Strategies and Measures to Prevent Spread of Invasive Species

Gustavo Darrigran and Cristina Damborenea

Abstract On the basis of the fouling caused by the golden mussel, *Limnoperna fortunei,* in industrial facilities and power plants (human environment), and its impacts on the ecosystem (natural environment), several strategies and measures for the mitigation of problems and prevention of further spread are discussed. At the local level, monitoring and early detection of the golden mussel can be accomplished through different methods, including those aimed at juveniles and adults, and also those aimed at their planktonic larvae. Priorities for designing bioinvasion management strategies should focus on generation of scientific knowledge, management, and actions at the sociopolitical level. Mitigation measures are closely related to the invasion phase, whereby the cost increases and the probability of eradication decreases over time.

Keywords *Limnoperna fortunei* · Golden mussel · Biological invasion · Prevention · Monitoring

Introduction

The golden mussel, *Limnoperna fortunei* (Dunker 1857), is one of the most aggressive freshwater invaders to have spread in Asia (Korea, Japan, Taiwan, Indochina) (Morton and Dinesen 2010) and in South America (Argentina, Bolivia, Brazil, Paraguay, and Uruguay) (Darrigran 2010). This invasive species has negative impacts on man-made structures (the "human environment" hereinafter), including power plants and industrial facilities. Plants are faced with problems derived from the blockage of pipelines, decreased water velocity, accumulation of shells, contamination of water by dead mussels, and clogged filters (Darrigran and Damborenea

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2005; see "Impacts of *Limnoperna fortunei* on Man-made Structures And Control Strategies: General Overview" in this volume).

The golden mussel also has an ecological impact on the "natural environment," where it affects the water column and changes the structure of available substrata. As a result, it impacts other members of the biota and their interactions, and it consequently modifies overall ecosystem processes, thus fulfilling the role of an "ecosystem engineer" (Jones and Lawton 1994; Darrigran and Damborenea 2011; Boltovskoy and Correa 2015).

Figure 1 summarizes the mechanisms by which an alien species can be transported elsewhere with the aid of man. For *L. fortunei,* expansion is associated with both "transportation-related" pathways and "commerce in living organisms." In the first case, there are two primary vectors. One is ballast water. Introduction of the mussel from Southeast Asia to South America is believed to have happened via bal-
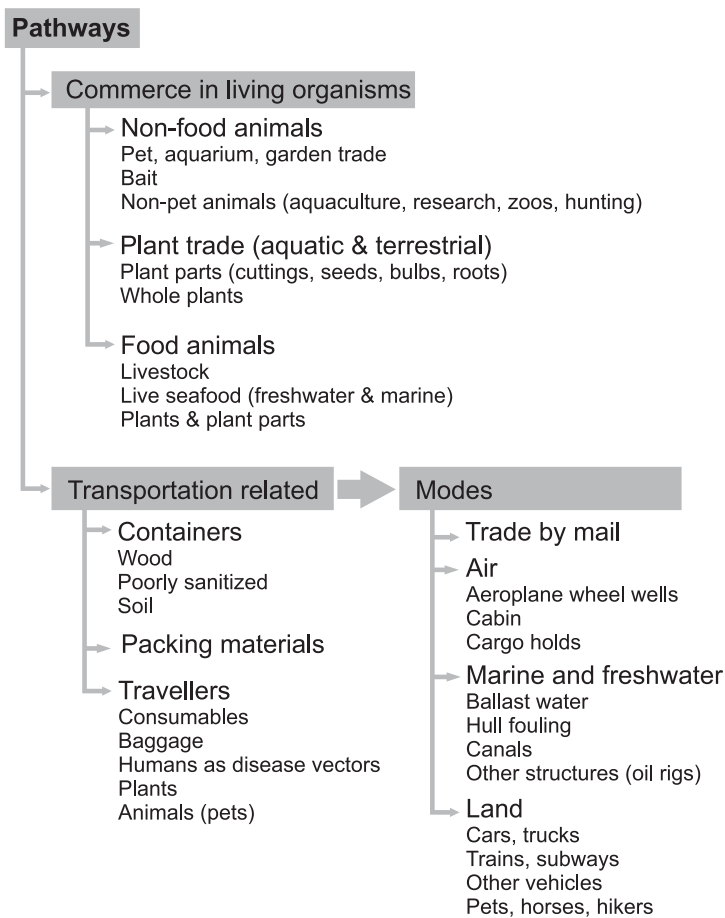
Pathways

