VECTORS of Change in Oceans and Seas Marine Life, Impact on Economic Sectors

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VECTORS Overview

‘VECTORS seeks to develop integrated, multidisciplinary research-based understanding that will contribute the information and knowledge required for addressing forthcoming requirements, policies and regulations across multiple sectors.’

Marine life makes a substantial contribution to the economy and society of Europe. In reflection of this VECTORS is a substantial integrated EU funded project of 38 partner institutes and a budget of €16.33 million. It aims to elucidate the drivers, pressures and vectors that cause change in marine life, the mechanisms by which they do so, the impacts that they have on ecosystem structures and functioning, and on the economics of associated marine sectors and society. VECTORS will particularly focus on causes and consequences of invasive alien species, outbreak forming species, and changes in fish distribution and productivity. New and existing knowledge and insight will be synthesized and integrated to project changes in marine life, ecosystems and economies under future scenarios for adaptation and mitigation in the light of new technologies, fishing strategies and policy needs. VECTORS will evaluate current forms and mechanisms of marine governance in relation to the vectors of change. Based on its findings, VECTORS will provide solutions and tools for relevant stakeholders and policymakers, to be available for use during the lifetime of the project.

The project will address a complex array of interests comprising areas of concern for marine life, biodiversity, sectoral interests, regional seas, and academic disciplines and especially the interests of stakeholders. VECTORS will ensure that the links and interactions between all these areas of interest are explored, explained, modeled and communicated effectively to the relevant stakeholders. The VECTORS consortium is extremely experienced and genuinely multidisciplinary. It includes a mixture of natural scientists with knowledge of socio-economic aspects, and social scientists (environmental economists, policy and governance analysts and environmental law specialists) with interests in natural system functioning. VECTORS is therefore fully equipped to deliver the integrated interdisciplinary research required to achieve its objectives with maximal impact in the arenas of science, policy, management and society.

www.marine-vectors.eu
**Executive Summary**

The marine environment is facing rapid change in marine life. VECTORS has aimed to elucidate drivers, pressures and vectors of these changes. WP2.1 has particularly focused on delivering new information to:

a) Document the distribution of invasive alien species (IAS) and outbreak forming species (OFS);

b) Determine the stochastic and deterministic drivers of proliferations and ecophysiological features boosting regime shifts of marine communities;

c) Discriminate sources and vectors of bioinvasions;

d) Foresee consequences of bioinvasions in terms of impact on ecosystem functioning and services.

All tasks have been successfully achieved. Outcomes include AquaNIS which represents the most updated database resource available to scientists, coastal managers, stakeholders, and policy makers dealing with aquatic IAS in European seas. A DNA/tissue repository bank of invasive alien species and outbreak-forming species has been established. It will enable future research using molecular tools for investigating marine bioinvasions. Novel data have been produced on OFS/IAS ecophysiology, behaviour, reproductive patterns, species interactions, ecological impacts, species growth rates, habitat susceptibility, drivers of invasions.

This new information from VECTORS has wide implications going far beyond the various study species and geographical areas, representing a model integrative approach towards a better understanding of the mechanisms of bioinvasions. Key findings can be summarised as follows:

- Large shifts in environmental abiotic parameters do not have uniform consequences on IAS and OFS biota, but biotic interactions play a key role in modifying local communities as a result of bioinvasions;
- Climate-driven warming is expected to exacerbate the potential trophodynamic impacts of invaders;
- Depending on whether thermal windows are exceeded at single-species level, the productivity of endemic jellyfish species in the North Sea will not inevitably increase with sea temperatures;
- In the eastern Mediterranean Sea, water temperature provides a mechanistic explanation for the replacement of indigenous species by thermophilic invaders in that region, similar future effects in the western Mediterranean can be anticipated;
- Return time of extreme jellyfish outbreaks in the Mediterranean Sea has been estimated as approx. 6.5 years;
- Jellyfish outbreaks should not increase in the Mediterranean region simply as a consequence of environmental fluctuations. Extreme outbreak events are likely to occur in the near future at sites in the proximities of marine canyons. Cross-shore advection by geostrophic flow represents an important aggregating force of jellyfish. Ability to anticipate these extreme events in the future will require a better understanding of how environmental drivers affect demographic and population processes in jellyfish species;
- Substantial insights on a key jellyfish species in European Seas, *Pelagia noctiluca*, have been achieved in terms of ecophysiology, reproductive potential, trophic interactions, and population genetic connectivity. An Individual-Based Growth Model (the first bioenergetic model ever developed for a jellyfish species) has been developed, providing a new tool to predict the ecological impact of *P. noctiluca* outbreaks;
- New species of conspicuous alien jellyfish have been identified and described for the first time in European seas;
- Risk of species translocations by shipping, aquaculture, sea food trade in European seas are still high, requiring reinforcement of surveillance and monitoring tools for prevention of new alien introductions; movement and marketing of live trade species should adhere to strict controls similar to those proposed for other pathways in order to prevent unintentional non-indigenous species introduction.
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- The recent enlargement (2010) of the Suez Canal is revealed to be critical for maximizing invasion success of Erythraean biota in the Mediterranean Sea; implications of future increase of the Canal’s depth or width are manifest. The many recent records and rapid spread of several invasive aliens are documenting a Mediterranean Sea climate change signature.

Altogether, the outcomes of VECTORS will represent a fundamental toolkit for application of the Marine Strategy Framework Directive in European Seas and for everyone dealing with the integrative coastal zone management and the protection of marine ecosystems processes and services.
**Introduction**

VECTORS research in WP2.1 has been dedicated to identifying mechanisms of species outbreaks and invasions and introduction vectors as both symptoms and drivers of change in European marine ecosystems as well as coupling distributional databases, life histories, and biological traits to environmental conditions fostering outbreaks/invasions of native or alien species (Box I). These studies have provided a substantive contribution to the understanding of the ecological conditions in which outbreaks and alien invasions are more likely to occur, and the determination of critical thresholds/tipping points that mark alternative regime shifts in community structure and organization.

In particular, research undertaken in WP2.1 has:

- Created an inventory of outbreak-forming species (OFS) and invasive alien species (IAS) in European seas, and a repository DNA/tissues bank (D2.1.1);
- Improved the overall understanding of generic mechanisms/drivers of species outbreaks and alien invasions, with special attention to selected species, especially jellyfish, crustaceans, fish, and other key OFS / IAS taxa;
- Defined the environmental situations and biological traits fostering outbreaks/invasions;
- Facilitated prediction of habitats/communities suitability for outbreaks/invasions.

WP2.1 research aimed to link invasions and outbreaks with the direct and indirect pressures of change (using outputs of WP1), support determination of synergistic and antagonistic effects among these pressures to boost understanding of local impact, support ecological and socio-economic assessment and develop forecasting models (WP2 understanding and knowledge has also been used for research carried out in WP3); provide baseline data essential to analysis of control/mitigation countermeasures (in WP5) and for development of management operational strategies for stakeholders and policy makers (in WP6).

**BOX 1 – OUTBREAK-FORMING SPECIES AND INVASIVE ALIEN SPECIES**

Outbreak forming species (OFS) are indigenous or alien species with invasive potential, undergoing pulse-like, periodic exponential population growth (usually days to months) during which they have an impact on biological diversity, ecosystem functioning, socio-economic values or human health.

Non-indigenous (i.e. alien, exotic, non-native) species (NIS) are introduced organisms outside their natural (past or present) range of distribution, and outside their natural dispersal potential, which might survive and subsequently reproduce, threatening biodiversity. Species of unknown origin that cannot be ascribed as being native or alien are termed cryptogenic species. In many cases, non-indigenous species do not harm the regional ecology and economics. However, in certain cases, non-indigenous species can become “invasive” species and have enormous and long-lasting impacts on the region. The Marine Strategy Framework Directive lists NIS as Descriptor 2 for the evaluation of Good Environmental Status (GES) of marine ecosystems, stating that NIS should be at levels that do not adversely alter the ecosystem.

Invasive alien species (IAS) are a subset of established NIS able to spread in invaded regions and impact biological diversity and/or ecosystem functioning, socio-economic values, or human health.

The goal of this work package was to understand the mechanisms of species outbreaks and invasions as both symptoms and vectors of change in marine ecosystems, and their potential to drive ecosystems. A suite of integrated targets were selected aiming to:

- document outbreak/invasions occurrence, spread and abundance by database compilation and collection of new descriptive data;
- identify biological features and environmental changes apt to boost outbreaks and invasions, by both iterative analysis of literature reviews and experimental work;
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- discern correlations with environmental indexes and drivers, distinguish critical thresholds of change;
- quantify spatial and temporal level of probabilities of outbreak/invasion events, by multiple regressions and environmental bootstrapping methods;
- discriminate the sources of changes and foresee future trends of invasions due to main vectors (i.e., ballast waters and fouling, aquaculture, waterways and global warming) and to understand their implications in ecosystem functioning, so to incorporate them in ecosystem-based approaches to biodiversity conservation and management, as required by MSFD.

Specific interactions with regional sea case studies in WP4 supported validation of correlations and statistical models for definitions of critical habitats and thresholds, highlighting regional sea differences and exceptions to the generic understanding.

Research in WP2.1 established an improved knowledge of changes caused by OFS/IAS in European seas, also considering climate change scenarios. Detailed case histories have been built on target species to document the potential for ecological and economic impacts of OFS/IAS and their linkages to climate change, as well as for developing risk assessment and ballast water management support tools. An integrated, multidisciplinary methodology has been developed to identify target OFS/IAS and to allow comparisons of different marine ecosystems in terms of susceptibility to biological invasions and to community changes.

The present D2.1.2 report has been structured to deliver a synthetic synopsis of outstanding results obtained across the six tasks of WP2.1:

2.1.1. Establishing the history, distribution and abundance of OFS and IAS

2.1.2. Establishing the biological traits and ecophysiological optima of OFS /IAS

2.1.3. Determining the environmental conditions that lead to outbreaks and invasions

2.1.4. Data analysis to test the role of aquaculture as a vector for invasions

2.1.5. Data analysis to determine the expansion of thermophilic invasives

2.1.6. Developing European guidelines on molecular tools for deciphering mechanisms of invasions and outbreaks
1. Task 2.1.1 Establishing the history, distribution and abundance of IAS

This task has been achieved through a variety of activities that each aim to provide data, tools and/or analysis methods for establishing the history, distribution and abundance of IAS, such as an innovative citizen science campaign and a highly functional NIS database. The activities led to historical reconstructions of bioinvasion pathways and determination of the current distribution and abundance of IAS in European Seas. Furthermore, citizen science campaigns are increasingly growing public awareness of environmental issues and of the scientific effort to preserve the integrity of ecosystems.

