

Harmonia⁺ and *Pandora*⁺: risk screening tools for potentially invasive plants, animals and their pathogens

Bram D'hondt · Sonia Vanderhoeven · Sophie Roelandt · François Mayer ·
Veerle Versteirt · Tim Adriaens · Els Ducheyne · Gilles San Martin ·
Jean-Claude Grégoire · Iris Stiers · Sophie Quoilin · Julien Cigar ·
André Heughebaert · Etienne Branquart

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Abstract Given the large number of alien species that may potentially develop into invasives, there is a clear need for robust schemes that allow to screen species for such risks. The *Harmonia*⁺ framework presented here brings together 30 questions that refer to distinct components of invasion. Together, they cover the stages of introduction, establishment, spread, and multiple kinds of impacts, viz. referring to the health of the environment (including wild species), cultivated plants, domesticated animals and man. In a complete assessment, input is provided by choosing among predefined ordinal answers and by supplementing these with textual clarification. Uncertainty is covered by indicating levels of confidence. By

converting answers into scores, which are then condensed into summary statistics, *Harmonia*⁺ allows for quantitative output on stage-specific and general risks. Test assessments on five species emerging in Belgium showed the perceived environmental risks of *Procambarus clarkii* to be highest (0.72), and that of *Threskiornis aethiopicus* to be lowest (0.13). Given the considerable parallels that exist between invasive alien species and emerging infectious diseases, we additionally created *Pandora*, which is a risk analysis scheme for pathogens and parasites. It consists of 13 key questions and has the same structure as *Harmonia*⁺. Since diseases play a paramount role in biological invasions, results of *Pandora* assessments may feed into *Harmonia*⁺ through a slightly adapted, host-specific version named *Pandora*⁺. *Harmonia*⁺, *Pandora* and *Pandora*⁺ may be used both for prioritization purposes and for underpinning detailed risk analyses,

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B. D'hondt · S. Vanderhoeven · J. Cigar ·
A. Heughebaert
Belgian Biodiversity Platform, Belgian Science Policy
Office, Avenue Louise 231, 1050 Brussels, Belgium
e-mail: bram.dhondt@inbo.be

B. D'hondt · T. Adriaens
Research Institute for Nature and Forest, Kliniekstraat 25,
1070 Brussels, Belgium

S. Vanderhoeven · E. Branquart (✉)
Département d'Étude du Milieu Naturel et Agricole,
Service Public de Wallonie, Avenue Maréchal Juin 23,
5030 Gembloux, Belgium
e-mail: etienne.branquart@spw.wallonie.be

S. Roelandt
Veterinary and Agrochemical Research Centre,
Groeselenberg 99, 1180 Brussels, Belgium

F. Mayer · J.-C. Grégoire
Biological Control and Spatial Ecology, Université Libre
de Bruxelles, Avenue F. Roosevelt 50, 1050 Brussels,
Belgium

V. Versteirt · E. Ducheyne
Avia-GIS, Precision Pest Management Unit, Risschotlei
33, 2980 Zoersel, Belgium

and can be consulted online through <http://ias.biodiversity.be>.

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Introduction

Species are termed ‘alien’ if they have become transported outside of their natural range through human actions. If these species are subsequently perceived as causing harm in their alien range, they are further referred to as ‘invasive alien species’ (COP 2002; Simberloff et al. 2013). The costs that come with invasive species damage and control are considerable: e.g., for The Netherlands, European Union, and globally, reported estimates are 1.3 billion (van der Weijden et al. 2007), 12.5 billion (Kettunen et al. 2008) and 1.4 trillion euro per year (Pimentel et al. 2001). Attempts to counteract alien species invasions are therefore undertaken worldwide, and a great deal of dialogue is needed between invasion biology experts and policy makers for these attempts to be successful (Young et al. 2014).

The sooner invasive alien species become tackled during invasion, the more cost-effective measures against them will be (Keller et al. 2007). But before such prevention or early eradication measures can take place, it is essential to first identify those species that pose the highest risk. Given the huge and increasing number of species that become transported and may potentially develop into invasive species, such a prioritization must allow for a high number of species from different taxonomic groups to be assessed in a relatively short time (Hulme et al. 2009). In these respects, it is helpful to have tools available that allow

to condense species information into their perceived risks according to a common framework.

Several years ago, Branquart (2007) launched an advisory quick-screening tool for invasive alien species in Belgium: the Invasive Species Environmental Impact Assessment (ISEIA). It consisted of a simple scheme to address the spread capacity and environmental impacts of alien species based on invasion histories in Belgium and neighbouring areas (instead of a life history approach, thereby classifying as a prioritization rather than a predictive system sensu Randall et al. 2008; Essl et al. 2011). It was subsequently applied by an expert panel to reach consensus scores for the risk that 100 species from different taxonomic groups pose to the Belgian territory. The resulting list was incorporated in an online database named *Harmonia* (Belgian Biodiversity Platform 2014a). Outside of Belgium, the ISEIA protocol has, among others, been used to inform risk analyses of particular species in the Netherlands (e.g. Gyimesi and Lensink 2010, van de Koppel et al. 2012), black lists of vascular plants in Luxembourg (Ries et al. 2013), and a horizon scan of potentially invasive species in Great Britain (Roy et al. 2014a). Although the field of risk analysis of invasive alien species has seen much progress in recent years, a considerable scope for improvement is still left (see reviews by Leung et al. 2012; Kumschick and Richardson 2013).

In particular, the following emerging issues were found to be unsatisfactorily covered in ISEIA. First, frameworks have been constructed that disentangle and define invasion processes better. These frameworks at minimum include stages of transport, establishment, spread and impact (Blackburn et al. 2011; Leung et al. 2012). Second, impacts of invasive alien species range well beyond ecological issues alone, referring also to economic activities or human health (European Environment Agency 2012). In fact, many infectious pests of cultivated plants and domesticated animals are alien organisms, and the same is true for several nuisance organisms of human health concern. Considering also potential impacts on infrastructure, recreation activities, aesthetics, and others, there is much room to improve the integration of these very diverse domains in a common risk analysis framework. Third, there has been an increasing awareness on the roles that parasites and pathogens play in biological invasions. The infections they cause have been identified as a key contributing factor to the

G. San Martin
Walloon Agricultural Research Centre, Rue de Liroux 9,
5030 Gembloux, Belgium

I. Stiers
Plant Biology and Nature Management, Vrije Universiteit
Brussel, Pleinlaan 2, 1050 Brussels, Belgium

S. Quoilin
Belgian Scientific Institute for Public Health, Rue J.
Wytzman 14, 1050 Brussels, Belgium

invasion success of many host species (Dunn and Perkins 2012). In addition, infectious agents hosted by alien species also directly contribute to the latter's suite of impacts, potentially affecting the health of plants, animals or humans (zoonoses; see Taylor et al. 2001). It is therefore critical that risk assessments of alien plants or animals consider the likelihood of entry, exposure and consequence of exotic pathogens and parasites.

