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Improvement of protocols for the early-detection of alien wood-boring beetles in Italian ports

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RIASSUNTO

I porti internazionali sono considerati i punti a alto rischio di introduzione di insetti esotici xilofagi. Le specie esotiche invasive sono in grado di causare, ogni anno, danni per miliardi di euro nel settore agricolo, ortofrutticolo e in quello forestale. Per questo motivo, la tempestiva intercettazione di insetti esotici diventa della massima importanza. L'obiettivo principale di questo studio è quello di migliorare e ottimizzare i protocolli di monitoraggio già esistenti per le intercettazioni di insetti esotici xilofagi. In particolare, si è voluto analizzare: a) come il grado di isolamento delle trappole all'interno dell'area portuale possa influenzare la ricchezza di specie e il numero di individui catturati dalle trappole stesse; b) se e come la ricchezza di specie e il numero di individui di insetti xilofagi cambi all'interno di uno stesso anno e tra anni diversi. Lo studio è stato condotto in cinque porti italiani che sono stati monitorati per tre anni (2012-2014): Genova (44°24'00"N 8°55'12"E), Ancona (43°37'39"N 13°30'09"E), Salerno (40°39'52"N 14°44'41"E), Ravenna (44°29'00"N 12°17'00"E) e Napoli (40°50'00"N 14°15'00"E). Tre trappole del modello multi-funnel sono state attivate con una miscela di sostanze generiche (a-pinene, etanolo, ipsdienolo, ipsenolo, metilbutenolo) e sono state posizionate all'interno di ogni porto a distanza di almeno 50 metri. Gli insetti appartenenti alle famiglie Cerambycidae, Buprestidae e Scolytidae catturati sono stati identificati a livello di specie. I risultati hanno evidenziato che: a) il numero di individui e di specie esotiche catturate sono risultati essere simili tra gli anni, dimostrando che la quantità e il tipo di merce importata è rimasta probabilmente inalterata; b) l'efficacia delle trappole all'interno dell'area portuale non è risultata essere influenzata dal grado di isolamento ma, più probabilmente, dalla vicinanza ad aree di stoccaggio di materiali e imballaggi legnosi; c) l'abbondanza di individui e la ricchezza di specie esotiche risultano variare casualmente all'interno di uno stesso anno, suggerendo come sia fondamentale che le trappole vengano mantenute attive durante tutto il periodo nel quale gli insetti possono risultare attivi. In conclusione, il presente studio conferma l'importanza dei programmi per l'intercettazione delle specie esotiche e sottolinea come ulteriori studi dovranno investigare in modo più approfondito il flusso dei materiali legnosi all'interno dell'area portuale per capire quali siano i punti migliori per il collocamento delle trappole.

ABSTRACT

International ports are considered as sites at high-risk for the introduction of alien wood-boring beetles. Invasive alien species cost billions of euros every year, the early detection has become of utmost importance. The main aim of this study is to improve and optimize the protocol already existing for alien wood-boring beetles interceptions. In particular we investigated: a) how the degree of isolation of the traps within the port area can affect alien and native wood-boring beetles richness and abundance; b) if and how the wood-boring beetles species richness and abundance change in time, both within the same year and among years. We selected five international Italian ports that were monitored for three years (2012-2014): Genova (44°24'00"N 8°55'12"E), Ancona (43°37'39"N 13°30'09"E), Salerno (40°39'52"N 14°44'41"E), Ravenna (44°29'00"N 12°17'00"E), and Napoli (40°50'00"N 14°15'00"E). Three multi-funnel traps baited with a multi-lure blend (α-pinene, ethanol, ipsdienol, ipsenol, methylbutenol), were set up in each port. We identified both alien and native Scolytinae, Cerambycidae and Buprestidae beetles. The results demonstrated that: a) the exotic species richness and abundance were similar among years, indicating that the type and quantity of imported goods did not clearly change in time; b) captures of exotic species are probably influenced more by the proximity of the traps to storage locations of woody materials than to their degree of isolation; c) the exotic species richness and abundance change randomly within years, indicating that traps installation period should cover the entire period when wood boring beetles can be active (April-October). In conclusion, this study confirmed the importance of such protocols for the early-detection of alien wood-boring beetles and underlined that a better comprehension of how woody material are stocked in ports is necessary to decide the optimal location of traps.

1. INTRODUCTION

1.1 Invasive species

Invasive alien species are animals, plants or other organisms introduced into places located out of their natural range of distribution, where they established and spread, generating several negative impacts on local ecosystems (IUCN 2011). This means that a species is defined as "invasive" or "alien" when it is introduced in an environment where it has never been previously present (IUCN 2000). The phenomenon of alien organisms introduction is considered, from different points of view, as a global threat. In the last years several studies on invasive species have been conducted and this issue has been addressed during several conferences held around the world. "Urge governments and donor agencies to increase funding to facilitate the development of prevention, management and monitoring programmes, essential research, and economic analysis on invasive alien plants" (Declaration of Méze 2005), this is what has emerged from one of the last conferences held in France about invasive alien species issue. Unfortunately, this is a normal and common phenomenon that takes place since centuries. Starting from the beginning of the Age of Discovery, man has voluntarily and involuntarily displaced organisms among continents (Mack et al. 2000) and this has resulted in the breakage of those physical barriers that in millions of years of evolution have diversified and made independent the biomes of the world (Holmes et al. 2009). It is difficult to determine when this process began, but surely we can say that, during the last 25 years, we have been witnessing a sharp increase in the number of alien species that has been introduced. The phenomenon of alien species introduction is first of all related to the globalization. The international trade of goods is the first cause of the introduction of invasive alien species as well as their spread is facilitated by increasing trade, travel and the transporting of goods, as these organisms may "hitchhike" on ships, containers, cars, soils, etc. An inventory of the phytosanitary interceptions in Europe on wood and wood products during the period 1995-2005 revealed that wood-boring beetles largely dominated the insects community associated with these pathway (Roques and Auger-Rozenberg 2006). This is therefore a global problem that requires international cooperation and action (IUCN 2011). Nevertheless the climate change might also have a huge impact on this phenomenon. The factors mentioned above have facilitated, in an integrated way of action, the spread and survival of exotic organisms in new environments resulting in an even higher rate of introduction of alien species (Hulme 2009). Whatever the cause, invasive organisms can in many cases inflict enormous environmental damage (Mack et al. 2000). The impacts of alien invasive species are immense, insidious, and usually irreversible. They are causing significant damage to ecological, economic and health levels. From an environmental point of view, they are able to

threaten the biological diversity through a reduction of genetic variability, change the trophic relationships among native organisms and, in the most serious cases, determine the extinction of native species and the alteration of habitats and ecosystems (Hulme 2007; Mooney and Cleland 2000) (Fig.1). From an economic point of view, it may cause serious damage to agriculture, horticulture and forest habitats (Pimentel et al. 2005; Vilà et al. 2009) (Fig.2). The greater or lesser aggressiveness of an introduced species in the new environment depends on many factors and the dynamics are often complex (e.g. the presence of vacant or unused niches, the absence of parasites and predators, limited local biodiversity, possible disturbances occurred before or during the invasion, etc.) (Mack et al. 2000).



Fig. 1: Forest damaged by wood-boring beetles
(source <http://www.oggi.it/blog-on-the-road/>)



Fig. 2: Female and larval galleries

As a matter of fact, alien species can compete with native species, act as pests or pathogens for cultivated or domesticated species, or even disseminate allergic or infectious agents. Animal invaders, as in this case of study, can cause extinction of vulnerable native species through different processes such as predation, competition and habitat alteration. Since the discovery of America, 10.000 alien species were approximately recorded in Europe, including taxonomic groups represented by plants and insects (DAISIE 2008). Among the latter, the largest group is represented by wood-boring beetles (mostly Scolytinae, Cerambycidae and Buprestidae) (Kirkendall & Faccoli 2010; Sauvard et al. 2010; Marini et al. 2011) which is considered as one of the most serious threats to forests worldwide (Brockerhoff et al. 2006a). These insects can be easily transported through international trade of wood products, such as logs, stumps and especially timber and wooden packaging or pallet (Fig.3) where they may evade the phytosanitary inspections and overcome the adverse weather conditions that occur during displacements (Brockerhoff et al. 2006b).



Fig. 3: Wood-packing material and pallets stacked in an Italian port

In addition, any preventive measure such as debarking, fumigation, irrigation, treatments with heat or chemicals, is not able to completely prevent the risk of new infestations of woody material (Skarpaas and Økland 2009). For these reasons, wood-boring insects are considered as the most successful group of invasive alien species (Haack et al. 2006). Moreover, from the economic point of view, these species appear to have the highest potential impacts, considering their ability to damage woody material, possibly leading host plants to death and the high cost incurred for their restraint (Aukema et al. 2009). Since the general phenomenon is expected to grow in the next years, techniques and valid protocols for the interception and monitoring of exotic species inside the highest risk points of new introduction, such as ports and airports, have become essential (Fig.4).



Fig. 4: Port of Naples, one of the ports involved in this study

1.2 Wood-boring beetles

The term wood-boring beetle encompasses many species and families of beetles whose larval or adult forms eat and destroy wood, meaning that they are xylophagous (Hickin et al. 1958). In general wood-boring beetles are known to attack weakened or dead trees and they are important as primary decomposers of trees within the forest ecosystem, making sure that the nutrients locked inside the wood can be recycled (Hicks et al. 2011). However some of them can become economic pests by changing forest ecosystems as most of the invasive alien species do (Fig.5). In recognition to the threat posed by untreated wood packaging materials, an international standard (ISPM 15) was first approved in 2002 and revised in 2013 (IPCC 2013). Although ISPM 15 has reduced the rate of infestation, some treatments may be improperly applied and live borers are still found occasionally in treated wood packaging materials and therefore the risk of biological invasions through wood pathway still exists (Haack et al. 2014).



Fig. 5: Example of xyломicetophagous species galleries

Wood and wood products used to support, brace, or package commodities during shipment provide a pathway for global transport of wood boring beetles. Storage of commodities packaged or shipped near forested and natural lands or landfills further provide an avenue for introduction and establishment of non-indigenous beetle taxa (Cline et al. 2011). Some data state that introductions of non-native species into the US cost about 2.1 billion per year in forest product revenue. For these reasons, the development of early detection methods for alien species is a crucial step when implementing rapid response systems, effective eradication and suppression protocols for invasive pests (Pluess et al. 2012). If alien wood-boring and bark beetles are quickly detected, site-specific phytosanitary measures can be implemented and a timely action plan can be produced. At this regard, as underlined above, Scolytidae, Cerambycidae and Buprestidae are the most commonly intercepted families of wood boring beetles.

1.2.1 SCOLYTINAE

The bark beetles are a subfamily of weevils insects which include a group of about 6,000 species distributed in 181 genera that are morphologically very similar, but different in their relations with the host plants and the environment in general (Fig.6). These beetles are usually divided, according to their feeding habits, in phleophagous, xylomicetophagous and spermatophagous. The species belonging to the first group, also known as "bark beetles", use phloem tissue as food and mating site (Fig.7) and are characterized by a relatively high host specificity. The species belonging to the second group, also known as "ambrosia beetles", dig galleries inside the wood and use symbiotic fungi cultivated inside the galleries as nourishment. Ambrosia beetles are recognized to be relatively polyphagous species; the species belonging to the last group, also known as "seed borers", develop at the expense of seeds or woody fruits.

