Eduardo Dopico and Yaisel Borrell (editors).

**ALERTOOLS Workshop:**

*Science & Educational Strategies for Early Detection of Bioinvaders*

Scientific & Educational Strategies for Early Detection of Biological Invasions

Avilés, Spain. 13th, 14th and 15th October 2016

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Index

Workshop Summary 5

Analytical tools: Sensors and warning signals 9
Juanes F. The potential use of visual and acoustic sensors for early detection of invasions 10
Jiménez-Ruiz J. Remote sensing as a tool to detect biological invasions in riparian ecosystems 12
Parrondo M. New chances for the early detection of the monster wels catfish Silurus glanis in freshwater ecosystems 17
Rech S. Rafting biota on anthropogenic marine litter along the Spanish Atlantic coast 18
Ardura A. Developing tools for early detection of marine invertebrate invaders: gaps and priorities in current databases 19

Analytical tools: Detecting the invisible 20
Zaiko A. Seeing the unseen: Molecular methods for biosecurity research and monitoring 21
Fernández Fernández S. Rainbow trout (Oncorhynchus mykiss) detection and distribution studied through environmental DNA in Nalon River, Northern Spain 22
Mauvisseau Q. Using environmental DNA (eDNA) to record the distribution of the invasive red swamp crayfish (Procambarus clarkii) in freshwater ponds 23
Rey A. Implementing genetic tools for ballast water monitoring: the trial of RNA metabarcoding to detect viable organisms 25
Clusa L. An easy and fast method to detect the five commonest invasive fish species in Europe from environmental DNA 26
Borrell YJ. Metabarcoding and post-sampling strategies to discover exotic species: a case study in Asturias estuaries 27

Analytical tools: Genetic methodologies 28
Moran P. Cytogenetic analysis on the invasive mussel Perna perna and Brachidontes sp 29
Gómez Agenjo M. Barcoding as a tool for early detection of hitchhiking invasive species on floating marine debris in the Cantabrian coast 30
Montes M. Molecular tools applied for exotic seaweed identification in Cantabrian coasts, Bay of Biscay 32
Muñoz-Colmenero M. Detection of the invader Ficopomatus enigmaticus directly in water before being seen: a new molecular marker 33
Skukan R. Species demarcation in green algae genera Codium using DNA-barcoding
Ardura A. PCR-based assay for Mya arenaria detection from marine environmental samples and tracking its invasion in coastal ecosystems
G. Valdecasas A. Colonization or facilitated invasion? The molecular answer

Educational and citizen-science approaches
Rayón F. Marine debris, a socioenvironmental approach for new solutions
Arboleya E. The Game of The Sea: a multidisciplinary game to learn about seas and oceans while playing
García-Vazquez E. Barcoding and citizen science for monitoring changes in fish parasites: insights for marine fish management and conservation in the Bay of Biscay
Pevida Llamazares D. “They came to stay”. How to identify invasive species. A PBL Project
Skukan R. ‘Pokemon Algae’ a game based on citizen science to study marine invasive algae
Miralles L. Citizen Landscape perception and marine conservation in the Asturian coast (North Spain)
Miralles L. Citizens for the control of biological invasions: a game-based training
Arboleya E. Searching IAS-birds around hometown skies
Dopico E. Citizen Science: active participation of citizens in science research would enhance science literacy?

Ongoing projects on strategies for early detection and management of invasive species
Consuegra S. AQUAINVAD-ED Project
Miralles L. Seawatch Surfers: The creation of a citizen network to detect and to alert coastal environmental problems
Díaz González T. The project LIFE+ARCOS: In situ and ex-situ innovative combined techniques for coastal dune habitats restoration in scs of northern Spain. Plant biodiversity conservation in coastal arenas cantabrian
Borrell YJ. Prevention and control of biological invasions in the port of Gijón, Bay of Biscay (FUO-95-2016)
Osendi García P. Proyecto Cangrejo: elimination program of the red crab in Narcea River
García-Vazquez E. Multidisciplinary tools for alert and control of marine bioinvasions: genetic markers, eDNA, ICT and citizen science

Conclusions
Workshop Summary

The Global Village or Globalization concepts have become a relevant part of our current lives. No doubts one of the associated consequences is the hotspot phenomenon of biological invasions. The spread of invasive species (NIS, non-indigenous species) has become so extensive (and global) that the ecological impacts of such invasions represent today a major threat to global biodiversity. The introduction of non-native species alter existing ecosystems and is now ranked second only to habitat destruction in terms of potential ecological catastrophe. When ecosystem goods and services are compromised by nuisance NIS this implies expenses of millions of dollars on remediation policies on the ecosystems.

After establishments in a new location, eradicating invasive species is extremely challenging, costly and in many cases not feasible. Actions for controlling biological invasions are the most efficient at the early stage of incursion. Preventing the introduction of invasive alien species is one of the main lines of action that have been stated by the European Commission growth strategy. In order to perform qualitative and reliable risk assessments, evaluate environmental status, ensure adequate border control and post-border detection, there is a need for innovative, rapid, and cost-effective diagnostic tools that be able to identify and quantify the full range of NIS. The efficiency of NIS prevention, detection and rapid response actions can be fortified by raising public awareness and engaging active citizens to be involved in NIS surveillance, notification programs, and eventually mitigation of spread and eradication actions.

The ALERTOOLS 2016 event, held at the Niemeyer center in Áviles, Spain, gathered together researchers, teachers, students and citizens coming from the UK, New Zealand, Canada, Lithuania and Spain, all of them concerned about the hot topicality of biological invasions. Organized together by the Marine Observatory of Asturias and the Cluster of Environment and Energy of the Campus of Excellence of the University of Oviedo, was coordinated by Dr. Yaisel J. Borrell (Biology) and Dr. Eduardo Dopico (Education Sciences) under the scientific direction of Prof. Eva Garcia-Vazquez.

First morning session (October 13th) was dedicated to analytical tools for early detection of non-indigenous individuals. Professor Francis Juanes (Cânada) (chair of the session) explained the great potential behind the use of visual and acoustic sensors for detecting NIS. Personal mobile phones, use of classical and camera traps together with remote access to proceed to automated identification of collected images, remote sensing by use of drones, satellites and video technology and automated identification of collected sound files can indeed help in early detection of NIS. Other four authors talked about the use of remote sensing in riparian ecosystems. The importance of fishermen web forums as a relevant source of early reports about
the presence of problematic species such as the wels catfish *S. glanis* in Spain, about the use of routine screening on marine rafting as a useful tool to detect NIS, and about the need to improve biological databases to help in NIS identifications.

The Second scientific session on October 13th was led by Dr. Anastasija Zaiko (Lituania/New Zealand) (chair of the session) and focused on molecular methods for biosecurity research and monitoring using environmental DNA (eDNA). The use of Next Generation Sequencing (NGS) methodologies and of the species-specific primers for early detection of NIS in eDNA was deeply discussed by six authors. NGS screening of eDNA and eRNA from Port and estuaries waters and species specific tools studies on eDNA coming from freshwater ponds and rivers to detect invasive trouts, crayfish, and other species such as Gambusia sp, *M. salmoides*, *Ameiurus sp*, *L. gibbosus* and *P. boraparva* were also addressed.

The evening session on October 13th was focused on classical genetic methodologies for NIS identification. Professor Paloma Moran (University of Vigo) started explaining how cytogenetic analyses can help to identify NIS. Other six studies set out that the use of barcoding techniques allowed accurate genetic identification of red and green algae species and also of hitchhiking and, human mediated introduced species, such as *M. arenaria* and *F. enigmaticus* or other species associated to NIS such as mites via parasitisms or comensalisms.

The next day, October 14th Citizen Science was very present. This science strategy linking scientist and citizens in a participative inquiry-based project offers a new framework for fieldwork in the scientific research. At the same time, it provides an outreach channel for research and an adequate space for citizenship awareness about sciences issues of common interest. Thus, we have seen how marine debris were arousing great concern among the Asturian coastal population and how a citizen science strategy would help to reduce the litter generation.

The ocean and coastal ecosystems conservation is a shared responsibility and scientists can enhance the citizens involvement in that task proposing simple card-based games showing amazing secrets of marine life and the risks of bioinvasions while they learn by playing. Didactics games are a good teaching resource that facilitates learn by doing. Thus, it was presented a marine invasive algae game based on currently famous Pokemon cards to attract the attention of citizens (mainly primary and secondary school children) and teach them about basic environmental issues caused by exotic species. The aims of the game were to teach kids in distinguishing invasive algae and to collect detached samples that can be found lying on the sand of surrounding beaches for scientific research.

A new game was also presented. This time the game was a essential part of the recruitment and training phase of volunteers for eradicating an invasive mussel
(Xenostrobus securis) from a north Iberian estuary in Spain. The results of this Citizen sciences experience suggested high efficacy of game-based training for increasing knowledge and promoting environmentally conscious attitudes, especially in young age groups.

Targeted specifically to students aged 12-16 from the subject Natural Sciences in Compulsory Secondary Education, an educational dynamics based on project-based learning (PBL) which revolves around biodiversity and ecosystems was presented. This educational activity encouraged students for spreading their work through infographics, QR documents and an explanatory video uploaded in a web video platform.

Another citizen science methodology was showed talking about parasites, natural components of aquatic communities. Identification of parasites requires specialized knowledge and can be a burden for already endangered artisanal fisheries. The costs of systematic monitoring of fish parasites can be relieved if citizens collaborate in sampling and easy DNA Barcoding protocols.

To conclude this section of the workshop, we discussed about the participation of the public in general in the scientific studies. Balancing the tension between scientific efforts and educational needs, citizen science connects science, education and citizenship, involving citizen participation in genuine scientific research. Citizens acting like scientists have stronger positive attitudes toward the environment. Besides, Citizen Science initiatives take advantage of citizen's points of view, not mediated by individual scientific background, to provide intuitive solutions to complex problems. In the Citizen Science approach, it is critical that the design of a research project that includes citizen science should match the needs and interests of the participants with those of scientists. Citizens need to understand scientific messages, ongoing research, its implications on their lives, and action taking on socio-ecological issues.

