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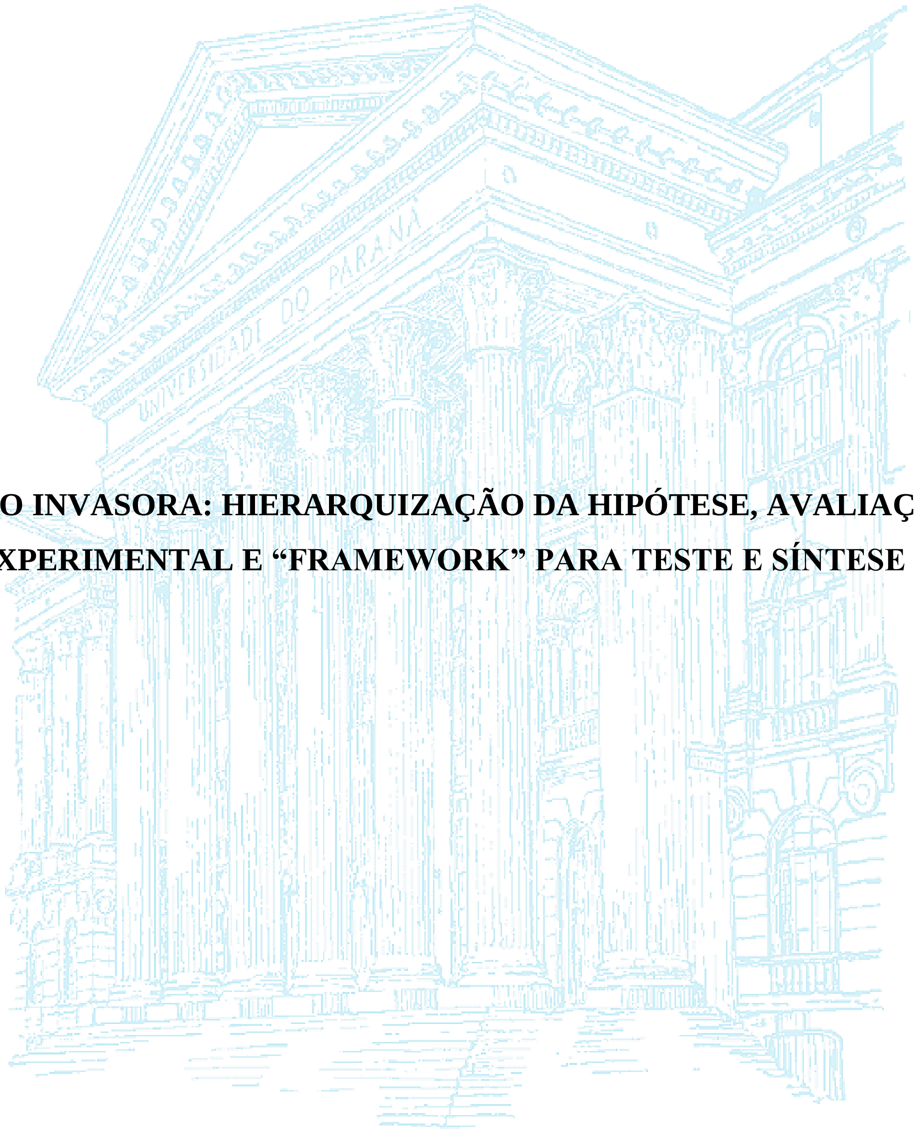

FUSÃO INVASORA: HIERARQUIZAÇÃO DA HIPÓTESE, AVALIAÇÃO EXPERIMENTAL E “FRAMEWORK” PARA TESTE E SÍNTESE

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FUSÃO INVASORA: HIERARQUIZAÇÃO DA HIPÓTESE, AVALIAÇÃO EXPERIMENTAL E "FRAMEWORK" PARA TESTE E SÍNTESE

Tese apresentada ao Programa de Pós-Graduação em Ecologia e Conservação – PPGECO da Universidade Federal do Paraná – UFPR, como requisito parcial para obtenção do título de Doutor.
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PARECER

Os abaixo-assinados, membros da banca examinadora da defesa da tese, a que se submeteu **Raul Rennó Braga** para fins de adquirir o título de Doutor em Ecologia e Conservação, são de parecer favorável à **APROVAÇÃO** do trabalho de conclusão do candidato.

Secretaria do Programa de Pós-Graduação em Ecologia e Conservação.