Commerce in living organisms

Non-food animals
Pet, aquarium, garden trade
Bait
Non-pet animals (aquaculture, research, zoos, hunting)

Plant trade (aquatic & terrestrial)
Plant parts (cuttings, seeds, bulbs, roots)
Whole plants

Food animals
Livestock
Live seafood (freshwater & marine)
Plants & plant parts

Transportation related   **Modes**

Containers   **Trade by mail**
Wood
Poorly sanitized   **Air**
Soil   Aeroplane wheel wells
                                     Cabin
Packing materials   Cargo holds

Travellers   **Marine and freshwater**
Consumables   Ballast water
Baggage   Hull fouling
Humans as disease vectors   Canals
Plants   Other structures (oil rigs)
Animals (pets)
                                     Land
                                     Cars, trucks
                                     Trains, subways
                                     Other vehicles
                                     Pets, horses, hikers

Fig. 1 Pathways responsible for the introduction of alien species

last water (Darrigran and Pastorino 1995), and its secondary spread to the Guaíba basin in Brazil likely occurred via ballast water originating in Argentina (Darrigran and Mansur 2009). The other primary vector is hull fouling. Hull fouling is a key mechanism for intra- and interbasin expansion (see "Distribution and Colonization of *Limnoperna fortunei*: Special Traits of an Odd Mussel" in this volume; Boltovskoy et al. 2006; Belz et al. 2012). Commerce in living organisms (Fig. 1), in turn, is thought to have been instrumental in the introduction of the golden mussel to Japan, presumably from China and/or Korea with edible freshwater Asian clams (*Corbicula fluminea*) (Nishimura and Habe 1987; see "Colonization and Spread of *Limnoperna fortunei* in Japan" in this volume). Secondary spread is also likely when larvae are inadvertently transported between basins with live fish bait of sport fishermen, or in the guts of fishes that swallow but do not digest mussels (Belz et al. 2012).

In South America, physiographic, hydrosedimentological, and chemical conditions of the large floodplain rivers and associated water bodies of the Río de la Plata watershed are particularly favorable for the spread of *L. fortunei* (Darrigran et al. 2012). "Jump dispersal," which combines upstream "jumps" of adults byssally attached to ship hulls followed by downstream passive drift of planktonic larvae (MacIsaac et al. 2001), has likely been instrumental in the swift dispersal of *L. fortunei* throughout this watershed (Boltovskoy et al. 2006). This mechanism has allowed the golden mussel to disperse upstream at a rate of approximately 240 km per year along the Paraná–Paraguay rivers since its introduction (Darrigran and Damborenea 2011).

It is now clear that *L. fortunei* has come to stay in both Asia and South America, and we must learn to live with it. This involves developing appropriate strategies to handle this bioinvasion. An adequate management program should take into consideration that *L. fortunei* has invaded two types of environments: the human environment and the natural environment. In both cases, the ultimate goal should be prevention of new invasions and controlling the spread of existing ones. Actions should be implemented at various levels, from the highest global level to the local level (DePoorter 2009).

Actions in the Human Environment

Invasive species can be harmful to environmental services, with negative effects on food production, biodiversity, the health of plants, animals, and man, public infrastructures, and therefore, human welfare (Pimentel 2002). The golden mussel is no exception. It interferes with fish culturing activities (e.g., in China, Uruguay, and Brazil), and water potabilization and irrigation systems, and it affects the operation of cooling systems in power plants and industries (Darrigran 2010; see "Impacts of *Limnoperna fortunei* on Man-made Structures And Control Strategies: General Overview" in this volume). Thus, every installation using raw water from water bodies in areas affected by the invasion must take measures to prevent and control

biofouling of *L. fortunei*. Planning and implementation of these measures require knowledge of the mussel's biological traits, in particular its life cycle, and the climate of the region, as well as a thorough understanding of structural and functional details of the plant in question (Mackie and Claudi 2010, Darrigran and Pereyra 2011).

Early detection of *L. fortunei* is critical in establishing prevention and control measures. Due to the biological characteristics of this species (planktonic larval stages and sessile benthic adults), monitoring for the presence of the golden mussel can be accomplished through various methods: (1) detection of adults, either by direct observation of suitable existing substrata where the mussels develop, or through deployment of artificial substrata that provide surface for colonization and (2) detection of the larval planktonic stage with the aid of plankton samples subsequently analyzed under the microscope or processed using molecular methods.