The last session of ALERTOOLS 2016 (October 14th) was dedicated to show details of funded projects working on the prevention and management of biological invasions. Professor Sonia Consuegra (UK), who also led the EU Project Aquainvad-ed, led this session. This last European project recruited eight PhD students from all Europe currently working in scientific institutions from three different countries (Spain, Italy and UK) and addressing several questions and tasks to prevent invasions in aquatic ecosystems. Professor Tomas Díaz (University of Oviedo) explained the Project LIFE+ARCOS that focus on dune habitats restorations and Professor José Rico (University of Oviedo) did the same with the Project FUO-095-2016 where the detection of invasive species in the Gijon Port Authority is the main objective. Pablo Osendi, from The Banzao Fishermen Guild, made a nice talk about a successful mitigation/eradication project for the red crayfish *P. Clarki* in the Narcea River. Finally,
Professor Eva García-Vázquez (University of Oviedo) summarized main results of the Project MINECO CGL-2013-42415-R, where almost all the aquatic invasive species from the Spanish catalogue of invasive species, RD 630/2013 of 2-August 2013 have been targeted with the goal of their prevention, identification and successful management using scientific, but also educational, strategies.
Analitical tools: Sensors and warning signals

Juanes F. The potential use of visual and acoustic sensors for early detection of invasions

Jiménez-Ruiz J. Remote sensing as a tool to detect biological invasions in riparian ecosystems

Parrondo M. New chances for the early detection of the monster wels catfish Silurus glanis in freshwater ecosystems

Rech S. Rafting biota on anthropogenic marine litter along the Spanish Atlantic coast

Ardura A. Developing tools for early detection of marine invertebrate invaders: gaps and priorities in current databases
COMMUNICATION: The potential use of visual and acoustic sensors for early detection of invasions

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Abstract:

As the threat of global invasions increase it is critically important to develop tools for early detections of invasive species. The earlier such species can be detected the more feasible their control and potential eradication will be. Unfortunately most new invasive species are detected by accident when making general surveys often when it is too late for rapid response or in a location where capacity for response is limited. Early detection as applied to invasive species is a system of active and passive surveillance to find and verify the identity of new invaders as quickly and efficiently as possible. Research is also needed to facilitate the incorporation of technological advances into existing methods such as sensors. A sensor is a device that detects or measures a physical or biological property (here an invasive species), and records, indicates or responds to it. In this talk, I summarize what features of visual and acoustic sensors make effective tools for early detection of invasives but do not include chemical methods such as environmental-DNA which are covered elsewhere.

I first define a good sensor as one that is dependable, accurate, flexible, targeted, automated and inexpensive. Clearly all of these traits will not be possible with every sensor, instead their importance will be context-specific. I first start with visual sensors, that include many of the traditional methods of invasive detection, but that have also more recently incorporated technology to automate them. They are also by far the dominant mode of detection, particularly in terrestrial habitats. At small scales, personal mobile phones are now being used to document the presence and location of invasive species. This information is rapidly transmitted and if the web of users is extensive enough such a method can produce accurate and wide-ranging maps.

Traps of all sorts are routinely used to detect and count wildlife species, but to date are rarely used for early detection of invasives. However use of traps, for invasive rodents, and camera traps for larger invasive wildlife are now beginning to develop with the advent of remote access to and automated identification of collected images. Similar image acquisition technology is being developed for use of early detection and identification of invasive species from aquatic samples. On a broader geographic scale remote sensing by use of drones, satellites and video technology is being developed to map invasive species habitats in both terrestrial and aquatic environments. There is large potential in the use of acoustic methods for early detection of invasives, but such technology is still in its infancy. Examples of the use acoustics, including automated
identification of the collected sound files to species, exist for invasive cane toads, birds and fishes. I conclude by suggesting that the use of visual and acoustic sensors hold much promise for detecting and monitoring invasive species in both terrestrial and aquatic environments, particularly in remote settings. Their efficiency will increase with the growth of observatories, especially in marine systems. Their use will increase and their cost decrease as technology improves and automation becomes more feasible. In my opinion, growth of the use of sensors for early detection of invasives will be driven primarily by the development of automatic detection and identification procedures, allowing the efficient processing of large amounts of data at time scales where rapid responses are still possible and before such species colonize and naturalize in their new habitats.
COMMUNICATION: Remote sensing as a tool to detect biological invasions in riparian ecosystems

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Abstract:

Exotic plants are common in rivers and riparian environments for several reasons. On the one hand, because the funds riparian basin sediments accumulate and are naturally rich in water and nutrients, whose concentration is further, increased by human activities. Furthermore, because it is altered by human activity, introducing deliberately or accidentally these species in an environment that favors dissemination of the invaders, and finally, because the disturbances that occur in these ecosystems provide liberated spaces (Planty-Tabacchi et al., 1996) ready to be colonized. When all these elements come together is easy to understand why these environments are, in the words of Hood and Naiman (2000) "ecosystems disproportionately susceptible to invasion". In these cases, invasions are carried out by plants able to exploit resources opportunistically (Jiménez-Ruiz et al., 2011). In fact, this type of behavior coupled with a tendency to establish monotypes, vegetation where exotic species on native species dominates and excludes or subordinates are features shared by many of the plants that invade wetlands (Zedler, 2004).

The main purpose of the study was to evaluate the possibilities of detecting relevant parameters to the management of water courses and detect the biological invasion from one of the most dangerous species in the Mediterranean rivers, *Arundo donax* (Lowe et al., 2000). The research was carried out in three sections of the Segura River, using a hyperspectral remote sensor. To this, it has been necessary lifted spectral images, adjust digital signatures and perform a supervised classification to characterize the variables of interest in each section. The fieldwork to perform has the following objectives: estimate the spectral reflectance of soil, to estimate the spectral reflectance of the parameters to identify and identify areas of training parameters to identify known, in order to validate and evaluate the results. To fulfill these objectives, previously fieldwork arises based on taking the following data:

1. For each of the sections, identify at least 10 horizontal floor areas (at least 5x5 m) distributed evenly along the entire stretch, half with greater reflection of light (clear and white surfaces, such as dry white sand) and the other half with the greater light
absorption (e.g. signature dark asphalt mix). For each of these areas is necessary at least three measurements with a field spectroradiometer, if possible during the flight or in the near future on a clear day and in the same time slot in which the flight is conducted. With three measurements for each surface it is sufficient to obtain the average spectral radiance of each.

2. For each of the species that are to be identified, required at least 3 geographical locations in each section, and at least 5 measurements of spectral reflectance with field spectroradiometer in the same conditions described in the previous section, which makes a number of measures reflectance 15 species. Of each of the individuals identified and located, its vigor or data that can influence it, such as the presence of irrigation estimated.

3. For each of the plant species identification of at least 6 ROIs (Regions of Interest Region Of Interest) is required.

4. Several water samples along both sections of which will be analyzed in the laboratory chlorophyll and turbidity. These samples will be taken in places unshaded and exposed to direct sunlight. In addition we will try to sample areas with different colors looking heterogeneity and contrast.

**Sensor Features**

Hyperspectral images were acquired with an optical imaging spectroradiometer. The geographical location of each scan line is accomplished through a direct positioning system capable of reading the altitude and displacement of the aircraft simultaneously and terrain the geographical coordinates with a frequency sweep from 0.005 s. The following table shows details of the sensor configuration:

<table>
<thead>
<tr>
<th>Sensor type</th>
<th>Scanner of continuous scan</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Technical aspects</strong></td>
<td></td>
</tr>
<tr>
<td>Spectral range</td>
<td>400 a 970 nm</td>
</tr>
<tr>
<td>Number of bands</td>
<td>62</td>
</tr>
<tr>
<td>Ancho de banda</td>
<td>9,2 nm</td>
</tr>
<tr>
<td>Field of View (FOV)</td>
<td>35,92º</td>
</tr>
<tr>
<td>Dimensión CCD</td>
<td>1024x1024 pixels</td>
</tr>
<tr>
<td>CCD pixel size</td>
<td>12 μm x 12 μm</td>
</tr>
<tr>
<td>Focal distance</td>
<td>18 mm</td>
</tr>
<tr>
<td>SNR</td>
<td>350:1 – 1400:1 (It depends of the configuration of bands)</td>
</tr>
<tr>
<td>Radiometric resolution (bits per pixel)</td>
<td>16 bits per spectral band per pixel = 992 bits per pixel</td>
</tr>
</tbody>
</table>
Digital number of Spectral Radiance Units: 1000=1 μW / (sr.nm.cm²)

Digital number of Spectral Reflectance Units: 1000=1

*Image preprocessing*

The image preprocessing is performed following eight basic steps:

Step 1. Calibration of angular bias.

Step 2. Radiometric correction, atmospheric calibration data. The software used were: CALLIGEO (supplied by SPECIM ltd) and ENVI.


Step 4. Construction of a mosaic RGB.

Step 5. Registration and training ROIs (Region of Interest) on the mosaic.

Step 6. Reverse Georectification of images classified.

Step 7. Georectification of classification results. The software used is: PIMHAI-PLATFORM.

Step 8. Construction of georectificado resulting mosaic. The software used was: ENVI

For all steps in the preprocessing of images, it has been used a specific software, except for some operations which used the ENVI software for its ease of use.

The calculated average spectrum for each of the eight classes and their respective ROIs are presented below:

![Vegetation Spectrum](image)

*Figure 1: Average spectrum for each of the eight classes*
Results and conclusions

This work shows that the contribution of hyperspectral analysis for the study of vegetation, both aquatic and non-aquatic, in both areas is considered a promising challenge, allowing detect incipient populations of invasive grass and well established populations.