1.1 AquaNIS - Information system on aquatic non-indigenous and cryptogenic species (KUCORPI, EMI-UT, GoCONSULT, UNIPV, IOLR)

Biological invasions attract increasing attention from scientists, policy makers and various management authorities. Consequently, the knowledge-base on non-indigenous species (NIS) continuously expands and the number and availability of web resources on NIS rises. Many of these databases began as inventories of NIS, but evolved to include information on NIS origin, introduction history, pathways, vectors, biological traits.

VECTORS has developed an advanced information system dealing with aquatic NIS introduced to marine, brackish and coastal freshwater environments of Europe and adjacent regions: “Information system on aquatic non-indigenous and cryptogenic species” (AquaNIS; please see http://www.corpi.ku.lt/databases/index.php/aquanis). This new database includes the most updated information on aquatic invasive alien species of the European Seas. AquaNIS differs substantially from existing NIS information sources in its organizational principles, structure, functionality, and output potential for end-users, e.g., managing aquaculture or ship’s ballast water. The system is designed to assemble, store and disseminate comprehensive data on both cryptogenic species and NIS, and assist the evaluation of the progress made towards achieving management goals. Information is organized in four interrelated major data blocks: Introduction event, Species, Geography and Impacts (Figure 1).

Figure 1. AquaNIS: two main (Introduction events and Species), one supporting (Geography) and one composite (Impacts) data blocks and attributes within them. Numbers in parentheses indicate how many predefined values are covered by each attribute; “N” means that numerical values are entered; “x” indicates a matrix of possible choices. Attributes indicated by open bullet points are under development (Olenin et al. 2013).

Further, data within blocks are grouped according to attributes, e.g., development trait, pathways and invasion vectors. The “Introduction event” data block: the basic data entry in AquaNIS is an introduction
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event record, documenting a species introduction into a recipient region. The recipient region is a country and/or country sub-area within a Large Marine Ecosystem (LME) or LME sub-region. The date of the first record indicates when a species presence was noticed in a region, according to different levels of certainty (year, decade or century). In AquaNIS only the first record of a NIS arrival to a recipient region is recorded. The “Species” data block contains information on a NIS from native and invaded areas worldwide, includes taxonomy, native origin, recorded biological traits, etc. Taxonomy is based on the updated accounts in a global organism-specific database; the Word Register of Marine Species (WoRMS).

The “Geography” data block supports all geographic information used for entries in “Species” and “Introduction event” data blocks. Information is arranged in a hierarchical order ranging from oceans, ocean sub-regions, Large Marine Ecosystems (LMEs), sub-regions of LMEs to smaller entities, from which a user can make a selection. The “Impacts” data block consists of two parts, containing different sets of data. One part (General impacts) includes information on species impacts from any world region, while the other one (Regional impacts) involves data on species impacts recorded in a particular recipient region (Olenin et al. 2013).

The AquaNIS database provides a vital data source for establishing the history, distribution and abundance of IAS. To date, it represents the most updated database resource available to scientists, coastal managers, stakeholders, and policy makers dealing with aquatic IAS in European seas.

1.2 ORMEF - A geo-referenced database for the presence and distribution of alien fish species in the Mediterranean Sea (CNR, CSIC-ICM)

The current change in biological species geographical distribution is one of the most noticeable symptoms of a changing biodiversity worldwide (Perry et al., 2005), altering ecosystem processes and their resilience to environmental change. A spectacular example of this phenomenon is provided by the recent modifications of the Mediterranean marine biota due to the ongoing incoming of species of extra-Mediterranean origin. A major convenient division of these immigrants separates “true” exotic species, introduced outside their dispersal potential, from non-native organisms which have extended their distribution by natural means (Olenin et al., 2010). The former classification would include Lessepsian species entering through the Suez Canal and other human mediated introductions (for example, ballast water or aquarium escapes). The second type of immigrants is basically represented in the Mediterranean by Atlantic species, entering the Mediterranean through the Straits of Gibraltar. In VECTORS, data on exotic fishes were extracted from a total of 457 published sources, spanning from 1896 to 2013. Records of occurrence were geo-referenced using ArcGIS 9.3 the Geographical Information System (GIS). Documentation of biological, ecological, taxonomic information, introduction pathways, bibliographic references and other relevant data for each species was also compiled. The database was named ORMEF (Occurrence Records of Mediterranean Exotic Fishes). The ORMEF database currently gathers 2,456 geo-referenced observations (Fig. 2). Out of these, 2,019 were extracted from published presence records; 377 from checklists and 60 from grey literature. The database includes information on 85 Lessepsian species, 20 human mediated introductions and 44 recent immigrants from the Atlantic, 23 questionable and cryptogenic fish species with a total number of 172 fish taxa.
Figure 2. ORMEF (Occurrence Records of Mediterranean Exotic Fishes). The total of 2,456 geo-referenced observations is shown. Red crosses indicate records of Lessepsian species; blue crosses indicate records of Atlantic species entering through Gibraltar and white crosses are human mediated translocations. Flags indicate the first records of a species in the Mediterranean Sea.

An example of how the ORMEF database can be utilised in order to increase our understanding of the optimal conditions for particular species invasions by tracing their invasion history has been completed for the bluespotted cornetfish, *Fistularia commersonii* (Rüppell, 1838). Originally distributed in the Indian and Pacific Oceans, today it is one of the most successful invaders of the Mediterranean Sea. Twelve years ago, in January 2000, this species was recorded for the first time along the Mediterranean coasts of Israel, entering from the Red Sea via the Suez Canal. Genetic studies demonstrated that this successful invasion was the result of a single introduction episode that produced a severe bottleneck in the Mediterranean population. VECTORS used records of the presence of *F. commersonii* in the Mediterranean Sea, from January 2000 (first detection) to October 2011 to reconstruct the chronology of the invasion (Fig. 3), assess spread rates, and estimate the environmental suitability of different areas in the Mediterranean to this Lessepsian invasion (Azzurro et al. 2013). A total of 191 observations were used to reconstruct the invasion sequence, to provide estimates of the rate of spread and to construct an environmental suitability model based on six biophysical variables and the maximum entropy approach. The results showed that colonization of the Mediterranean Sea proceeded in parallel along the southern and northern rim of the Basin at speeds that reached 1,000–1,500 km year\(^{-1}\) with a clear decrease in the rate of spread at the Sicily Strait. The most important explanatory variables for describing the distribution of *F. commersonii* were mean depth (explaining 32.4 % of the data variance), chlorophyll-a (29.3 %), and salinity (18.4 %).

Coastal areas with relatively low chlorophyll-a concentrations and high salinity were the preferred habitat of the bluespotted cornetfish in its invaded range. Conversely, extreme productivity (highly eutrophic or highly oligotrophic), low salinity and cold temperatures provided abiotic resistance to this invasion. Areas of high environmental suitability were identified along the northern coasts of the Levantine Sea, Dodecanese, Sicily Strait and Tyrrhenian Sea. In contrast, the north Aegean Sea, the Adriatic and the Alboran Sea, the Nile Delta, the western coasts of Egypt and Cyrenaica were unfavourable for the invasion. Despite some limits due to the model’s resolution scale, these general predictions provide new insights into the *F. commersonii* invasion, indicating abiotic factors of primary importance in shaping the distribution of this species in its invaded range.
1.3 Alien species in the Baltic Sea: annual-scale dynamics in response to environmental variability (EMI-UT)

Environmental variability can affect species distribution and population sizes, and is therefore expected to influence alien species. VECTORS investigated the temporal variability of 11 alien species representing different trophic levels and ecological functions in two gulfs (Gulf of Finland and Gulf of Riga) of the brackish Baltic Sea in relation to environmental change. The following species were investigated: the cirriped Balanus improvisus, the gibel carp Carassius gibelio, the predatory cladoceran Cercopagis pengoi, the zebra mussel Dreissena polymorpha, the Chinese mitten crab Eriocheir sinensis, the cladoceran Evadne anonyx, the amphipod Gammarus tigrinus, the polychaete Marenzelleria neglecta, the soft-shelled clam Mya arenaria, the round goby Neogobius melanostomus and the New Zealand mud snail Potamopyrgus antipodarum. Dependent on data availability and invasion time, some of the time-series go back to 1957.

Our analysis of the environmental characteristics since 1990 showed that a clear shift in environmental conditions occurred in 1995/1996 both in the Gulf of Riga and in the Gulf of Finland. Such changes were mainly attributed to an increase in mean sea surface temperature and a decrease in North Atlantic Oscillation (NAO) index. However, independent of the invasion time, organism group or the life-history stage, abundance and/or biomass of the investigated alien species was either stable or displayed abrupt increases over time (Figure 4). Timing in population shifts was species-specific and exhibited no generic patterns, indicating that the observed large shifts in environmental abiotic parameters have no uniform consequences on the alien biota. This points to the importance of biotic interactions in modifying local communities as a result of bioinvasions. In general, the inter-annual dynamics of alien and native species was not largely different, though native species tended to exhibit more diverse variability patterns compared to the alien species.

Generally, correlations between the studied abiotic environmental variables and biota were moderate in the Gulf of Riga, except for pelagic species (C. pengoi and B. improvisus nauplii) and fish (C. gibelio) that showed high matches with environmental variability. In the Gulf of Finland, pelagic species (E. anonyx and B. improvisus nauplii) also tended to display better links with environmental variability than benthic alien invertebrates. Therefore, there were no key environmental factors that affected most of the alien species, instead, the effects varied among the studied gulfs and species. While both benthic and pelagic invertebrates were affected by a combination of temperature, salinity, wind and ice conditions, and water chlorophyll level, alien fish dynamics were best related to water temperature in spring. It was
concluded that temperature should be considered as a common significant forcing factor for the population dynamics of most of the investigated alien species. Our analysis also demonstrated that pelagic alien invertebrates were a less stable component of an ecosystem compared to benthic alien invertebrates (Ojaveer et al. 2011).

Fig. 4. Long-term abundance dynamics of three alien zooplankton species in the Gulf of Riga (filled diamonds) and the Gulf of Finland (open diamonds). The lines indicate abundance regimes (Ojaveer et al. 2011).