The presence of adults indicates that the invasion is already underway, also furnishing information on the population dynamics of the mussel. Monitoring of the larvae, on the other hand, may allow detection of the species before it has become firmly established and achieved extensive spread.

Detection of Juveniles and Adults

Invasion of the golden mussel created a new scenario for many ecosystem compartments, and particularly for the benthos (Darrigran 2002; Boltovskoy and Correa 2015; see "Relationships of *Limnoperna fortunei* with Benthic Animals" in this volume). Monitoring of golden mussel populations in order to quantify densities, establish their structure and reproductive status, and describe their growth can be carried out in the vicinity of water intakes that feed the cooling systems of industrial facilities, or even within the cooling systems themselves (see Pereira et al. 2012 for a selection of applicable methods).

Artificial substrates provide suitable surfaces for colonization by benthic organisms and are widely used in the study of freshwater sessile macroinvertebrates (see see Fig. 2 in Chapter "Population dynamics and growth of *Limnoperna fortunei*" in this volume). Experimental frames of various designs and materials (polyvinyl chloride (PVC), concrete, wood, ceramics, various plastics, nettings, fabrics, etc.) were used in several studies, both in South America (Boltovskoy and Cataldo 1999; Fontes et al. 2002; Darrigran et al. 2007; Queiroz et al. 2007; Sylvester et al. 2007; Santos et al. 2008; Sardiña et al. 2008; Bergonci et al. 2009; Mansur et al. 2009; Belz et al. 2010; Pereira et al. 2010; Volkmer Ribeiro et al. 2010; Bonel 2011) and in Asia (Morton 1977; Ohkawa et al. 1999; Matsui et al. 2001, 2002; Nagaya et al. 2001; Nakano et al. 2010, 2011; Xu et al. 2013). The use of artificial substrates has both advantages and disadvantages. Among the former, ease of sample retrieval and density estimates, as well as the ability to ascertain the initial time of colonization, are important. On the other hand, they can be vandalized, lost, broken, exposed to air thereby killing adhering organisms, or undergo excessive siltation thus precluding mussel settlement or even causing death of already established colonizers (Sylvester 2006). In order to preclude predation of the mussels settling on the exposed artificial

substrata (predation can eliminate up to over 90 % of *L. fortunei* biomass: Sylvester et al. 2007; Nakano et al. 2010), they can be protected with mesh or plastic netting. These enclosures can either be deployed barren of mussels, allowing the protected substrata to be colonized by drifting veligers and develop mussel beds, or stocked with mussels whose number and sizes have been pre-established, prior to deployment.

Detection of Larvae

Light Microscopy

Larvae of *L. fortunei* in the water column can be sought and quantified using plankton samples, obtained either by towing a net or by filtering a known volume of water with the aid of a suction pump (recommended when quantitative data are needed; see Boltovskoy 1981; Harris et al. 2000; Alder and Morales 2007; Suthers and Rissik 2009; Santos et al. 2012 for details on zooplankton sampling methods). This approach has been used extensively, usually to investigate seasonal changes in the reproductive activity of the mussel (see "Reproductive Output and Seasonality of *Limnoperna fortunei*" in this volume). However, precise abundance estimates are time consuming, and may require that large volumes of water be filtered, especially when larvae are scarce. If other molluscan species with planktonic larvae are present in the area, identification of different species will be also necessary (Ezcurra de Drago et al. 2006; Mansur et al. 2012a).

Molecular Methods

Pie et al. (2006) and Boeger et al. (2007) described a polymerase chain reaction (PCR)-based molecular method for the detection of *L. fortunei* larvae (see Tscha et al. 2012 for a detailed description). This method is very sensitive, detecting as little as 0.041 ng of *L. fortunei* DNA (a single larva yields ~28 ng of DNA), or the equivalent of one larva in 200 L of water (Pie et al. 2006; Tscha et al. 2012). Typical larval densities range around 1 to >20 larvae/L (see "Reproductive Output and Seasonality of *Limnoperna fortunei*" in this volume). Thus, this technique allows confirmation of the presence of mussels at a very early stage, before they have been detected by direct observation (Darrigran et al. 2009).