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DEDICATÓRIA

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RESUMO GERAL

A introdução de espécies não-nativas possui reconhecido impacto em todos os níveis ecológicos e constitui hoje uma importante ameaça à diversidade biológica nos mais diversos ecossistemas. Com o elevado número de espécies não-nativas nos mais diversos ambientes, é esperado que muitas dessas formem novas interações positivas, facilitando umas as outras de diversas formas e causando então um aumento do impacto dessas e até mesmo acelerando o número de novas invasões. Esse processo foi chamado de fusão invasora. Dada a relevância teórica e prática da hipótese de fusão invasora, se faz necessária uma síntese das evidências existentes. Sendo assim, através do método de hierarquização de hipóteses foi realizada uma divisão da hipótese em sub-hipóteses mais facilmente testáveis. A maioria das sub-hipóteses apresentou um domínio de evidências a favor da hipótese de fusão invasora. Adicionalmente, ficou clara a escassez de estudos que avaliem experimentalmente o impacto sinérgico entre espécies não-nativas resultante de interações positivas entre essas. Aliando isso ao fato de que diversas espécies não-nativas começaram a ser registradas nas últimas décadas na planície do Alto rio Paraná, o impacto sinérgico de três espécies não-nativas foi avaliado através de um experimento em mesocosmos. Relações de facilitação entre as três espécies analisadas leva a crer que essas possam fazer parte de um processo de fusão invasora apesar de não ter sido encontrado impacto sinérgico entre elas. Apesar disso, alterações populacionais e ecossistêmicas foram encontradas, ressaltando a importância de entender como espécies não-nativas interagem no ambiente invadido. Após análise dos estudos sobre a hipótese de fusão invasora, de críticas e debates sobre essa, percebe-se a necessidade de um melhor detalhamento e distinção dos principais aspectos da hipótese. Para o processo de fusão invasora, especial atenção deve ser dada às espécies generalistas, facilmente engajadas em interações mutualísticas; engenheiras ecossistêmicas por causarem grandes mudanças que desestabilizam a comunidade nativa facilitando a chegada de outras não-nativas; e predadoras de topo que podem complementar o impacto uma das outras. Por fim foi criado um “framework” para o teste da hipótese, que distingue níveis de suporte de acordo com o tipo de evidência encontrado.

Palavras-chave: espécies exóticas, interações interespecíficas, síntese, resistência biótica, invasibilidade.

GENERAL ABSTRACT

Non-native species introduction has widely known impacts across all levels of biological organization and therefore is an important threat to biodiversity. With the large number of non-native species found in nearly all ecosystems it is expected that new positive interactions arise among them, facilitating one another in various ways, increasing their impacts and/or accelerating the number of new successful introductions. This process has been called invasional meltdown. Given the theoretical and practical relevance of the invasional meltdown hypothesis it is now necessary to synthetize existing evidence. To do so, we used the hierarchy of hypotheses method to separate the broad definition into more testable sub-hypotheses. The majority of sub-hypotheses selected had a dominance of evidence supporting the invasional meltdown hypothesis. It is clear that there is scarce evidence experimentally testing the synergistic impact of non-natives resulting from their positive interaction. Additionally, several non-native species have been detected in the last decades in the Alto rio Paraná floodplain, so we tested through a mesocosm experiment, the synergistic impact of three non-native species now occurring in the region. We found several ways through which these three species facilitate each other. Therefore these species might be part of an invasional meltdown process even though no synergistic impact was found among them. We also detected population and ecosystem alterations due to species invasions, which strengthen the importance of understanding how non-native species interact in the invaded range. After analyzing published evidence for the invasional meltdown hypothesis, critics and debates, it is necessary to distinguish main aspects of the hypothesis for a better understanding of the process, what will allow more accurate tests and detection of the problem. In regard to the invasional meltdown hypothesis, special attention should be given to generalist species which are more prone to form new mutualistic interactions; ecosystem engineers which might cause severe changes to the environment, disrupting the native community and facilitating the establishment of other non-natives; and top predators which can have synergistic impact upon shared native preys. At last a framework for testing the invasional meltdown hypothesis was created distinguishing level of support according to type of evidence found.

Keywords: exotic species, interspecific interactions, synthesis, biotic resistance, invisibility.

PREFÁCIO

Invasões Biológicas

A perda da biodiversidade mundial já ultrapassou o seu limite máximo operante, tendo consequências irreversíveis para os mais diferenciados ecossistemas (Rockström *et al.* 2009). Dentre os principais fatores contribuintes para perda de biodiversidade está a introdução de espécies não-nativas, com reconhecidos impactos em todos os níveis ecológicos (Parker et al. 1999, Lockwood *et al.* 2007, Simberloff & Rejmánek 2011). O aumento do número de espécies não-nativas ao redor do mundo pode causar também o fenômeno de homogeneização biótica (genética, taxonômica ou funcional). Em nível de comunidades, por exemplo, isso pode causar um aumento da similaridade entre biotas inicialmente distintas, ao substituir espécies nativas raras e/ou endêmicas por espécies não-nativas generalistas (Soulé 1990, McKinney & Lockwood 1999, Rahel 2002).