1.4 Citizen science on jellyfish blooms (CONISMA, CSIC)

Building comprehensive databases of species distribution and abundance, with large geographical and long historical coverage, is a problematic task due to funding limitations. Thanks to easy internet access and new tools for disseminating information (e.g. smartphones), volunteer monitoring by citizen scientists is now increasingly contributing to projects on environmental monitoring or conservation biology and ecology. Citizen science campaigns are now a valuable instrument supporting bioinvasion monitoring by providing a wealth of updated information on propagule dispersal, habitat invasibility, ecosystem pressures, and species interactions. The advantages are represented by: a) public involvement in science, b) coverage of large areas almost indefinitely, c) minimal costs, d) large amount of data, e) easy documentation through pictures, f) if a species is recorded, it means that it occurs at a given place and at a given time, and g) if a species is not recorded when other species are recorded, chances are good that that species was really absent. Conversely, the disadvantages are: a) great efforts in mass media involvement, requiring good communication skills; b) non homogeneous data quality; c) unknown research effort: if no species are recorded, it does not mean they were really absent (negative data can be due to absence of observers); and d) records are mostly based on shoreline observations. As an example, VECTORS adopted a citizen science approach to the mapping of the presence of jellyfish in the Mediterranean Sea through several levels of outreach intervention.
Outreach Activities:
**Poster** (Fig. 5) - A highly impacting poster with the main members of the Mediterranean gelatinous plankton has been updated and distributed, with new graphics and artwork in respect to previous releases. All of the drawings have been done anew with digital art.

Figure 5. Gelatinous taxa of the Mediterranean Sea. Poster developed for the citizen science campaign. Dissemination across the Mediterranean basin was promoted by a number of projects, public bodies, and magazines adhering to the goals of VECTORS research.

**Calendar** - The images of the poster have been used to set up a calendar of all of the species, and also new places explaining the reasons for the increased presence of jellyfish in the Mediterranean Sea and also in the world ocean. The legends to the figures are in both Italian and English and strongly support the information given in the poster.

**Web Page** - The web page of the popular science magazine Focus hosts Meteomeduse, with a map of the Mediterranean and the locations of all the sightings delivered by the citizen scientists. On the web page there is an interface from where the citizens can send their records.

**Smartphone app** - A smartphone app has been launched, containing all of the information available in the Focus Web page. Citizens can take pictures of their sightings and send the records directly through their smartphones.

**Media coverage** - The campaign has been promoted with intervention in the main Italian media, with articles in the newspaper La Stampa, La Repubblica, Nuovo Quotidiano di Puglia, etc. and in local and national magazines. Appearances in TV and radio programs, both national (Pianeta Mare, Superquark) and local have further promoted the initiative. Parallel initiatives were also developed in Spain and in other Mediterranean countries.

Special attention has been made to involve scientists who are not directly involved in jellyfish research but who then act in the same way as individual citizens. The appearances in the media and also in specialized scientific journals and monographs have attracted the attention of marine scientists who started to send records whenever they encountered jellyfish during their research in the field. This part
of the program is particularly useful to gather information from the open sea and also during the winter, when far fewer citizens visit the coast.

Citizen science records provide a lot of rewarding feedback. In 2009, they documented the first appearance of the ctenophore *Mnemiopsis leidyi* and the cnidarian medusa *Phyllorhiza punctata* in the Western Mediterranean and the expansion of their distribution in following years. In 2011, they detected the westernmost expansion of the Lessepsian immigrant *Rhopilema nomadica* in Maltese waters, and in 2013 its occurrence in Tunisia. In 2013, the jellywatch citizen campaign allowed reporting of a massive thaliacean bloom (*Salpa maxima*) in the Southern Adriatic and a bloom of an undescribed species of *Pelagia* that suddenly appeared with a large population in the Northern Adriatic. The report on the thaliacean bloom is published in F100 Research, and the description of the new species of jellyfish, *Pelagia benovici* sp. nov. in Zootaxa (Fig. 6).

Citizen science has proved crucial in covering a vast area over a long period of time. It has enhanced the outreach of the project, allowed recording of the presence of massive proliferations of gelatinous plankters and even led to the discovery of a new species. The manpower available to any scientific project would never lead to such results. Of course scientists used this information to focus on special areas and species whenever they became aware of the occurrence of significant and interesting phenomena. As remarked by Boero (2013), the availability of records of unusual events such as salp proliferations will contribute to understand anomalies in marine community dynamics or even anomalies in the functioning of whole ecosystems.

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**1.5 Reconstructing the invasion history of the worst IAS: the case of *Mnemiopsis leidyi* in the Mediterranean Sea (CONISMA, CSIC).**

Knowledge of the source and pathways of alien species introductions is essential for developing management strategies to prevent bioinvasions. The invasive ctenophore *Mnemiopsis leidyi* entered the Black Sea in the early 1980s. The invasion was followed by the Azov, Caspian, Baltic and North Seas, and, most recently, the Mediterranean Sea. Previous studies identified two distinct invasion pathways of *M. leidyi* from its native range in the western Atlantic Ocean to Eurasia. However, the sources of newly established populations in the Mediterranean Sea remained hitherto unclear. Here sequence variation was investigated in both mitochondrial (Cytochrome c Oxidase subunit I) and nuclear (Internal Transcribed Spacer) markers in *M. leidyi*, encompassing five native and 11 introduced populations, including four from the Mediterranean Sea. Extant genetic diversity in Mediterranean populations (n=8, Na = 10) preclude the occurrence of a severe genetic bottleneck or founder effects in the initial colonizing population. Our mitochondrial and nuclear marker surveys revealed two possible pathways of introduction into Mediterranean Sea. In total, 17 haplotypes and 18 alleles were recovered from all surveyed populations. Haplotype and allelic diversity of Mediterranean populations were comparable to
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populations from which they were likely drawn (Fig. 7). The distribution of genetic diversity and the pattern of genetic differentiation suggest initial colonization of the Mediterranean from the Black-Azov Seas (pairwise FST = 0.001–0.028). However, some haplotypes and alleles from the Mediterranean Sea were not detected from the well-sampled Black Sea, although they were found in Gulf of Mexico populations that were also genetically similar to those in the Mediterranean Sea (pairwise FST = 0.010–0.032), raising the possibility of multiple invasion sources. Multiple introductions from a combination of Black Sea and native region sources could be facilitated by intense local and transcontinental shipping activity, respectively. The Mediterranean Sea receives an enormous flow of global shipping (Seebens et al 2013). The tropical Western Atlantic Ocean is a source of trade to the Mediterranean Sea, and places it at risk of future invasions from discharged ballast water. Moreover, high shipping activity within the Mediterranean Sea itself poses additional risk of translocation of *M. leidyi* and other alien species throughout the basin. The case study of *M. leidyi* provides evidence that the achievement of Good Environmental Status in European Seas will require reinforcement of current regulations to achieve a successful prevention of new alien introductions.

![Venn diagram illustrating shared haplotypes/alleles of *Mnemiopsis leidyi* between regions.](image)

**Figure 7.** Venn diagram illustrating shared haplotypes/alleles of *Mnemiopsis leidyi* between regions. Venn diagram showing COI haplotypes (A) and ITS alleles (B) sharing between Mediterranean and possible source populations from North America and Black-Azov Seas. Note that haplotype M101 and allele G from South America are excluded (from Ghabooli et al. 2013).

1.6 Spatio-temporal dynamics of alien and native outbreak forming species in the Dutch S Delta Waters: the case of *Mnemiopsis leidyi* and *Aurelia aurita* (NIOZ)

Monitoring of jellyfish at 17 stations in Lake Veere during several years revealed seasonal and year to year variations in the abundance of *Aurelia aurita* and *Mnemiopsis leidyi* (Fig. 8). Spatial analysis revealed that *M. leidyi* successfully settled in the semi-enclosed salt water lake in 2010. In the following years the species attained its peak biomass in autumn, after the maximum abundance of *A. aurita* in spring. There seems to be a trend of a decrease in average peak densities of *M. leidyi* over the last 3 years. However, this trend might be caused by an extended cold period during spring 2013, and consequent late development of the *M. leidyi* population. The monitoring will be maintained in 2014 to further investigate this trend.
2. Task 2.1.2 Establishing the biological traits and eco-physiological optima of OFS and IAS

Several key species have been studied to establish the biological traits and eco-physiological optima of OFS and IAS. Studying these renowned species offers insights into the characteristics of OFS and IAS that can be used to manage future outbreaks. This new information has wide implications going far beyond the several study species and geographical areas, representing a model integrative approach towards a better understanding of the mechanisms of bioinvasions by delineating the environmental and ecophysiological envelopes promoting the success of OFS/IAS. Altogether, the outcomes of VECTORS will represent a fundamental theoretical and methodological toolkit for application of MSFD in European Seas and for everyone dealing with integrative coastal zone management and the protection of marine ecosystems processes and services.

2.1 Thermal controls of a biological invader: Mnemiopsis leidyi in the southern North Sea (UHAM)

In summer/fall 2006 *Mnemiopsis leidyi* (warty comb jelly) was identified in the North and Baltic Seas and its establishment represents one of the most potentially dramatic marine bioinvasions in northern European Seas in recent decades. The species has an “r” life history strategy and can increase rapidly in favourable conditions due to a combination of high rates of growth and reproduction that are fuelled by extremely high rates of feeding. This species is therefore capable of (and perhaps adapted to) rapid colonization of available niches. *Mnemiopsis leidyi* is a generalized feeder that consumes microplankton (e.g., protists) during the larval phase and a wide variety of taxa including holo-and meroplankton, as well as the eggs and larvae of fish during the adult life phase. This invader now occurs at relatively high abundances during summer in the southern North Sea but its potential impact on the plankton community and food web dynamics in that system are poorly studied. To examine this potential impact, the specific growth and clearance rates (food ingestion rates) of 1.5-mm tentaculate larvae offered 2-day old copepod (*Acartia tonsa*) nauplii at a concentration of 110 mg C l⁻¹ were measured at eight different temperatures between 6°C and 30°C. Second, specific growth and prey clearance rates were examined at six different prey concentrations between 40 and 135 mg C l⁻¹ at 25°C.