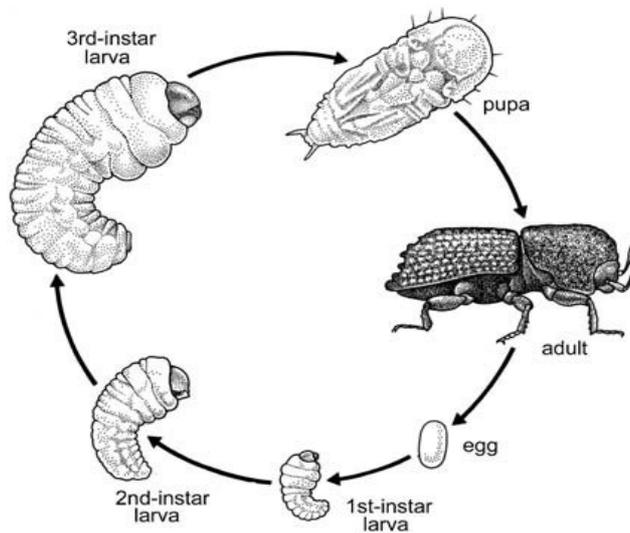


Fig. 6: Life cycle of a wood-boring beetle



Fig. 7: An adult of *Ips typographus*, one of the most important pest for Euroasiatic forests

Bark and ambrosia beetles are able to travel mainly in fresh, often debarked, wood and timber (Fig.8,9), (Haack 2001; Allen & Humble 2002; Colunga-Garcia et al. 2009; Haack & Petrice 2009). Only a few scolytid species are likely to be transported in plants or plant parts. Some examples are given by the cut stems of *Dracaena* shipped to Europe from Central America, which have been found to be infested by species belonging to the genus *Xyleborus*. Moreover, seeds and nuts are often colonized by *Coccotrypes*, *Dactylotrypes* and *Hypothenemus*, whereas orchids have been found as vectors of the ambrosia beetle *Xylosandrus morigerus* (Kirkendall & Faccoli 2010).



Fig. 8: Wooden boxes for packing goods from an Italian port



Fig. 9: Pile of logs waiting to be moved.

1.2.1.1 Phleophagous beetles

The adults of phleophagous species represent the 90% of the total species in temperate areas. These beetles survive the winter under the bark of infested trees or in the needle litter. When the spring air temperature reaches 15°C, the beetles begin to be active. Once the temperature climbs above 25°C, they leave their wintering grounds and swarming begins. They begin to fly on to the weakened trees that have reduced ability to shed resin trees. Then, the male begins to eat the bark in order to create a “wedding chamber”. During this activity, on the bark surface small pieces of sawdust, that have been thrown by the beetles out of their wedding chamber, can be observed. This is the first sign of an ongoing infection. A male takes 2-4 days to burrow the “wedding chamber”. After having completed this activity, it begins to release pheromones into the air in order to attract females for mating. Bark beetles are polygamous species, which means that the male mates with more than one female, usually with 2 to 3. The mating is performed inside the “wedding chamber” and, after that, the females begin to dig galleries where eggs are laid on. The female's galleries are characterized by

having a constant diameter without any kind of debris. The larvae will hatch soon thereafter and feed digging galleries perpendicular to the female's gallery. The larvae's galleries are characterized by diameters that increase with the size of the individuals and presence of excrement and sawdust (Battisti et al. 2013) (Fig.10a). After reaching maturity, the larvae dig a pupal chamber in which they conclude the metamorphosis. The development of a generation takes about 6-8 weeks. The set of maternal and larval galleries is called reproductive system. The neo-adult, before starting with a new cycle, spend a period of time, which is necessary for the maturation of the gonads, feeding directly on the substrate in which the larvae have developed: in polygamous species, the adults feed on the remains phloem left by the larvae, while in monogamous species they move in search of new and fresh substrates, such as buds or branches belonging to the same host plant (Battisti et al. 2013). At the time of mating, in polygamous species, the male dig the pupal chamber and only then it is reached by the females, whereas in monogamous species the opposite trend occurs. (Balachowsky 1949; Chararas 1962).

1.2.1.2 Xylomicetophagous beetles

Xylomicetophagous species, thanks to a complex of symbiotic fungi and bacteria, are able to develop inside the inner woody tissues, with very low nutrient content. In xylomicetophagous species, females dig long tunnels deep into the wood. The galleries are ramified in order to form chambers where eggs are laid (Fig.10b). Once born, the larvae don't eat directly the wood, but they feed on symbiotic fungi cultivated by the female inside the galleries, where there are suitable conditions for their growth, both in terms of darkness and humidity. As soon larvae are developed, the new adults emerge and go backward along the tunnels dug by the individuals of the parental generation. During this path they complete the maturation of the gonads and they get in contact with the spores which will subsequently be transported towards new hosts.

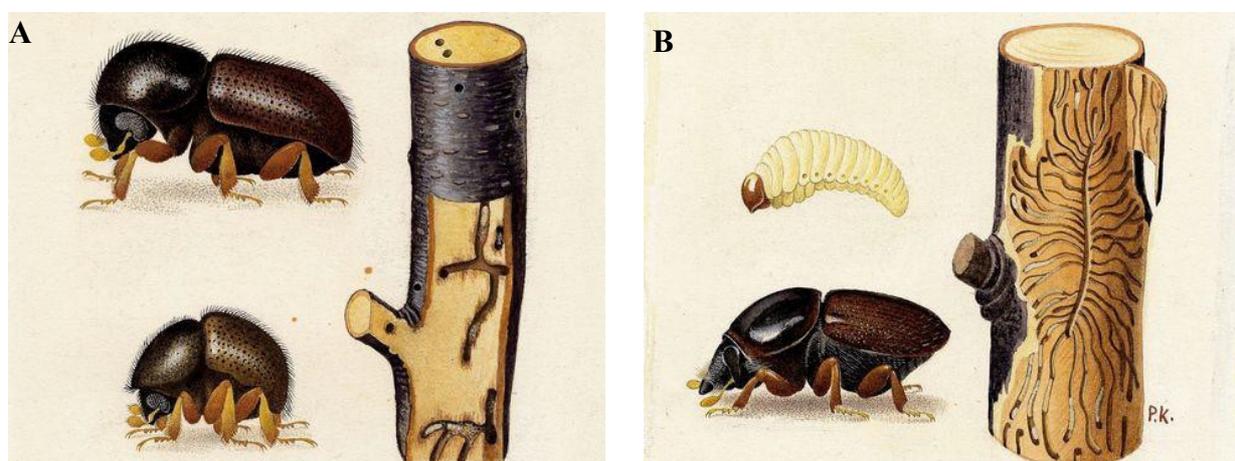


Fig. 10: Comparison between xylomicetophagous beetles on the left side (A) and phleophagous beetles on the right side (B)

1.2.2 CERAMBYCIDAE

The long-horned beetles are a cosmopolitan family of beetles characterized by extremely long antennae (Fig.11). Some of them are serious pests recognized as a problem in forest ecosystems. Their attack may cause economic losses on naturally damaged or felled timber (Evans et al. 2004) and they can be vectors of very harmful pests, such as the pine wood nematode *Bursaphelenchus xylophilus* transmitted by *Monochamus spp.* (Schroder et al. 2009). The family is composed by more than 35.000 species and 4.000 genera. They can develop in highly decomposed wood, herbaceous plants or roots. Larvae of most species develop for about one year in the trunk or branch of living or dead trees (although some species can have two broods per year and other species may take five years or more for maturation). Adults are relatively short lived.



Fig 11: An adult of *Anoplophora chinensis*, one of the most dangerous insect introduced in Italy

Cerambycids can be easily transported inside live plants and wood packing material used in international cargo (Hu et al. 2009; Haack et al. 2010). For example, the bonsai trade was the pathway of introduction for the citrus longhorned beetle (*Anoplophora chinensis*) in USA.

1.2.3 BUPRESTIDAE

Buprestidae is a family of beetles with about 15,000 species and 450 genera. They are characterized by having iridescent colors (Fig.12). The larvae bore in roots, logs, stems and leaves of various types of plants ranging from trees to grasses. The wood-boring types generally favor dying or dead branches. Although only two species of exotic buprestids of minor importance (*Buprestis decora* and *Chrysobothris dorsata*) have so far established in Europe (Denux & Zagatti 2010), some of them are serious pests capable of killing trees and cause major economic; moreover other species have to be considered as potential threats to European forests (Cappaert et al. 2005).



Fig. 12: An example of how adult buprestids look like

The most important pathway for the introduction of these species is the transportation of wood for industry and firewood. Larvae can also be introduced with bonsais (Fig.13).

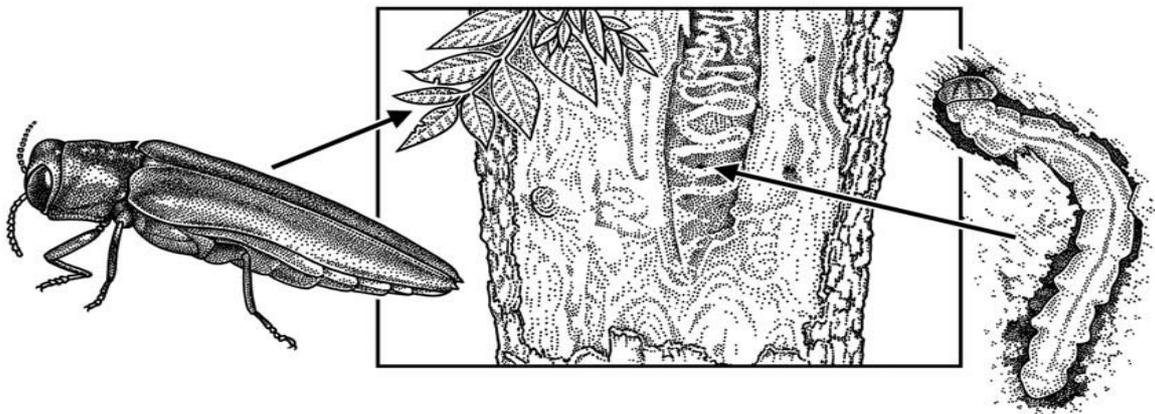


Fig. 13: Larval galleries of buprestids

1.3 Monitoring of invasive xylophagous insects

The monitoring and the early detection of alien species in high risk points for new introduction are of primary importance to increase the possibility to stop the process of invasion and, therefore, prevent and limit the huge economic and environmental costs that would be needed for the eradication and/or containment of these organisms. In Europe, preliminary checks at ports and airports are usually performed through specific inspections. Security services, coordinated by the National Plant Protection Organization with the cooperation of international bodies such as IPPC and EPPO, have developed protocols to intercept and identify quarantine pests. Nevertheless, these protocols show large discrepancies between the number of species intercepted and the exotic species established during the same period (Humble and Allen 2001; Roques 2010; Marini et al. 2011). For this reason, USA, Australia and New Zealand have decided to use, in addition to traditional control methods (e.g. evaluating presence and number of holes or sawdust on woody material (Fig.14), verifying the certified ISPM-15 mark), other kinds of tools such as traps, which allow to increase the chances of alien species interceptions (Haack et al. 2001; Tkacz 2002;

Brockerhoff et al. 2006a; Rabaglia et al. 2008). In this context, the trapping method can assume decisive importance, especially during the early stages of biological invasions, both to collect information on alien species arriving through international trades (Wylie et al. 2008) and to try stopping them, considering that an exotic species is able to establish only if the number of introduced individuals is higher than a defined threshold (Allee effect). Anyway, considering the low number of individuals that usually characterize the first stage of the biological invasion (Liebhold and Tobin 2008), it is essential to have reliable tools that are able to capture emerged individuals even at low population density. To reach this aim, there are three main variables that must be considered

- 1) the type of trap,
- 2) the type of attractive lures
- 3) the position of the traps within ports and in their surrounding areas.