Although the molecular PCR-based method allows assessing the presence of *L. fortunei* larvae with high accuracy, larval densities cannot be quantified. In the past decade, the use of real-time quantitative PCR (qPCR) became widespread for detecting and quantifying specific DNA in a DNA complex solution. This technique can be used for the quantification of planktonic organisms, including larvae of *L. fortunei* (Endo et al. 2009). Field-collected plankton is subjected to DNA extraction and qPCR analysis using specific primers for the golden mussel (developed from CO1). Highly specific amplification, formed by 138 bases, indicates the presence of

larvae in a sample and the number of larvae can be assessed based on the amplification factor (Endo and Nogata 2012).

These molecular techniques are operationally faster than traditional methods, more accurate, and they do not require taxonomic knowledge for the identification of larvae. On the other hand, the economic costs of these analyses, especially when considering the hardware required, are significantly higher.

Actions in the Natural Environment

The mechanisms involved in biological invasions comprise two complementary components: one is associated with the ability of a given species to invade a new range ("invasiveness"), and the other is the susceptibility of a given environment to be invaded ("invasibility") (Hicks 2004). Both must be taken into account when designing suitable strategies for curtailing bioinvasions.

Criteria for Defining an Invasive Species

Scoring of harmful invasive species as a function of their nuisance may be necessary when priorities in resource allocation are unavoidable. Thus, management efforts focus on the most problematic invasive species. However, deciding which species should be fought first is often not a straightforward issue. Increasing awareness of bioinvasions and the problems they cause have resulted in a growing volume of scientific publications on this topic (Kolar and Lodge 2001), as well as an accumulation of technical terms, often synonymous (Colautti and MacIsaac 2004; Lockwood et al. 2007). This has hindered both investigation and decision-making processes associated with the management of bioinvasions. The Convention on Biological Diversity defines invasive species as those that thrive unaided by humans and threaten natural or seminatural habitats outside of their normal area of distribution. Some native species can become invasive when they are transported to other areas within the same country (Simkanin et al. 2009), or even to nearby regions within the same ecosystem, especially if conditions for their survival, growth, and expansion have been modified (see "Distribution and Colonization of *Limnoperna fortunei*: Special Traits of an Odd Mussel" in this volume) (Xu et al. 2014).

Valéry et al. (2008) pointed out that the most widespread criteria to define invasive species have been the biogeographic criteria and the criteria related with their impact. The biogeographic approach is fairly practical, since it is based on an objective assessment of geographic distribution. An introduced species is one that manages to overcome a geographic barrier. This criterion thus allows for fast implementation of management options at an early stage of transfer from the original range. The impact criterion, on the other hand, requires that, in order to be considered invasive, a species must have a larger impact in the invaded ecosystem than the one it has in its native range. Assessment of the magnitude of impact, however, is unclear and questionable.

A more comprehensive definition of invasive species should be based on a series of requirements that the invasive species meets. As pointed out above, a biological invasion occurs when a species, aided by human activities, overcomes a number of barriers (Fig. 3), colonizes a new area where it acquires a competitive advantage, and grows rapidly in density and distribution. This may result in functional dominance of the system, which in turn can threaten native biodiversity and cause economic damage

Designing a Plan for Sustainable Management of Invasive Species

Cowie (2004) proposed three basic components for the conservation of biodiversity, which can also be useful for strategies of management of bioinvasions. The components are: (1) generation of scientific knowledge, (2) management, and (3) sociopolitical environment.

Scientific knowledge must reach the level of local and regional environmental organizations in order to help shape an adequate management plan. However, even when the requirements of scientific knowledge and management are met, without social awareness and support from environmental policies, conservation of biodiversity and management of bioinvasions can only succeed in small areas and for short periods. Social awareness is a sine qua non condition for active social involvement in the problem. Without it, success will be regionally restricted and temporally short. The social component is obviously the most complex of the three.

Generation of Scientific Knowledge

The results of scientific research aimed at assessing the risk of invasion should be explored and used by managers and decision makers. After the pioneering work of B. Morton in Hong Kong (Morton 1973, 1975, 1977, 1982; Morton et al. 1976), most subsequent information on *L. fortunei* has originated from South America (Argentina, Brazil, including several review books: Penchaszadeh 2005; Darrigran and Damborenea 2006, 2009; Mansur et al. 2012b) and Japan. In this context, investigations oriented at evaluating invasion risk and pathways, as well as possibilities of secondary spread (e.g., Darrigran and Pereyra 2011; Belz et al. 2012; Campos et al. 2012; Darrigran et al. 2012; Sylvester et al. 2013) are of particular significance. As noted by Hicks (2004), work on predicting future invasions is strongly lagging behind when compared to efforts dedicated to assessing impact of invasions that have already occurred. Investigations aimed at prediction, pathway analysis, and ecological scenario building that can be used in decision support systems are urgently needed (Hicks 2004). Some of this type of work has been done with *L. fortunei* (e.g., Kluza and McNyset 2005; Belz 2006, 2009; Oliveira et al. 2010; Campos 2014), but much more is needed.