On these grounds, we constructed a protocol that allows for a rapid screening of the diverse risks of potentially invasive alien species, and prioritizes them accordingly. The protocol consists of two tools, *Harmonia*⁺ and *Pandora*. These are named after the notoriously invasive Harlequin ladybird *Harmonia axyridis* and the important entomopathogenic fungus *Pandora*, the spread of which seems to be accelerated by *H. axyridis* (Roy et al. 2008). The *Harmonia*⁺ protocol applies to any potentially invasive plant or animal species. The *Pandora* protocol applies to potentially risky parasites and pathogens, the results of which may (but not necessarily need to) feed into *Harmonia*⁺. Both tools incorporate the elements outlined above, among other improvements.

The aim of this paper is to present the different schemes in detail and how they were realized. As an illustration, we also included the results of a preliminary assessment performed on a handful of species and pathogens currently emerging in Belgium.

Methods

We set up a collaboration among experts of different Belgian institutes through a project that lasted from November 2012 to March 2014. These partners drafted the protocol together, each providing input from their field of expertise (cf. spatial modelling, agriculture, forestry, veterinary health, human health or biodiversity conservation; see list of authors).

Furthermore, three groups of collaborators helped steering the project (Table A1 in the Supplementary Material). First, a committee of end users was assembled at the start of the project, as to provide input to maximize the project's relevancy for policy makers. The end-user committee was 25 people strong, representing 15 different administrations or institutes.

Second, a panel of 22 risk analysis experts, representing 21 different institutes from eight different countries, joined a two-day workshop in the 11th month of the project, where a draft version of the protocol was tested and commented on. Experts were divided into small groups according to their expertise (environmental, plant, or human and animal health), then applying the protocol to several test species.

Third, during the 16th month of the project, 30 scientists were asked to individually complete an updated version of the questionnaires for one or a few selected organisms as a proof of concept. Concerning *Harmonia*⁺, these were five species for which the environmental impacts in particular are currently debated for Belgium: *Lithobates catesbeianus* (American bullfrog), *Ludwigia grandiflora* (Water primrose), *Nyctereutes procyonoides* (Raccoon dog), *Procambarus clarkii* (Louisiana crayfish) and *Threskiornis aethiopicus* (Sacred ibis). Concerning *Pandora*⁺, these were Bluetongue virus, Classical Swine Fever, *Echinococcus multilocularis* (Fox tapeworm), *Batrachochytrium dendrobatidis* (Amphibian chytrid fungus) and Ranavirus. Moreover, these scientists were asked to provide comments on the protocols, and also to anonymously score their appraisal of the protocols' clarity, consistency and completeness through an online questionnaire.

The *Harmonia*⁺ protocol

We here present the general structure of the protocol and some of its key elements. The full *Harmonia*⁺ protocol can be consulted and applied online at <http://ias.biodiversity.be> (Belgian Biodiversity Platform 2014b).

Conceptual bases for invasion and risk

Generally, *Harmonia*⁺ operates within the framework of risk analysis, striving for maximal compliance with internationally accepted standards set out through the International Plant Protection Convention (FAO 2006) and the World Organisation for Animal Health (OIE 2013). Here, risk analysis is considered to consist of risk assessment and risk management, and *Harmonia*⁺ only deals with the former.

The framework for invasion is a modified version of that from Blackburn et al. (2011). These authors distinguished a series of stages with, for each stage,

barriers that need to be overcome for an organism to pass on to the next stage (Transport, Introduction, Establishment and Spread). However, we integrated Transport into Introduction because not all organisms encounter the barriers of both stages (i.e. those that become transported unintentionally, Blackburn et al. 2011). Also, we added Impacts at the end. We refer to these stages as *modules*, the definitions of which are given in Table 1.

Entities that potentially bear impacts from the organism are referred to as *targets*. Sectors that deal with specific targets are collectively referred to as a *domain*. We distinguish among the *environmental domain* (wild plants and animals, habitats and ecosystems), *plant domain* (cultivated plants), *animal domain* (domesticated animals), *human domain* (humans) and *other domain* (targets not included elsewhere; Table 1).

Our framework of risk is based on Kinney and Wiruth (1976). 'Risk' indicates the chance that a particular hazardous event (e.g. competition, hybridization) may actually cause damage, and is regarded as a product of three factors: exposure x likelihood x consequence. For instance, whether the poisonous alien mushroom *Leucocoprinus birnbaumii* is of human health concern is a question of (1) whether humans actually encounter *L. birnbaumii* (exposure), (2) whether it becomes eaten, when exposed (likelihood), and (3) the severity of illness, when eaten (consequence).

In effect, exposure is the end result from the successive actions of Introduction, Establishment and Spread, whereas likelihood and consequence together form the backbone of the Impacts. Yet, not every single question on Impacts necessarily asks for both likelihood and consequence, as it may pertain to one of these components more (Daehler and Virtue 2010).

Questions and answers

The questions reflect criteria that are considered important for, and together constitute, each of the invasion stages. In total, 25 questions are included that may contribute to score calculation, as shown in Table 1. The full protocol also includes questions that identify the organism and area under assessment and the person(s) doing the assessment, as well as text fields to comment on each of the answers provided.

Alternative answers to questions are pre-defined and on an ordinal basis (type 'low' < 'medium' < '-high'). Heikkilä (2011) listed some good practice suggestions for prioritization protocols, including the

use of about five alternative answers, naturally depending on the question. We included five answers only when cut-off values are precise, or when two sub-questions become combined into one, and otherwise opted for three alternative answers. Some impact questions include an 'inapplicable' option, when the hazard referred to is theoretically senseless (e.g. interbreeding between plants and animals) and could thus invoke unfair species comparisons. Inapplicable is different from the lowest possible answer in that it omits the question from calculation.