Somados aos potenciais e reais problemas, o crescente número de pessoas e/ou produtos em deslocamento de um país a outro ao redor do mundo significa que os riscos de bioinvasões também estão crescendo (Perrings *et al.* 2005). Além do impacto ecológico, há o impacto econômico que nos EUA até o final da década de 90 já ultrapassava os 120 bilhões de dólares por ano (Pimentel *et al.* 2005), chegando a 1.4 trilhões de dólares no mundo inteiro, o que correspondia a 5% da economia mundial (Pimentel *et al.* 2001).

Fusão Invasora

Comumente os estudos de invasões biológicas focam apenas no registro destas no ambiente ou no impacto delas sobre espécies nativas através de predação ou competição, deixando inexploradas interações como mutualismo, comensalismo e amensalismo entre espécies não-nativas (Simberloff & Von Holle 1999, Ricciardi 2001, 2005). Ao revisar estas relações entre espécies não-nativas, Simberloff e Von Holle

(1999) propuseram a hipótese da "fusão invasora" (*Invasional meltdown*). Esta hipótese remete a um processo de facilitação entre espécies não-nativas, e foi definido pelos autores como: "*um processo pelo qual um grupo de espécies não-nativas facilita a invasão uma das outras de várias maneiras, aumentando a possibilidade de sobrevivência e/ou impacto ecológico, e possivelmente a magnitude deste impacto".* O termo facilitação remete a um processo de interação em que uma espécie tem um efeito positivo na persistência ou no crescimento populacional de outra espécie (Ricciardi 2005). Simberloff (2006) ressalta que este processo de fusão invasora vai além da simples interação entre espécies não-nativas, sendo um processo em nível de comunidades em que o efeito conjunto de facilitações leva a um aumento do número de espécies não-nativas estabelecidas no ambiente aumentando o impacto causado por estas. Além disso, um ecossistema com alta pressão de propágulos se tornará progressivamente instável e cada vez mais susceptível a novas introduções (Ricciardi 2005). Estas interações são tão ou mais comuns que aquelas em que há prejuízo para uma das partes (Ricciardi 2001, Simberloff & Von Holle 1999).

Ricciardi (2001) mostra dois possíveis caminhos para o processo de fusão invasora: (*i*) a facilitação direta, onde os efeitos de uma espécie não-nativa beneficiam diretamente a outra, por exemplo, por fornecer mais nutrientes ou abrigo e proteção; (*ii*) facilitação indireta, onde o benefício é indireto, por exemplo, por reduzir a população de uma espécie predadora, ou aumentar a de presas da outra espécie não-nativa.

Em outra abordagem, Grosholz (2005) ressalta que interações positivas entre espécies não-nativas ocorrem de três maneiras: (*i*) conforme o número de espécies não-nativas aumenta em uma comunidade, elas desestabilizam as populações nativas facilitando novas invasões no novo ambiente; (*ii*) espécies não-nativas já estabelecidas modificam qualitativamente o ambiente facilitando o estabelecimento de novas espécies; (*iii*) invasões mais recentes podem alterar o ambiente de tal maneira que resulta em um aumento e/ou expansão de uma espécie não-nativa que já estava estabelecida. Este último caso citado por Grosholz (2005) constitui uma das explicações possíveis para o fenômeno do atraso da invasão (do inglês, *lag time phenomenon*) (Simberloff 2009). Muitas espécies não se tornam invasoras imediatamente após a sua introdução, se mantendo em baixas densidades e inofensivas até mesmo por décadas, e repentinamente sua população aumenta se tornando uma peste. O gatilho para este rápido crescimento e dispersão pode ser a introdução de outra espécie não-nativa facilitando a sobrevivência e/ou a dispersão uma das outras (Simberloff 2009).