Fig. 14: Phytosanitary visual inspections verify the ISPM 15 mark and the possible presence of holes or sawdust indicating the presence of alive insects

1.3.1 TRAP MODELS

The models most commonly used for trapping wood-boring insects are the multi-funnel trap, the cross-vane trap and the german slot trap (Petrice et al. 2004) (Fig.15). Several studies were performed in the field to compare the efficiency of these trap models in trapping xylophagous species, but the results vary within Scolytidae (Czokajilo et al. 1999; Fletchmann et al. 2000; Stone et al. 2010), Cerambycidae and Buprestidae families (Morewood et al. 2002; De Groot & Nott 2003). Despite the multi-funnel traps are the most widely used for the monitoring in high risk areas both in the USA and New Zealand (Brockerhoff et al. 2006a; Rabaglia et al. 2008), few studies have been performed to assess the effectiveness of different trap models in areas such as ports and airports, where environmental conditions are significantly different from those in the forest (Fletchmann et

al. 2000; Rassati et al. 2014a). However, the last studies have shown that the effectiveness of cross-vane and multi-funnel traps is very similar, but at the same time the authors emphasizes how the multi-funnel model seem to be, actually, the most suitable for the use in ports and airports, since they are more resistant to adverse weather conditions (eg. strong winds) and faster and easier to assemble and install (Rassati et al. 2014a).

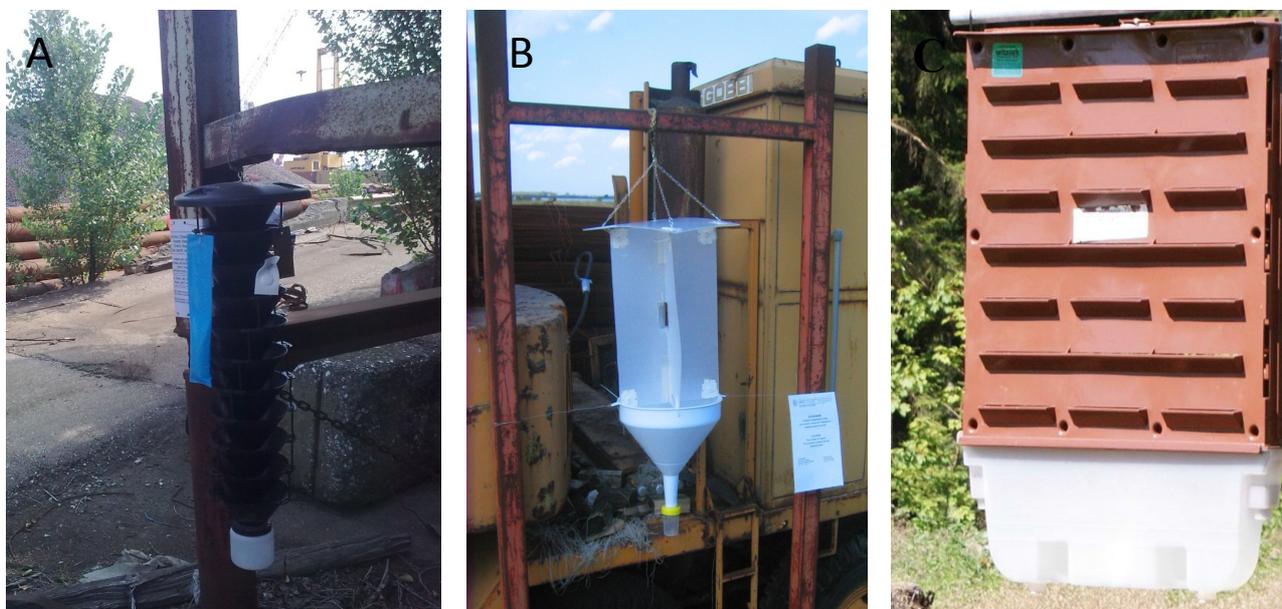


Fig. 15: Trap models commonly used for monitoring wood-boring beetles: A) Multi-funnel, B) Cross-vane and C) German slot

1.3.2 ATTRACTIVE LURES

The second most important aspect that must be considered to improve the efficiency of monitoring protocols is the use of attractive lures. When monitoring programs are aimed to obtain information about the phenology and density of a given target species, species-specific lures are commonly used (i.e. sex or aggregation pheromones). Instead, when the monitoring program aims to intercept a range of species that is as wider as possible, often belonging to different families, the situation changes. In fact, this requires a high number of lures and traps, causing an increase of associated costs. To reduce the overall costs and to render the job of phytosanitary inspectors easier, the use of a combination of different generic lures and specific pheromones seems to be the best option: this is the so called multi-lure technique (Schwalbe & Mastro 1988 Brockerhoff et al. 2012). The monitoring of xylophagous insects is normally carried out through the use of a generic blend, consisting of kairomones - especially α -pinene and ethanol - which simulate the volatiles emitted by decaying or dying plants (Brockerhoff et al. 2006a), and beetles pheromones (eg ipsenol or ipsdienol). Although some studies demonstrated a negative effect when different lures are used together (Schroeder & Lindelöw 1989 Miller & Rabaglia 2009), other works identified multi-lure trap as the best technique. The use of this technique allows the number of traps to be reduced, saving time on trap

checking, decreasing the general costs for materials and manpower (Brockerhoff et al. 2012) and reducing problems related to the possibilities of finding suitable and safe places for hanging the traps within the ports.

1.3.3 TRAP POSITION

A monitoring program should provide information not only on the arrivals but also on the possibility of stabilization of alien species (Fig.16). Considering that alien insects captured within the ports do not provide this kind of information, some authors have pointed out the importance of regular assessments of healthy forest conditions in natural environments near the high risk site (Bashford 2008; Wylie et al. 2008; Britton et al. 2010) and of the use of traps integrated with some susceptible plants called "sentinel trees" (Wylie et al. 2008). Some authors have also proposed a classification based on the risk of introduction of new species that aims to identify appropriate sites for monitoring activities (Bashford 2008). In this way, the opportunity to compare the captures obtained with traps within the high-risk sites with those in the surrounding areas, could give useful information about the establishment of alien species in natural environments as well as an assessment of the reliability of the monitoring program carried out within the ports or airports. However, it is still under investigated how the position of the trap can affect the efficacy of trapping programs within the



Fig. 16: Multi-funnel trap positioned inside Ravenna port

ports area. A previous study conducted in New Zealand (Brockerhoff et al. 2006) revealed that, in areas surrounding ports, the traps located near trees showed a reduced efficiency in catching beetles because of the competing attraction of the adjacent host trees, but it was unclear if this could compromise the sensitivity of the trapping programme. Moreover this study suggested that in ports, trap

positioned near solid fences, walls, or even inside a warehouse are expected to be less effective at disseminating pheromones and less accessible than traps placed in an open area. Anyway, it's still unclear whether the trap position is a relevant factor within port areas.

1.4 Monitoring in Italy

Except for some sporadic works carried out locally (Cola 1971; Francardi et al. 2006), a nationwide and continuous monitoring program for alien xylophagous species has started only in 2012, thanks to the collaboration between the University of Padova and the regional phytosanitary agencies. Fifteen Italian international ports and the surrounding forests were monitored with multi-funnel traps baited with a multi-lure blend, three in each port and three in forests located 3–5 km away from the port (Rassati et al. 2014). During 2012 of monitoring fourteen alien species, among which four new to Italy, were trapped. The simultaneous use of traps in ports with large volume of imported commodities and in their surrounding broadleaf forests strongly increased the probability of alien wood-boring beetle interceptions (Rassati et al. 2014). This demonstrated how the identification of sites where the arrival and establishment of alien species is more probable, combined with an efficient trapping protocol, can substantially improve the efficacy of early detection (Rassati et al. 2014). Similar approaches may be used in other countries as early warning systems to implement timely measures to eradicate or contain alien invasions at the European scale (Rassati et al. 2014). In fact, we know that Italy appears to be, together with France and Great Britain (DAISIE 2010) at high risk of alien species introduction due to the particular suitable environmental and climatic conditions. There are several exotic species known to be established in our country, including among Scolytidae, *Ambrosiodmus rubricollis* Eichhoff (Faccoli et al. 2009), *Xyleborus atratus* Eichhoff (Faccoli et al. 2008), *Coccotrypes dactyliperda* Fabricius (Targioni-Tozzetti 1984), *Cyclorhipidion bodoanum* Reitter (Audisio et al. 2008), *Dactylotrypes longicollis* Wollaston (Sampò and Olmi 1975), *Gnathotrichus materiarius* Fitch (Faccoli 1998), *Hypothenemus eruditus* Westwood (Balachowsky 1949) *Monarthrum mali* Fitch (Kirkendall et al. 2008), *Phloeotribus liminaris* Harris (Plume et al. 2004), *Xyleborus pfeilii* Ratzeburg (Francardi et al. 2006), *Xylosandrus crassiusculus* Motschulsky (Plume et al. 2003), *Xylosandrus germanus* Blandford (Stergulc et al. 1999), *Xylosandrus morigerus* Blandford (Kirkendall and Faccoli 2010) and among the longhorn beetles *Xylotrechus stebbingi* Gahan (Sama 2006) and *Phoracatha recurva* Newman (Sama and Bocchini 2003). Most of them are species from Asia, Far East and Americas, but it is very difficult to determine the exact date of introduction. Anyway the high number of stabilized exotic species confirms the wide adaptability of xylophagous organisms to survive even in environments completely different from the ones of their origin.

2. AIM OF THE STUDY

The main aim of this study is to improve and optimize the protocol already existing for alien wood-boring beetles interceptions. In particular we investigated :

A) how the degree of isolation of the traps within the port area, with reference to the physical elements that surround them, can affect alien and native wood-boring beetle richness and abundance;

B) if and how the wood-boring beetle species richness and abundance change in time, both among weeks within the same year and among years.

We expected, first, that captures are affected by the trap position, with traps placed in "open spaces" having a greater efficacy than those surrounded by buildings or infrastructures; second that captures do not follow a precise seasonality since the arrival of goods in port areas is a random phenomenon and it is not concentrated in a particular time-span within the same year; third that captures can change among years according to changes in the amount and type of imported goods.

3. MATERIALS AND METHODS

3.1 Experimental sites

We selected five international Italian ports that were monitored for three years (2012-2014): Genova (44°24'00"N 8°55'12"E), Ancona (43°37'39"N 13°30'09"E), Salerno (40°39'52"N 14°44'41"E), Ravenna (44°29'00"N 12°17'00"E), and Napoli (40°50'00"N 14°15'00"E).