Figure 2: Classification map of the vegetation.

References


COMMUNICATION: New chances for the early detection of the monster wels catfish Silurus glanis in freshwater ecosystems

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Abstract:

One of the most important threats to Spanish freshwaters ecosystems are non-indigenous species (NIS). They could produce a high impact on the survival of native species as a consequence of competition for limited resources and environmental changes. The giant wels catfish *Silurus glanis* was intentionally introduced in Spain in 1974. Since then it has had an alarming and hidden spread in Spanish basins due to its consideration as a fishing trophy, specially in the last fifteen years. This silurid can affect the survival of native species as a consequence of disease transmission, predation of native species as cyprinids, mollusks, crayfish or birds; and change the structure of the food web in some regions. Due to the absence of consensus among the official and non-official information about the spread and presence of *Silurus glanis* in Spain, we had updated the invasive pattern of *S. glanis* through Spanish freshwater ecosystems since the first introduction in the Segre river (Ebro's basin) until nowadays. For that purpose blogs, forums, web pages, videos, magazines and newspapers, scientific papers and all the official information from the Ministry of Agriculture, Food and Environment of Spain about the spread/presence of *S. glanis* in Spain until June 2016 were consulted, scrutinized and summarized. A graphic interface representing all the information included in this database was designed using QGIS 2.14 Essen. The current situation reflects its presence in six of the seven main river basins in the Spanish territory. Recently, the Spanish Catalog of Alien Invasive Species has undergone some changes adding new species. This has provoked great conflicts between fishermen, scientists and institutions. Prevention, detection, correct managements actions and a considerable change in minds about the relevant issues such as how to deal with invasive species are really important. Considering the situation, this method can be a helpful tool in the fight against this controversial and dangerous invasive species. It can be used for early report allowing fast and effective stakeholders management actions.
COMMUNICATION: *Rafting biota on anthropogenic marine litter along the Spanish Atlantic coast*

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**Abstract:**  
Alien invasive species (AIS) and marine anthropogenic litter pose major threats to global ecosystems. Anecdotal recordings and some localized studies show that a large variety of species are able to use man-made floating litter as a transport vector both for large scale (trans-oceanic) and small-scale (along coastlines) dispersal. Recent findings point to an important role of marine litter rafting in AIS introductions. However, there are no comprehensive studies about the processes of colonization and transport by floating items on a large geographical scale. In this work, anthropogenic litter and attached biota along the coastline of Asturias, central Bay of Biscay, Spain, were analyzed. Stranded anthropogenic litter was quantified and classified on 17 beaches along the 200 km coastline of Asturias. Systematic monitoring for litter items with attached macro fauna was conducted from February to March of 2016. Anthropogenic litter was present on all beaches in varying abundances. Litter composition differed between beaches and gave important clues about the most important litter sources. Genetic barcoding, combined with visual taxonomic analysis, revealed a variety of species of goose barnacles, barnacles, bivalves, gastropods, polychaetes and bryozoan colonies attached to stranded litter, including several invasive species, such as the Mediterranean mussel (Mytilus galloprovincialis), the giant pacific oyster (Crassostrea gigas) and the Australian barnacle (Austrominius modestus). Plastics were the most common litter items on the sampled beaches and the most frequent carriers of attached biota, with fishing and aquaculture related items being particularly important. Such sea-based activities seem to be the main sources of stranded litter items with attached biota along the Asturian coastline. Future research and management actions should concentrate on these litter sources and involve citizens and stakeholders. Fully biodegradable alternatives for floating plastics and extended producer responsibility need to be promoted and litter management in the source sectors needs to be optimized.
COMMUNICATION: Developing tools for early detection of marine invertebrate invaders: gaps and priorities in current databases

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Abstract:

Marine alien species invasions are global phenomenon which is the main drivers for ecosystem change; they can often drastically affect structure and function of a novel ecosystem. The early detection is of prime importance for preventing the species’ establishment and potential dispersal, its eradication and control are more efficient, and less costly, when the species is present in very low densities. However, species at low density generally require intensive sampling efforts to be detected. An important shortcut to reduce the sampling effort is to analyze environmental DNA. Eukaryotic organisms leave traces of their presence in the water where they live. DNA presents in the water is extracted and amplified by PCR target markers to identify the species that inhabit therein. Besides, using species-specific markers the presence of one target species DNA from the water samples can be detected using PCR and a simple electrophoresis in agarose gel. This is a good and convenient approach when the target species is known and the focus is put on its detection rather than general biodiversity assessment. The suggested method is reproducible, fast, and cost-efficient. However, the principal problem of this methodology, is the design of appropriate species-specific markers, they must be specific of one target species, without any cross-amplification even in close species, but with a good amplification in eDNA from water samples, in which the DNA could be degraded. For this purpose, is essential a complete DNA database, with a lot of different species, genetic markers and haplotypes per each species and marker. The principal aim of this work is to analyze the state of the art of the databases, in this case GenBank that is the most complete database in number of markers and species, in order to describe the best region to design species-specific primers for invasive species early detection.
Analitical tools: Detecting the invisible

Zaiko A. Seeing the unseen: Molecular methods for biosecurity research and monitoring

Fernández Fernández S. Rainbow trout (Oncorhynchus mykiss) detection and distribution studied through environmental DNA in Nalon River, Northern Spain

Mauvisseau Q. Using environmental DNA (eDNA) to record the distribution of the invasive red swamp crayfish (Procambarus clarkii) in freshwater ponds

Rey A. Implementing genetic tools for ballast water monitoring: the trial of RNA metabarcoding to detect viable organisms

Clusa L. An easy and fast method to detect the five commonest invasive fish species in Europe from environmental DNA

Borrell YJ. Metabarcoding and post-sampling strategies to discover exotic species: a case study in Asturias estuaries
COMMUNICATION: Seeing the unseen: Molecular methods for biosecurity research and monitoring

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Abstract:

Non-Indigenous Species (NIS) are one of the greatest threats to marine biodiversity and ecosystem. Efforts are increasingly focused on development of effective surveillance methodologies for early detection of new introductions and monitoring the dynamics of the established populations. Most current marine surveillance programs rely predominantly on taxonomic expertise, are laborious, and often fail to identify cryptic species or those at the dispersal stage (eggs or larvae). Therefore, current approaches are often insufficient to deliver timely information on NIS distribution and advice on rapid response action plans. Developing an effective surveillance tool for NIS presents a major challenge to biologists as it requires accurate detection of species at low densities and often in early life-stages. By complementing the conventional research methods with molecular techniques different biosecurity objectives can be effectively addressed: e.g. identifying risks while species are still at the pathway, early detection of their spread and further surveillance of existing populations. The results of a few case studies performed in different geographical regions suggest that employment of molecular methods have evident advantages and provide a powerful tool for biosecurity research in diverse and heterogeneous marine ecosystems. The applicability of these methods will expectedly increase even more with further development of technologies and improvement of our baseline knowledge on genetics of marine biota.
COMMUNICATION: Rainbow trout (Oncorhynchus mykiss) detection and distribution studied through environmental DNA in Nalon River, Northern Spain

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Abstract:

Rainbow trout (Oncorhynchus mykiss) has been nominated among 100 of the "World's Worst" invaders. It has been introduced into at least 99 countries and evidences of its negative impacts have been reported worldwide. Self-sustaining populations of Rainbow trout are not described in Spain, but it is still present in streams, especially in areas close to fish farms from where individuals frequently escape. These escapes can have severe negative impacts in local fish fauna, mainly in small streams with small populations. One of their potential threats is the competition with other salmonid species for space and food. The present study is focused on Nalon River, in Asturias, northern Spain, where some rainbow trout farms are located. If escapes occur, Rainbow trout could be affecting local fishes as brown trout (Salmo trutta L. 1758), an appreciated species in the region. Through environmental DNA samples (genetic material obtained directly from environmental samples as soil, sediment or water), Oncorhynchus mykiss DNA has been detected and quantified along Nalon River, associated to reservoirs and also in different parts of running waters. Its occurrence far from rainbow trout farms suggests there is a self-sustained population in Nalon River basin.
COMMUNICATION: Using environmental DNA (eDNA) to record the distribution of the invasive red swamp crayfish (Procambarus clarkii) in freshwater ponds

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Abstract:

Environmental DNA (eDNA) is a powerful method for assessing the presence and the distribution of both native and invasive species. By sampling the environment, we can detect species without physical capture or visual confirmation. This tool is extremely effective for the early detection of aquatic invasive species in order to monitor and prevent their spread. We used the eDNA method in order to detect several invasive crayfishes Procambarus clarkii, Orconectes limosus and Pacifastacus leniusculus present or likely to invade the fishponds of the Natural Regional Park of Brenne. This park, covering 166 000 hectares with more than 2000 lakes and fishponds, is one of the most important wetland in France and listed as an International RAMSAR wetland zone since 1991. Invasive crayfish species, especially recently introduced are drastically reducing the biodiversity of different sites. This is why there is an urgent need to better understand the current situation of the invasion that comprises information about what species are being impacted by it as well as how the structure of the habitats (i.e. the fishponds) could be affected. Monitoring invasive species in the Park using eDNA will improve their management. We designed specific primers targeting short portion of the COI gene for each crayfish species, and set up an experimental aquarium approach to confirm the specificity of the primers, the sampling protocol, and the persistence of crayfish eDNA. We confirmed in silico and in vivo with DNA from invasive crayfish species (Procambarus clarkii, Orconectes limosus and Pacifastacus leniusculus) and native crayfish species (Astacus astacus, Astacus leptodactylus and Austropotamobius pallipes) the specificity of our primers. The mesocosm experiment highlighted that crayfish eDNA can be detected in aquatic ecosystem after one day of presence. However, eDNA persistence varied after the removal of individuals. Detection results during mesocosm experiment were obtained by performing qPCR with SybrGreen and Taqman assay. Same results were obtained with both assays. In a second step, we analysed samples taken in the Natural Regional Park of Brenne in 2013 and 2015. The overall detection was respectively
70% in *P. clarkii* and 66% in *O. limosus*. However, there were no statistical differences between the detection rate of these two species. We were also able to detect *Pacifastacus leniusculus* eDNA in a pond in the south of the Natural Regional Park of Brenne. The field experiment has proven the reliability of the eDNA detection method. Field experiments showed that qPCR using SybrGreen protocol with the same primers gave better reliable results that with TaqMan protocol. After optimization of the eDNA detection in water samples, we conclude that sampling must be made during the main period of activity of crayfish, i.e. in summer. This method is a powerful tool for establishing the presence or absence of invasive crayfish species in the numerous fishponds in the National Regional Park of Brenne and will allow to develop further a sustainable monitoring system based on the knowledge of how one established species spread and of where a new introduced exotic species is early detected. The environmental DNA method could even show better results by using new detection method such as the digital PCR or involving volunteers by setting up citizens’ programs.
COMMUNICATION: Implementing genetic tools for ballast water monitoring: the trial of RNA metabarcoding to detect viable organisms