Relevância teórica: hipóteses e teorias conflitantes

A hipótese da fusão invasora entra diretamente em conflito com outra hipótese amplamente difundida na ecologia de invasões, a hipótese da resistência biótica (Elton 1958). Charles S. Elton propôs que comunidades com uma maior riqueza de espécies são mais resistentes (i.e. habilidade de uma comunidade em resistir a mudanças frente a uma força potencialmente perturbadora) à invasão que outra semelhante, porém com uma menor riqueza. Isto ocorreria por que em comunidades mais ricas os recursos limitantes ou nichos seriam amplamente utilizados, deixando poucos recursos ou nichos disponíveis para indivíduos de novas espécies recém-chegadas. Também haveria uma maior probabilidade de existirem predadores nativos, patógenos, parasitas, e outras espécies previamente introduzidas que atuem como competidoras com as recém-chegadas. Sendo assim, conforme aumenta o número de espécies não-nativas em uma comunidade, esta se torna mais resistente a invasões subsequentes (Fig. 1). É importante ressaltar que há uma ampla gama de estudos que corroboram esta hipótese, assim com estudos que são contrários a ela, sendo este assunto ainda bastante discutido na literatura (ver exemplos em revisão de Levine & D’Antonio, 1999).

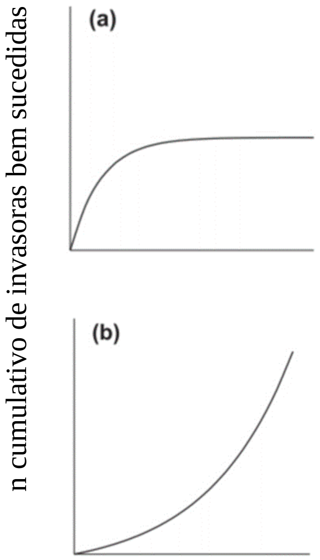

Figura 1. Tendência temporal do número cumulativo de espécies invasoras de acordo com (a) modelo de resistência biótica e (b) modelo de fusão invasora (adaptado de Ricciardi 2001).

A teoria do equilíbrio de biogeografia de ilhas de MacArthur & Wilson (1967) também possui ideias contrarias a hipótese da fusão invasora. O modelo de Robert MacArthur e Edward Wilson propõe que a chegada de uma espécie em um novo ambiente é compensada pela extinção de outra espécie já presente, sendo assim o número total de espécies é mantido constante. Os autores consideram a competição como principal força controlando o número de espécies no ambiente e assim não consideram possíveis interações positivas que podem levar a um aumento da riqueza (Simberloff & Von Holle 1999). Outro importante fator a ser considerado é a identidade das espécies, não somente a riqueza, que sabidamente pode ser crucial no estabelecimento ou não no novo ambiente (Crawley *et al.* 1999).

Justificativa

Dada a relevância teórica e prática da hipótese de fusão invasora para a ecologia de invasões e o alto número de estudos citando Simberloff & Von Holle (1999) (mais de 700 citações de acordo com a base de dados do ISI Web of Science em novembro de 2015), se faz necessária uma síntese das evidências existentes. Heger & Jeschke (2014) sugerem a utilização de três passos para a síntese de evidências de uma dada hipótese ecológica. Primeiramente uma revisão sistematizada do assunto deve ser conduzida para identificar os artigos relevantes. Devido a natureza abrangente de uma hipótese, muitos estudos encontrados na revisão sistematizada terão testado aspectos diferentes da mesma hipótese e consequentemente podem chegar a conclusões diferentes, não devendo então ser comparados entre si. Então, em um segundo momento, deve-se utilizar o método de hierarquização de hipóteses para dividir essa em diferentes sub-hipóteses mais específicas que então poderão ser comparadas entre si. Em um terceiro momento meta-análises podem ser feitas para as diferentes sub-hipóteses. Com essa sequencia é possível descartar aspectos com baixo nível de suporte, e direcionar futuros estudos para aqueles aspectos pouco estudados, mas com alto nível de suporte. Dessa forma, o presente trabalho realiza os dois primeiros passos (revisão sistematizada e hierarquização de hipóteses) para a hipótese de fusão invasora (Capítulo I).

Analisando os estudos encontrados na revisão sistematizada, fica clara a escassez de estudos que avaliem experimentalmente o impacto sinérgico entre espécies não-nativas resultante de interações positivas entre essas. De fato, há muitas evidências para interações positivas entre as espécies invasoras (e.g. Ricciardi 2001, O’Dowd et al. 2003, Grosholz 2005, Relva et al. 2010), o que sugere que pode ocorrer uma fusão invasora e os impactos serem potencializados. Nesse sentido, experimentos bem delineados com tal propósito é a maneira mais clara de se testar a hipótese de fusão invasora. Tendo em vista que diversas espécies não-nativas começaram a ser registradas nos últimos anos na planície de inundação do Alto rio Paraná este sistema apresenta um bom modelo para a avaliação de potenciais efeitos sinérgicos entre essas espécies. Três das espécies que mais chamaram atenção foram: o Oscar *Astronotus crassipinnis* (Heckel, 1840), o mexilhão dourado *Limnoperna fortunei* Dunker 1857, e a macrófita submersa *Hydrilla verticillata* (L.F.) Royle (1977). Sendo assim, o resultado do impacto individual e sinérgico dessas espécies foi avaliado através de um experimento em mesocosmos (Capítulo II).