The full *Harmonia*⁺ protocol also provides general guidance on how to answer questions (Belgian Biodiversity Platform 2014b). Key guidelines are, firstly, to base answers as much as possible on evidence and not on a purely hypothetical or speculative basis. Second, to always employ the precautionary principle; e.g., by taking the worst-case scenario when different scenarios are possible. This is in line with a primary principle from the Convention on Biological Diversity (COP 2002). Third, to use cases that are similar in biology or geography when direct evidence appears lacking (the higher the similarity, the better). To minimize ambiguity with regard to the questions and their potential answers, every single question is furthermore provided with ample guidance, including definitions, conceptual underpinnings, cut-off values and specific examples (cf. Gordon et al. 2010).

Score calculation

The methods for calculating scores from the answers provided were chosen to be simple, based on concerns raised by the end-user committee on the tractability of output. A score is calculated for each module separately, and these can then be aggregated into higher-level scores (Fig. 1). We here distinguish among default and alternative operations, the effects of which are discussed below.

The alternative answers within each question are ranked and their ranks re-scaled to a [0,1]-scale (0 = lowest, 1 = highest). The default method within each module then is to take the (arithmetic) mean of the answers provided, optionally with different weights allocated to the questions. The maximum is suggested as an alternative method.

Introduction, Establishment and Spread scores are combined in a joint score that expresses Exposure, by taking the geometric mean. This multiplicative

Table 1 An overview of the modules and questions included in the *Harmonia*⁺ protocol**Introduction**

Questions from this module assess the risk for *The Organism* to overcome geographical barriers and -if applicable- subsequent barriers of captivity or cultivation. This leads to Introduction, defined as the entry of *The Organism* within the limits of *The Area* and subsequently into the wild

- (1) The probability for *The Organism* to be introduced into *The Area*'s wild by natural means is [low | medium | high]
- (2) The probability for *The Organism* to be introduced into *The Area*'s wild by unintentional human actions is [low | medium | high]
- (3) The probability for *The Organism* to be introduced into *The Area*'s wild by intentional human actions is [low | medium | high]

Establishment

Questions from this module assess the likelihood for *The Organism* to overcome survival and reproduction barriers. This leads to Establishment, defined as the growth of a population to sufficient levels such that natural extinction within *The Area* becomes highly unlikely

- (4) *The Area* provides [non-optimal | sub-optimal | optimal] climate for establishment of *The Organism*
- (5) *The Area* provides [non-optimal | sub-optimal | optimal] habitat for establishment of *The Organism*

Spread

Questions from this module assess the risk of *The Organism* to overcome dispersal barriers and (new) environmental barriers within *The Area*. This leads to spread, in which vacant patches of suitable habitat become increasingly occupied from (an) already-established population(s) within *The Area*

- (6) *The Organism*'s capacity to disperse within *The Area* by natural means is [very low | low | medium | high | very high]
- (7) *The Organism*'s frequency of dispersal within *The Area* by human actions is [low | medium | high]

Impacts: environmental targets

Questions from this module qualify the consequences of *The Organism* on wild animals and plants, habitats and ecosystems

- (8) *The Organism* has a(n) [inapplicable | low | medium | high] effect on native species, through predation, parasitism or herbivory
- (9) *The Organism* has a [low | medium | high] effect on native species, through competition
- (10) *The Organism* has a(n) [no / very low | low | medium | high | very high] effect on native species, through interbreeding
- (11) *The Organism* has a [very low | low | medium | high | very high] effect on native species, by hosting pathogens or parasites that are harmful to them
- (12) *The Organism* has a [low | medium | high] effect on ecosystem integrity, by affecting its abiotic properties
- (13) *The Organism* has a [low | medium | high] effect on ecosystem integrity, by affecting its biotic properties

Impacts: plant targets

Questions from this module qualify the consequences of *The Organism* on cultivated plants (e.g. crops, pastures, horticultural stock)

- (14) *The Organism* has a(n) [inapplicable | very low | low | medium | high | very high] effect on plant targets, through herbivory or parasitism
- (15) *The Organism* has a(n) [inapplicable | very low | low | medium | high | very high] effect on plant targets, through competition
- (16) *The Organism* has a(n) [inapplicable | no / very low | low | medium | high | very high] effect on plant targets, by interbreeding with related organisms or with the target itself
- (17) *The Organism* has a [very low | low | medium | high | very high] effect on plant targets, by affecting the cultivation system's integrity
- (18) *The Organism* has a(n) [inapplicable | very low | low | medium | high | very high] effect on plant targets, by hosting pathogens or parasites that are harmful to them

Impacts: animal targets

Questions from this module qualify the consequences of *The Organism* on domesticated animals (e.g. production animals, companion animals)

- (19) *The Organism* has a(n) [inapplicable | very low | low | medium | high | very high] effect on individual animal health or animal production, through predation or parasitism
- (20) *The Organism* has a [very low | low | medium | high | very high] effect on individual animal health or animal production, by having properties that are hazardous upon contact
- (21) *The Organism* has a(n) [inapplicable | very low | low | medium | high | very high] effect on individual animal health or animal production, by hosting pathogens or parasites that are harmful to them

Table 1 continued

Impacts: human targets

Questions from this module qualify the consequences of *The Organism* on humans. It deals with human health, being defined as a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity

- (22) *The Organism* has a(n) [inapplicable | very low | low | medium | high | very high] effect on human health, through parasitism
- (23) *The Organism* has a [very low | low | medium | high | very high] effect on human health, by having properties that are hazardous upon contact
- (24) *The Organism* has a(n) [inapplicable | very low | low | medium | high | very high] effect on the health of human targets, by hosting pathogens or parasites that are harmful to them

Impacts: other targets

Questions from this module qualify the consequences of *The Organism* on targets not considered in previous modules

- (25) *The Organism* has a [very low | low | medium | high | very high] effect on causing damage to infrastructure

The full version (Belgian Biodiversity Platform 2014b) also includes

1. Questions on the biological and geographical context (viz. ‘*The Organism*’ and ‘*The Area*’)
2. A question on the level of confidence for each relevant question
Answer provided with a [low | medium | high] level of confidence
3. A comment box for each question
4. Extensive guidance for each question
5. Examples for each question