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Abstract:

Ballast water is one of the major vectors of Non Indigenous species (NIS) introduction in marine environments. To prevent the risk of biological invasion, the Ballast Water Convention establishes, among other regulations, limits on the amount of viable organisms a ship shall discharge at their arrival to port. Currently, compliance of this regulation is tested by visual identification, epifluorescent microscopy or culture based methods, depending on the size class of the target organisms. Yet, these approaches are expensive, laborious and do not provide data on species composition. Alternatively, genetic based approaches may provide cost-effective and accurate biodiversity assessments and hold great potential for early detection of NIS in ballast water. Here, we provide a critical evaluation of the pros and cons of genetic tools for ballast water monitoring and management, focusing on the integration of DNA and RNA metabarcoding in the Ballast Water Convention. Contrary to DNA, which can be detected after cell death, RNA is rapidly degraded, meaning that this molecule could be used to identify living organisms. In order to test the validity of RNA as a proxy for cell activity, we designed an experiment based on samples of known proportions of live/dead zooplankton individuals and compared the species composition obtained from DNA and RNA metabarcoding. As a result, the potential of appealing genetic tools to provide required data for the Ballast Water Convention is proposed and the integration of these tools in biological invasion surveillance is discussed.
COMMUNICATION: An easy and fast method to detect the five commonest invasive fish species in Europe from environmental DNA

AUTHORS: Laura Clusa

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Abstract:

Biological invasions are a global threat to biodiversity, and thus for aquatic resources. The five species described here have been introduced into streams worldwide by different ways. Micropterus salmoides and Ameiurus sp mainly for recreational fishing; Gambusia holbrooki and Gambusia affinis for mosquito control with the intention of stopping the development of some infectious diseases, like malaria; Lepomis gibbosus as an ornamental species; Pseudorasbora parva was acquired as a contaminant of fish consignments. The effects of these invasive species are numerous. They compete with native fauna for resources in invaded habitats. They prey upon small native fauna, such as fishes and amphibians, sometimes resulting in the decline or extinction of such species. They are also known host of many parasites, like the rosette agent. All these five species occur in all Europe. Pseudorasbora parva is native to eastern Asia and the other four are native to North America. From EASIN, IUCN and DAISIE databases, these species are the most cited as invasive in all European countries. Here it is described an easy method to detect the different species directly from water samples. Specific primers for each species were designed within the 16S rDNA region. The panel of specific primers was validated from tissue as well as from water samples from aquariums where there have been the invasive species. With this tool and a simple PCR protocol followed by visualization in agarose gel or capillary electrophoresis it is possible to detect these species in environmental DNA. This powerful and economical tool will be useful to control the European invasions of these species.
COMMUNICATION: Metabarcoding and post-sampling strategies to discover exotic species: a case study in Asturias’s estuaries

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Abstract:

Estuaries are among the most productive habitats in Earth, producing more organic matter than other areas comparables in size like forests, meadows or agricultural lands. In addition, estuaries show high, and precious, biodiversity levels. These areas are among the most affected ecosystems by human activities. In this study environmental DNA (eDNA) and Metabarcoding analyses in the two most important estuaries in Asturias in terms of food production (Ría del Eo and Ría de Villaviciosa, Asturias, Northern Spain) were carried out in order to assess the usefulness of these novel tools to unravel changes in biodiversity levels and/or possible introductions of alien species as a consequence of anthropogenic activities in so valued ecosystems. The eDNA from Asturian estuaries water samples was obtained using the Power Water MOBIO DNA extraction kit. PCRs products, obtained using COI gene primers from Gellers et al. (2013) and adapters, were pyrosequenced using 454 (Roche). Quality controls of raw data and blasts against local COI gene database (evalue 0.001 and 90% of identity) were conducted for OTUs detections. A strategy of Post-NGS samplings was conducted for confirming NGS results. Significant changes in mollusks and crustaceans diversity indexes were detected in human-influenced areas, like factories, ports and harvesting zones. Besides this, the presence of alien genera (Crepidula, Lymnaea, Macrobrachium) that could be parasitaring cultured species in these estuaries were also detected. Post-NGS samplings and classical barcoding genetic analyses confirmed the presence of Crepidula fornicata in Asturian estuaries. Though metabarcoding has still unsolved problems such as the lack of universal primer pairs (Barcodes) or the availability of reference sequences for all the species in databases, it already represents a powerful tool that can help in the monitoring and management of these relevant ecosystems.
Analitical tools Genetic methodologies

Moran P. Cytogenetic analysis on the invasive mussel Perna perna and Brachidontes sp

Gómez Agenjo M. Barcoding as a tool for early detection of hitchhiking invasive species on floating marine debris in the Cantabrian coast.

Montes M. Molecular tools applied for exotic seaweed identification in Cantabrian coasts, Bay of Biscay.

Muñoz-Colmenero M. Detection of the invader Ficopomatus enigmaticus directly in water before being seen: a new molecular marker

Skukan R. Species demarcation in green algae genera Codium using DNA-barcoding

Ardura A. PCR-based assay for Mya arenaria detection from marine environmental samples and tracking its invasion in coastal ecosystems

G. Valdecasas A. Colonization or facilitated invasion? The molecular answer
COMMUNICATION: Cytogenetic analysis on the invasive mussel Perna perna and Brachidontes sp


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Abstract:

The mussels of the genus Perna, in addition to their great commercial importance, are well known for their ability to invade new habitats. These species have been repeatedly introduced in, amongst other regions, the Atlantic coast of America, from Brazil to Florida, provoking serious damages to both artificial structures and local ecosystems such as mangroves and seagrass. Among the native benthic species displaced by Perna sp. are some mussels of the genus Brachidontes. This genus is characterized by a great genetic variability that includes the presence of some cases of cryptic speciation. In the present work a comparative cytogenetic study of introduced P. perna and the native Brachidontes exustus, B. puniceus and B. rodriguezi was undertaken. Chromosome spreads were obtained from gonads and gills after hypotonic treatment and fixation with ethanol/acetic acid. The presence of GC rich and poor regions was analyzed by differential staining with CMA/DAPI/PI and the distribution of heterochromatic regions were evaluated by C banding. rRNA and histone gene clusters were located by fluorescence in situ hybridization using a fragment of the 28S rDNA, the entire 5S rDNA unit and the whole histone H3 gene as probes. Our results showed interspecific differences among both the number and the morphology of chromosomes and in the distribution of the gene clusters analyzed. The results, supplemented with phylogenetic analysis on mitochondrial sequences (16S rDNA and COI), were compared with previously available data in other mussels in order to understand the evolutionary patterns within the family Mytilidae.
COMMUNICATION: Barcoding as a tool for early detection of hitchhiking invasive species on floating marine debris in the Cantabrian coast

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Abstract:

Million tons of debris are spilled every year into the ocean affecting marine communities, driving them to change their habitat or even to the extinction. Plastic items, such as bottles, occupy the first position on the marine litter list, whereas fishing gear are the largest artifacts in the water column. This floating devices are additional hard surfaces that have the ability to provide a new habitat for several organisms. Most common ones are gooseneck barnacles, mussels, bryozoans and polychaete worms. This process helps species’ spread and proliferate in non-native areas, putting in hazard the ecosystem dynamics and the population decline of endemic individuals. Furthermore, climatic change intensifies habitat disruption which enhances invasive species establishment. Consequences of species new distribution can lead, not only to economic losses, but also to significant biodiversity changes. Hence, it is not surprising that biological invasions are becoming one of the major concerns of our time.

The principal aim of this study was to create social awareness about the relationship between marine debris and the enhancement of invasive species spreading. Thereby, owing to that there is no enough data from the Cantabrian coast, an exhaustive sampling process was performed along 22 Asturian beaches. Bottles and fishing gear found on the shore with attached organisms, were collected and the number of bottles and rope pieces was recorded per beach. Molecular techniques were used to identify the specimens to species level. Concepts of “prevalence” and “mean intensity” were applied in this study in order to calculate the proportion of NIS colonized items and the mean number of non-indigenous individuals carried per NIS colonized item. Results were compared with those obtained from the statistical analysis approach.
A total number of 777 bottles were counted, 8 of them carrying biota, and approximately 1000 rope pieces, 19 of them with attached fauna, were noted down. From the 17 identified species, 2 were cosmopolitan, 7 native and 8 not indigenous, including 3 reportedly invasive species: barnacles from Australia, *Austrominius modestus*; Pacific oysters *Crassostrea gigas* and the barnacle *Amphibalanus amphitrite*. Prevalence and mean intensity of both type of items showed similar values, which agreed with statistics, allowing to conclude that, almost in this study, there were no significant differences among bottles and ropes from the point of view of non-indigenous adhered biota. Finally, although NIS traceability could not be estimated here, molecular techniques would not be able to correctly identify one individual as native or NIS.