Após análise dos estudos sobre a hipótese de fusão invasora, de críticas e debates sobre essa, percebe-se a necessidade de um melhor detalhamento e distinção dos principais aspectos da hipótese. Aliado a isso, ligar os principais aspectos da hipótese a exemplos existentes na literatura, identificar cenários de alto risco e esclarecer como essa ocorre nos diferentes níveis ecológicos, permite que um "framework" para teste seja criado. Esse é um importante passo para possibilitar testes mais específicos da hipótese (Capítulo III).

Apresentação da tese

Os três capítulos são apresentados em forma de artigo científico. Devido a isso cada capítulo apresenta sua própria apresentação de um tema comum, o que pode ter levado a uma redundância de informações entre os capítulos. O capítulo I foi desenvolvido durante o doutorado sanduíche na "Freie Universität Berlin" em parceria também com o "Leibniz-Institute of Freshwater Ecology and Inland Fisheries" (IGB), ambas as instituições na cidade de Berlim, Alemanha. É de coautoria de Lorena G. Aparicio, Sidinei M. Thomaz, Jean R. S. Vitule e Jonathan M. Jeschke e está formatado para ser submetido ao periódico "Oikos". O capítulo II foi realizado em parceria com Programa de Pós-Graduação em Ecologia de Ambientes Aquáticos da Universidade

Estadual de Maringá (UEM). Os experimentos foram realizados nas dependências do Núcleo de Pesquisa em Limnologia, Ictiologia e Aquicultura (Nupélia) no município de Porto Rico-PR. É de coautoria de Vanessa M. Ribeiro, Juliana Wojciechowski, Igor de Paiva Affonso, Eduardo R. Cunha, André A. Padial, Sidinei M. Thomaz e Jean R. S. Vitule, e está formatado para ser submetido ao periódico "Ecology". É importante ressaltar aqui que análises laboratoriais do experimento foram realizadas por terceiros e devido a não entrega dos resultados de zooplâncton, esse está faltando no artigo aqui apresentado. O capítulo III é de coautoria de Sidinei M. Thomaz, Daniel Simberloff e Jean R. S. Vitule. Está formatado para ser submetido ao periódico "Biological Invasions".

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CHAPTER I

THE INVASIONAL MELTDOWN HYPOTHESIS AS A HIERARCHY OF HYPOTHESES

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ABSTRACT

Negative interactions have long been suggested as a major barrier for species arriving in a new habitat. More recently, positive interactions caught the attention both from community assembly theory and biological invasions. The invasional meltdown hypothesis (IMH) brought the idea that positive interactions among non-native species could facilitate one another's invasion in one (A→B) or both (A↔B) directions, even increasing their impact upon the native community. Many studies have addressed the IMH in the last ca. 15 years, but with contrasting results. Here we use the hierarchy-of-hypotheses (HoH) approach to differentiate key aspects of the IMH, organizing and linking empirical studies to sub-hypotheses of the IMH. We also assess the level of empirical support for each sub-hypothesis based on the weighted evidence reported in the studies. We considered all studies citing Simberloff and Von Holle (1999) and identified 150 relevant studies addressing the IM. In general, there are more supporting than questioning studies. Most studies supporting the IMH were conducted at the level of individuals or populations, whereas supporting studies at the community or ecosystem level are currently rare. Only few sub-hypotheses are questioned by more than 50% of the weighted evidence. This is the case for non-native species affecting each other's survival, growth, reproduction, abundance, density or biomass in A↔B interactions. The IMH is supported by the majority of studies, but few studies present truly strong evidence. With the HoH presented here, it is possible to monitor progress in testing the IMH. For instance, more tests at the community and ecosystem level are needed in the future.

Keywords: Facilitation, mutualism, review, non-indigenous, exotic.

INTRODUCTION

Upon arriving in a new environment, non-native species have to deal with a new set of interacting species that may represent barriers for their survival and establishment. This ecological barrier imposed to newcomers is attributed to negative interactions (e.g. direct and indirect competition, predation) with native species, but it is possible that new positive interactions (e.g. mutualism) between the arriving non-native and a native species or even with other non-natives arise (Simberloff and Von Holle 1999). Although less attention has been given to positive as compared to negative interactions (Lortie and Callaway 2009, Stachowicz 2001, Bruno et al. 2003), it is now acknowledged that positive interactions play a decisive role in shaping communities and regulating ecosystem structure and function (Halpern et al. 2007, Brooker et al. 2008).