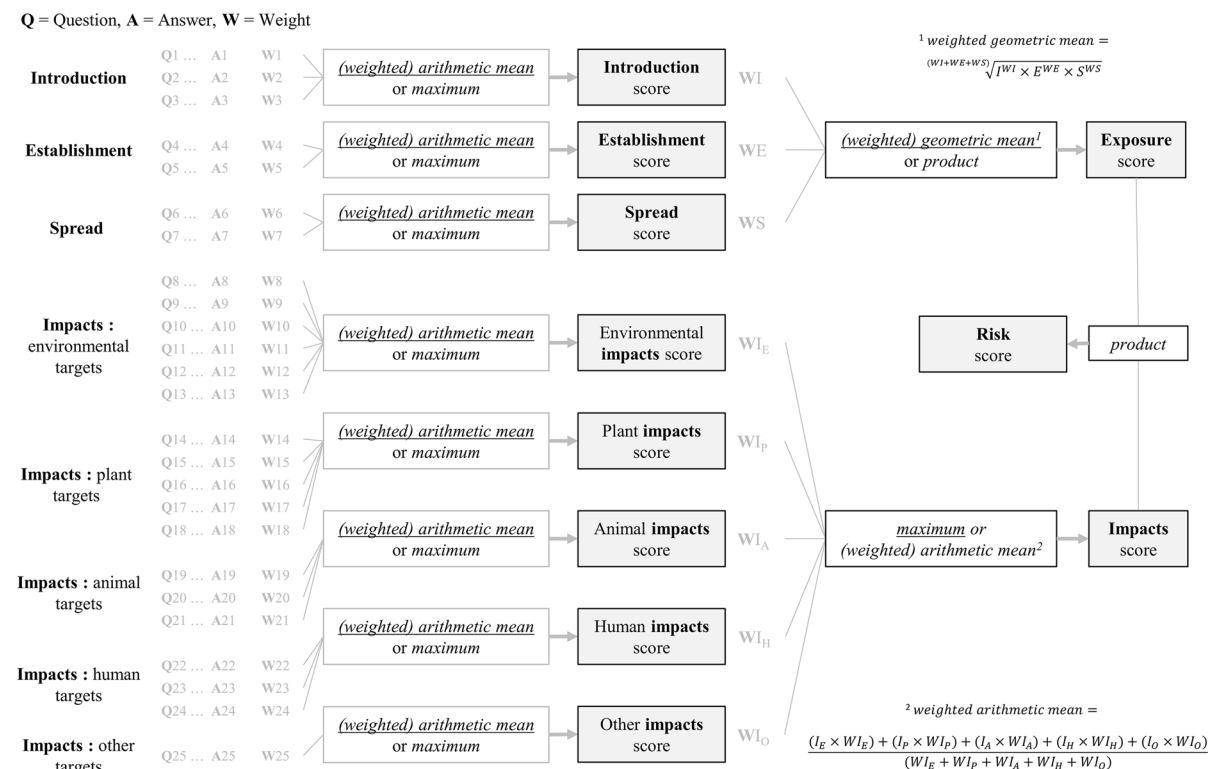


Fig. 1 The mathematical backbone of the *Harmonia+* protocol. The operations proposed as default are *underlined*

approach follows the invasion concept, with the score being zero as soon as any of its three constituent processes fail (Blackburn et al. 2011). The geometric

mean also allows for the processes to be given different weights. Here, use of the product is provided as an alternative option.

The scores from the different Impact modules (i.e., the Environmental, Plant, Animal, Human and Other impact scores) can become aggregated into a general Impact score, either by taking the maximum if the user considers the highest risk as defining (default), or by taking the (weighted) arithmetic mean if the user considers risks to be additive.

Finally, Exposure and overall Impact can become multiplied to yield an overall Risk score for the species at hand, following the risk framework above (Kinney and Wiruth 1976).

Uncertainty

Any component of invasion risk is liable to uncertainty, which in fact lies at the very base of performing risk analyses (Leung et al. 2012). For every relevant question, an assessor using *Harmonia*⁺ is therefore asked to provide a level of confidence with his/her answer provided ('low', 'medium', 'high'). Following Mastrandrea et al. (2011), this level of confidence reflects a combination of the average robustness of pieces of evidence ('limited', 'medium', 'robust'), and the degree of agreement between pieces ('low', 'medium', 'high'). Module and higher-level scores that summarize the overall level of uncertainty are calculated parallel to, and by the same operations as, the scores above.

The Pandora protocol

Pandora is a risk screening protocol designed for emerging or re-emerging, pathogenic or parasitic micro-organisms. Given the many parallels with biological invasions, *Pandora* essentially uses the same structure and mode of operation as *Harmonia*⁺. Yet, the stages covered are referred to as Entry, Exposure and Consequence, in accordance with the risk assessment steps set out by the World Organisation for Animal Health (OIE 2013). The former modules determine the Emergence status of the pathogen at hand, while the Consequence modules again refer to concerns for the environmental, plant, animal, human and other domains. In total, 13 questions were identified that together constitute the perceived risk of a pathogen, as shown in Table 2. Guidance, cut-off values and examples in *Pandora* are taken from veterinary to human health science. The reservoir in which the pathogen resides does not need to be specified in *Pandora*, and may include plant or animal species.

Pandora⁺

Since the risk of an alien plant or animal species (dealt with by *Harmonia*⁺) is often linked to the risk of a pathogen or parasite being hosted by that species (dealt with by *Pandora*), we furthermore created a version of *Pandora* that is restricted to one specific host organism. This so-called *Pandora*⁺ protocol thus asks for the risk of a particular pathogen or parasite to be introduced by a particular host species. It thus bridges the two other protocols, and its resulting scores may feed directly into a *Harmonia*⁺ assessment.

The full *Pandora* and *Pandora*⁺ protocols can be consulted online through the link provided above (Belgian Biodiversity Platform 2014b).

Results

Use of *Harmonia*⁺

For the assessments from the scientific panel, it was chosen beforehand to apply all 'default' operations and consider all question and module weights equal. The results for the *Harmonia*⁺ assessments are shown in Table 3. The accompanying confidence scores for the modules are shown in Table A2 in the Supplementary Material.