Fig. 2 Stages in the invasion process of a nonnative species, pointing out activities that need to be carried out at each stage

Species in native range

Prevention

TRANSPORTED
and released alive

ESTABLISHMENT
of a viable population in new location

Eradication

RESTING PERIOD

SPREAD

Control or restoration

ECOLOGICAL IMPACT
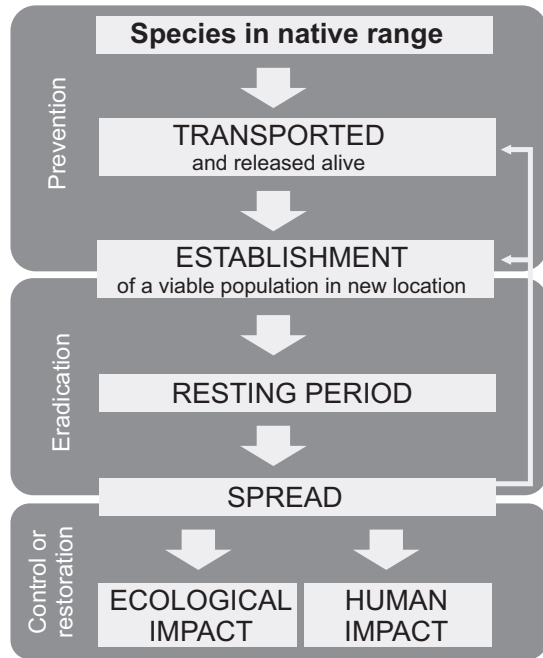
HUMAN IMPACT

Management Plan

Human actions in response to impacts derived from biological invasions are often too late and too weak to significantly mitigate harm (Lodge et al. 2006). This is especially true for the golden mussel, where more than two decades after the introduction and extensive damage to many industrial installations (see "Impacts of *Limnoperna fortunei* on Man-made Structures And Control Strategies: General Overview" in this volume), none of the South American countries colonized by the mussel has developed a centralized, coordinated management strategy, and investment into research and management of this mussel remains very low. In Japan, no mussel quarantines have yet been implemented (see "Colonization and Spread of *Limnoperna fortunei* in Japan" in this volume).

Reversal of this trend will require coordinated management at various levels (regional, global) with federal or national leadership and the close cooperation of state and local government bodies (DePoorter 2009). Effectiveness in the early detection and prevention of biological invasions must increase significantly, thus allowing quick responses to new, potentially harmful introductions, and reducing the spread of existing invasions (Lodge et al. 2006). Actions and investments should be centralized and coordinated by a national center to maximize cost-effectiveness and sustainability of the control efforts.

Analysis of the components of an invasion (invasiveness and invasibility, Hicks 2004), and the stages of the invasion process (Figs. 2 and 3), indicate that each stage is characterized by very dissimilar attributes as to the feasibility and cost of
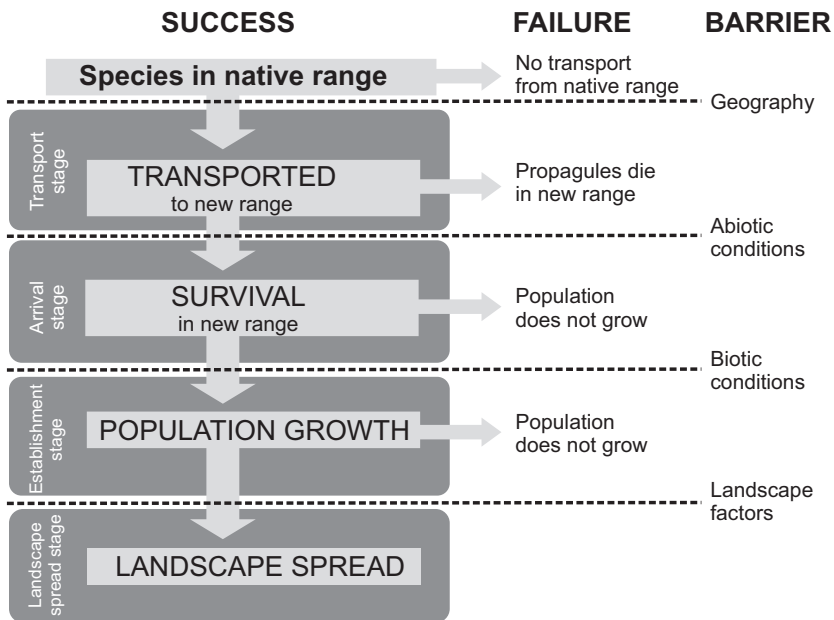
Fig. 3 A conceptual model of the invasion process of a species, highlighting the transition steps between the four stages of invasion