The port of Genoa (44°24'00"N 8°55'12"E) is the largest Italian port in terms of extension. The port covers a surface area of 7.000.000 m² (140.000 m² occupied by warehouses) and features 80 berths with drafts up to 18 m alongside 21.900 m of quay-line and 5.000.000 m² of stretches of water with a depth between 8 and 15 m. Moreover, the port of Genoa ranks as the premier Italian port in terms of total traffic, handling over 56 million tons per year, and offering over 150 liner services to ports worldwide (Assoport 2011). It is a multi-service port, catering to all key commodity sectors, and it's also the most important in terms of employment. Genoa benefits from its strategic geographic position as the logical Southern European maritime gateway for the major consumer and industrial centres in Northern Italy and Central Europe, specifically, Switzerland, Germany and Austria. Its historic international importance, coupled with its strong multi-functionality (the 29 dedicated terminals are equipped to handle all key commodities such as solid and liquid bulk, conventional cargo, perishable goods, steel and forest products) make it the capital of Italian shipping (Assoport 2011).

The port of Ravenna (44°29'00"N 12°17'00"E) is the only Italian port-canal. Nowadays, in addition to oil and chemical products, traffic within the port involves raw materials and finished goods from the ceramics district, iron goods, timber and agro-food production. On average the overall movement of goods has exceeded 26 million tons per year (Assoport 2011). Today the port overall avails of about 24 km of quays, of which 16 km of operational quays, with a canal bed depth of 10.50 m, 2.800.000 m² of warehouses, 1.400.000 m² of yards and 1.000.000 cm of tanks/silos and areas in the interior of the port perimeter measuring 2.080 hectares, of which over 1.500 already urbanised or in the process of being urbanized (Assoport 2011).

The port of Ancona (43°37'39"N 13°30'09"E) has very good performances in international ferry-boats traffic and it is leader in Italy for number of passengers, with more than 1.5 million transits per year from/to Greece, Croatia, Albania, Montenegro and Turkey. On these ferryboats, together with the passengers, there are 200.000 trucks every year carrying goods and it has been estimated that almost 25% of Greece import/export traffic passes through Ancona port toward and from Europe (Assoport 2011). Quays have modern and efficient infrastructure which are able to handle any kind of goods. Bulk goods are mainly coal, cereals and steel. Cargo traffic was estimated

to be about 8.500.000 tons per year (Assoporti 2011).

The port of Naples (40°50'00"N 14°15'00"E) is one of the most important multipurpose call in the Mediterranean Sea. This is due to its geographical position and traffic, in terms of both quantity and wide range, as well as to the quality of services rendered to vessels. It extends on a surface of about 1.500.000 m² with more than 70 berths, quays for an extension of 12 km with depths up to 15 m. All in all the port has storage areas for about 330.000 m² – about 50.000 m² of which are warehouses (Assoporti 2011). Container traffic with a total throughput of about 445.00 teu a year, liquid and dry bulks for 5 millions tons each and general cargo for 20 millions tons, are among the main commercial activities (Assoporti 2011). Every year 8 millions passengers pass through this port. Shipbuilding industry activities are also of considerable importance for the port economy. Cruise, commercial and shipbuilding industry activities are expected to increase.

The port of Salerno plays a strategic and important role within the industrial and commercial system in central and southern Italy. Many maritime lines link up Salerno with the Mediterranean ports and, in a special way, with the Tyrrhenian ports, with the main European ports. The Port of Salerno has a total areas of 1.7 million m², 15 moorings on 5 piers, covering a total length of 3 km, with 11.5 m. water depth. The quays have a total areas of 500.000 m² (Assoporti 2011). The Port of Salerno is the 5th regional port in Italy for container handling. The cargo handled in recent years have reached the threshold of 12.000.000 tons of goods (Autorità Portuale di Salerno, 2013).

3.2 Type of traps

The multi-funnel traps produced by the Spanish company Econex® were used. These traps are made of 12 black funnels placed in succession along the vertical with a collector cup screwed to the base of the last funnel. We used the version defined "dry", that is provided with a hole on the bottom of the collector cup which may favor the rainwater runoff. The traps were hung at about 2 m above the ground, using facilities (wire fences, griders, piles, buildings steel bars, etc.) as support. Moreover, each trap has been marked with a proper identification code.

3.3 Attractive lures

All traps were baited with a generic multi-lure blend composed of (-) a-pinene (Ultra High Release, release rate of 2 g day⁻¹; 90 days field-life at 20 °C), ipsenol (+50/50; release rate of 04 mg day⁻¹; 90 days field-life at 20 °C), ipsdienol (release rate 04 mg day⁻¹; 90 days field-life at 20 °C), 2 methyl-3-buten-2-ol (release rate of 11 mg day⁻¹; 90 days field-life at 20 °C) and ethanol (release rate of 03 mg day⁻¹; 90 days field-life at 25 °C). All these substances were provided by Contech Enterprises Inc. (Victoria, BC, Canada). These lures had been tested earlier and attract a wide

variety of wood-boring beetles (Rassati et al. 2014a). We did not add any liquid to the collection cups, and therefore used an insecticide (FERAG IDTM; SEDQ, Spain) to quickly kill the insects and to prevent their escape, predation events and any phenomena of cannibalism that would have made the subsequent identification difficult. The substances were replaced during the season based on their expected field-life (3 months).

3.4 Experimental scheme

In each of the selected sites, three traps were set-up, keeping a distance of at least 50 m between them. The choice of traps position was made based on the permission given by the managers of the different ports and considering strategic points of arrival of woody material or other goods with the presence of wooden packaging materials. Trapping occurred from early May to late September of each year (Fig.17).

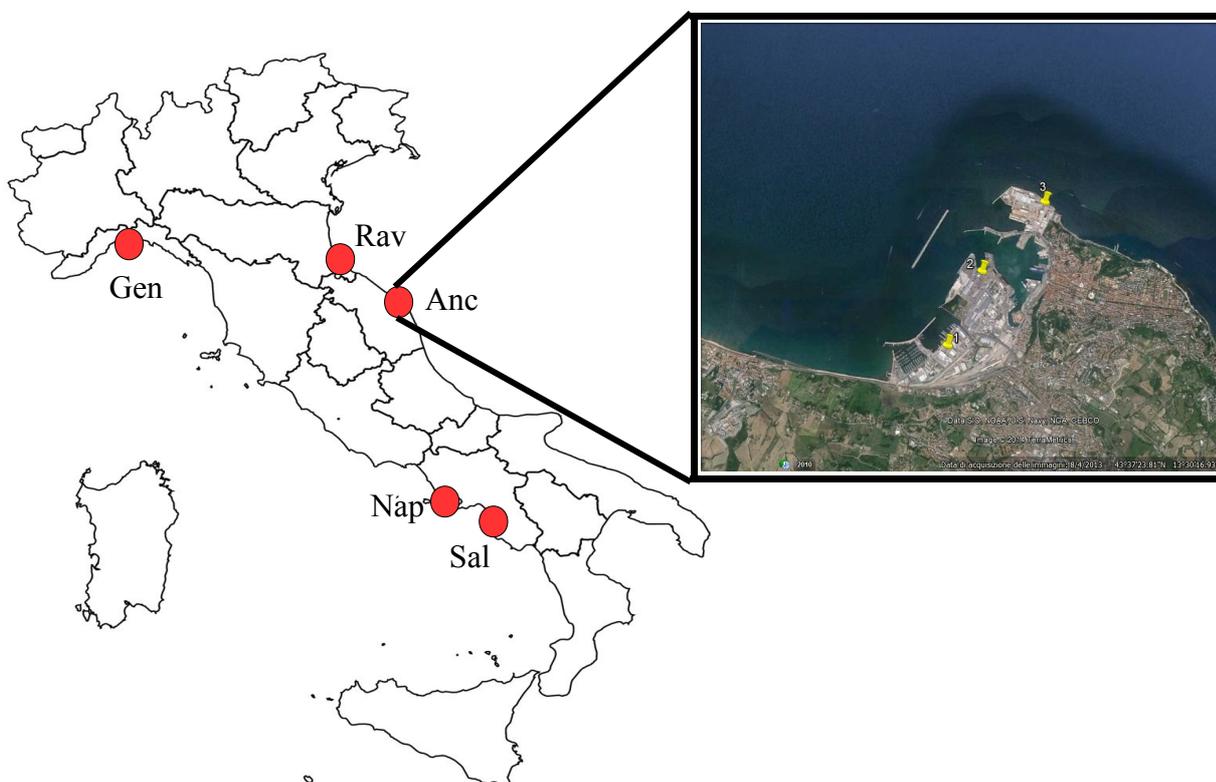


Fig. 17: Geographical distribution of the 5 Italian selected ports and an example of how the traps were set up in each site. Port name abbreviations: Ancona (Anc), Genova (Gen), Napoli (Nap), Ravenna (Rav) e Salerno (Sal).

3.5 Sample collection and storage

The collection of the material found inside the traps was carried out, on average, every 14 days. The trapped insects were placed inside special containers on which the identification number of each trap, the collection date and the port name were recorded. The collected material was then sent by

mail to the University of Padova, where adults of the target insects (Scolytinae, Cerambycidae, and Buprestidae) were stored in alcohol until morphological identification. Then trapped individuals were first carefully cleaned from debris, dust and dirt, using brushes. Colour and size of these insects make this step difficult to complete in a short time. Then the interested xylophagous insects were separated from the rest and identified, in order to separate native and exotic species of bark beetles (Fig.18). They were counted and classified according to their morphological characteristics using specific keys and optical microscopes. In a few cases, we used molecular techniques to identify the trapped insects. In particular, DNA extraction was carried out following a salting out protocol based on the differential solubility of proteins and DNA at high salt concentrations (Patwary et al. 1994). Species were classified either as native or alien according to the available literature (Wood & Bright 1992; Curletti 1994; Bense 1995; Pfeffer 1995). We considered as alien all those species that are not native to Italy. This category can include species that are already established, previously intercepted but not yet established, or never intercepted before.



Fig. 18: Morphological classification of some bark beetles under an optical microscope

3.6 Estimate of isolation degree

Through the use of GIS software, traps located in the five selected Italian ports were geo-referenced and the environment around these traps was analyzed. In particular, we classified the position of the various traps through a rating system that takes into account the degree of isolation of each trap with reference to the physical elements that surround it. In order to analyze how the position of the traps within ports can affect the quality and quantity of captures, we first digitized the polygons, then, using photoshop, we superimposed a circle of about 100m radius (Fig.19) on the image and we visually estimated the surface covered by building or other infrastructures.