Within all laboratory trials, clearance rates and ingestion rates were between 0.01 and 0.16 l ind⁻¹ d⁻¹ and 0.3 and 17.9 µg C d⁻¹, respectively, and both rates were highest at 25°C. Carbon-specific ingestion rates were between 13.6 and 209.1% C d⁻¹ and the highest rates were measured at 27°C. Larval specific-growth rate (SGR) was between 2 and 22% body C d⁻¹ and significantly increased with increasing temperature until 27°C. Growth rates were negative at 6°C and 30°C. Between 8 and 27°C,
the increase in ingestion rate and SGF could be described by Q10 values of 5.35 and 2.74, respectively. Gross growth efficiency (GGE) was similarly (mean 13%) between 10 and 27°C but GGE was negative at 6 and 30°C. No increase in ingestion or growth rates was observed at prey concentrations between 40 and 135 µg C l⁻¹. However, ingestion and growth rates were significantly lower at the highest food concentration (135 µg C l⁻¹, p < 0.05) likely due to an oversaturation effect at this high prey concentration.

Laboratory results defined the thermal window supporting positive rates of feeding and growth in the larvae of this invasive ctenophore. Temperatures < 8°C were sub-optimal for feeding and growth of larvae. Based upon long-term, model-derived weekly temperature conditions in the North Sea (1996-2006, ECOHAM), Mnemiopsis larvae are expected to grow little and have little to no impact on local zooplankton populations from mid-January to the end of April. The concentration of prey limiting feeding is not known, however, ECOHAM zooplankton carbon estimates indicate prey concentrations >40 µg C l⁻¹ from April to the middle of December. In the Dutch coastal zone, M. leidyi has been reported to start spawning in May and increase 3 orders of magnitude in abundance reaching seasonal peaks in mid-June and August. Abundance declined in late October. Laboratory estimates provide a mechanistic, cause-and-effect understanding for these seasonal patterns. Climate-driven warming is expected to exacerbate potential trophodynamic impacts of this invader in the southern North Sea.

2.2 Temperature-dependent settlement characteristics of scyphozoan planulae (UHAM)

Ongoing pressures and state changes in European regional seas may increase the risk of jellyfish outbreaks in these areas. For example, warming water temperatures may favour the reproductive success of scyphozoan jellyfish in the North Sea. Their metagenetic life cycle includes a benthic polyp stage arising from the settlement of planula larvae released from pelagic medusae. The abundance and settlement success of scyphozoan planula larvae and the reproductive potential of polyps are critical not only for the persistence of populations but also for the mass occurrences of these jellyfish. To better understand how climate change could affect jellyfish populations, the influence of temperature was investigated on settlement proficiency and success of planulae of the scyphozoan jellyfish Cyanea lamarckii, a common jellyfish encountered within the North Sea. In the first experiment, larvae were equally distributed to settlement chambers maintained at 12 temperatures between 9 and 27°C and settlement was determined every day (n = 5 replicates / temperature). Settlement occurred over 55 days at the coldest temperature, but the majority of settlement occurred within five days at all temperatures. Maximum and minimum settlement success was 56% at 27°C and 31% at 24°C. Warm temperatures were found to induce earlier settlement, which could affect the extent of wind-driven dispersal of planula larvae after they are released from medusae. A second experiment examined how long settlement could take place (settlement proficiency) for larvae maintained in the water column. When provided access to settlement habitat, planulae settled most rapidly at the warmest temperature and larvae were proficient for 20, 31 and 14 days at 11.4, 13.3 and 19.6°C, respectively.

In the North Sea, planula larvae may experience sea surface temperatures between ~9 and 17°C (May to August, ICES 2013) during periods of time when this scyphozoan species is normally observed in spawning condition. Therefore, settlement success will not be deleteriously affected if warming occurs. Based upon drift modelling of larvae released at 5 m depth throughout the North Sea (ECOHAM-HAMSOM, 20 km grid) during the peak reproduction period of C. lamarckii (middle of May and June), the majority of larvae would settle between 10 and 35 km from their release location (assuming 5 day settlement). Given that larvae are proficient to settle until roughly 300 degree-days (°C x time in days) after their release, larvae could be advected 100 to 150 km. This, to the best knowledge, is the first estimate of potential dispersion distances based upon laboratory measurements of settlement dynamics of planula larvae.

2.3 Oxygen consumption rates of scyphozoan polyps at different temperatures (UHAM)

To examine thermal windows of polyps, oxygen consumption rates were measured in congeners including A. aurita (North Sea and Baltic Sea population), A. labiata (Pacific origin) and A. limbata
(Pacific origin) as well as *Cyanea capillata* (Baltic Sea population) at six temperatures between 7 and 20°C. Individual polyps were measured at six different temperatures: 7, 10, 12, 15, 18 and 20°C. Oxygen consumption rates, corrected to a mean polyp body size of 345 µg, were lowest in *A. limbata* (53.6 ng O₂ polyp⁻¹ h⁻¹ at 7°C) and were highest for Baltic Sea individuals of *A. aurita* at 18°C (354 ng O₂ polyp⁻¹ h⁻¹). In general, there was considerable overlap among the rates at the same temperatures for different species. Interestingly, a gradual increase in oxygen consumption as temperatures increased was not observed but, rather, a significant (p < 0.05) increase in oxygen consumption rate after a threshold temperature was exceeded. This threshold temperature appears to be species-specific and was between 12 and 15°C for *A. aurita* (Baltic), *C. capillata*, *A. labiata* (Pacific), and 10 and 12°C for *A. aurita* (North Sea) and 7 and 10°C for *A. limbata* (east Pacific). The highest O₂ consumption rates were not always observed at the warmest temperature. In three of the five groups, oxygen consumption rates were highest at either 12 or 18°C and were lower at 20°C which may be an indicator of metabolic stress at warm temperatures. This research sheds light on the optimal windows for polyp production and suggests non-linear impacts of temperature on polyp metabolism. Our research also suggests that warming will not inevitably increase the productivity of endemic jellyfish species, it will depend upon whether thermal windows are exceeded.

### 2.4 Metabolic scope in a native and an invasive fish species: the effect of temperature (CNR)

Climate change is affecting the sea surface temperature with dramatic consequences for animal life in the near future. Direct effects of temperature changes include fish distribution shifts towards higher latitudes and depths and increases in extinction rates. This appears to be induced by the strong effect that temperature has on fish metabolism, which, in turn, affects growth rate, survival, and reproduction. In the Mediterranean Sea, warming conditions are considered to be facilitating the arrival and spread of tropical invaders. The increasing success of these species may be partly related to the fact that their optimal temperature, in terms of maximizing metabolic scope, is higher than that of indigenous species (Fig. 9).

![Normalized values of MS in Sarpa salpa and Siganus rivulatus](image)

*Figure 9. Normalized values of MS in Sarpa salpa and Siganus rivulatus*

VECTORS research determined the effect of temperature on the metabolic scope of two fish species that occupy a similar ecological niche: the native *Sarpa salpa*, and the invasive *Siganus rivulatus*, that entered the Mediterranean from the Red Sea through the Suez Canal. A large difference was found in the optimal temperature for aerobic scope (T<sub>opt</sub>) between *S. salpa* (i.e. 21.8°C) and *S. rivulatus* (i.e. 29.1°C) highlighting the importance of water temperature in shaping the energy availability of these two species. If one considers together the great difference in T<sub>opt</sub> for aerobic capacity between *S. salpa* and *S. rivulatus* and the increase in water temperature in the eastern side of Mediterranean Sea, this provides a mechanistic explanation for the replacement of *S. salpa* by *S. rivulatus* species in that region. As a consequence, it is possible that in the near future the increase in the water temperature that is occurring in the western side of the Mediterranean Sea will bring a shift in the siganids distribution.
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towards this area and the siganids may out-compete the S. salpa species. This result provides new insights into potential future fish distribution in relation to an increase in water temperature in the Mediterranean Sea and the consequences of their adaptive responses.

2.5 Ecophysiology, trophic ecology, reproductive biology and bioenergetics of Pelagia noctiluca (CONISMA, CSIC)

In recent years, jellyfish blooms have attracted considerable scientific interest for their potential impacts on human activities and ecosystem functioning, with much attention paid to jellyfish as predators and to gelatinous biomass as a carbon sink. The mauve stinger Pelagia noctiluca (Forskål, 1775) (Scyphozoa) can be considered as one of the dominant jellyfish species in the Mediterranean Sea. Due to its high abundance and painful stings P. noctiluca outbreaks are generally described in negative terms, because the medusae may cause damage to economic activities, such as fisheries, mariculture and tourism, and to the ecosystem by affecting the structure and organization of plankton communities. Several studies on P. noctiluca have been carried out in the Mediterranean Sea, but many questions related to jellyfish population ecology and biology still remain unsolved, preventing a mechanistic understanding of jellyfish blooms dynamics and of their impacts on the marine pelagic ecosystem.

The work carried out in VECTORS aimed to increase knowledge of the ecophysiology, reproductive biology, trophic ecology and bioenergetics of P. noctiluca from the Strait of Messina, an important proliferation and reproduction area for this species, due to the favourable temperature range and high productivity. The reproduction was investigated by histological analysis of gonads (size distribution of oocytes), the calculation of gonadosomatic and fecundity indexes, and the biochemical composition of gonads. Trophic ecology was studied by isotopic and fatty acid analyses, and by studies of gut contents. A bioenergetic model based on respiration, consumption, assimilation, and growth was developed for the ephyra, juvenile and adult stages in P. noctiluca’s life cycle. Finally, the effect of predation by a coastal fish (Boops boops) on P. noctiluca was studied over a year cycle.

The results showed that P. noctiluca can reproduce throughout the year, although the ability to form a bloom is limited to two main events of reproduction, one in autumn and another in spring (Fig. 10). Females of P. noctiluca produce great amounts of small-sized mature eggs (diameter < 200 μm) during high food availability, whereas a reduced number of larger eggs (diameter > 200 μm) are produced when prey availability is low. Protein contents stored within the gonads seasonally change, with the highest concentrations during the pre-spawning periods. Pelagia noctiluca is a generalist predator, with a wide diet. Stable isotope and fatty acids analysis highlighted that its diet varied over the year, according to prey availability in the environment, and then varied its trophic level, too. Analysis of fatty acids trophic tracers suggested the transfer of key molecules from somatic tissue to gonads to enhance reproductive success. Biochemical, reproduction and observational data are in agreement with the hypothesis of seasonal vertical migratory patterns. An Individual-Based Growth Model has been developed in VECTORS, representing the first modelling approach on jellyfish, aiming to predict daily growth rate and daily carbon ingestion of P. noctiluca under natural conditions. This model will represent an essential tool to measure the impact of P. noctiluca outbreaks on planktonic community. Finally, this work has increased general awareness that P. noctiluca should not be considered as an energy dead-end in the ecosystem. The demersal fish Boops boops foraged on jellyfish all year long, with different intensity among jellyfish body parts, depending on their relative energy content.
3. Task 2.1.3 Determining environmental conditions that lead to outbreaks and invasions

To identify habitats potentially susceptible to outbreaks and to determine the probability that an outbreak will occur at specific places and times, past history events have been coupled with current data collected in Tasks 2.1.1 and 2.1.2. VECTORS has examined trends and covariances between invasion events and environmental drivers in space and time. Inter- and intra-population genetic studies also contributed to improve knowledge on the biological mechanisms causing invasions of pelagic species. Taking into account the specific ecological features of different ecosystems, this task contributed to determining the susceptibility of these ecosystems (or their internal elements) to IAS/OFS occurrence, and to foreseeing species invasiveness in different environmental situations.