All five species are currently emerging in Belgium, which is reflected in the Exposure scores (Table 3). Taking the inter-assessor disagreement into account, it is clear that these species fall in two distinct groups according to their perceived environmental risks: while *P. clarkii*, *L. catesbeianus* and *L. grandiflora* are attributed relatively high risk scores, *N. procyonoides* and *T. aethiopicus* are deemed relatively low-risk species. Their environmental impacts are further itemized according to the different criteria in Table 4, with a summary of the textual support provided by assessors. Note that the guidance for environmental impacts asks users to select higher scores if the impacted species or ecosystems are of conservation concern, i.e. threatened, keystone or emblematic species, or natural ecosystems (Belgian Biodiversity Platform 2014b). Noteworthy impacts from the other modules relate to disease transmission from *N. procyonoides* to animal targets and humans, and effects on watercourses by *P. clarkii* and *L. grandiflora* (Table 3). More elaborate

Table 2 An overview of the modules and questions included in the *Pandora* protocol

Entry

Questions from this module assess the likelihood for (re)emerging pathogenic agents to be (re)introduced into the environment of *The Area*

- (1) The probability of *The Pathogen* to be introduced into *The Area* is [low | medium | high]

Exposure

Questions from this module assess the pathways necessary for exposure of pathogenic agents to targets in *The Area*

- (2) *The Pathogen* has a [low | medium | high] probability to be maintained and spread in *The Area*
 (3) The probability for *The Pathogen* to be transmitted from its reservoir to individual targets is [low | medium | high]

Consequence: environmental targets

Questions from this module qualify the consequences of *The Pathogen* on wild animals and plants, habitats and ecosystems

- (4) *The Pathogen* has a [low | medium | high] effect on native species individuals
 (5) *The Pathogen* has a [no / very low | low | medium | high | very high] effect on native species populations

Consequence: plant targets

Questions from this module qualify the consequences of *The Pathogen* on cultivated plants (e.g. crops, pastures, horticultural stock)

- (6) *The Pathogen* has a(n) [inapplicable | low | medium | high] effect on individual plants
 (7) *The Pathogen* has a(n) [inapplicable | no / very low | low | medium | high | very high] effect on plant populations

Consequence: animal targets

Questions from this module qualify the consequences of *The Pathogen* on domesticated animals (e.g. production animals, companion animals)

- (8) *The Pathogen* has a(n) [inapplicable | low | medium | high] effect on the health (physical well-being and welfare) of individual animals
 (9) *The Pathogen* has a(n) [inapplicable | no / very low | low | medium | high | very high] effect on the health (physical well-being and welfare) or production of animal populations

Consequence: human targets

Questions from this module qualify the consequences of *The Pathogen* on humans. It deals with human health, being defined as a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity

- (10) *The Pathogen* has a(n) [inapplicable | low | medium | high] effect on the health (physical, mental or social well-being) of individual humans
 (11) *The Pathogen* has a(n) [inapplicable | no / very low | low | medium | high | very high] effect on the health (physical, mental or social well-being) of the human population

Impacts: other targets

Questions from this module qualify the consequences of *The Pathogen* on targets not considered in previous modules

- (12) *The Pathogen* has a(n) [inapplicable | low | medium | high] effect on international trade and tourism
 (13) *The Pathogen* has a(n) [inapplicable | low | medium | high] effect on public attention and perception

The full version (Belgian Biodiversity Platform 2014b) also includes

1. Questions on the biological and geographical context (viz. '*The Organism*' and '*The Area*')

2. A question on the level of confidence for each relevant question

Answer provided with a [low | medium | high] level of confidence

3. A comment box for each question

4. Extensive guidance for each question

5. Examples for each question

information on these particular species can be found in risk analysis reports for Belgium (Adriaens et al. 2013; Baiwy et al. 2013; Delsinne et al. 2013; Robert et al. 2013; Vanderhoeven 2013).

All results have been reported back to the scientific panel with a call to comment on the validity of the output with regard to their species, but no critiques or disagreements were received.

Table 3 Example output of a quantitative risk assessment performed with *Harmonia*⁺

Taxon assessed	Area assessed	Number of assessors	Introduction score	Establishment score	Spread score	Exposure score
<i>Procambarus clarkii</i> (Louisiana crayfish)	Belgium	3	0.56	0.83	0.54	0.62
<i>Lithobates catesbeianus</i> (American bullfrog)	Belgium	3	0.39	1.00	0.58	0.60
<i>Ludwigia grandiflora</i> (Water primrose)	Belgium	3	0.78	0.92	0.67	0.75
<i>Nyctereutes procyonoides</i> (Raccoon dog)	Belgium	3	0.44	0.92	0.46	0.56
<i>Threskiornis aethiopicus</i> (Sacred ibis)	Belgium	3	0.44	0.75	0.58	0.57

Taxon assessed	Environmental impacts score	Plant impacts score	Animal impacts score	Human impacts score	Other impacts score	Impacts score	Risk score
<i>Procambarus clarkii</i> (Louisiana crayfish)	0.72	0.17	0.17	0.08	0.42	0.72	0.48
<i>Lithobates catesbeianus</i> (American bullfrog)	0.61	0.00	0.19	0.04	0.00	0.61	0.37
<i>Ludwigia grandiflora</i> (Water primrose)	0.52	0.06	0.00	0.00	0.92	0.92	0.68
<i>Nyctereutes procyonoides</i> (Raccoon dog)	0.22	0.03	0.22	0.29	0.00	0.29	0.16
<i>Threskiornis aethiopicus</i> (Sacred ibis)	0.13	0.06	0.10	0.04	0.17	0.22	0.13

Shown is the mean score from the assessors. Default operations used; all module and question weights considered equal. The species are ranked according to their environmental impacts score

Use of Pandora and Pandora⁺

The results for the *Pandora* assessments are shown in Table A3 in the Supplementary Material. These, for instance, show that the general risk of Bluetongue virus to Belgium is considered higher than that of Classical Swine Fever because of a higher emergence risk, whereas their perceived consequences on animal health are the same. The test assessments using *Pandora*⁺ are shown in Table A4. As these assessments were made with regard to invasive alien species as host organisms (viz. *N. procyonoides* and *L. catesbeianus*), the risk scores tabulated here can be used to feed questions 11, 18, 21 and 24 in assessments of their hosts with *Harmonia*⁺ (Table 1).

Feedback from assessors

The majority of assessors that completed the anonymous survey granted *Harmonia*⁺ medium to high scores for clarity, consistency and completeness, and this was somewhat less for *Pandora/Pandora*⁺ (Fig. 3).