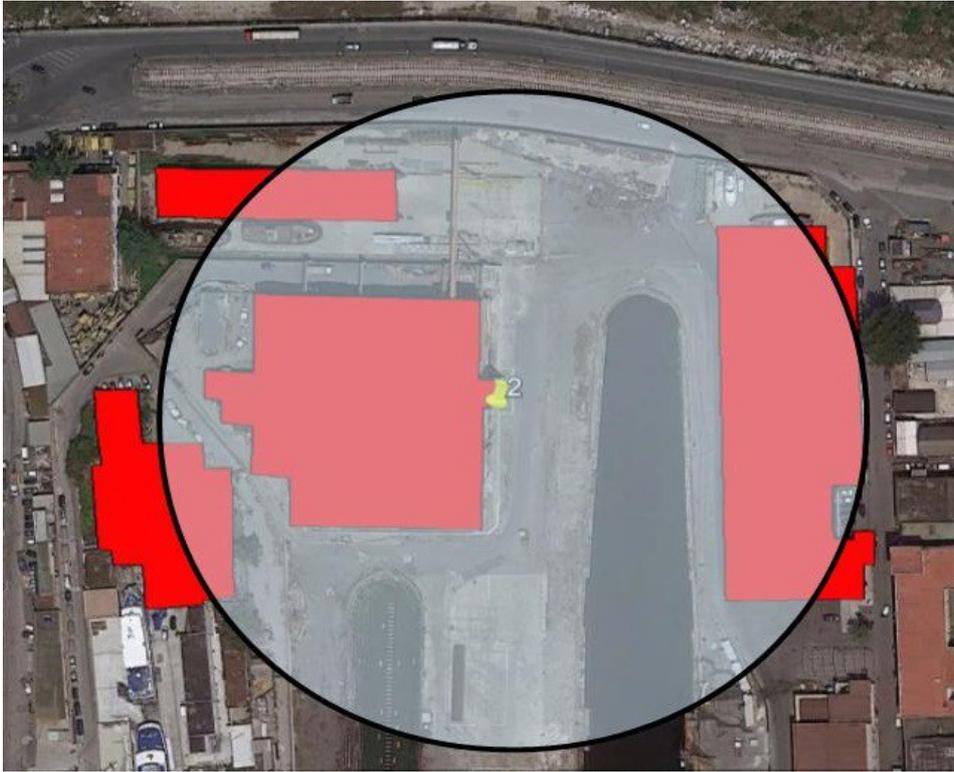


Fig. 19: An example of isolation degree calculation

3.7 Data analysis

To evaluate differences in exotic and native species richness and abundance among years and among check date within years we used general linear mixed-effects models (GLMM). The mean number of exotic and native species or individuals trapped per site and per trap ,standardized for the duration of the trapping, was the response variables. The model included the site as random factors to account for the spatial and temporal dependence of the sampling. To evaluate the effect of the degree of isolation on the number of trapped exotic and native species and individuals we used a linear regression (LM). The total number of exotic and native individuals trapped by each trap was the response variables. All the analyzes were performed using the software R.

4. RESULTS

4.1 General results

During three years of monitoring, 6772 individuals divided in 51 different species, belonging to 3 different families, were trapped in the five ports considered for this study. Among the trapped species, 44 were native and 7 were exotic species. Ancona was the port where the highest value of abundance occurred, with 2197 captured individuals. In particular 2190 native and 7 exotic individuals, divided respectively between 16 native and 1 exotic species, were trapped. Moreover, Ancona represented the site with the highest value of native individuals belonging to native species. Instead the lowest number of individuals occurred at the port of Genova (546) among which 537 native and 9 exotic individuals, divided respectively in 18 native and 2 exotic species. Genova was also the port with the lowest value of native abundance. Napoli is the port with the lowest value of native richness, with 14 trapped species. Salerno, instead, represented the site with the highest value of native richness, with 22 trapped species. Ancona was the site with the lowest number of exotic species richness (1) and abundance (6 individuals). On the contrary, Ravenna was the site with the highest exotic richness (5 species) and abundance (18 individuals). Among the three families of wood-boring beetles taken into account, the most numerous was Scolytidae, with 38 species (32 native and 5 exotic species), followed by Cerambycidae, with 11 species (9 native and 2 exotic species) and Buprestidae, with 2 species (both native). The most numerous native species were: *Ips sexdentatus*, *Hylurgus micklizzi* and *Orthotomicus erosus* with respectively 1161, 1485 and 3365 individuals. Instead, the most numerous exotic species was *Xylotrechus stebbingi* with 20 captured individuals.

4.2 Differences among years

We did not find significant differences neither in standardized exotic species richness (GLMM, $P=0.63$) nor abundance (GLMM, $P=0.59$) among the three years of monitoring. Regarding native species, we found a significant difference in the number of species among years (GLMM, $P<0.05$) but not in the number of individuals ($P=0.55$). In particular, the number of native species was significantly higher in 2013 and 2014 than in 2012 (Fig.20).

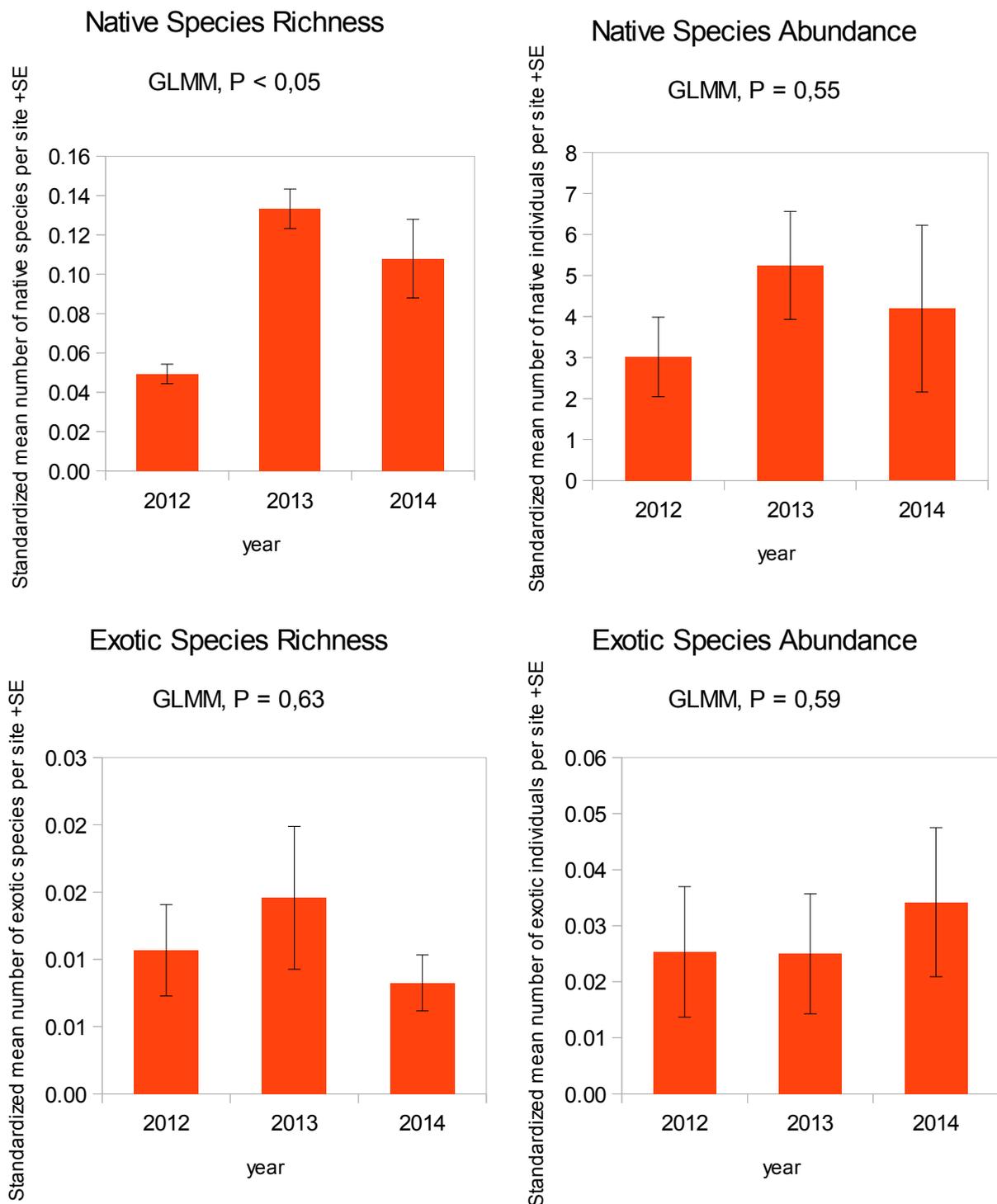


Fig. 20: Differences among years both for native and exotic species

4.3 Effect of isolation degree

Regarding the effect of the isolation degree on traps efficiency, we did not find any significant effect neither on the number of native nor exotic species richness and individuals (GLM, $P > 0.05$). However, for exotic species we found that the more a trap was surrounded by buildings or infrastructures, the lower was the number of trapped species and individuals (Fig. 23,24), whereas the opposite trend was found for native species (Fig. 21,22).

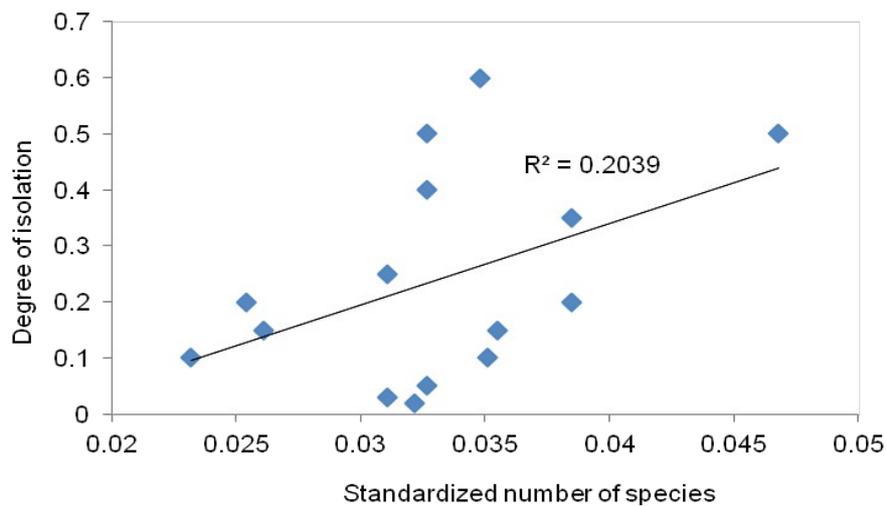


Fig. 21: Standardized native richness in relation to the isolation degree per trap

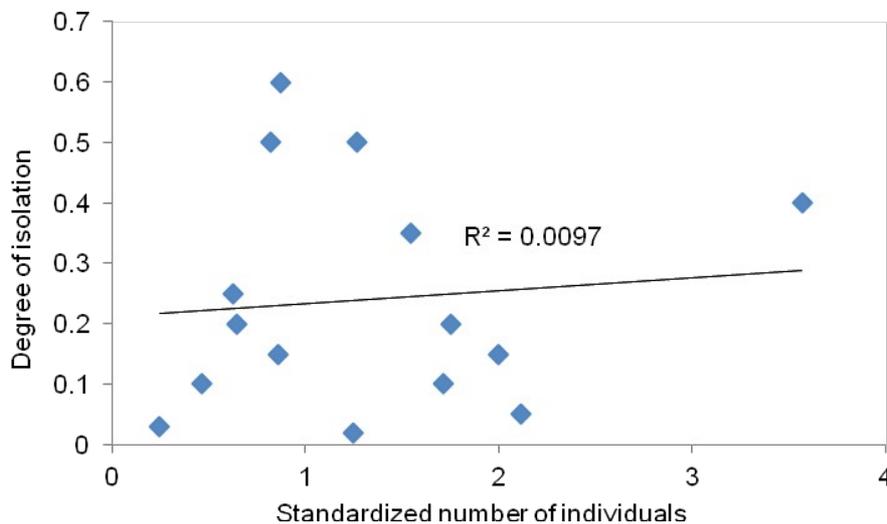


Fig. 22: Standardized native abundance in relation to the isolation degree per trap