3.1 Niche selection associated to high invasiveness of the alien species Gammarus tigrinus in the Baltic Sea (EMI-UT)

Recently, the Baltic Sea, especially in its northern part, has witnessed an unprecedented increase in the number of biological invasions. Although the exact cause of such a situation is unknown, the evidence suggests an intensified or modified invasion vector associated with ballast water and hull fouling. Among the recent non-indigenous species the gammarid amphipod *Gammarus tigrinus* is one of the more aggressive invaders in the Baltic Sea. Recent experimental evidence indicates the species has a strong potential to modify benthic community structure and functioning in the whole coastal zone of the northern Baltic Sea.

Quantitative sampling of the shallow water habitats of the Gulf of Finland showed that *G. tigrinus* has become established in the whole coastal zone of the basin. The abiotic variables studied explained...
slightly over 50% of the variability of G. tigrinus abundance and biomass. Exposure to waves was by far the most important variable in the Boosted Regression Trees modelling followed by average water salinity, water transparency, maximum chlorophyll a and average water velocity. Sediment type described only a minor proportion of the variability of G. tigrinus. Amphipod abundance and biomass decreased in a logistic form with increasing exposure. Similarly, reduced salinities favoured the invasive amphipod and increasing water velocity at a site increased both abundance and biomass of G. tigrinus. The relationship between chlorophyll a and G. tigrinus abundance was unimodal with maximum G. tigrinus abundance estimated at around 10–55 mg Chl a m⁻³ and biomass at around 10–35 mg Chl a m⁻³. Above and below this range, G. tigrinus abundance sharply decreased. The effect of exposure on the abundance and biomass of G. tigrinus depended heavily on the values of eutrophication-related variables and the salinity level with high abundance and biomass at low chlorophyll a when either salinity or exposure values were also low. Similarly, the effects of exposure on G. tigrinus depended on water velocity values with highest abundance and biomass of G. tigrinus recorded in areas characterized by low exposure and high water velocity (Fig. 12). The nMDS analysis showed that the distribution range of G. tigrinus was relatively broad and similar to those of native gammarids. Only the native Gammarus duebeni occurred primarily close to the waterline where other gammarid amphipods were rare. Thus, G. tigrinus is exceptional among the invasive species as all other invasive species in the Gulf of Finland form two distinct groups in terms of their habitat requirement. Gammarus tigrinus may occasionally share habitat with those invasive species but currently such habitat overlap was negligible in the Gulf of Finland. Native gammarid species were found at higher abundances and biomasses in the areas where G. tigrinus was rare or lacking. Without G. tigrinus the abundance of the native gammarids was an average of 880 ind m⁻² while with G. tigrinus their abundance was about three times lower. In the areas already invaded by G. tigrinus the abundance of native gammarids never reached over 1000 ind m⁻². Without G. tigrinus, however, values over 2000 ind m⁻² and occasionally even over 5000 ind m⁻² were recorded (Kotta et al. 2013).

Figure 11. Three-dimensional partial dependence plots in the Boosted Regression Tree model for the abundance (top) and the biomass (bottom) of Gammarus tigrinus in the Gulf of Finland. Note how the effect of one variable is dependent on another (Kotta et al. 2013)
3.2 Vertical migration patterns of the OFS jellyfish *Pelagia noctiluca* (CONISMA, CSIC)

VECTORS undertook a historical background review of the proliferations and the climatic, oceanographic and biological forcing for blooms of the most abundant stinging jellyfish in the Mediterranean Sea, the mauve stinger *Pelagia noctiluca* (Canepa et al. 2014). Over recent decades, man’s expanding influence on the oceans has begun to cause change in some regions, including in the Mediterranean Sea. New proliferations of jellyfish may be occurring in the Mediterranean Sea, possibly in response to the cumulative effects of some of these anthropogenic impacts. Many of these “proliferation events” are due to *Pelagia noctiluca*, an oceanic scyphozoan that has become very abundant along the coasts. *Pelagia noctiluca* is usually considered to be the most important jellyfish species in the Mediterranean Sea due to its widespread distribution, abundance, and ecological role and also because of its negative interaction with humans. Climatic conditions that favour enhanced reproduction by *P. noctiluca* and probably also determine optimal conditions for the formation of blooms are characterized by mild winters, low rainfall, high temperature, and high-atmospheric pressure.

![Image](image.png)

*Figure 12. Seasonal vertical migration of *Pelagia noctiluca* in proximity of marine canyons.*

Data on beach strandings along the Spanish Catalan coast revealed that jellyfish occur in greatest concentrations along the northern Catalan coast and on beaches located close to marine canyons. The arrival of *P. noctiluca* at the coast depends firstly on the offshore production of jellyfish. Oceanographic structures like fronts, which enhance and maintain high levels of biological production and provide ideal conditions for feeding, growth, and reproduction of the jellyfish are present in the NW Mediterranean. The weakening of the front results in large numbers of *P. noctiluca* being driven into the coast by southeast winds. In the NW Mediterranean Sea, *P. noctiluca* exerts top-down control over a variety of prey including fish eggs and possibly the invasive ctenophore *Mnemiopsis leidyi*. *P. noctiluca* is also responsible for the majority of the stings incurred by bathers along the Mediterranean coasts, with remarkable socio-economic impact on human activities in coastal areas.
3.3 Understanding ecological extreme events: deterministic and stochastic drivers of jellyfish outbreaks in the NW Mediterranean (UPISA, CSIC, CONISMA)

There is increasing evidence that climate extreme events such as droughts, storms and floods are becoming more frequent with climate change. These environmental extremes may provoke extreme ecological responses in species assemblages and cause severe impacts to natural ecosystems. Species outbreaks (SO) are examples of extreme ecological events that may be driven by extreme climate conditions. SO occur when the alignment in space and time of certain environmental drivers (abiotic and biotic), result in particularly favourable conditions for population renewal processes. Defining these conditions quantitatively and understanding when and where they will occur are key tasks to forecast SO. The environmental envelope that provides favourable conditions for population renewal is just one realization of a virtually infinite number of possible combinations of environmental conditions. To deal with this complexity, it is convenient to think of the set of conditions that lead to SO as the result of a combination of deterministic and chance events. In VECTORS, deterministic and stochastic drivers of jellyfish outbreaks (JO) were examined in the NW Mediterranean.

The dataset consisted of semi-quantitative measures of the abundance of eight species of jellyfish collected at 243 sites along the Catalan coast (Spain). The data have been collected through a Citizen Science program under the supervision of the Catalan Water Agency on a daily basis from May to September from 2007 to 2010. An outbreak was defined as the occurrence of at least one jellyfish m$^{-2}$, corresponding to an abundance class of 2 in the dataset. Sea surface temperature (SST), Chlorophyll a, geostrophic currents (zonal and meridional components) and distance from the nearest canyon were used as environmental predictors. The deterministic contribution of the environmental drivers to JO was evaluated through a Bayesian hierarchical analysis based on the INLA approach (Integrated Nested Laplace Approximation) that ensured efficient computation. Spatial dependencies in JO were modelled through a Gaussian Markov Random Field (GMRF) and temporal autocorrelation as an autoregressive AR(1) process. The GMRF representation was implemented using the SPDE method (Stochastic Partial Differential Equation). JO were more common in May and June compared to the other months, particularly in 2009 (Fig. 13). The analysis indicated that JO were more likely to occur at sites in proximity of canyons, supporting the recently proposed hypothesis that when occurring near the coast, these physiographic features of the deep sea environment can operate as a conveyer belt driving jellyfish blooms during upwelling. The analysis also highlighted a negative relation between JO and SST, reflecting a general decline in JO from May to September.

The importance of stochastic events (e.g., the chance alignment of favourable SST and current conditions) was examined through the environmental bootstrap. This approach consists of randomization of the stochastic component of short time series of environmental data (i.e. the residuals obtained after removing the seasonal trend) to generate new hypothetical sets of environmental conditions. These new sets of environmental data were used iteratively with the Bayesian model (10000 simulations) to determine the probability of observing a jellyfish outbreak under stochastic environmental fluctuations. Results indicated that chance events were not as important as deterministic processes in driving JO. Nevertheless, the environmental bootstrap predicted a return time of 6.5 yrs for the most extreme event in the dataset, which was six days with JO in a month (Fig. 14). Overall, these results suggest that extreme events are likely to occur in the near future at sites close to canyons. As an alternative to stochastic environmental fluctuations, population processes and demographic stochasticity may explain temporal variation in JO and are worth exploring in the future.
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Fig. 13. Timing of occurrence of jellyfish outbreaks on the Catalan coast. Bars from black to light grey correspond to sampling months from May to September in each year.

Fig. 14. Empirical cumulative (a) and probability density (b) distributions and return time plot (c) for extreme jellyfish outbreak events obtained from the environmental bootstrap analysis. Calculations are done for tow month separately (May: dash-dot line; June: continuous line.)
3.4 High levels of relatedness and inbreeding in *P. noctiluca* populations reveal spatial and temporal reproductive subunits (CONISMA).

*Pelagia noctiluca* is one of the most venomous jellyfish in the Mediterranean Sea and its recurrent outbreaks, reported since the seventeenth century, earned this species a reputation as a plague, attracting special interest and concern since the late seventies. Aggregations of hundreds of thousands or millions of individuals are not rare for this species, whose population abundances show large fluctuations. Indeed, sudden demographical outbreaks lasting one or more years are normally followed by abrupt collapses, apparently without any symptom of distress. Previous population genetics and phylogeographic studies focusing on *P. noctiluca* at a large scale in the Mediterranean Sea and eastern Atlantic Ocean indicated that high levels of gene flow allows for a great connectivity across very large areas, maintaining a substantial panmixia. To elucidate the *P. noctiluca* population genetics behaviour in a more itemized way, VECTORS research developed, for the first time in Scyphozoa, a set of species-specific microsatellite markers and used them to genotype a total of 488 individuals from 10 geographic locations in the Mediterranean Sea and one site in the northeast Atlantic Ocean. Moreover, the sampling was replicated in time for two populations in the southern Tyrrhenian Sea: for three consecutive years (2010-2011-2012) in one location (Ustica Island) and for two consecutive years (2011-2012) in another one (Strait of Messina).