Summing up, due to the results obtained in this study, it can be concluded that current online databases should be updated with new reference sequences in order to improve the reliability of barcoding techniques.

It is a reality that marine debris are potential vectors of non-indigenous species dispersal, contributing to reinforce the biological invasions issue. Therefore, there is a need to improve the current waste management measures, otherwise, most part of land produced debris will end up in the ocean, enhancing the occurrence of alien species.
COMMUNICATION: Molecular tools applied for exotic seaweeds identification in Cantabrian coasts, Bay of Biscay

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Abstract:
The introduction of exotic species is considered the third biggest threat to global biodiversity. Anthropogenic activities (e.g.: shipping and aquaculture) are the main entry point for exotic species introductions into marine ecosystems. In order to enforce the security of marine ecosystems, controls that allow a rapid and correct identification of introduced species, especially the ones with imminent invasive capabilities, are to be performed. Species identification via morphological analysis is not precise and efficient enough to maintain this control, especially in the case of cryptic species introductions. Molecular tools turn out to be a necessary requirement for correct exotic species evaluation. The best tool for species identification is DNA barcoding. On the shores of the Cantabrian Sea (North of Spain) there are no routine examinations of seaweeds combining molecular and morphological methods.

Samples all around Cantabrian coast were collected and old FCO herbarium samples, mainly focused in red algae (which include several seaweed species complexes with many cryptic species) in order to evaluate the real status of red algae introductions as well as the capability of barcoding as a better tool for the identification of cryptic species, using the rbcL and COI genes. These results confirm the presence of the exotic seaweeds, even from previously identified samples from the herbarium, hinting to overlooked cryptic introductions, and some of these being first introductions ever to be reported in North European coasts (Grateloupia imbricata, Pachymeniopsis gargiuli, Polysiphonia scopulorum). This study demonstrates the success in combining morphological and genetic methods for early detection of exotic algae. However, DNA barcoding with COI gene is less effective compared rbcL gene as COI Databases are still incomplete and imprecise for a wide range of seaweed species-level identifications.
COMMUNICATION: Detection of the invader Ficopomatus enigmaticus directly in water before being seen: a new molecular marker

AUTHORS: Marta Muñoz-Colmenero (1), Alba Ardura (2), Laura Clusa (1), Laura Miralles (1), Fiona Gower (3), Anastasija Zaiko (3), Eva Garcia-Vazquez (3)

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Abstract:

The marine polychaete *Ficopomatus enigmaticus* has been defined as a problematic invader which distribution is increasingly expanding worldwide. This species find a good opportunity to establish itself in vulnerable habitats, as those modified by the humans. One example of that are the port areas, where there are high anthropic contamination and artificial structures that can be used as new niches. In these areas, *F. enigmaticus* causes damage to structures and sewage systems because it is an active reef builder. In addition, its bioconstructions cause changes in the habitat and its dynamic. It is because is especially important to detect the species before it is able to settle down. In our study we have designed a PCR-based species-specific molecular marker within the cytochrome oxidase subunit I gene (COI). The new marker was validated in vitro and with real samples: of tissue from reference samples of the wormtube, and water samples obtained in Bay of Biscay ports (north of Spain). The detection by the new marker was successful and the wormtube was detected in Villaviciosa port before settlement, where it was absent one year before. The individuals found were identified visually during the sampling, and then taxonomically, and by Barcoding molecular tool, verifying the species. In view of the good results and the efficiency of the new and cheap molecular marker, this can be used in the routine monitoring of coastal areas in order to prevent the invasions through the early detection.
COMMUNICATION: Species demarcation in green algae genera Codium using DNA-barcoding

AUTHORS: Roberta Skukan $^{1,2,3}$, Jose M. Rico$^2$, Marcos Montes $^{1,2}$, Fidel Delgado$^3$, Ignacio Albert$^3$, María Álvarez$^3$, Yaisel J. Borrell$^1$

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Abstract:

*Codium fragile* ssp. *fragile* (green sea finger) is a notorious invader in the coastal rocky shores of the marine environment and listed as one of the 100 worst invading species in Europe. As some of taxa in the genus *Codium* are cryptic, the necessity for molecular tools utilization is inevitable in order to avoid unserviceable risk assessments and false assumptions about ecological consequences of invasive species. We carry out DNA barcoding to *Codium* specimens from two sampling location in a west (cold-temperate) and east (warm-temperate) points of Asturias (Spain) using plastids elongation factor TU (tufA) and RuBisCO gene. Both of genetic markers revealed two distinct species in two phylogenetic groups, one confirming the presence of invasive *Codium fragile* ssp. *fragile* and other that did not match any of the sequences in the *GenBank* database. The thoroughgoing molecular analysis will be complement with morphological characteristics of invasive *Codium fragile* ssp. *fragile* along the coast of Cantabrian Sea and Atlantic coast of Europe to reveal species diversity, spatial and temporal distribution in the era of ongoing climate change.
COMMUNICATION: PCR-based assay for Mya arenaria detection from marine environmental samples and tracking its invasion in coastal ecosystems

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Abstract:

Invasive species is one of the most important factors implicated in the native biodiversity loss. Their early detection is indispensable to control and management, because after their incursion and establishment is too difficult their eradication. The Mediterranean Sea is one of the world’s hotspot of biological invasions but with a very big deficiency of monitoring programs. In this work, our study is focused on the soft shell clam Mya arenaria, considered among 100 Mediterranean Worst Invasive Species. We have designed a species-specific marker in silico that was further tested on environmental samples. The sensitivity and specificity of the new marker was validated in vitro on samples containing different concentrations of M. arenaria DNA and mixtures of DNA extracted from other phylogenetically close species. The new marker allows amplifying small fragments of 280 pb and detecting at least 0.00103ng/µl of M. arenaria DNA. Besides, no cross-amplification was detected. We suggest application of the newly designed marker as an easy method for target species detection from environmental samples, that can be also adopted for the citizen science programmes as requires minimal technical skills and reasonable amount of expenditures.
COMMUNICATION: Colonization or facilitated invasion? The molecular answer

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Abstract:

Field biological dynamics often exceeds our limited models and scenarios. A particular case is the colonization–invasion dichotomy presented by the invasive alien hydrophyte Ludwigia grandiflora, the widely distributed mosquito Culex pipiens and the water mites Arrenurus novus. The concerted evolution of these three actors are summarized as follows: the plant Ludwigia invades ditches, channels and shallow rivers and at the same time promotes the spread of Culex mosquitos to previously vacant areas. Coupled with the mosquito, of which it is an external parasite at the larval stage, the water mite Arrenurus colonizes new areas in its usual way. These organisms are important actors in the medical and veterinary field, as the plant plays an important role in the uncontrolled proliferation of mosquito populations, the mosquito species is a potential vector of organisms that cause diseases in humans and animals, and mites could act as biological control agents. Knowing the species identity of all the actors is decisive is human actions are to be taken to stop the spreading of invasive taxa. The invasive plant and the mosquito are well characterized and known in all their life stages. The water mite has been diagnosable only in the adult stage. “In this work we show how molecular methods have helped to identify all the life cycle stages of the water mite, and complete the characterization of the main actors of this play, as well as we emphasized how important it is this kind of identifications.
Educational and citizen-science approaches

Rayón F. *Marine debris, a socioenvironmental approach for new solutions.*

Arboleya E. *The Game of The Sea: a multidisciplinary game to learn about seas and oceans while playing*

Garcia-Vazquez E. *Barcoding and citizen science for monitoring changes in fish parasites: insights for marine fish management and conservation in the Bay of Biscay*

Pevida Llamazares D. *“They came to stay”. How to identify invasive species. A PBL Project*

Skukan R. *'Pokemon Algae' a game based on citizen science to study marine invasive algae*

Miralles L. *Citizen Landscape perception and marine conservation in the Asturian coast (North Spain)*

Miralles L. *Citizens for the control of biological invasions: a game-based training*

Arboleya E. *Searching IAS-birds around hometown skies*

Dopico E. *Citizen Science: active participation of citizens in science research would enhance science literacy?*
COMMUNICATION: Marine debris, a socioenvironmental approach for new solutions

AUTHORS: Fernando Rayón Viña

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Abstract:

Marine debris (especially plastic litter) has become a main problem to the coastal environments all over the world. The increase of production and use of synthetic materials instead of natural and easy-degradable ones has rose the litter that can end up on the seas and coasts. Marine debris favours the introduction of invasive species by two ways: degrading the potential recipient environments and acting as vectors of hitch-hiking species. An integrated coastal zone management is a better approach because it takes into account all the stakeholders. The perception of local populations about the problematic of marine litter as carrier of invasive species has to be known for development and implementation of measures acceptable by beachgoers and locals equally. The state of marine litter in the coast of Asturias (North Spain) is largely unknown. A study of the marine debris and social awareness about this problem on the region was performed. Marine debris was sampled and classified from 21 beaches along the coast of Asturias beaches to know the quantity and type of materials polluting the zone. Questionnaires (N=214) were performed in 9 beaches to understand the local social perception of this litter and its effect on the environment. The data showed that all beaches had some level of littering but the quantity and materials were different. The questionnaire revealed that marine litter was commonly perceived as a problem for the beaches. However, some differences in the perception of its harmful effect were detected between zones and types of beach. The relationship between litter and invasive species was rarely known by the general population. This knowledge will allow to design sound awareness campaigns to increase citizen’s knowledge about coastal pollution, to diminish litter generation from its source, and hopefully to reduce littering, which is crucial to rid this problem away.