Discussion

Any risk analysis scheme on biological invasions taking a format such as the one used here, essentially

serves as a template for collecting proof on the invasion risk of a particular species. This meets a ‘prudential’ rather than ‘evidential’ burden of proof (Pigliucci and Boudry 2014), because the potential costs of misclassifying a riskful species as risk-free are significantly different from the reverse situation. This cost asymmetry, together with the subjectivity on what is perceived as ‘costly’, inevitably makes the construction of such risk analysis schemes a somewhat perilous undertaking. We therefore involved as many different contributors as possible in the realization of *Harmonia*⁺ and *Pandora*, attempting to end up with relevant schemes for assessing the risk of potentially invasive organisms.

The *Harmonia*⁺ protocol encapsulates all elements that were already present in its predecessor ISEIA (Branquart 2007). If only a quick assessment of spread and environmental impacts is needed, ISEIA may therefore still be the method of choice. Yet, *Harmonia*⁺ includes a considerable number of new elements, many of which were suggested in recent reviews of risk assessment methods, such as the inclusion of non-environmental impacts (Verbrugge et al. 2010), the inclusion of all invasion stages (Leung et al. 2012) and the separation of likelihood from magnitude (Kumschick and Richardson 2013). Overall, *Harmonia*⁺ allows for fairly complete information, as well as an increased resolution, compared to ISEIA.

Table 4 Assessed environmental effects of the example species by Belgian experts using the *Harmonia*⁺ criteria

Taxon assessed	(8) predation, parasitism or herbivory	(9) competition	(10) interbreeding	(11) pathogens or parasites	(12) abiotic properties	(13) biotic properties
<i>Procambartus clarkii</i>	[NA L M 3H] Heavily predate on invertebrates, amphibians, and fish eggs and larvae	[L M 3H] Strong interspecific competition with native crayfish species	[3N/VL L M H VH]	[VL L M H 3VH] Asymptomatic carrier of pathogen that are detrimental to <i>Astaculus astaculus</i> , incl. the crayfish plague	[1L M 2H] Increases water turbidity and affects nutrient and energy flows	[1L M 2H] May strongly alter the trophic structure of freshwater communities through tow-down and bottom-up effects
<i>Lithobates catesbeianus</i>	[NA L M 2H] Displaces native amphibians by predation	[L M 2H] Strong interspecific competition with native amphibians	[3N/VL L M H VH]	[VL L M 2H 1VH] Carrier of pathogens that are detrimental to native amphibians, incl. ranavirus and chytrid fungus	[2L M 1H] Tadpoles may affect nutrient cycling and the production rate of phytoplankton in water bodies	[L M 2H] Cascading effects in the food web enhanced by facilitation with non-native fish
<i>Ludwigia grandiflora</i>	[3NA L M H] May cause local decline of amphibian populations; behaves more as a scavenger than an active predator	[L M 3H] Forms dense monospecific stands outcompeting most native aquatic plants	[2N/VL L M H VH]	[3VL L M H VH] May locally outcompete red fox through spillback of rabies and canine distemper viruses	[L M 2H] Dense mats alter waterflow, accelerate siltation and favour eutrophication and anoxic water conditions	[L 2M 1H] Dense mats may affect invertebrate and fish communities (understudied)
<i>Nyctereutes procyonoides</i>	[NA L 3M H] May cause local decline of amphibian populations; behaves more as a scavenger than an active predator	[2L M H] Limited impact on populations of native carnivores	[3N/VL L M H VH]	[VL IL M H VH] May locally outcompete red fox through spillback of rabies and canine distemper viruses	[3L M H]	[2L M H]
<i>Threskiornis aethiopicus</i>	[NA IL 2M H] Rare and anecdotal predation events on protected bird species (terns)	[2L M H] Anecdotal disturbance of heron nesting colonies	[3N/VL L M H VH]	[1VL IL M H VH] May contribute to the dissemination of native pathogens	[3L M H]	[3L M H]

The number of attributed scores is given, together with a summary of comments provided (NA inapplicable, N/VL no/very low, VL very low, L low, M medium, H high, VH very high)

The *Harmonia*⁺ and *Pandora* schemes allow for different strategies of use, and the schemes are particularly flexible in this regard. The numerical analysis outlined above allows for a clear ranking of species' overall risks. As such, the schemes can be used both for horizon scanning of emerging species and for prioritization schemes of already-present species. Since it is not compulsory to include all modules for *Harmonia*⁺ to yield output, and since the scores can be combined in different ways, it should be fit for many different purposes; whether this is to inform prevention measures, define exposure, compare different risks, or rather emphasize the general risk. At the other extreme, the schemes may facilitate the formulation of a detailed risk analysis report, if emphasis is put on the textual clarification that comes with the answers rather than on a quantitative digest. Indeed, it may not always be informative nor desirable to condense existing information in only a handful of numbers.

Though any user is essentially free to use the protocols as (s)he wishes, our envisioned process for optimal use would be through a multi-expert, two-stage approach. First, assessments are performed individually by several experts, allowing each of them to formulate his/her personal ideas. Second, apparent disagreements are openly discussed to look whether these trace back to linguistic or epistemic uncertainties that can be solved, or persist as different opinions (Leung et al. 2012). Overall, this process should increase the level of confidence for an assessed species, yet is also considerably time- and resource-demanding. Also, solving ambiguity proves challenging even when appropriate tools, definitions and sources are provided, a case which was illustrated by Strubbe et al. (2011) for risk perception in birds (e.g. for *T. aethiopicus*, included here as well).

However the protocols are used, it is strongly encouraged to set both the mathematical operations and the weights beforehand, and by a different panel than the assessors. The operations and weights should reflect what is considered fit for purpose to end users, and should thus ideally be chosen by them. It may be questioned whether the assessors should even be informed on these parameters, as to avoid biased answers.