The samples of the geographically restricted area of southern Tyrrhenian Sea were first selected to investigate the processes governing the genetic behaviour of the single “bloom units”, namely the fluctuating agglomerates of jellyfish considered as separate populations. *Pelagia noctiluca* blooming in the southern Tyrrhenian Sea exhibited significant deviation from the Hardy-Weinberg equilibrium due to a large excess of homozygotes across 8 of 9 microsatellite loci, leading to high inbreeding coefficients (FIS). The results of sibship and parentage analyses supported the high inbreeding values by detecting intra-population relatedness higher than expected by chance (P<0.05) in at least three samples and siblings in at least 5 out 8 samples, 4 of which contained full-sib in addition to half-sib dyads. The genetic differentiation among populations was globally small (FST =0.01714; P<0.0001) but highlighted: a) a spatial genetic patchiness uncorrelated with distance between sampling locations, and b) a significant (P<0.0001) genetic heterogeneity between samples collected in the same locations in different years.

These findings were concordant with those of the large spatial scale study, carried out to infer the population structure of *P. noctiluca* across the Mediterranean Sea and northeast Atlantic Ocean. In fact, almost all the populations of the study area exhibited high levels of inbreeding, as highlighted by substantial and positive FIS values. The spatial genetic structure analysis did not recognize any strong genetic structure in the whole study area, confirming that the great dispersal potential of *P. noctiluca* individuals guarantees high gene flows among the populations in a large geographic area. Nevertheless, a low (but statistically significant) and apparently unexplainable genetic differentiation (overall FST= 0.01149) was found between some populations in the study area.

The patterns of genetic differentiation, such as those found in the small and large scale population genetics studies in *P. noctiluca*, are known as “chaotic genetic patchiness” or “fluctuating genetic mosaics”, commonly found in species characterized by high dispersal potential, as benthic marine invertebrates with a pelagic larval stage. Variability in reproductive success of the single individuals and genetic drift linked to high mortality rates in early life stages are usually considered the most important causes of these kinds of genetic patterns. In *P. noctiluca*, some specific features were identified as responsible for the genetic patchiness:

a) each mature female jellyfish spawns oocytes in a sticky mucus ribbon, holding eggs together for several minutes before its dissolution. This peculiarity may favour fertilization of the whole set of oocytes by sperms released by a single or a few male mates, producing a large amount of full sibs;

b) fusion of gametes produced by related individuals and the resultant formation of inbred offspring may be favoured by aggregative swimming behaviour of jellyfish. In fact, related individuals born from the
same parental group at the same time have a reasonable probability of remaining together in the native bloom unit and hence also during subsequent spawning events, increasing the probability to produce inbred offspring (Fig. 15);

c) maintenance of kin-related jellyfish aggregation along marine currents may be influenced by small-scale hydrodynamic and oceanographic patterns conducive to limited individual mixing despite the high dispersal potential. Hydrogeographic features such as eddies, gyres or upwelling fronts could promote the aggregation of some groups of medusae in confined areas, preventing an extensive mixing with individuals belonging to other aggregations and allowing the fusion between gametes produced by related individuals (Fig. 16).

*Figure 15. Sexual reproduction of Pelagia noctiluca. A) Mating-like behaviour favouring synchronous spawning; B) release of eggs clumped in mucous threads; C) close-up of B.*

These physiological (a), behavioural (b) and environmental (c) factors might generate a strong variance in the reproductive success of *P. noctiluca* individuals. Indeed, if just a few males achieve the objective of reproduction, and the probability of union between gametes is not random (as indicated by the strong inbreeding), the generated recruit pools can have an unbalanced genetic composition with respect to the rest of the population.

According to these results, *P. noctiluca* in the Mediterranean Sea and Atlantic Ocean does not maintain a single perfectly homogenous population (i.e. panmictia), even if the high gene flow avoids the establishment of strong genetic structures. Rather, the non-random mating among individuals and the influence of stochastic environmental factors shape the population genetics of this species generating a spatially and temporally fluctuating genetic patchiness.

This work was based on available samples deposited at the VECTORS repository DNA/tissue bank at the University of Salento (see Deliverable 3.2.2 for further information on the repository).
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Figure 16. Hydrogeographic features such as eddies, gyres or upwelling fronts may promote the aggregation of outbreak subunits of *Pelagia noctiluca* in restricted coastal areas.

3.5 Molecular recognition of moon jellyfish across the Mediterranean Sea and first identification of the recently introduced sp.1 population in the Varano coastal lagoon (Adriatic Sea) (CONISMA)

The moon jellyfish (*Aurelia* spp.) is likely the most studied taxon of scyphomedusae, as well as the one with the widest distribution worldwide, since it has been found from polar to tropical latitudes. The taxonomy of this genus is currently in a state of upheaval, since recent molecular phylogenetics studies redrew it as composed of a few morphologically distinguishable species (*A. aurita*, *A. limbata* and *A. labiata*) and several other cryptic species (i.e. species not distinguishable using classical morphological characters). Many of the *Aurelia* species are geographically restricted, but some of them have a disjointed distribution likely due to multiple human mediated introductions. Nevertheless, information about the *Aurelia* geographic distribution in the Mediterranean Sea is still poor and fragmented. Through VECTORS research, a DNA barcoding study was carried out with the purpose of improving the knowledge about the distribution of *Aurelia* spp. in the Mediterranean Sea and identifying, for the first time, the *Aurelia* population recently introduced in the Varano Lake, a coastal lagoon situated along the Italian Adriatic coast. The mitochondrial Cytochrome Oxidase subunit I (COI) sequences obtained for all the study specimens were aligned with COI sequences from several previously identified *Aurelia* species present in GenBank and analysed using DNA barcoding techniques. The 54 sequences of *Aurelia* spp. collected across the Mediterranean Sea and northern European Seas comprised four genetically distinct species of *Aurelia*: *Aurelia aurita* (specimens from British Islands); *Aurelia* sp. 1 (Varano Lake, Italy; Empuriabrava, Spain); *Aurelia* sp. 8 (Bizerte, Tunisia; Porto Cesareo, Italy); *Aurelia* sp. 5 (Mljet Lake, Croatia) (Fig. 17). All data were supported by a robust “barcoding gap”, namely a well-defined difference between intra-specific and inter-specific pairwise genetic distances. These results first confirmed *Aurelia* sp. 5 as a species confined in the Mljet Lake and *Aurelia aurita* as a stable inhabitant of the European North Atlantic Ocean and North Sea coasts. They also confirm the “Lessepsian” distribution of *Aurelia* sp. 8, which was identified as the moon jellyfish species inhabiting the southern Mediterranean and Ionian coasts (Bizerte and Porto Cesareo).
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Moreover, all of the three species in the Mediterranean Sea cohabit in the Adriatic Sea. Until now, only *Aurelia* sp. 5 and *Aurelia* sp. 8 were considered to stably inhabit the coastal waters of this small basin, not considering the moon jellyfish population in the Varano Lake which was identified as *Aurelia* sp. 1. Another interesting finding of this study was the occurrence of *Aurelia* sp. 1 both in the Varano Lake and in the touristic harbour of Empuriabrava, namely two sheltered areas highly affected by human activities. *Aurelia* sp. 1 was probably introduced in the Empuriabrava marina by ships and it may also be one of the sources from which the species spread along the north-west Mediterranean Sea. Whereas a likely explanation of the *Aurelia* sp. 1 presence in the Varano Lake is the regular import of mussels and shrimps from other localities for aquaculture restocking purposes that may have served as vectors for the introduction of polyps or ephyrae. Nevertheless, the failed colonization of these kind of environments by other species already present in the Mediterranean Sea (for example *Aurelia* sp. 5 or *Aurelia* sp. 8, which bloom in the Adriatic Sea), suggests that *Aurelia* sp. 1 might be ecologically advantaged in coastal basin environments in the Mediterranean Sea. The hypothesized adaptability of *Aurelia* sp. 1 to environments often subjected to sharp environmental changes (i.e. coastal lagoons and harbours) might represent the main reason for its ubiquitous distribution worldwide. This work was based on available samples deposited at the VECTORS repository DNA/tissue bank at the University of Salento.

### 3.6 Influence of jellyfish biomass on bacterial dynamics in the lake of Varano (CONISMA)

Jellyfish blooms were increasingly reported in recent years and interpreted as the direct effect of multiple drivers of change in marine ecosystems, including global warming. Much attention has been paid to the mechanisms of bloom formation, the predatory impact of jellyfish on food webs and ecosystem processes, and the jellyfish-related hazards for human activities in coastal areas. Conversely, much less information is available on the fate of decaying gelatinous biomasses (jelly-falls) following bloom events. The rapid decay of jellyfish bodies after the completion of generally seasonal or annual life cycles leads to the rapid and massive release of nutrients into the water column. This event may produce an important trophic shift in the receiving marine food webs, related to qualitative/quantitative substantial changes in the microbiome, with consequences for the overall functioning of aquatic ecosystems. As part of VECTORS research a PhD project was developed to evaluate the impact of jellyfish biomass produced by blooms of the scyphozoan *Aurelia* sp. on the
dynamics of different microbial populations in the marine coastal lagoon of Varano (Southern Adriatic coast, Italy).

Quantitative analysis of changes in relative abundances of the microbial community due to jellyfish biomass was carried out by traditional microbiological methods. In parallel, a qualitative analysis of the microbial community was carried out using a combination of traditional and modern methods: a standard selective isolation and culturing methodology combined with PCR-based molecular barcoding of the main taxonomic units, and Next Generation Sequencing metagenomic analysis. Finally a “blue growth” approach was dedicated to the identification of bioactive compounds with antibacterial properties isolated from gelatinous biomasses of the jellyfish *Aurelia* sp.

In the presence of jellyfish the abundance of some microbial pollution indicators, heterotrophic bacteria and vibrios, was higher than in the control samples (absence of jellyfish). In May, coincident with the peak of jellyfish blooms, the bacterial biomass and abundance were higher in the zone with *Aurelia* sp. than in the control zone. Nonetheless, most of the examined parameters showed their highest peaks in the presence of decaying gelatinous biomasses (*jelly-falls*). The highest values of bacterial biomass and abundance were recorded in September simultaneously with the jellyfish decaying.