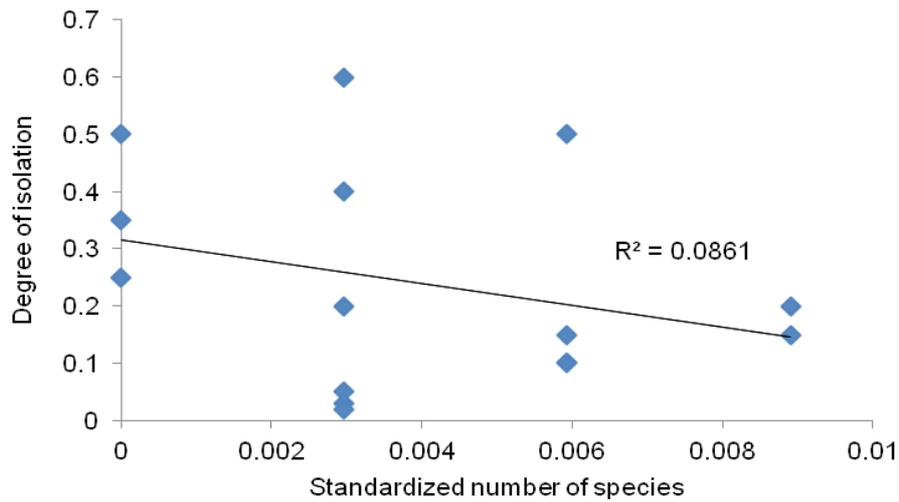


Fig. 23: Standardized exotic richness in relation to the isolation degree per trap

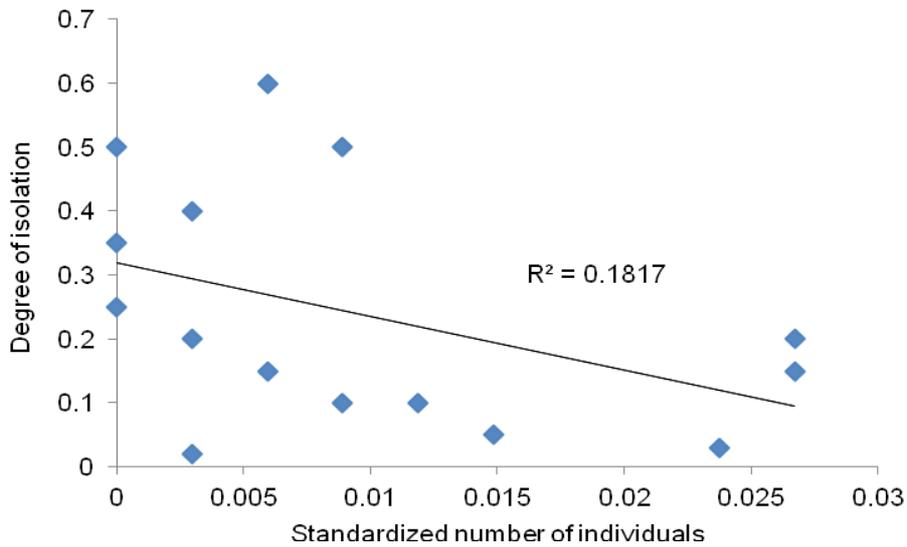


Fig. 24: Standardized exotic abundance in relation to the isolation degree per trap

4.4 Differences within years

Despite we are not able to describe a clear trend, but, in general, catches seemed to be concentrated mainly between late July and early September for both native and exotic species. Exotic species were not trapped all the years and in all ports: only the port of Ravenna and Salerno have recorded exotic captures during all the monitored years. On the contrary, the other ports have recorded exotic species just in two out of the three years of monitoring.

The port of Genova showed significant differences (GLM, $P < 0.05$) among trap checks carried out within the same year only in terms of native species abundance, whereas the native species richness remained constant (Fig. 25 A,B,C). On the contrary, the trapping events involving

exotic species were less frequent (one in 2013 and two in 2014, Fig. 26 A,B) and showed differences only in their abundance.

The port of Ravenna showed a similar trend. Regarding native species, we found again significant differences (GLM, $P < 0.05$) among trap checks carried out within the same year only in terms of native species abundance, whereas the native species richness remained constant (Fig. 25 D,E,F). On the contrary, the trapping events involving exotic species were more numerous than in the previous port, with differences in both species richness and abundance occurred only in 2012 (Fig. 26 G,H,I).

Also the port of Ancona showed significant differences (GLM, $P < 0.05$) among trap checks carried out within the same year only in terms of native species abundance, whereas the native species richness remained constant (Fig. 25 G,H,I). Regarding the exotic captures, in 2012 we didn't find exotic individuals, whereas, in the last two years, there was a general increase in the abundance of exotic individuals with a stable exotic richness within years (Fig. 26 C,D).

The port of Napoli showed significant differences (GLM, $P < 0.05$) among trap checks carried out within the same year in terms of native species abundance in 2012 and 2013, whereas the number of trapped individuals remained constant in 2014 (Fig. 25 L,M,N). The native richness was instead constant within years. On the contrary, the trapping events involving exotic species were less frequent (one in 2012 and two in 2013, Fig. 26 E,F) with differences only in their abundance.

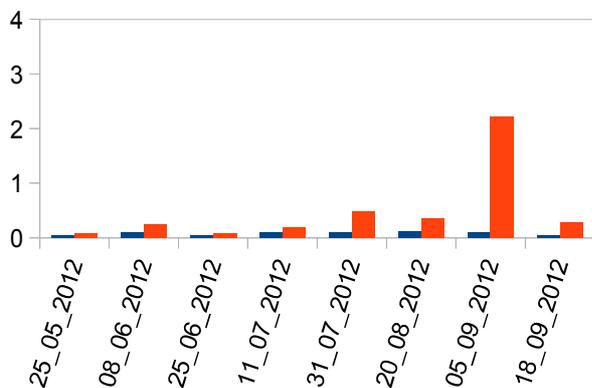
The port of Salerno showed significant differences only in native species abundance (GLM, $P < 0.05$) (Fig. 25 O,P,Q), whereas the trapping events involving exotic species were more frequent than in the other ports, despite the species richness and abundance were similar within years (Fig. 26 L,M,N).

In general, the results underlined that if for native species the species richness remain more or less constant within year, the species abundance tends to change in time. Moreover, regarding exotic species, the results demonstrated that the latter were trapped randomly within the same year, and it is not possible to identify a clear trend.