Marine debris (specially plastic litter) has become a main problem to the coastal environments all over the world. The increase of production and use of synthetic materials instead of natural and easy degradable ones has increased the litter generation that can end up on the seas and coasts, affecting negatively on these ecosystems. Marine debris; along with other environmental and human impacts such as animals and plants death or intoxication, niches and landscape degradation, or tourism and fishing affection, favors the introduction of invasive species by two ways:
degrading the potential receptor environments, easing the introduction of alien species; and acting as vectors of hitch-hiking species, allowing potential alien species reach new regions. An integrated coastal zone management aims to be a better approach than the classical measures. For that, there are two issues to consider: baseline knowledge of the state of the debris in the beaches of the zone to know the problem magnitude; and population appreciation about this problematic has to be known, granting implementation of measures acceptable by beachgoers and locals equally.

Due to the lack of information about the state of the Cantabrian sea and the coast of Asturias (North Spain), a study of the marine debris and social awareness on the region was performed by sampling marine debris in 21 beaches along the coast of Asturias and performing questionnaires in 9 of these 21 beaches in order to know the quantity and materials of the litter and the social perception of this litter and its effect on the environment. The data showed that all beaches had, in general, a low level of littering with the exception of the beaches of Navia, Bayas and Xagó, and the quantity and composition were quite different between beaches and zones. Besides, the questionnaire results showed a general good knowledge about the state of marine debris and the beaches and the negative effects and risks associated with plastic pollutants, but their knowledge and awareness about invasive species is poor or vague, and also the relationship between marine debris and invasive species. Also, all this knowledge will allow to set better awareness campaigns to increase the citizens knowledge, diminishing the litter generation on its source and also reducing its actual quantity, which is crucial to rid this problem away.
COMMUNICATION: *The Game of The Sea: a multidisciplinary game to learn about seas and oceans while playing*

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**Abstract:**

Seas and oceans are unknown by most part of society although they cover the 71% of the Earth. Sea, ocean and coastal ecosystems conservation should be incorporated in educational curricula and also in public awareness. New educational tools such as games can help to change that, for instance by showing both amazing secrets of marine life and the risks of biological invasions while entertaining.

‘The Game of The Sea’ could be considered a kind of these mentioned tools. It is a multidisciplinary game. It involves different educational areas such as Biology, Math, Physic or even History and Literature, and different sort of activities like science experiments, stories’ creation or popular games. At the same time, it is a multiage game and it encompass people in a huge range of age, from children to adults, but it is more focused in school and high school students. In this fun way citizens will increase their knowledge about seas and oceans while playing.

On the one hand, the basic materials for playing the game are very easy to find or to make, as it is preferred. They are some pieces in different colors, no more than five, because each piece matches to a team. Each team can be formed from one to six participants, approximately. In this particular case the pieces are seashells painted in blue, yellow, pink, red and green. Another basic material is the board of the game, this has been designed with sixty eight squares in four different colors. Those colors are the same as the four set of question cards to be employed along the game, another material that is required. Each color matches to a specific educational are. Thus, blue cards are about biological invasions and their risks and the main characteristics of seas, oceans and coastal ecosystems. Green cards consist of questions about marine life and scientific experiments. Cards in red join to Literature, History and similar subjects. Finally, cards in yellow contain popular games. Moreover, dice and papers for stickers, where there is a wave formed by empty gaps, are necessary as well.

On the other hand the rules of the game are very simple and similar whichever board-card game. A wide number of participants can be able to play at same time. However, it is advisable no more than thirty. Otherwise, each team will choose a piece in the color it prefer, the dice will be rolled, the squares will be counted and finally the team will take a card in the same color as the square where its piece will be. After that, the team will work together to give the correct answer, create a story, guess what marine things are in a special box, think about how and why a coin on a little stick could fall into a water bottle, or whichever thing they would have to do to score. The more
correct answers the more stickers the team will get to complete the empty gaps from
the wave in the paper for stickers. Thanks to its simply methodology this game could
be available to teach other topics different from this, so that children could reach other
significant learnings too. It is necessary to point out that there is no limited time to play.
It means, people can play until they get bored, when they reach the last square of the
board game or even when they complete the wave from their paper for stickers.
Eventually, the game was played by seventy-eight students from first grade of
compulsory secondary education (aged 12-13). About fifteen participants were playing
each time. Small groups allowed researches to take an efficient measurement of the
improvement of students' knowledge about seas, oceans and coastal ecosystems.
To accomplish this task and to confirm children learnings, a test was designed. It
consisted of three pair of questions about ocean information that appear along the
game. In particular, each couple was regarding to the themes of cards in colors red,
blue and green (question cards in yellow did not contain information related to the
main topic of the game). The test consisted of six multiple-questions and each one
includes four options, based on Likert scale. Children answer it before (pre- test) and
after (post- test) the game, during no more than ten minutes. All the answers were
collected and analyzed with statistics support.
As a result citizens’ knowledge about seas and oceans shows a high increase after
playing. For the two questions about oceans and biological invasions the knowledge
increase were 88%. Since before playing both questions were answered correctly by
less than 4% of participants however, after the game more than 92% of children
choose de correct answer in these two questions. The second pair of questions
regarding to science and marine life had also good knowledge improvement but with
some differences. Around 9% of students choose the correct option before playing
‘The game of the sea’ but, after that, more than 70% did. However, the second
question was successfully answered by more than 91% of students before and after
playing. It was due to the contents of this question were already included in the
educational curricula. It happened the same with the last two questions which were
about History, Literature and similar. Question number one was correctly answered by
more than 38% of children before and by more than 65% after playing. The scores
increased in the second question, so more than 92% of students choose the correct
answer before the game and the 100% did after it.
Therefore, apart from the motivation to learn, using games as a learning tool is a good
way to approach seas and oceans to society, in this particular case. Moreover, it can
help in citizens’ awareness of how important oceans are and the value to preserve
them.
COMMUNICATION: Barcoding and citizen science for monitoring changes in fish parasites: insights for marine fish management and conservation in the Bay of Biscay

AUTHORS: Garcia-Vazquez E, Ardura A, Borrell YJ, Dopico E, Duarte C, Gea B, Roca A, Miralles L

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Abstract:

Parasites are natural components of aquatic communities; however, if their communities change or increase, they may endanger host populations. Early alert of parasite changes in particular fishing zones may be used to readdress local fisheries management to help affected fish populations and increase sustainability. Identification of parasites requires specialized knowledge and can be a burden for already endangered artisanal fisheries. The costs of systematic monitoring of fish parasites can be relieved if citizens collaborate in sampling and easy DNA Barcoding protocols, following a strategy of citizen science. In this study we have assayed a pilot program to test the viability of such strategy in the central south Bay of Biscay. Volunteers provided parasite samples found from fish products they consume regularly. The parasite species was determined from DNA based on a simple PCR protocol for amplifying the COX2 gene. The proportion of valid samples provided by citizens was circa 90%. Changes in parasite anisakis communities of different fish species were determined, and management recommendations suggested from these results.
COMMUNICATION: “They came to stay”. How to identify invasive species. A PBL Project

AUTHORS: Dolores Pevida Llamazares and Dolores Ocejo Alvarez

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Abstract:

Spanish Law 42/2007 of 13 December on Natural Heritage and Biodiversity defines an invasive alien species (IAS) as “that one that is introduced or established in an ecosystem or a natural or semi natural habitat and is an agent of change and threat to native biological diversity, either by their invasive behavior, or the risk of genetic contamination”. The Royal Decree 630/2013, of 2 August, regulates the Spanish Invasive Alien Species Catalogue, it strengthens protection for the natural environment and biodiversity in Spain. Besides, codes of conduct to prevent trade in alien invasive species propose a set of rules to help us to develop, raise awareness and adopt voluntary measures to control invasive alien species.

With these references, we intend to develop an educational dynamics based on project-based learning (PBL) which revolves around biodiversity and ecosystems, from the context of Natural Sciences in Compulsory Secondary Education (students aged 12-16). The aim is to identify invasive species in our local habitat. Because of the age of the students and educational intentions aimed at changing social attitudes, pets are also involved in the project. Classroom and outdoor activities have the challenge to detect alien and invasive species. Through knowledge the environment, the analysis and description of native species and alien and with ICT support, teachers and students design a map geotagging invasive/alien species detected through apps, by using QR codes to storing and sharing information. This educational activity concludes with students spreading their work through infographics, QR documents and an explanatory video uploaded in a web video platform.

The methodology used is supported in the work in cooperative teams, using cooperative learning techniques and other active methodologies which make our students the principal actor of their learning process, favouring their responsibility in it. When we put the students in charge of certain activities and assign them responsibilities in the process we always, or usually, achieve a great implication, which results in academic, personal, social or community success.

The internal management and work of the teams facilitates the development of a research project structured in the following:
1. Awareness. - through cooperative techniques (1-2-4), bonding activities, and images and videos that can catch the attention of students, surprise and to detect previous ideas.

2. The research work. – It is started with the concepts of biome and ecosystem.

The structure of the class into cooperative teams allows us to organize the work by the “Jigsaw” technique. We form base working teams and expert teams who will investigate and work with tasks about the different biomes, their geographical location and the description of them in a world map. From there, we work with the balance existing in nature between species, providing stability, and discover animal species and their environment conditions, vegetal species and their evolutionary adaptations. This balanced vision of our environment will allow us for discovering non-autochthonous, exotic, invasive vegetal and animal species, which break the nature balance. Therefore, our students will learn to identify, locate and place invasive species from an outline elaborated with seven characteristics that help us in the identification.

3. This learning process can not be developed exclusively in the classroom. Therefore, we plan fieldwork, with a visit to the Atlantic Botanical Garden in Xixón, where we will be able to see and study more easily a large number of invasive species.

For this fieldwork to settle in the lives of our students we propose a global activity that will make them put in place all the knowledge acquired. Through field itineraries (previously established by teachers) that will take place around the Institute, different teams will identify the species found, take pictures of them and locate them by using the GeoLocator in their mobile phone.