The diversity of the culturable heterotrophic bacteria was determined by taxonomic identification of the culturable heterotrophic isolates corroborated by a standard 16S rDNA barcoding. The standard qualitative analysis showed that γ-proteobacteria represented the main component of the bacterial community in the lake of Varano during the jellyfish bloom and that the *Vibrionaceae* family was a key component. The analysis of microbiome diversity of the Varano lake was also attempted for the first time through the application of a metagenomic (Next Generation Sequencing) Illumina-based protocol targeting the universally conserved 16S ribosomal RNA gene. In the presence of jellyfish a significant increase of some rare families (*Halomonadaceae*, *Shewanellaceae*, *Comamonadaceae*, *Simkaniaceae*, *Micrococcaceae*) was recorded along with a slight increase of *Oceanospirillaceae*. Jellyfish biomass therefore favoured the growth and dominance of specific bacterial assemblages more rarely occurring in ambient waters, thus inducing large changes in community composition. To analyse the potential causal mechanisms regulating jellyfish impacts on the bacterial community, VECTORS also investigated the occurrence of bioactive metabolites with antimicrobial activity contained in different components of *Aurelia* sp. (tentacles, umbrella and exudates). In each examined compartment an antibacterial lysozyme-like activity was detected (especially in tentacles homogenate). Isolation and characterization of related compounds might lead to the development of new biological approaches in the preparation of antibiotics.

**4. Task 2.1.4. Data analysis to test the role of aquaculture as a vector for invasions (UNIPV, IOLR, NIOZ)**

The mechanisms of change involving IAS has been reviewed on the basis of the existing data (Mediterranean and Atlantic) from areas of intensive aquaculture farming, in particular mollusc culture, to establish the ecological consequences of the introduction of target farming species and of their associated specific and/or non-specific symbiotic organisms (e.g. epibionts, commensals, and parasites). Analysis focused on comparison of distribution and abundance of IAS and native species and provided new insight on invasion dynamics, estimated current trends of invasions, and identified new putative hotspots for invasives in European seas. These data represent an important knowledge-base from which to analyse the evolution of the benthic community following new species introduction. VECTORS is therefore recommending that movement and marketing of live trade species should adhere to strict controls like those proposed for other pathways (Ojaveer et al., 2014) in order to prevent further unintentional NIS introduction.

Aquaculture is one of the main pathways for the introduction of NIS in Europe, accounting for high percentages of introduced species (Galil et al., 2013, 2014; Ojaveer et al., 2014). Several activities were
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conducted within this task with the aim of quantifying the role of aquaculture as a vector for NIS invasion in a well-known European site hosting aquaculture facilities: the Lagoon of Venice (Italy).

The Lagoon of Venice is (i) a well-studied environment for which long term data series are available; (ii) a hotspot of introductions in Italy and in the whole Mediterranean Sea; (iii) hosts facilities of shellfish culture, in particular Manila clam (*Ruditapes philippinarum*) farms which account for 50% of the Italian clam production, one of the largest in the world. Data on the role of aquaculture in the pan-European context were obtained from AquaNIS (Olenin et al., 2014)

In order to update the list of NIS in the Lagoon of Venice, an extensive sampling survey of this lagoon was performed in July 2012, targeting some supposed preferential areas for NIS introduction: shellfish farms of the Manila clam, commercial and touristic harbours, marinas, and sites of seafood processing and seafood trade. Control areas without anthropogenic activities were also surveyed. The benthic macrofauna was collected from both hard bottoms (wooden poles) and soft bottoms. Macroinvertebrates were identified to species level and the number of NIS over total number of species was measured in each sampling site. A total of 51 samples were examined. Data was obtained on the current abundance and distribution of NIS in the three hydrographic basins within the lagoon.

As regards the pathway “seafood trade”, analysis focussed on the fish market of Chioggia, one of the major crossroads for trade and commercial exchanges in Europe, located within the southern basin of the Lagoon of Venice. Loading and unloading of packaged seafood takes place directly on docks, very close to the inner canals where lagoon waters circulate. The director and the business manager of the Chioggia fish market were interviewed about the import-export movement of marine fish and shellfish species in 2011, in order to gather information on the main species traded as seafood. Data was collected on: native and non-native species arriving at Chioggia in 2011 and sold to national customers, total quantities of imported products (t y⁻¹), live or dead imports, and national and international market vendors. For all species of the Chioggia market list, a literature search was then carried out in order to check the range of species origin. For the non-native species already known to have been introduced to other European and Mediterranean countries, information on their introduction history was researched. Furthermore, biological traits of non-indigenous species were analysed, with the aim of assessing whether, in the event of accidental escape following trade operations and transport, these species could find a suitable habitat and hence a chance to establish a wild population in the northern Adriatic Sea.

Data extracted from AquaNIS revealed that aquaculture is responsible for about 50% of all NIS introductions in the Baltic Sea; 35% in the Western European Margins (with high inter-country variability - France displaying the highest percentages); and 10% in the Mediterranean Sea, where the Suez Canal is by far the most important pathway of introductions (Galil et al., 2014).

In the Lagoon of Venice, the number of NIS has dramatically increased since the 1980’s (about 320%) (Figure 18a), and most of them have been attributed to the pathway “culture” (Figure 18b).

![Figure 18 – Timeline of NIS introductions in the Lagoon of Venice. (a) Cumulative number of NIS, showing an increase of 320% from 1980 to 2014; (b) cumulative number of NIS introduced by different pathways. “Culture” and “Culture, Vessels” account for 67% of all introductions.](image-url)
Only 3 NIS were intentionally introduced for cultivation purposes (the clam *Ruditapes philippinarum* and the oysters *Crassostrea gigas* and *Saccostrea glomerata*, the latter now being extinct in the Venice Lagoon); all the remaining ones are unintentional introductions, mainly macroalgae and benthic invertebrates that have arrived with oysters and clams.

The analysis of benthic samples collected from artificial hard substrates (wooden poles) revealed that about 10% of macroinvertebrates present in the Lagoon of Venice (about 200 species) are NIS (Figure 19a): no significant difference was observed in distribution and abundance of NIS among sampling sites (harbours, marinas, etc.), showing that the NIS now represent a permanent component of the Lagoon community. Analysis of years of first records in Venice lagoon and other European and Mediterranean countries suggested that more than 50% of the NIS that were unintentionally introduced with shellfish transfers were probably transferred from other European shellfish culture sites (Fig. 19b).

**Figure 19.** A) NIS as a percentage of the total number of species (about 200) observed during a survey of macroinvertebrates in the Lagoon of Venice. B) Percentages of likely source regions of NIS unintentionally introduced via shellfish trade in the Lagoon of Venice.

Venice is not only a sink but also a source of NIS that have been spreading towards other Mediterranean and Atlantic localities (Minchin et al., 2013, Marchini et al., 2014a,b). In particular, sites that have imported shellfish from Venice for farming purposes now exhibit a NIS community with elements in common to Venice. The lagoon of Olbia, in Sardinia, is the most relevant case (Marchini et al., 2014a). An emblematic example of NIS introduced to Venice that has spread elsewhere is the newly recorded North Pacific isopod *Paranthura japonica* (Richardson, 1909, Marchini et al., 2014b), originally described from Japan and Russia, which has recently been observed in the Bay of Arcachon, France, Atlantic coast. Hundreds of specimens of *P. japonica* were collected at several sites in the Lagoon of Venice, during the VECTORS sampling survey of July 2012. The analysis of samples collected in 2010-11 in Olbia and La Spezia (Italy, Western Mediterranean) revealed that the species has also been introduced there. A likely hypothesis to explain the presence of *P. japonica* in several regions of coastal Europe is a series of aquaculture-mediated introduction events during the last decades of the 1900s: (i) first stage, from Japan to Arcachon (France), perhaps with oyster spat transfers occurred in the 1970's; (ii) second stage, from France (or elsewhere in Europe) to Venice, perhaps with Manila clam transfers occurred in the 1980's; (iii) a third stage, from Venice to the Western Mediterranean, perhaps with mussel transfers. Shipping and recreational boating could also be responsible for the secondary spread of this species, which is well-adapted on artificial hard substrates of harbours and marinas.

The analysis of Chioggia fish market datasets showed that about 20 species are regularly traded live for the fish market and handled near the lagoon canals (Ferrario et al., 2013). They are: *Agropecten purpuratus*, *Buccinum undatum*, *Ensis directus*, *Ensis macha*, *Mytilus edulis platensis*, *Patinopesten yessoensis*, *Pecten maximus* (molluscs) and *Homarus americanus*, *Jasus sp.*, *Linuparus sp.*, *Litopenaeus vannamei*, *Metacarcinus magister*, *Panulirus gilchristi*, *Pandalus borealis*, *Panulirus sp.*, *Paralithodes camtschaticus*, *Pleoticus muelleri*, *Pleoticus robustus*, *Portunus (Portunus) sanguinolentus*, *Portunus (Posidon) validus*, *Puerulus sp.*, *Scyllarides herklotsii* (crustaceans). The analysis of biological traits of these species revealed that should they be accidentally released from Chioggia fish market, they are likely to successfully settle in the Venice Lagoon. For instance, the...
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decapod *Homarus americanus* and the bivalve *Ensis directus*, already recorded with stable populations along the Atlantic coasts of Europe since the 1960s and the 1970s, are a possible threat in the Lagoon of Venice because they are euryoecious species able to grow on every kind of substrate, to thrive in low salinity and to endure a broad spectrum of water temperatures. As regards *Homarus americanus*, its release/escape from live imports has been speculated as a possible pathway of introduction in the eastern Atlantic Ocean, although other vectors cannot be excluded (Ferrario et al. 2013). Such marketing experiments should be monitored by a careful scientific assessment in order to take into account their possible ecological consequences. Therefore, movement and marketing of live trade species should adhere to strict controls like those proposed for other pathways (Ojaveer et al., 2014) in order to prevent unintentional NIS introduction.

The trend of NIS introduction in the lagoon of Venice is still increasing and can be reversed only if authorities, stakeholders and citizens will apply responsible management of live food import, recreational boating, commercial navigation and shellfish farming. It is critically important to raise general awareness of actions addressing the problem of marine NIS introductions. These results are of interest for the MSFD – GES descriptor 2 Indicator 2.1.1: “Trends in abundance, temporal occurrence and spatial distribution in the wild of NIS, [...] in risk areas, in relation to the main vectors and pathways of spreading of such species.”