Linking the importance of positive interactions and factors influencing non-native species success, Simberloff and Von Holle (1999) quantified how frequently positive interactions occur compared to negative ones. Based on the widespread occurrence and the importance of positive interactions between non-native species, Simberloff and Von Holle (1999) coined the term ‘invasional meltdown’ (IM) “for the process by which a group of nonindigenous species facilitate one another’s invasion in various ways, increasing the likelihood of survival and/or of ecological impact, and possibly the magnitude of impact”. A large amount of evidence for and against the invasional meltdown hypothesis (IMH) arose since the publication of Simberloff and Von Holle (1999), which has received 763 citations to date in the ISI Web of Science database (data checked at 01/14/2016).

An evaluation of six major hypotheses in invasion biology showed that the IMH had the highest level of support among the evaluated hypotheses, but it was also one of the least addressed, with only 30 studies testing its predictions (Jeschke et al. 2012). On the other hand, a recent meta-analysis showed that invaders most commonly reduce one another's performance rather than facilitating each other (Jackson 2015). The discrepancy between these and other studies might be due to the fact that the definition of the IMH is broad, and several different aspects of the hypothesis might be tested in different studies, leading authors to different conclusions. This has been pointed out as a major source of contradictory results for several hypotheses in invasion biology (Heger et al. 2013, Heger and Jeschke 2014). Words such as "various ways" which appear in the IMH definition (see in the previous paragraph) leave open different interpretations. However, being broad and imprecise is a characteristic of the majority of major hypotheses in ecology, and with few exceptions, they can only be tested if further specified (Heger et al. 2013, Heger and Jeschke 2014).

Three different types of interaction scenarios involving the non-native species are typically amalgamated under the umbrella of invasion meltdown. First, a non-native species can facilitate any aspect of another's invasion (e.g. survival, reproduction, resource acquisition), while the latter has no detected influence on the former (+/0 interaction). Second, both species have a reciprocal effect on one another (+/+ interaction; as mentioned in Simberloff and Von Holle's definition). And third, more than two species can interact and benefit each other, but with no mutual benefit between two of the involved species. For example, in a three-species scenario, species A can positively affect species B which positively affects species C, which in turn positively affects species A. One of these interactions might even be negative to one species but if a large number of non-native species are involved, the net outcome could be positive. In this case, it might be that no direct mutualism is involved but in the essence of the IMH idea, this would also be a strong case to consider. These three interaction scenarios described above are typically indiscriminately cited as IM in the literature. Due to the pronounced differences between these scenarios, we argue that differentiating them is important for better understanding and evaluating the IMH.

Studies on the IMH also differ in the ecological level they consider. For example, a study might report that one non-native suppresses a native species (e.g. through predation or competition), causing its abundance to decline and consequently, throughout competitor release, leading to an increase of another non-native species' abundance. This would be population-level evidence. Another study might report community and ecosystem alterations. For example, if in the above-mentioned scenario the benefited non-native species is a plant that alters soil properties and nutrient availability that leads to a compositional change in the community, we would have a much stronger evidence for the IMH. Simberloff (2006) already highlighted that most evidence available at that date was from the population level, but that true IM would be at the community level.

In order to fully understand and evaluate the IMH, we need to separate these different aspects of the hypothesis (interaction scenario and ecological level) into more specific sub-hypotheses. The hierarchy-of-hypothesis (HoH) approach (Jeschke et al. 2012, Heger et al. 2013, Heger and Jeschke 2014) is a new tool for research synthesis (along with systematic reviews and meta-analyses) that can be used for disentangling different key aspects of major hypotheses. The HoH approach can be seen as a special variant of a systematic review where available empirical studies for a given major hypothesis are linked to hierarchically divided sub-hypotheses of the major hypothesis. A HoH is a special case of ontology. "Ontologies are formal models that define concepts and their relationships within a scientific domain" (p. 160 in Madin et al. 2008), and we fully agree with Madin et al. 2008 that their wider usage would advance ecological research. Thus far, the HoH approach has been applied in detail to the enemy release hypothesis (Heger and Jeschke 2014; a first, more rough application to six invasion hypotheses has been presented by Jeschke et al. 2012). Systematic reviews and meta-analyses are the widely known tools used to synthesize particular fields and hypotheses in ecology, and the HoH approach is an additional, potentially useful tool (Lowry et al. 2013, Lortie 2014).

In view of the contradictory results present so far on the IMH, our objectives are: (i) to apply the HoH approach in order to differentiate key aspects of the IMH and represent these as sub-hypotheses of the IMH, (ii) link empirical studies on the IMH to these sub-hypotheses, (iii) separate the most relevant sub-hypotheses according to their level of support, and (iv) identify current gaps to guide future research.

