^{-1} for the EMED (Nykjaer, 2009). Abrupt rising temperature since the end of the 1990s has modified the potential thermal habitat available for warm-water species, facilitating their settlement at an unexpectedly rapid rate, and it has been shown that the introduction of tropical alien species has been exacerbated by the warming of the EMED (Raitsos *et al.,* 2010).

G. Data-shortage and data-uncertainties: our Achilles' heel

Data availability: The picture reflects the scientific effort including taxonomic expertise availability not only in discovering and reporting new species or mapping their distribution but even for compiling data at national levels.

Missing taxa: Our Mediterranean inventory of NIS is still incomplete since of the unicellular organisms only foraminiferans were considered. Bearing in mind that the diversity of marine unicellulars is scarcely known in wide areas of the Mediterranean Sea and that it can rarely be excluded that a suspected unicellular invader was already present as part of the rare, hidden and unsampled plankton, we refrain from citing a detailed list of plankton.

Uncertainty in taxonomy: In general, it is important to stress that recently strong efforts are being made for evaluating the real state of certain presumed widely distributed or cosmopolitan species. It is often demonstrated that such 'species' are actually complexes of species when carefully investigated using molecular tools or even fine morphological characters.

This applies to many groups including Bryozoa, Foraminifera, macroalgae and Polychaeta. For bryozoans, molecular analyses demonstrated a promising method for clade discrimination in some species belonging to *Bugula*, *Celleporella* and *Electra*. However, SEM analysis was also often revealed as a resolutive tool for distinguishing species showing close similarities (Rosso, 2004; Berning *et al.*, 2008), or to state the actual conspecificity of populations (Harmelin *et al.*, 2012), above all in concurrence with examination of species types. But, until now few taxa have been checked (see Harmelin *et al.*, 2011, for instance) and uncertainties in managing literature data in the absence of figures/material have to be considered. Consequently, it is difficult to make inferences, especially when using literature data, when species have not been figured.

Uncertainty of natural range: Resolving natural ranges and marine invasions in globally distributed species requires molecular analyses. Mitochondrial (mtDNA) and nuclear (nDNA) sequence data from different populations may confirm the source of the introduced population (Concepcion *et al.*, 2012; Schubart *et al.* 2012). Additional information and insights would be provided by past distributions, at least for species with the ability to be fossilized and whose fossil record is clearly evident and known. For instance, the interpretation of the cryptogenic state of the bryozoan *Catenicella paradoxa*, recently described from the CMED but belonging to a genus presently absent from the Mediterranean, is partly supported by the presence of Pleistocene fossil representatives of the same genus in southern Italy (see Rosso, 2009).

Uncertainty of introductions via Gibraltar: The status of some species with an eastern Atlantic origin in the WMED is difficult to determine, because (1) intense fishing and transport activities (goods, commercial passengers, tourists) occur between Africa and Spain or France (e.g. the ports of Algeciras, Malaga, Barcelona, Marseille), which represents a potential and continuous source of introduction of NIS, via ballast water and transfers of organisms attached to ship hulls as fouling or fishing discards, as recently exemplified by the introduction of the West African gastropod *Marginella glabella* into Málaga harbour (Luque *et al.*, 2012), (2) the Strait of Gibraltar is a boundary more or less permeable to Atlantic species that naturally increase their distribution range, and (3) there is a limited knowledge on the biodiversity from North African littoral. Therefore some of the species recently reported in the WMED and considered to have expanded their geographic range could be in fact introduced by man.

Effect of climate: Climate change can enhance establishment of some introduced NIS (see Por, 2010; Raitsos *et al.*, 2010). Consequently, it skews the real magnitude of the phenomenon attributed solely to anthropogenic intervention.

The way ahead

Although the European states have a comprehensive regulatory framework to protect economic interests against diseases and pests, these are often inadequate to safeguard against species that threaten native biodiversity. Moreover, the regulatory system pertains to pathogens while large sized species that may have considerable impact on health or the economy are not considered to date.

Taking into account the aim of the MSFD to achieve Good Environmental Status but also the gap in our knowledge on the processes and impacts of marine biological invasions in the Mediterranean, important areas of action and further research should be initiated:

- To create and keep updated an inventory of all marine NIS in the Mediterranean. As Grosholz (2002) stated, perhaps the largest obstacle to understanding broader patterns of invasion is to identify the species, due to the global decline of taxonomy in research projects, and the uncertainty on which species are native and which are NIS for the less studied groups, such as some marine and estuarine invertebrates (e.g. zooplankton, foraminifers). It is crucial to know the identity and spatial distribution for each species. Towards that end, molecular tools have been proven very useful to identify possible sources of the NIS and their degree of hybridization with their native counterparts. This is particularly interesting for marine species, most of which have planktonic larvae that permit long-distance dispersal and consequently gene flow among different populations.
- To search ecological and economic consequences of NIS at community and ecosystem levels. The presence of one or more NIS can strongly modify the structure of an entire community (e.g. the case of alien engineer-species). These changes are usually related to changes in trophic levels. Diversity of certain recipient communities has recently been shown to influence invasion success with more diverse communities being less easily invaded (Stachowicz *et al.,* 1999). However, this rule is still strongly debated. It seems to be necessary to have a base-line of the different native communities in the Mediterranean (e.g. algae, soft-bottom, litoral rocky bottom, etc.), including information on ecological and functional diversity, trophic webs, temporal dynamics, etc., in order to be able to assess the impact of any NIS on any particular community. In general there are few studies on the impact of NIS on ecosystem processes in marine and coastal ecosystems. This type of research must be multidisciplinary in order to take into consideration all the compartments related with the invader(s).
- Research on the NIS life cycles. A better understanding of the biological characteristics that favour or not the spread of NIS would help us to predict future invasions and to suggest management measures to mitigate their impact.
- Biological invasions are almost always large-scale processes, so tracking their onset followed by their subsequent spread is certainly challenging for the scientific community. To overcome this difficulty, one effective solution is to involve citizen-scientists and NGOs in the monitoring of NIS or in reporting historical information through national, regional, and European networks such as the CIESM JellyWatch Program, MAMIAS (UNEP-MAP-RAC/SPA, 2012), the European Alien Species Information Network (EASIN; Katsanevakis *et al.,* 2012), etc. Recently, participative actions have started to be experimented in the Mediterranean Sea (see Azzurro *et al.,* 2011 for fish species). These innovative methodologies can provide information that otherwise cannot be obtained and should be seriously taken into consideration due to our increasing need to be informed about the changing biodiversity.
- To develop species distribution models under present and future climatic conditions.

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