A comparable analysis of a historical data set (20+ years) of soft bottom macrozoobenthos of the Dutch S Delta Waters (managed by the Monitor Taskforce of NIOZ-Yerseke) revealed that the major part of the biomass consists of Non Indigenous Species (NIS) (Table 1). Often few or even a single NIS accounts for the major part of the local macrozoobenthic biomass. The different systems in these waters appear to have their own ‘specific’ outbreak forming species (except for the Oosterschelde and Westerschelde). In total 23 NIS have been recorded. The metadata will be fed into the AquaNIS database (see section 1.2). Based on literature data it can be concluded that aquaculture (shellfish industry) and transport (ship movement) are the main vectors of the NIS. This North Sea information reinforces the findings of the study on the impact of NIS introduced by aquaculture in the Venice Lagoon in the Mediterranean.

*Table 1. Overview of the dominant Non Indigenous Species (NIS) of the different systems of the Dutch S Delta Waters*

<table>
<thead>
<tr>
<th>System</th>
<th>NIS</th>
<th>% NIS biomass</th>
<th>Dominant NIS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grevelingen</td>
<td>16</td>
<td>~75 %</td>
<td><em>Crepidula fornicata</em></td>
</tr>
<tr>
<td>Eastern Scheldt</td>
<td>19</td>
<td>&lt; 45 %</td>
<td><em>Crassostrea gigas</em>¹ and <em>Ensis directus</em></td>
</tr>
<tr>
<td>(Oosterschelde)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lake Veere</td>
<td>14</td>
<td>~80 %</td>
<td><em>Mya arenaria</em>; <em>Crassostrea gigas</em>²</td>
</tr>
<tr>
<td>Western Scheldt</td>
<td>18</td>
<td>~11% (recently 45%)</td>
<td><em>Ensis directus</em></td>
</tr>
<tr>
<td>(Westerschelde)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

¹ Species out of scope for the monitoring programme
5. Task 2.1.5 Data analysis to determine the expansion of thermophilic invasives

This task successfully performed a trend analysis of distribution and abundance of the thermophilic non-indigenous species (or Erythrean aliens), whose many new records and rapid spread act as a Mediterranean Sea climate change signature. The analysis and the new VECTORS findings reveal there is no other vector of marine bioinvasions that delivers as high a propagule supply for so long to a certain locale as the Suez Canal into the Mediterranean. Future inventories of the biota of the Mediterranean will be dominated by many more thermophilic aliens, which will require region-wide surveillance and monitoring for their inclusion into integrated ecosystem-based management.

5.1 A larger canal, a warmer sea, a changed biota – alien thermophilic biota in the Mediterranean Sea (IOLR)

The Suez Canal has undergone several major enlargements in order to maintain its market share. The recent expansion, completed January 2010, increased its depth to allow passage of vessels up to a draft of 66 ft, and already the Suez Canal Authority (SCA) is evaluating the feasibility of increasing the Canal’s depth or doubling its width to attract the loaded very large crude carriers (VLCC’s) & ultra large crude carriers (ULCC’s) (www.suezcanal.gov.eg). The Canal’s typical cross-sectional area that was 304 m² in 1869, 1800 m² in 1962, and 3600 m² in 1980, is at present 5200 m². Propagule input is an important determinant of invasion success, characterized by size (large inocula are more liable to establish populations), frequency (risk of establishment increases with inoculation frequency) and duration. Thus the implications of a deeper, wider Canal combined with higher through-current velocities on transport of Erythraean biota through the Canal are all too clear. There is not another vector of marine bioinvasions that delivers as high a propagule supply for so long to a certain locale. Yet, conspicuously absent among the items enumerated in the SCA’s “Vision, Mission and Duties”, are references to its environmental responsibility (www.suezcanal.gov.eg). The last decades of the 20th century saw pronounced thermal fluctuations and a significant increase in the average temperature of the waters in the Mediterranean: the temperature in the upper layer has been increasing at an average (±SD) rate of 0.03 ± 0.008°C yr⁻¹ for the western basin and 0.05 ± 0.009°C yr⁻¹ for the eastern basin (Nykjaer, 2009). Indeed, the timing of the initiation of a significant increase in the number of invertebrate Erythraean aliens along the south-western Anatolian and the southern Aegean coasts was positively correlated with a more extensive inflow of the warm-water Asia Minor Current. A model of the evolution of the Mediterranean Sea during the 21st century under the IPCC A2 scenario showed a nearly spatially homogeneous increase in SST distribution (+3.1°C in 2099) (Somot et al., 2006). The warming trend seems already to have a significant influence on the establishment and distribution of thermophilic species (Fig. 20).
Rising seawater temperature could change the pool of species which could establish themselves in the Mediterranean, enable the warm stenothermal species (native and alien) to expand beyond their present distributions, and may impact on a suite of population characteristics (reproduction, survival) that determines interspecific interactions, and, therefore, the dominance and prevalence patterns of both native and alien species, and provide the thermophilic aliens with a distinct advantage over the native Mediterranean biota. The many recent records and rapid spread of several invasive aliens that have been reported within the VECTORS project might already be a Mediterranean Sea climate change signature. The interactions between the degradation of the littoral ecosystem, the increase in seawater temperature and salinity, and the monumental enlargement of the Suez Canal, which serves as a conduit for alien propagules, are critical for maximizing invasion success. Future inventories of the biota of the Mediterranean will be dominated by many more thermophilic aliens.

6. Task 2.1.6 Developing European guidelines on molecular tools for deciphering mechanisms of invasions and outbreaks (CONISMA)

This task was conceived to extend the use of molecular tools used by European research groups engaged in monitoring changes in the marine environment due to invasive alien species or the spread of native species with increasing competitive performance and outbreak potentials.

VECTORS aimed to gather world-wide renowned experts to jointly review scientific issues, existing methods, technical difficulties associated with development of sophisticated tools, and to consider the usefulness of DNA-based technologies for various applications relevant to invasive species monitoring. Currently used molecular tools include cytochrome oxidase subunit I (COI), ribosomal internal transcribed spacer (ITS) microsatellites or amplified fragment length polymorphism (AFLP) and molecular and cellular tools for studying various anthropogenic impacts on marine and aquatic organisms (e.g. microarrays, stress genes, comet assay, etc.). A call for voluntary, cost-effective sampling campaigns has been launched across VECTORS partners, scientific societies, individual scientists, and lay citizens (following the highly rewarding example of the citizen science programmes), to obtain specimens/tissues of IAS/OFS useful for RNA/DNA extraction and creation of a DNA repository at the CONISMA laboratory. This repository has been created and described by the Deliverable 2.1.1 (submitted at Month 14), and it is continuously implemented and integrated by new
specimens. The RNA/DNA bank provided baseline material already used by the scientific community for different goals including validations of morphological identifications and new species descriptions (Scorrano et al in preparation; Piraino et al 2014), assessment of genetic connectivity between populations (Aglieri et al 2014), reconstruction of temporal dynamics and spatial pathways of outbreaks/invasions (Ghabooli et al 2013), and identification of donor regions and vectors of introduction.

A workshop for VECTORS partners and additional European and non-European experts was held in September 2012 (Fig. 21) to set up a European forum for collaborative integration and to develop recommendations and guidelines for the promotion and use of molecular tools in monitoring and modelling programs of OFS and IAS. The workshop included two days of invited oral presentations, a poster session and discussions. Participants were introduced to the most up to date molecular methods used to identify organisms and investigate population structures and biodiversity changes of marine communities. Invited speakers and titles of keynote lectures were as follows:

- Giacomo Bernardi (University of Santa Cruz, CA) - Molecular tools to investigate Lessepsian bioinvasions, past, present, and future
- Ferdinando Boero (University of Salento, CoNISMa) – New threats for marine biodiversity
- Gisella Caccone (University of Yale, New Haven) - Using genomic tools to track the evolutionary history and the potential for success of invasive species
- Gary R. Carvalho (University of Bangor, Wales) - Molecular markers and marine invasions - Insights from molecular genetic analysis of population and species diversity
- Maurizio Casiraghi (Università Milano Bicocca) - DNA barcoding to investigate biodiversity - beyond a simple identification method
- John Darling (Environmental Protection Agency, USA) - How can genetic studies best inform the management of marine biological invasions?
- Mike Dawson (University of Merced, CA) - Using genetics to understand the causes of jellyfish blooms
- Marty Deveney (SARDI, Australia) - Molecular and traditional surveys for marine pests: uncertainties, validation and developing integrated systems for pest surveillance
- Bella Galil (IOLR, Haifa) – Exotics in the Mediterranean - Bioindicators for a sea change
- Esther Lubzens (Technion University, Haifa) - An "outsider's" view on molecular research of invasive species - where is it leading us?
- Tomaso Patarnello (Università di Padova) - Population transcriptomics: expression profiling in marine species
- Graziano Pesole (Università di Bari)
- Marc Rius (University of California, Davis, USA; and University of Southampton
- Constraints and possibilities in the study of genetic data of marine biological invasions.
- Daniel Simberloff (University of Knoxville, USA) - "Invasion biology: Where did it come from, where is it going, and how can molecular tools help?"
- Frederick Viard (Station Biologique de Roscoff, France) - From species identification to population analyses: the usefulness of molecular tools for the study of invasive ascidians.
- Filip Volckaert (University of Leuven, Belgium) - Molecular tools for monitoring the impact of marine alien and invasive species in Europe

The entire workshop was recorded and a DVD was produced for distribution to VECTORS participants and other European scientific institutions. The workshop organization included a young scientist support programme, which favoured participation of 20 students/youth scientists (up to 35 yr) by covering their registration fee and B&B accommodation. A workshop volume is now subject to editorial workflow with peer review of ten selected contributions to be published in a special issue of the international scientific journal BIOLOGICAL INVASIONS (expected by 2014). A position paper is being prepared including recommendation for incorporation of specific molecular tools, development of probes, methods for collection of samples, processing and presentation of results, in order to establish a unified, comprehensive, and comparative molecular data collection and analysis for predicting the outcome of marine bioinvasions in European seas.
The meeting offered an opportunity to discuss future collaborations between groups and the submission of joint research proposals. Expected outcomes of the meeting included joint collaborations (e.g. Rius and Darling, 2014) and formulation of recommendations and guidelines for the study of marine bioinvasions.

Fig. 21. Poster of the MOLTOOLS workshop held in Lecce, September 2012.
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References


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