METHODS

To identify empirical studies on the IMH, we searched the ISI Web of Science database for all publications citing Simberloff and Von Holle (1999) until 21 November 2014. Such a search is possible for the IMH, as the publication by Simberloff and Von Holle (1999) is clearly recognized in the field as the first and single paper coining the IMH, so most studies testing the IMH are likely referring to it. We did not consider books, which are often hardly accessible, nor theoretical studies as we restricted our analyses to empirical findings. We also excluded meta-analyses and reviews, as these articles do not provide original results, and including them would result in double-counting empirical findings.

In the first evaluation of the search results, titles and abstracts of the 637 papers citing Simberloff and Von Holle (1999) were screened. In this way, we identified 511 potentially relevant studies where we analyzed the full text. However, most of these papers cited the paper by Simberloff and von Holle (1999) with objectives others than testing the IMH. For example, a great majority of the citations were made in the introduction of the work, to justify more general aspects about invasion. After excluding these papers, 150 relevant empirical studies were maintained and included in the analysis. Following Jeschke et al. (2012b) and Heger and Jeschke (2014), we classified the evidence reported in each of the 150 studies as either supporting (i.e. evidence is in line with the hypothesis), questioning (i.e. evidence is conflicting with the hypothesis), or being undecided (inconclusive). Undecided studies were the ones that provided evidence both for and against a given sub-hypothesis.

The HoH was created by dividing the IMH into sub-hypotheses according to the following three criteria:

1) *Type of interaction,* classified as either: (1.1) A→B, where two non-native species interact and only one is affected, with no evidence for the second (e.g. simple facilitation); (1.2) A↔B, where two non-native species interact and both species are affected (e.g. mutualism); or (1.3) multi-species interaction, that is an interaction network between three or more non-native species (e.g. one species affects the interaction between the second and third species).

2) *Ecological level*, classified as either: (2.1) individual, (2.2) population, (2.3) community, (2.4) ecosystem level.

3) *Outcome of interaction:* This criterion relates to the effects of the interaction between the involved non-native species. We divided it according to the relevant ecological level (see 2):

a. Individual level: (3.1) resource (e.g. food source, feeding preference, predation, herbivory), (3.2) survival, growth or reproduction, (3.3) dispersal of individuals, (3.4) impact on individuals of native species.

b. Population level: (3.5) abundance, density or biomass, (3.6) population dispersal, (3.7) impact on native population.

c. Community level: (3.8) composition, (3.9) richness (3.10) diversity (3.11) impact on native community.

d. Ecosystem level (3.12): no further division was needed due to a lack of studies at this ecological level.

For 6 papers, it was not possible to classify the outcome of interaction, thus these were excluded from the analysis at this level (they were included for other analyses).

Regarding the focal non-native species involved, we additionally recorded whether they belong to the same native range (an indication of co-evolution), whether their interactions were direct or indirect, the taxonomic group (plants, algae, fungi, invertebrates (subdivided into: crustaceans, insects, mollusks, other invertebrates), vertebrates (subdivided into: fishes, amphibians, reptiles, birds, mammals), and eubacteria/archaea/viruses), and the number of non-native species investigated. We divided the studies per group to check whether the IMH support has been biased toward some specific group of animal or plant. We also recorded for each study the continent where it was performed or where the individuals came from in case of laboratory studies, the major type of habitat (terrestrial, freshwater, marine), . The identification of continents and of the type of ecosystem aimed to identify potential biogeographical and ecosystem biases, respectively, in the tests of the IMH. We also recorded which research method was applied (experiment or observation; conducted in the field, enclosure (incl. exclosure and common garden), or in the laboratory) and if the evidence provided was analyzed quantitatively with statistics, quantitatively without statistics (e.g. due to small sample size; studies that only graphically plot data and/or only include simple descriptive statistics such as means are included in this category) or only qualitatively (only non-numerical information is presented). These analyses were conducted to verify the robustness of the IMH tests: for example, controlled experiments employing quantitative (statistical) analyses can be considered more robust than purely observational studies based on descriptive analyses.