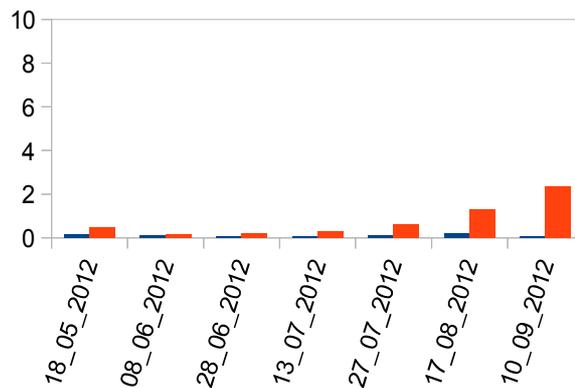
NATIVE

■ Native Richness
■ Native Abundance

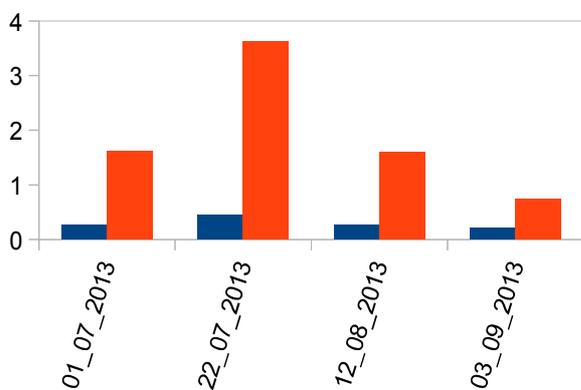
A NATIVE GENOVA 2012



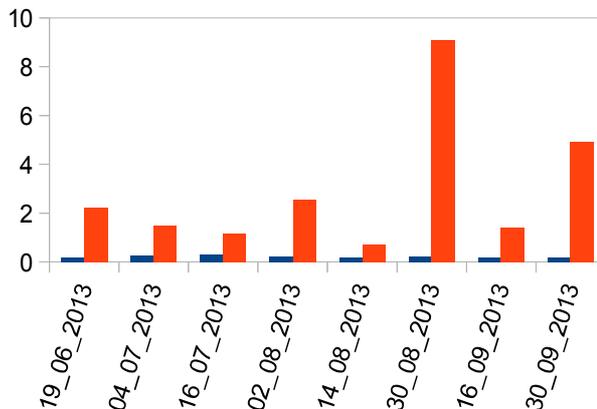
D NATIVE RAVENNA 2012



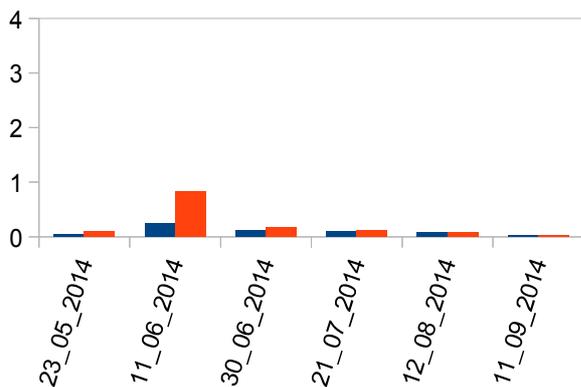
B NATIVE GENOVA 2013



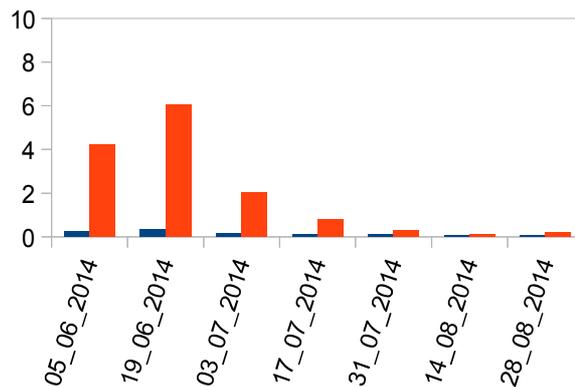
E NATIVE RAVENNA 2013



C NATIVE GENOVA 2014

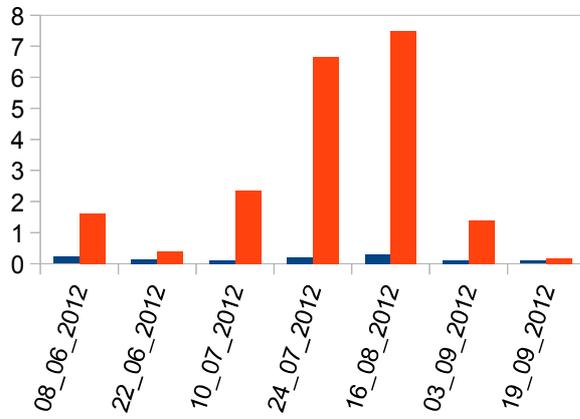


F NATIVE RAVENNA 2014

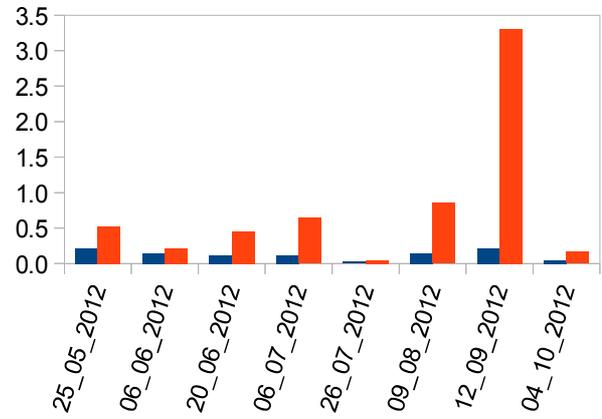


■ Native Richness
 ■ Native Abundance

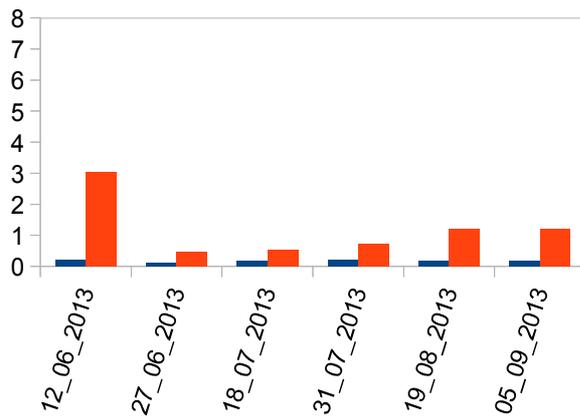
G NATIVE ANCONA 2012



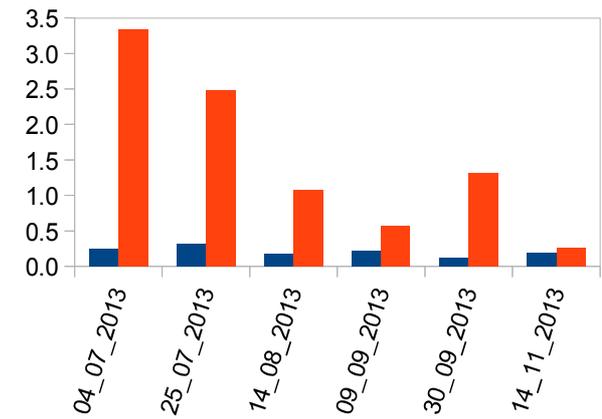
L NATIVE NAPOLI 2012



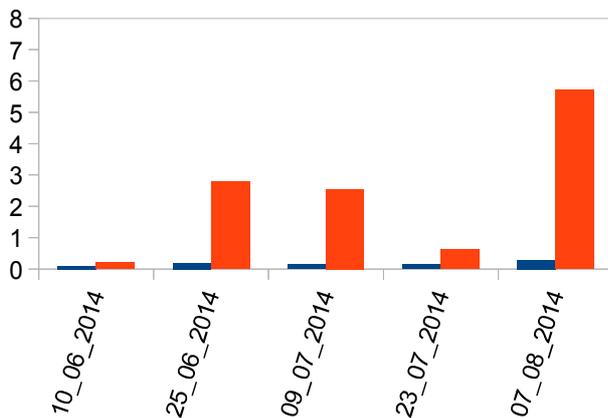
H NATIVE ANCONA 2013



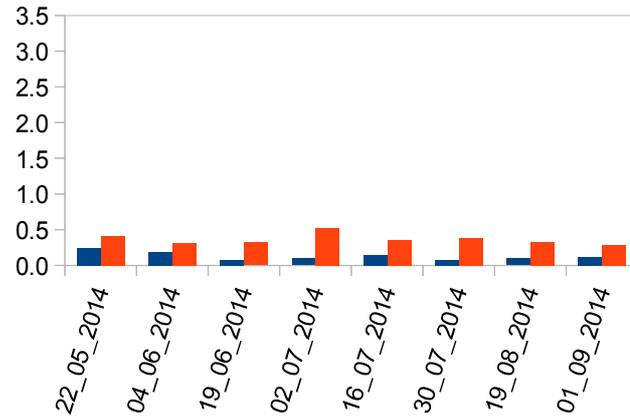
M NATIVE NAPOLI 2013



I NATIVE ANCONA 2014



N NATIVE NAPOLI 2014



■ Native Richness
 ■ Native Abundance

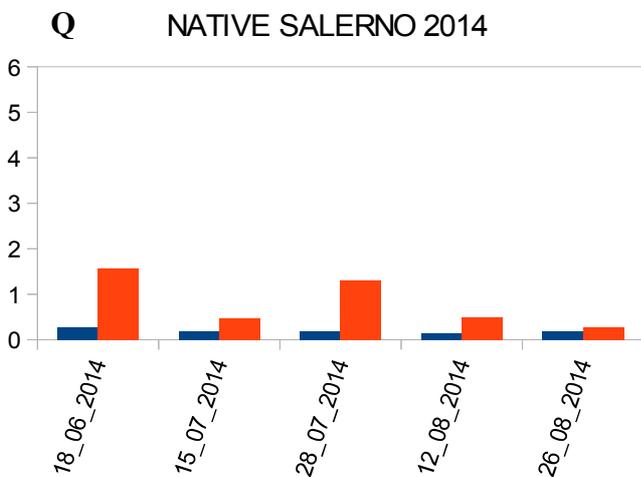
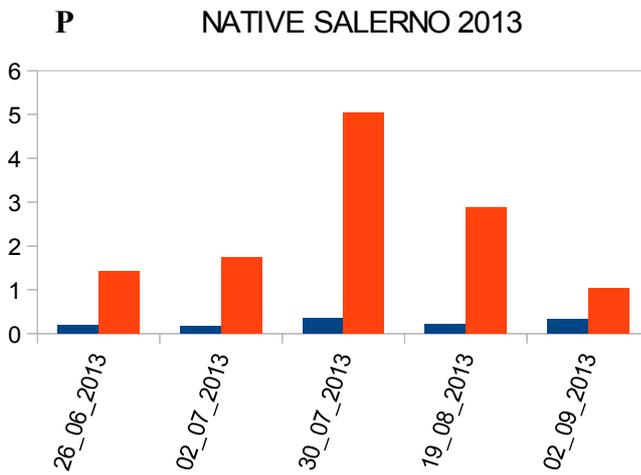
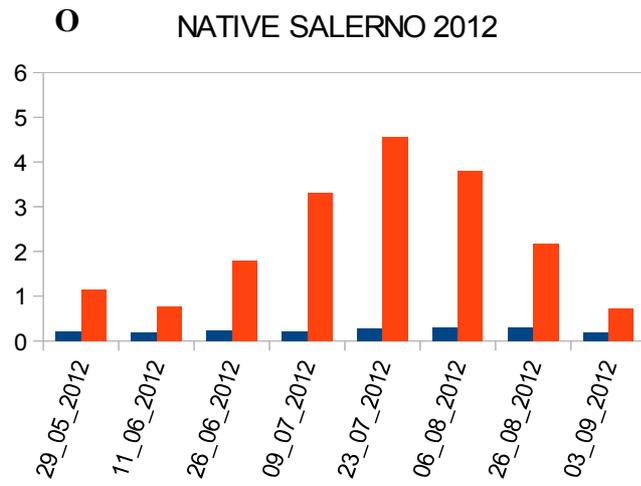
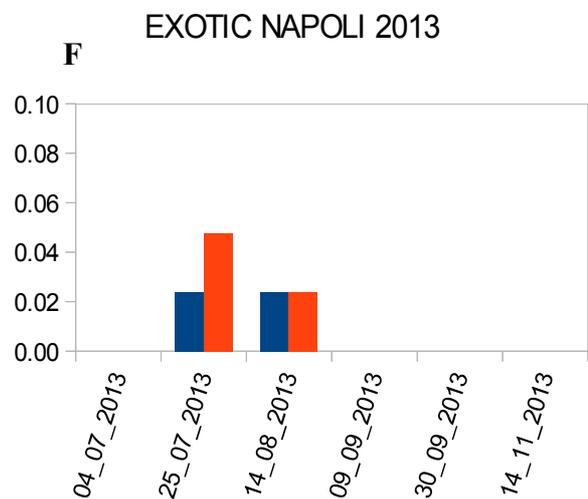
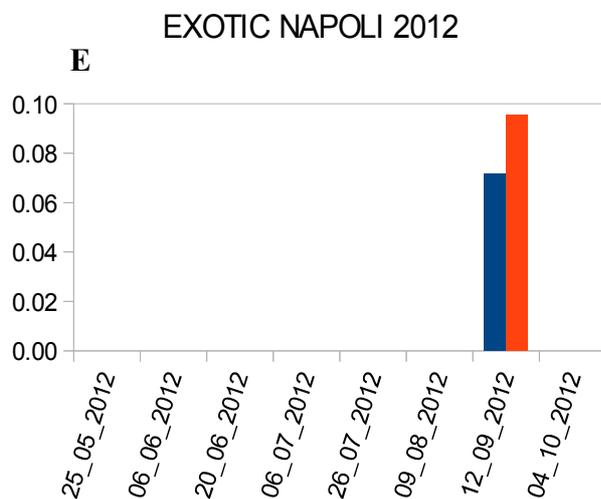
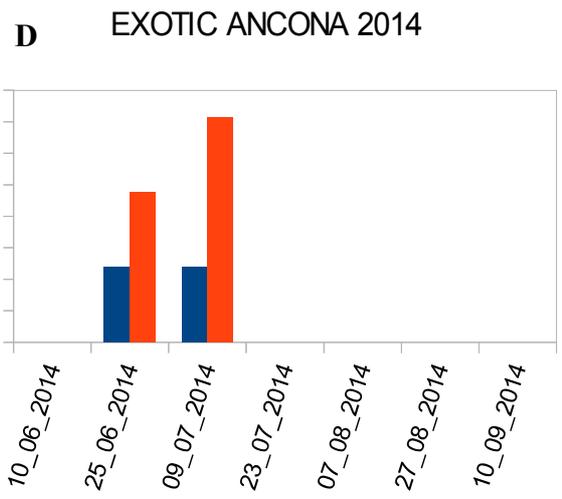
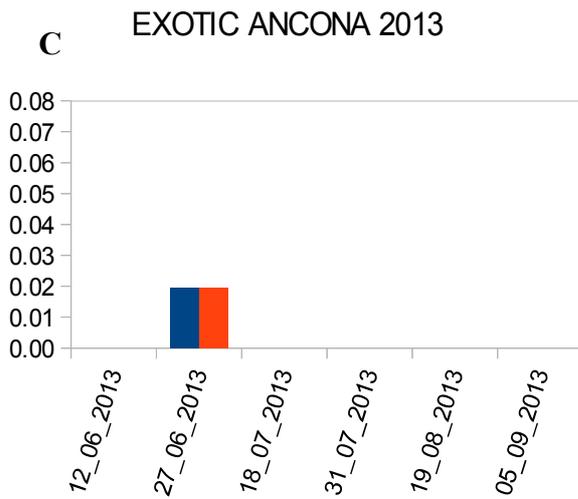
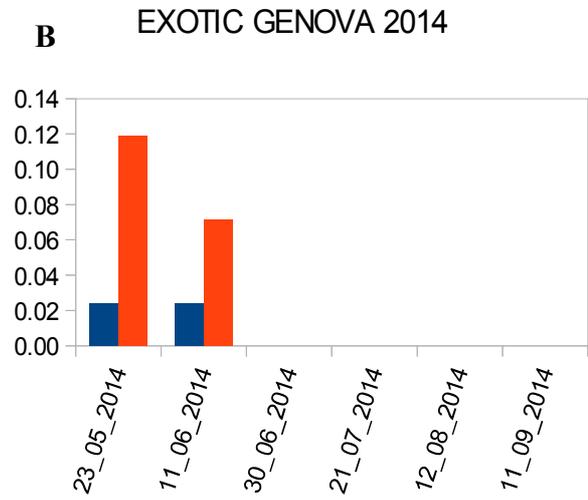
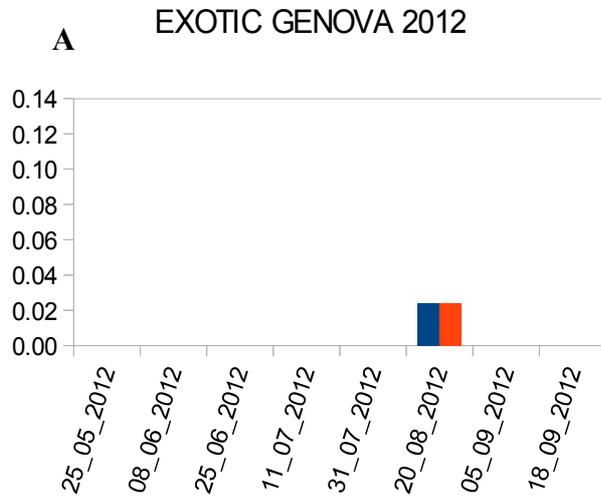