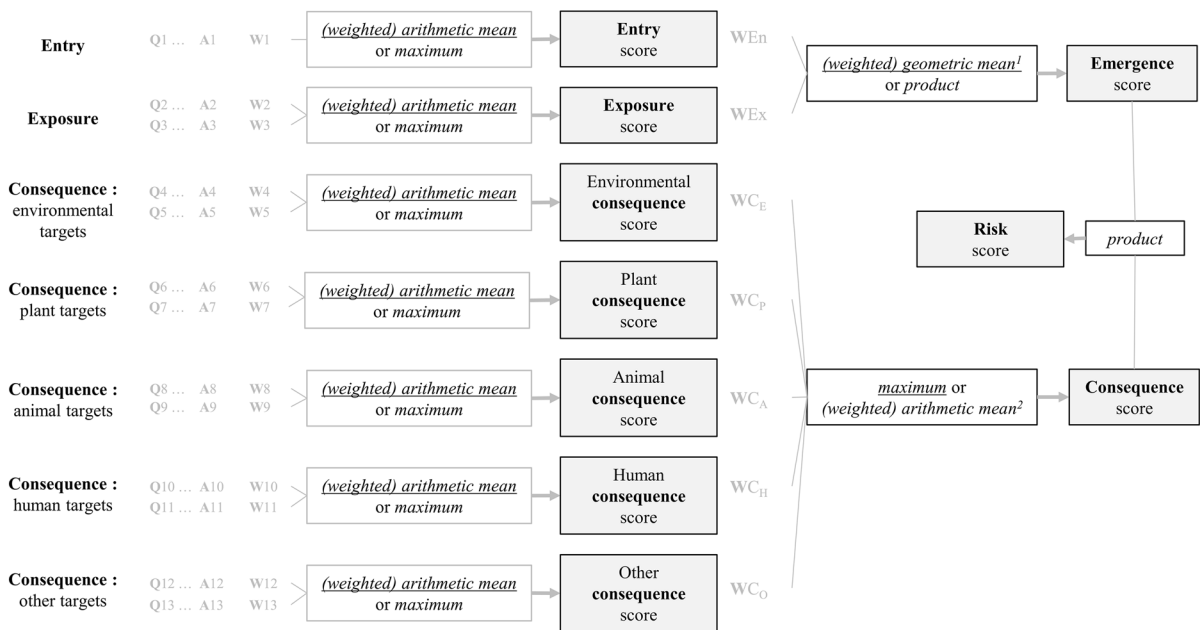
The operations should be chosen with care as they may yield very different results. The potential distribution of module output using our set of suggested

operations is shown in Fig. 4, as derived from simulations. The final Risk score tends to be left-skewed and very often zero, which in fact accords well with the observation that most alien species do not develop into invasives. Example output making use of alternative operations are shown in Figs. A1–A3 of the Supplementary Material. For instance, the maximum may be taken instead of the mean within each of the Impact modules (Fig. A1). Although this is a duly justified use of the precautionary approach, the simulations highlight several drawbacks in terms of the protocol's discriminative power. First, the number of potential module scores is limited to the number of potential answers. Second, module output tends to be highly skewed towards one, especially if the maximum is chosen also above the module level (the chance for none of the 18 questions to be answered by the highest possible alternative is close to zero). The mean, then again, favors intermediate scores (Fig. A2; Holt et al. 2006, but see Hughes 2008). Our set of suggested operations therefore reflects a trade-off between the use of the precautionary principle, discriminative power and flexibility. Nonetheless, differently chosen sets of operations may be found that also satisfactorily use output space (Fig. A3).

Weighting allows for particular questions or modules to be more emphasized than others, again in line with the end users' interests. For instance, a health care administration setting up a screening of alien mosquito species may consider environmental impacts to be worthwhile of inclusion, yet to merely be aggravating factors, whereas an environmental administration might reason the exact opposite. These administrations would increase or decrease the relative weight for the human impacts module, respectively. Note that the arithmetic and geometric mean allow for weights to be taken into account, whereas the maximum and product do not (Figs. 1, 2).

In their recent review, Leung et al. (2012) identified about 70 qualitative or semi-quantitative scoring methods for alien species risk assessment. Given this high diversity, it may be asked what added value a new protocol like *Harmonia*⁺ has. A closer examination of the listed methods, however, shows that Leung et al. (2012) consider only five methods to be really generic in the sense that they apply to a wide range of taxa. These are the UK non-native species risk assessment template (Baker et al. 2008), the German–Austrian Black List Information System (Essl et al. 2011), the

Q = Question, A = Answer, W = Weight



$$^1 \text{weighted geometric mean} = \frac{(W_{En} + W_{Ex})}{(W_{En}^{W_{En}} \times E_{Ex}^{W_{Ex}})}$$

$$^2 \text{weighted arithmetic mean} = \frac{(C_E \times W_{C_E}) + (C_P \times W_{C_P}) + (C_A \times W_{C_A}) + (C_H \times W_{C_H}) + (C_O \times W_{C_O})}{(W_{C_E} + W_{C_P} + W_{C_A} + W_{C_H} + W_{C_O})}$$

Fig. 2 The mathematical backbone of the Pandora protocol. The operations proposed as default are *underlined*

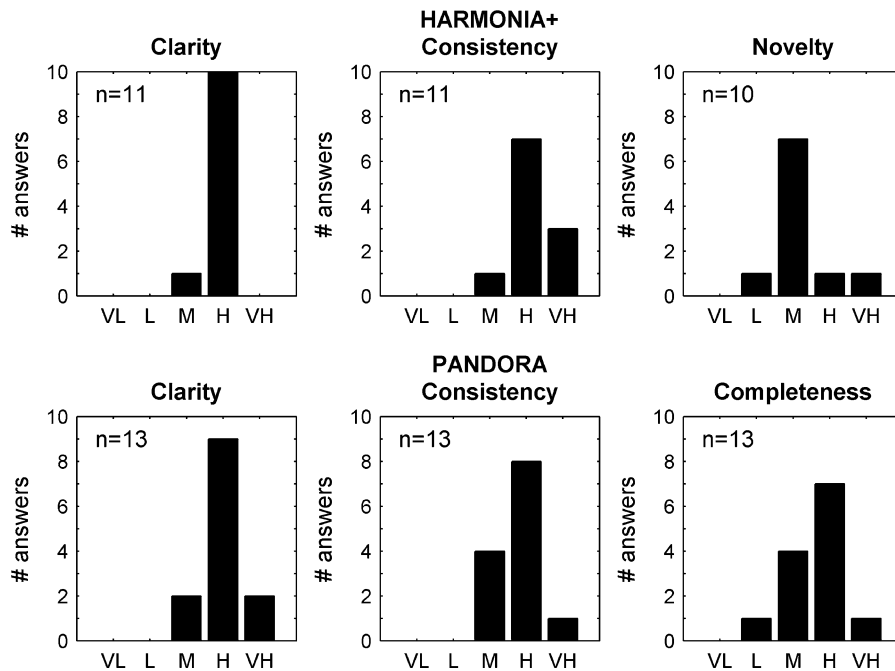


Fig. 3 Results from the anonymous survey among test assessors concerning their appraisal of the *Harmonia+* and *Pandora* questionnaires concerning its clarity and consistency (of how

questions and examples are phrased, and how concepts are presented), and completeness (of how risk is covered). VL very low, L low, M medium, H high, VH very high