eradicating the invader, which depend, among others, on the ecosystem involved. These stages obviously also determine the best management option available. Management criteria and options eventually adopted, however, are often flawed and ineffective, partly because they do not take these variables into consideration, and partly because prior knowledge based on ad hoc studies is ignored or overlooked. Failure to consider conditions typical of each stage of the invasion can finally result in a useless management strategy. As suggested by Hicks (2004), the longer it takes to start taking action after a new species is introduced, the lower are the probabilities of its eradication and the higher the costs of coping with it. Thus, the best cost-to-benefit combination and the highest chances of eradication are restricted to the early stages of this process, which underscores the importance of early detection. By contrast, once the species has become conspicuous and widespread, the period of high cost-to-benefit ratio has already expired, and the probability of successful eradication is close to zero. By this time, the only remaining option is management of the invasion. Management efforts may be significant when the invasion affects human activities. On the other hand, when biodiversity or other ecosystem attributes are negatively affected but perceived by managers as of minor practical importance, the resulting management efforts are often minor.

Actions associated with reduction of the risk of entry, establishment, and dispersion of invasive species, including *L. fortunei,* fall into three main categories: (1) pre-border actions (adopted by the potential donor country/area aimed at precluding export of the species), (2) border actions, and (3) post-border (emergency)

actions (Maynard and Nowell 2009). Each category, in turn, has two main components: physical (infrastructure, materials, finance) and human (legislation, procedures, capabilities). The first action arising from category (2) is quarantine. In order for quarantines to be effective, there must be a permanent compromise of physical and human resources guaranteeing infrastructure capacity, technical experience, communication capacity, and personnel training. Research on strategies aimed at mitigating bioinvasion-related problems often centers the attention on the receptor country or area, thus minimizing the responsibility of the donor side (Maynard and Nowell 2009). No safeguards are established enforcing the donor country to ensure that products and vectors (e.g., ships and trucks) leaving its ports comply with minimum requirements of safety as far as bioinvasions are concerned.

Lach et al. (2003) performed a survey exploring scientists' and the general public's expectations toward the role of scientists in communication, management, and policy. The five potential roles that research scientists might play were concluded to be: (1) reporting scientific results that others use in making decisions on natural resource management issues, (2) reporting and then interpreting scientific results for others who are involved in natural resource management decisions, (3) working closely with managers and others in integrating scientific results into management decisions, (4) actively advocating for specific and preferred natural resource management decisions, and (5) making decisions about natural resource management and policy. While scientists slightly preferred the interpretive role for themselves, other groups tended to prefer an integrative role for scientists. Most respondents were in favor of scientists getting involved in interpreting and helping to integrate the results of their science into policy decisions. This clearly is the best alternative for the sound management of many environmental issues, including bioinvasions. However, the administrative and political scenarios in many of the countries invaded by *L. fortunei* strongly hinder integration between administration and science.

Developing Social Awareness

Implementation of an integrated and sustainable management plan of biological invasions requires consideration of several components, including scientific knowledge and a blueprint for sound actions (Darrigran et al. 2008). Action must be taken at two levels: (1) in society in general, through formal and informal education with publicity strategies, documentaries, etc., seeking to generate demand at the following level and (2) among public officials, advocating for the development of norms and management programs to cope with bioinvasions in a timely manner and soundly.

An educational program aimed at the general public should include the following components: (1) it should inform society timely and thoroughly, making it aware of the ecological, economic, and social impacts that invasive species generate, including specifics as to the status and vulnerability of the different sectors endangered, (2) it should foster interinstitutional cooperation for the formation of specific work groups, timely participation of the communication media, and, above all, the co-

operation of the different sectors involved, including civil organizations, and (3) it should implement programs in environmental education aimed at generating social awareness that will help prevent new introductions and facilitate early detection of nonnative species.

The economic burden of these actions is often lighter than that of control and eradication programs.

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