4. Final products and spreading. This way they will get enough information to finish the job in the classroom. This work will be an idea-sharing session in which they will present a map of the area worked with localized species and QR codes that contain the information that each team collected, depending on the species. This sharing will be recorded as a graphic document of the project, which after a little editing work will serve to spread the project for peer evaluation.

5. The evaluation will be complemented by evaluation rubrics to be set in the interactions that occur in the equipment throughout the process (social skills, methodology, group process), in the acquired knowledge, in the final elaborated products and in the process itself.

6. We do not want to finish this project without taking a scope, a social projection that extends beyond the walls of the classroom. Now that we are...
already experts, we will elaborate a joint document to appeal to the awareness and social awareness and thus promote changes in attitudes and ways of behaviour. We offer our students five areas of intervention that will result in a global document.

The objective of this project is to awake the curiosity and motivation of the students, by changing the usual strategies of the classroom for an original project. Simultaneously the curricular contents related to biomes, ecosystems and invading species should serve as a bridge to generate increase awareness about the risks and challenges that the environment suffer. This should allow us to generate proposals concerning our closer educative environment.
COMMUNICATION: 'Pokemon Algae' a game based on citizen science to study marine invasive algae

AUTHORS: Roberta Skukan 1,2,3, Yaisel J. Borrell1, Jose Manuel Rico2, Laura Miralles1.

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Abstract:

Frequently, citizens (commonly know as citizen scientist) are the first encountering non-indigenous species however, this valuable data remain unnoted because commonly they do not have the required taxonomic expertise. From a global perspective, volunteers and citizen scientists are actively involved in solving this obstacle. Thus, the researchers from the University of Oviedo developed a marine invasive algae game based on currently famous Pokemon cards to attract the attention of citizens (mainly primary and secondary school children) and teach them about basic environmental issues caused by exotic species. The aims of the game were i) to teach kids in distinguishing invasive algae and ii) to collect detached samples that can be found lying on the sand of surrounding beaches for scientific research. The game took place in three different cities of Asturias: Luarca, Salinas and Llanes (Cantabrian Sea, Spain). Cards were made containing the most important information about the marine invasive algae in the Cantabrian Sea: Codium fragile subsp. fragile, Sargassum muticum, Colpomenia sinuosa, Asparagopsis armata, Grateloupia imbricata, Polysiphonia schneideri and Undaria pinnatifida. Children were seeking for herbarium specimens of invasive algae hidden randomly in a demarcated area with the help of cards. After that, they keep the cards and during 15 days, they can look for any detached invasive algae on surrounding beaches. All the different algae they found were stored in ethanol and analyzed by scientists in order to improve the knowledge of invasive species of the region. The citizen engagement was surprisingly very high, reaching almost 90% in one location (Salinas). Moreover, samples were relevant for the research and the citizen sampling can be comparable with scientific sampling done by the researchers in the same and adjacent areas. Hence, 'Pokemon Algae' game can be a useful tool for citizen science activities and can be applied for enrollment of junior citizen scientists.
COMMUNICATION: *Citizen Landscape perception and marine conservation in the Asturian coast (North Spain)*

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**Abstract:**

Human perception of the environment is a keystone for any successful conservation program. However, marine ecosystems used to be one of the most unknown environments by citizens. In this study, 102 multiaged volunteer citizens participated in a survey of marine landscape perception and marine conservation. Also 2 international ports and 2 natural protected areas of Asturias (North Spain) were sampled and sessile invertebrates were identified with DNA-barcoding. The two international ports revealed more biodiversity as well as higher species richness than the protected areas, despite the non-indigenous species found in there. Citizens do not expect that and they rather natural protected areas more than anthropogenic ones when asked. However, after a talk with the scientific results, 59.7 % of major and 27.8 of non-major in marine science changed their unconscious perception of landscape. Moreover, 91.7% of non-major and 62.9% of major changed their choice of a place to focus conservation efforts after the talk. All these results highlighted the importance of scientific diffusion of knowledge for citizens for marine conservation. Finally, the genetic monitoring of the area pointed at the ports as possible biodiversity reservoirs.
COMMUNICATION: Citizens for the control of biological invasions: a game-based training

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Abstract:

Citizens are essential for safeguarding ecosystems and also for combating environmental problems such as biological invasions. Examples of informed citizens volunteer to eradicate nuisance alien species are in many countries. The training period is key for both recruitment and constancy in the environmental volunteering. However, what are the best methods and tools to engage citizens in marine conservation? In this real experience we have recruited volunteers from two different age groups for eradication of alien invasive pygmy mussels (Xenostrobus securis) from a north Iberian estuary in Spain. Their training was based on a simple visual game with native and invasive mussels. We have evaluated their knowledge gain and attitudinal changes with a pre- and post- test based on a questionnaire scored in Likert scale. Significant positive correlation was found between knowledge gain and attitudinal change, being both greater in high school students than in adult lifelong learners. Similar recruitment was achieved in the two groups of age. The results suggest high efficacy of game-based training for increasing knowledge and promoting environmental-conscious attitudes, especially in young age groups.
COMMUNICATION: Searching IAS-birds around hometown skies

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Abstract:

Contents related to environmental education in educational curricula contribute to the development of citizenship competencies to maintain a friendly relationship with the environment. The ecological life space needs all our attention to preserve in sustainability, so society should be aware of which and how invasive alien species (IAS) can affect it in different ways and must all be vigilant to detect and hold them.

People used to think birds could not take part in biological invasive processes. However, now Spain is suffering from several bird biological invasions in many of its regions. In particular, Kramer’s parrot (*Psittacula krameri*) or the monk parakeet (*Myiopsitta monachus*) are the most harmful for birdlife.

In addition, citizens are aware of this dramatic situation and are able to participate in different sort of activities which contribute to developing preventive measures against some bird species. In this context, the Spanish Non-Governmental Organization SEO/Birdlife (Sociedad Española de Ornitología, it means, the Ornithology Spanish Society) promoted a census for these bird species whose main source of information are citizens. Media tools such mobile phone applications, social networks and so on, were provided to them to register alien species.

The census was carried out along the whole of Spanish regions. The citizen participation, apart from bird experts, was so successfully that two reports were published with the huge amount of data collected and analyzed. Moreover, they created two maps, for each bird species mentioned before, to represent this information. To sum up, Seville is the Spanish region whose natural environment is the most harmful by Kramer’s parrot (more than 1,000 birds). However, Madrid and Barcelona situation is worst, so that monk parakeet population is bigger than 5,000.

Despite this kind of activities and environmental laws, there is plenty of actuations to do to protect and improve environmental status regarding to bird invasive species and natural bird species. For this purpose, it was designed an outdoor *activity* for students from first grade of compulsory secondary education in a rural context, Pola de Siero (Asturias-Spain) to recognize birds from students’ hometown.

Previously, it was done a survey to 73 students in classroom (aged 12-13). The 10 items questionnaire was designed to collect what knowledge they had about their environment, including birdlife, and some tools that could be useful to improve it on their own, like mobile phone applications, for instance. Finally, it was very important to know their preferences in out-class activities.
On the one hand, the outcomes of the survey showed very interesting information about children environmental perception, so that 80% of students claim there were birds in their nearest natural environment. However, only one child (of the 73 interviewed) was able to identify European robins in his zone. On the other hand, the results showed that mobile phones could be successful tools in this kind of activities, since 90% of children, or their families, have smartphone or tablet and usually. More than 80% use them in their spare time, and even more, they want to use them as well at school as another learning material. Apart from all that was said before, more than 90% of the students want to do outdoors activities related to environment. The high score of this item is directly related to the students’ demand for ages. They want to do more outdoor activities and more learnings in connection with their immediately environment.

All the information collected and analyzed, allowed researchers to design the next step that was to do field work. In a citizen science dynamic, researchers and several teachers from a wide range of educational areas, joined to their students and their families. Together did trekking in the incredible environmental surroundings of the high school, at which this educational community belonged.

The main goal of the activity was to detect and recognize birds from their council. Thanks to the information available in birding apps and birds guides, related to the main birds’ body and life characteristics and their different types of singing as well, it was possible to recognize hometown’s birds. Thus, participants could check out whether this was its natural habitat, it was a migratory bird or was colonizing a foreign space.

Outdoor activities like this help us to know the species of birds that inhabit our environment and contribute to developing preventive measures against bird species like the Kramer’s parrot or the monk parakeet, that have already been mentioned. Also, and not least, it contributes to knowledge about and protection of our ecosystem and improves citizen skills as protectors and bird experts.
COMMUNICATION: *Citizen Science: active participation of citizens in science research would enhance science literacy?*

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**Abstract:**

Citizen Science describes the participation of the public in general in the scientific studies. To develop citizens’ critical thinking and responsible and credible decision making toward complex environmental issues, scientific literacy for citizenship and citizen science has gained much attention in school science and public education. Environmental education has an important role in this process. By emphasizing the connection of scientific knowledge to their everyday experiences in the world, the goal of scientific literacy aims to develop responsive and responsible citizens, as well as decision makers and problem solvers for the society and environment. Citizens acting like scientists have stronger positive attitudes toward the environment. There are many examples of successful programs where citizens gathered crucial data about global climate change, biodiversity inventory and biological invasions, among others. These programs take advantage of citizen’s points of view not mediated by individual scientific background to provide intuitive solutions to complex problems. In the citizen science approach, it is critical that the design of a research project that includes citizen science should match the needs and interests of the participants with those of scientists. Citizens need to understand scientific messages, ongoing research, its implications on their lives, and action taking on socio-ecological issues. Without a shared common ground of interests and meanings of problem contexts, citizens do not find their participation meaningful or responsible in any contexts. Balancing the tension between scientific efforts and educational needs, citizen science connects science, education and citizenship, involving citizen participation in genuine scientific research.
Ongoing projects on strategies for early detection and management of invasive species