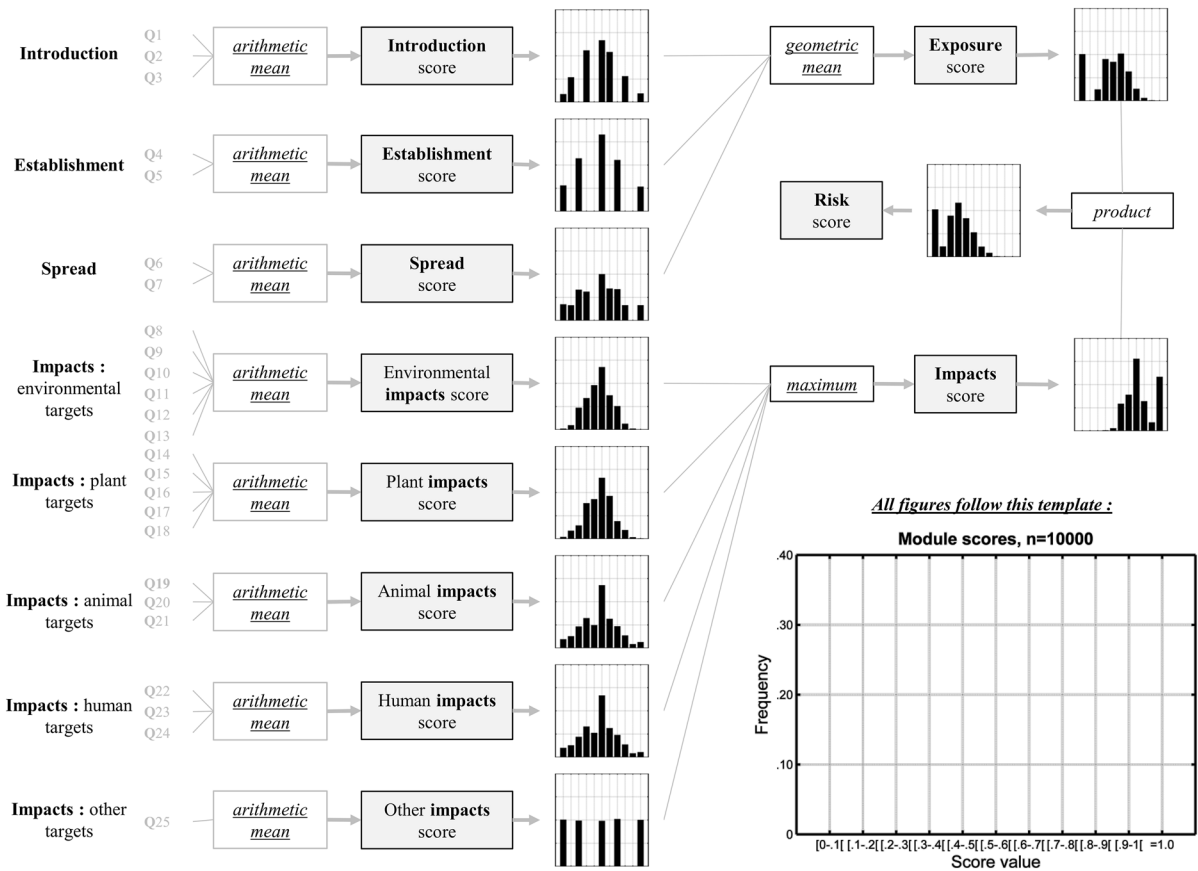


Fig. 4 Overview of the distribution of scores in *Harmonia*⁺ using the default operations. The entire protocol was run 10.000 times, with a randomly drawn answer for every question. All module and question weights considered equal

Norwegian black list (Gederaas et al. 2007) and impact assessment system (Sandvik et al. 2013), and the ISEIA protocol (Branquart 2007). However, the recent classification of Blackburn et al. (2014), and the system it is based on (Nentwig et al. 2010), should probably be added to that. Yet, none of these listed encompass the four basic invasion stages, incorporate uncertainty and consider socio-economic impacts in addition to biodiversity impacts. This is with the exception of the UK template, which thus relates to *Harmonia*⁺ the most (Leung et al. 2012; Roy et al. 2014b). It is noteworthy that the UK template has also been applied to four of our test species, and reaches a similar divide with *Ludwigia*, *Lithobates* and *Procambarus* as high-risk species on the one hand, and *Threskiornis* on the other (concluded risk is ‘medium’; GB Non-Native Species Secretariat 2014).

Accordingly, a panel of international delegates identified the development of rapid (national to

international) risk assessment methods to be a priority issue in Europe’s efforts for tackling invasive species (Caffrey et al. 2014). Indeed, the European Union recently approved a new regulation on the prevention and management of the introduction and spread of invasive alien species (European Commission 2014). Many of the regulation’s underlying principles (with regard to the pivotal role of prevention, the need for prioritization, and of risk analysis in general) also underlie *Harmonia*⁺, and our tool has indeed been found to be one of the few schemes that substantially comply with this regulatory framework (Roy et al. 2014b).

The *Pandora* scheme on risks of emerging pathogens and parasites is in line with different disease prioritization schemes that exist within the fields of animal and human medical science, such as those from Krause (2008), Cardoen et al. (2009), Havelaar et al. (2010) and Dufour et al. (2011). However, many, if not all,

prioritization schemes are restricted to vector-specific and/or domain-specific pathogens (e.g. zoonoses). Much like the case with *Harmonia*⁺, the main added value of *Pandora* therefore lies in the fact that it is very generic, incorporating potential impacts from very different types of pathogens in very different domains. Besides, it allows to inform *Harmonia*⁺ assessments in a clear-cut fashion, much like Copp et al.'s (in press) approach of infectious agents within the ENSARS protocol for species of aquacultural interest.

Harmonia⁺ and *Pandora* do not take into account positive impacts nor the feasibility of management, which is inherent to risk assessment schemes. However, it is to be acknowledged that decision makers eventually need to make a weighting among different lines of information, and opportunities for further work may therefore lie in the construction of tools that address these other stakes in ways that are compatible with current risk assessments schemes.

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