Fig. 25: Differences in native richness and abundance within years per site

EXOTIC

■ Exotic Richness
 ■ Exotic Abundance



■ Exotic Richness
 ■ Exotic Abundance

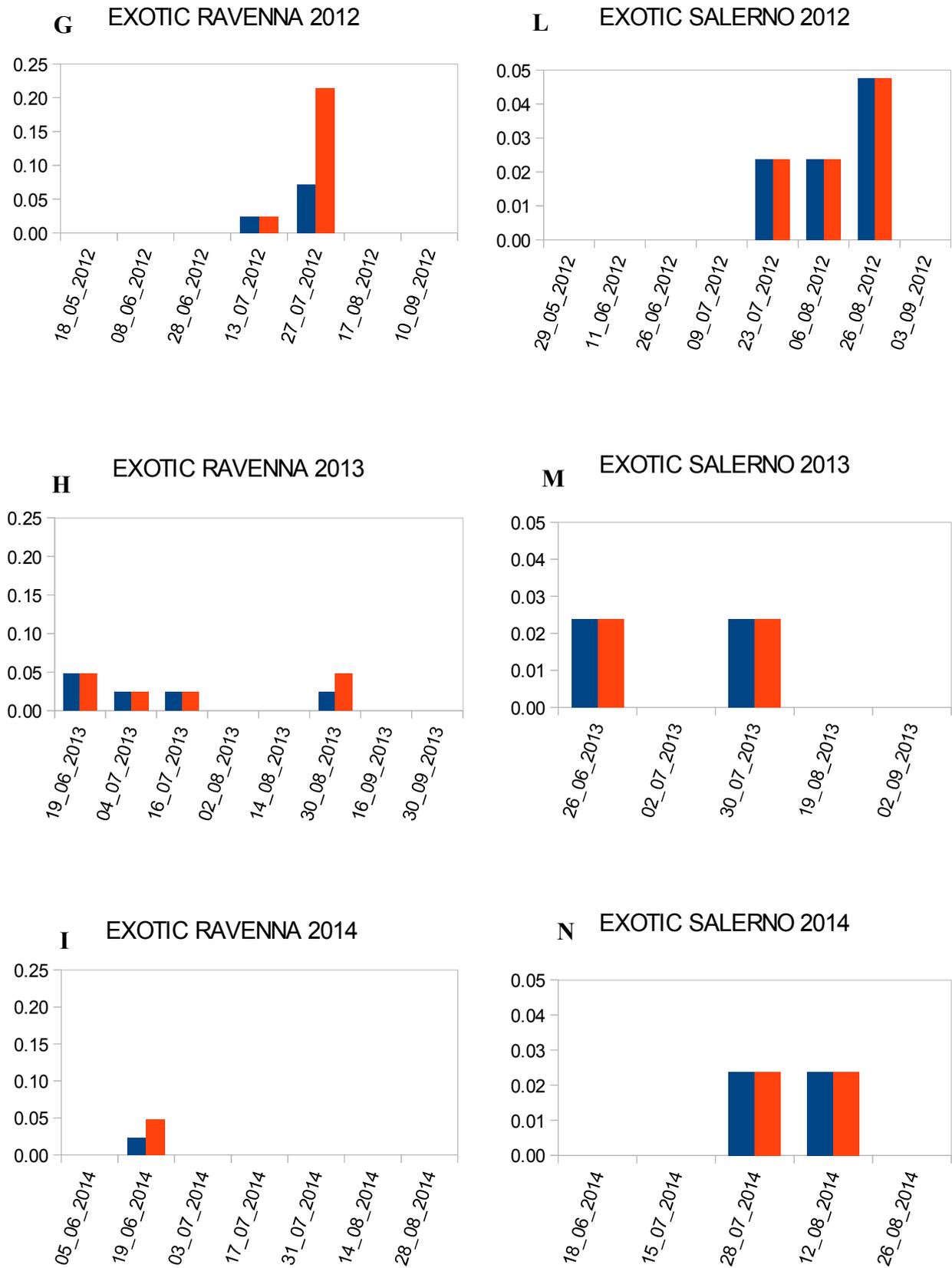


Fig. 26: Differences in exotic richness and abundance within years per site

5. DISCUSSION

The phenomenon of alien wood-boring beetles invasion is a worldwide problem and it's supposed to grow in the coming years (Hulme 2009). In fact, despite the measures undertaken to prevent the arrival of alien species, the risk of new introductions is still high and appears to be increasing with the raising volume of international cargo and the number of countries from which goods will be imported (Aukema et al. 2004; Kirkendall and Faccoli 2010; Colunga-Garcia et al. 2013; Haack et al. 2014). In fact, international trade represents the first cause of introduction of alien wood-boring beetles as these insects can be easily transported in associations with woody materials. Moreover, since the '80s, it became more and more evident that climate change may have important consequences on biological invasions (Ayres & Lombardero, 2000). The insects, thanks to the fast life cycles, the high potential of reproduction and the high capacity of physiological adaptation, are organisms that can directly and quickly respond to climate change (Crozier & Dwyer 2006). Higher temperatures, frequently associated with long periods of drought, generally determine the northward shift of thermophilic species of insects, increasing the intensity of infestation in the southern edge of natural range of specific trees. Moreover, climate warming may reduce the thermal limitations that are now hampering the establishment of species coming from tropical or sub-tropical regions (Rassati et al. 2014). For these reasons, the monitoring and the early detection of alien species in sites at high-risk of new introduction are of primary importance to increase the possibility to stop the process of invasion and, therefore, prevent and limit the huge economic and environmental costs that would be needed for the eradication and/or containment of these organisms.

We found that there were no significant differences neither in standardized exotic species richness nor in the abundance among the three years of monitoring. We can relate this result to the international commercial trade that has not significantly changed during the last three years of monitoring which means that the type and quantity of goods have not changed so clearly in order to justify a change in the communities of exotic species. Regarding native species, we found a significant difference in the number of species among years but not in the number of individuals. In particular, the number of native species was significantly higher in 2013 and 2014 than in 2012. During the winter 2012, Italy was hit by a cold snap which brought temperatures far below zero, with abundant snowfall throughout Italy, especially on the Adriatic coast. At the same time, summer 2012 was recorded as one of the warmest seasons over the last 60 years. This rather stormy weather condition may explain why in 2012 significantly lower values of native species were recorded. This could be probably explained considering the climate characterizing those years. Previous studies have demonstrated that the minimum temperature can strongly influence the spread of wood-boring

beetles influencing negatively their life cycle (Rice et al. 2008). In addition, we must say that native captures could be related to species that may actually fly from the local vegetation surrounding ports or emerge from wood-packaging materials that were associated with either national or international trade.

Regarding the effect of traps isolation degree, the results did not show any significant effect neither on the number of exotic nor native species richness and individuals. The final trend didn't meet our expectation, as we first expected that the more a trap would have been isolated, the more species and individuals could have been trapped. However, it is reasonable to think that captures of exotic species are strictly related to the random arrival of wood packaging materials than to their degree of isolation. In fact, the presence of woody materials in ports is not constant over time and space, with commodities periodically unloaded, shipped or moved (Stanaway et al. 2001) and the type of woody materials, their amount, and their storage locations are often unpredictable factors. In this regard, previous studies indicated that, as a general rule, high amounts of imported commodities in a given area increase the probability of alien species introduction, and this has been demonstrated at both continental (Mack et al. 2000; Haack 2001; Marini et al. 2011; Huang et al. 2012; Liebhold et al. 2013) and port scale (Rassati et al. 2014b). However, to increase the efficiency of the early detection it will be of utmost importance to better understand how wood packaging materials move inside ports as well as from ports to surrounded areas, as it will enhance the decision process about where traps should be located and where surveillance efforts should be focused. From our results it seems that the position of potential sources of alien and native wood boring beetles is more important than the degree of isolation of the traps. In fact, if for exotic species the efficiency of traps could be related to their proximity to storage locations of woody materials, for native species, the distance between the traps and natural areas surrounding the ports could be crucial. It is also necessary to consider the environmental conditions characterizing high-risk sites such as ports, in particular the wind, which could be very strongly favor the diffusion of pheromones.

Lastly we found that in general, catches seem to be mainly concentrated between late July and early September for both native and exotic species of insects. However, as we expected, for exotic species it's not possible to identify a clear trend due to the random captures occurred within the same year. As previously mentioned, especially for exotic species, captures are more influenced by the random arrival and storage position of goods within ports than traps isolation degree, and this trend seemed to be followed also within years. This fact underline that the traps should be used all year long or at least from early spring (April) to autumn (October), in order to cover the entire period at risk and increase, in this way, the chances of wood-boring beetles interception. Regarding native species, the differences that we found in terms of wood-boring beetle abundance could have

been related to both the natural life cycle of the trapped insects and their random arrival in association with national trades. In addition, future studies should focus on understanding if the native species, that were most commonly trapped inside these ports, are also the most prone to be transported within wood packaging materials and thereby constitute a pool of invaders that can be moved outside the country through international trade.

In conclusions this study demonstrated that:

- A) the exotic species richness and abundance were similar among years, indicating that the type and quantity of imported goods did not clearly change in time;
- B) captures of exotic species are probably influenced more by the proximity of the traps to storage locations of woody materials than to their degree of isolation;
- C) the exotic species richness and abundance change randomly within years, indicating that traps installation period should cover the entire period when wood boring beetles can be active (April-October).

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Nino