Consuegra S. *AQUAINVAD-ED Project*

Miralles L. *Seawatch Surfers: The creation of a citizen network to detect and to alert coastal environmental problems*

Díaz González T. *The project LIFE+ARCOS: In situ and ex-situ innovative combined techniques for coastal dune habitats restoration in scis of northern Spain. Plant biodiversity conservation in coastal arenales cantabrian*

Borrell YJ. *Prevention and control of biological invasions in the port of Gijón, Bay of Biscay (FUO-95-2016).*

Osendi García P. *Proyecto Cangrejo: elimination program of the red crab in Narceea River.*

Garcia-Vazquez E. *Multidisciplinary tools for alert and control of marine bioinvasions: genetic markers, eDNA, ICT and citizen science*
COMMUNICATION: AQUAINVAD-ED Project

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Abstract:

Recent research has shown that Aquatic Invasive Species (AIS) are on the rise due to the synergistic effects of climate change and habitat destruction. The impacts of AIS on biodiversity, human health, and loss of ecosystem services (food, tourism and water provision) have long been known, but their control and management has now become a worldwide priority. Successful management of AIS is challenging because it requires several steps in succession: (1) early detection, (2) identification of routes of introduction and pathways of dispersal, and (3) development of efficient control measures. Public awareness and stakeholder involvement is also critical for preventing new introductions and for mitigating the impact of existing ones. The main research goal of AQUAINVAD-ED is to exploit novel molecular advances combined with the power of crowd data sourcing (citizen science) to develop innovative methods of early detection, control and management of AIS. This will be achieved via a multi-disciplinary network of experts on invasion biology, ecology, marine and fresh water biotechnology, citizen science and environmental policy working in three different countries. The consortium has a strong inter-sectoral dimension and includes scientists and professionals from 3 universities, 1 technological institute, 2 government agencies, 1 NGO and 5 SMEs working in fundamental and applied aspects of AIS. AQUAINVAD-ED will catalyse research and commercial activity in the detection and management of aquatic invasive species, as well as in the implementation of codes of good practice for the European industry and Government agencies. This will be achieved by training the next generation of researchers on the principles of invasion biology, providing them with the tools and knowledge required to detect and quantify the ecological and socio-economic impacts of invasive species and the skills necessary to communicate science to the general public, across disciplines and sectorial boundaries.
COMMUNICATION: Seawatch Surfers: The creation of a citizen network to detect and to alert coastal environmental problems

AUTHORS: Laura Miralles 1, Carlos Meana 2

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Abstract:

Citizen awareness and participation are indispensable for any successful conservation program. The management of bioinvasions is more difficult and sometimes impossible in late than in early stages. Therefore, the collaboration of citizens for early detection of nuisance organisms is essential. However, not all citizens can provide useful information. In the case of coastal environments, surfers could be a good option since they used to go to the same surf spot several times per week or even per day. They know the beach they used to go by heart and they are one of the first citizens that can detect changes in the coast. For these reasons, to enroll surfers as environmental volunteers can provide a lot of valuable data. Different surf schools were contacted in the north coast of Spain (South Bay of Biscay) in order to create a network for early detection of coastal environmental problems such as invasive species. A multi-aged group of citizens were enrolled. All participants got one personal card that identifies them as ‘Seawatch Surfers’ and that contained the contact email for reporting possible invasive species. All age groups were highly participative during training activities such as workshops of invasive species identification. However, families (kids and their parents) reached a higher recruitment than kids or adults that went alone to the training activities. As an ongoing project, the network is still under construction however, 4 ‘Seawatch Surfers’ alerted of invasive oysters and algae in 2 different localities of Asturias (Salinas and Navia). These results suggested that ‘Seawatch Surfers’ can help in early detection of nuisance species and the citizen network can report a lot of valuable data.
COMMUNICATION: The proyect LIFE+ARCOS: in situ and ex situ innovative combined techniques for coastal dune habitats restoration in scis of northern Spain. Plant biodiversity conservation in coastal arenasales cantabrian

AUTHOR: Tomás Emilio Díaz González

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Abstract:

The Proyect “In situ and ex situ innovative combined techniques for coastal dune habitats restoration in SCIs of northern Spain. Plant Biodiversity Conservation in Coastal Arenales Cantabrian (LIFE+ARCOS). (LIFE13 NAT/ES/000883) focuses on the implementation of a series of measures to improve the conservation status of dune ecosystems along the Cantabrian coast. The main goal of this project is to ameliorate the conservation status of the threatened Cantabrian Coastal sand dunes by the restoration of 10 areas included in 10 selected Natura 2000 sites in order to achieve an optimum conservation status of these areas [of Asturias (Barayo, Verdicio and La Vega), Cantabria (Liencres, Somo and Berria) and País Vasco (La Arena, Laida, Santiago and Zarauz). Duration: Start: 01/07/14 End: 31/12/2018. Coordinating Beneficiary: Universidad de Oviedo and JBAG. Associated Beneficiaries: (1) Dirección General de Sostenibilidad de la Costa y del Mar (MAGRAMA) (2) Diputación foral de Guipuzkoa (3) Dirección General de Montes y Conservación de la Naturaleza del Gobierno de Cantabria, (4) Sociedad de Ciencias Aranzadi, (5) Ecología Litoral S.L. The Life+ARCOS objetives are aligned and focused on the conservation priorities proposed in "Priority Action Frame for the Natura 2000 in Spain". These objetives can be resumed as follows: 1) Contribute to the restoration, improvement and maintenance of the habitat types and species of Community interest presented in coastal sand dunes. 2) Contribute with the obtained results to the proposed plans that should be updated every six-years in order to maintain or restore the favourable status of conservation of habitats and species of interest associated to coastal dune ecosystems in the Atlantic Biogeographic unit (2nd and 3rd points of the General Priorities to the entire Natura 200 network in Spain (F.2. section), p. 30 of the MAC). 3) Contribute to the prevention, control and eradication exotic invasive species (16th point of the F.2 section in the MAC) 4) Promote the social participation in the conservation and restoration programs of habitats included in SCIs under the Natura 2000 network, improving the gobernance policies.
COMMUNICATION: *Prevention and control of biological invasions in the port of Gijon, Bay of Biscay (FUO-95-2016)*

**AUTHORS:** Yaisel J. Borrell1, Laura Miralles1, Marcos Montes1,2, Robertan Skukan1,2, Eva García-Vazquez1, Jose Manuel Rico2.

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**Abstract:**

Invasions of marine non-indigenous species (NIS) are today one of the greatest threats to biodiversity worldwide. Ports are main entry gates of exotic biota, principally through biofouling and ballast water. Since eradication is more difficult in late than in early invasion stages, new strategies are needed for effective prevention and early detection of nuisance organisms. The overall objective of this project is directly related to reinforce environmental safety policies in industrial seaports and it involves a genetic monitoring of exotic species in the Port of Gijon. This information is crucial for the development of a protocol for the prevention of unwanted introduction of alien species as a result of industrial activity seaports. Four different areas were sampled within Port of Gijon (Fig. 1). We sampled more than 40 different green, brown and red seaweeds and more than 200 marine invertebrates. We found from 5% to 17% of exotics species by kingdom of marine species. This work allows to identify and assess the risk of NIS introduction through a particular gateway (port) and timely detect a new incursion, in order to enable efficient response measures. Ports are the main entry of NIS in Europe and it’s just the time to include them in scientific and educational strategies to solve the problem of biological invasions.
COMMUNICATION: PROYECTO CANGREJO: Elimination program of the Red Crab in Narcea River

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Abstract:
The Crab Project was an experience developed by our association between 2009 and 2010 in the Narcea River (Principality of Asturias). The project was financed by the Fundación Biodiversidad, and had two principal aims: In a first time, the reduction of the red crab number in the Narcea River and the second time, and more importantly, to know the degree of the expansion in the basin of Narcea River and the implication in the fight against the invasive species. The project allowed the elimination of 42,855 red crabs in the two years and an important work or implication of the local anglers in this problem. The local anglers are our eyes in the river and they are a good tool in the location of invasive species. More information about the project in the next link: http://elbanzao-proyectocangrejo.blogspot.com.es/
COMMUNICATION: *Multidisciplinary tools for alert and control of marine bioinvasions: genetic markers, eDNA, ICT and citizen science*


**Affiliation:** University of Oviedo, Spanish Institute of Oceanography, University of Victoria, University of Klaipeda, Cawthron Institute

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**Abstract:**

The Spanish Royal Decree 630/2013 of August 2 2013 identifies invasive exotic species as one of the main causes of biodiversity losses, aggravated in vulnerable habitats such as islands and continental waters. In the project MINECO CGL2013-42415-R we have developed and validated new multidisciplinary methodologies that have a great potential of sensitivity and discrimination power: e-DNA and citizen science. Species-specific markers versus Metabarcoding from eDNA, and Internet-based tools versus active face-to-face search of volunteers strategies were assayed. The project focused on coastal aquatic invertebrate species catalogued in the RD 630/2013 that inhabit coastal and estuarine habitats of Asturias coast (Bay of Biscay). Specific markers PCR-amplified on DNA extracted from water samples were highly efficient for early detection of invasive species. On the other hand, volunteers recruited face-to-face and trained with innovative educational activities contributed successfully to controlling invasive species in a pilot study within the estuary of Aviles. The results are expectedly applicable at international level and could be employed for early detection and control of invasive marine and coastal species. The citizen science development will be useful for managing present and future biological invasions in the region.
Conclusion

ALERTOOLS 2016 was a great scientific, but also human, experience.

Gathering together scientists and citizens was a restorative idea that we really cannot wait to repeat.

Please, enjoy the reading of this E-Book and get informed, and even involved, with us at http://alertools.uniovi.es/

We really hope to meet you again, soon.

Prof. Yaisel J. Borrell and Prof. Eduardo Dopico

Editors.

To contact:

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