Each study differs in several important aspects (research method, number of species investigated, type of interaction, ecological level), and these are of major importance when evaluating the IMH. We thus weighted studies according to these aspects, adapting the formula suggested by Heger and Jeschke (2014) for study weight w:

$$w = m \times \sqrt{n} \times i \times j,$$

where, m is a score for the research method (1 for observational enclosure studies, 2 for observational field studies or experimental laboratory studies, 4 for experimental enclosure studies and 8 for experimental field studies), n is the number of focal non-native species (capped at a maximum value of 100), i is a score for the type of interaction (1 for A→B studies, 3 for A↔B studies, 8 for multi-species interactions) and j is a score for the ecological level (1 for individual studies, 2 for population studies, 6 for community studies and 8 for ecosystem studies). For the research method and ecological level, studies sometimes presented information on more than one category, and the highest value was used for weight calculations in these cases. These scores for calculating w were arbitrarily chosen based on our interpretation of importance to the IMH, and so different HoH approaches can use different scores. It is important to highlight that the HoH structure is not fixed, and we present one of many possible divisions of the IMH into sub-hypotheses. In our case, the weights varied from 3 to 1024 (Supplementary material Appendix 2 Fig. A1), and these values represent the extremes of a gradient of supporting levels of the IMH.

To avoid inflation of the sample size due to weight calculations, proportional weights were used by dividing the separate sum of weights supporting, questioning or being undecided for a given sub-hypothesis by the total sum of weights of that sub-hypothesis. This result was multiplied by the sample size number of the sub-hypothesis and rounded to full numbers (following Maletta 2007, Heger and Jeschke 2014).

To test whether empirical support differs between sub-hypotheses, we performed Mann-Whitney U-tests. Chi-square tests were performed to assess whether results supporting, questioning or being undecided deviate from an equal distribution within each sub-hypothesis. If statistically significant, post-hoc comparisons between supporting and questioning studies were carried out for this sub-hypothesis.

RESULTS

Of the 150 relevant empirical studies on the IMH that we identified, 63.3% supported the IMH, 23.3% questioned it and 13.3% were considered undecided, as they showed both evidence for and against the IMH (Supplementary material Appendix 2 Table A1). When the weight given for the studies were considered, a similar pattern was found with 63.5% supporting, 21.0% questioning and 15.5% being undecided (Table 1). The majority of studies were observational field studies (38.6%, n = 85), followed by experimental field studies (31.8%, n = 70), and most studies provided quantitative information together with statistics (90.7%, n = 136) (Supplementary material Appendix 2 Fig. A2 and A3).

Analyzing weighted and unweighted data for each type of interaction separately, A→B (n = 58) and multi-species interactions (n = 58) present the majority of studies supporting the IMH, whereas for A↔B (n = 34) there was no statistical difference in the amount of studies supporting and questioning the IMH (Table 1, and Supplementary material Appendix 2 Table A1). In addition, the level of support varied between these three sub-hypotheses (Fig. 1). When sub-hypotheses are divided by ecological level, most studies still support the IMH within each hierarchical level (Fig. 2, Table 1, and Supplementary material Appendix 2 Table A1). The majority of studies found were on individual (n = 89) and population levels (n = 87), followed by community level (n = 21); only 3 studies were done at the ecosystem level. Finally, the support for the IMH considering the outcome of interaction was also significant for all comparisons made, i.e. where more than five studies were found (Table 1).

A significantly lower level of support was found when non-native species coevolved as compared to no coevolution (n = 43) (Fig. 3). However in nearly half of the studies (49.7%, n = 75), this information was not available. We furthermore compared whether evidence comes from direct (n = 87) or indirect (n = 56) interactions between non-native species. In both cases, there were significantly more studies supporting than questioning the hypothesis for weighted data (Table 2), and there was no significant difference between direct and indirect effects (Fig. 4).

Taking into account particular habitats, most empirical tests of the IMH were carried out in terrestrial ecosystems (63.1%, n = 94). Freshwater and marine habitats were only studied in 32 (21.5%) and 23 (15.4%) tests, respectively. Although no difference in support level was found among habitats, they all presented a significantly larger amount of studies supporting than questioning or undecided ones for weighted data (Fig. 5, Table 2). Regarding taxonomic groups, plants and algae (n = 98) and invertebrates (n = 83) had more studies supporting than questioning, whereas for vertebrates (n = 51) there was no significant difference (Table 2). Overall, invertebrate studies showed a significantly higher level of support than vertebrate studies (Fig. 6).

For unweighted data there was a significantly higher number of supporting studies for all taxonomic groups (Supplementary material Appendix 2 Table A2).

The HoH illustrates the number of studies and level of support for different sub-hypotheses (Fig. 7). Although most sub-hypotheses of the IMH are empirically supported, the A↔B type of interaction at population level is not supported (Fig. 7). These studies are the ones where two non-native species negatively affect each other's abundance, density and/or biomass. At the individual level, studies showing two species negatively affecting each other's survival, growth and/or reproduction are the majority in their category, thus also questioning the IMH (Fig. 7).

Table 5. Weighted evidence from empirical tests supporting, questioning or being undecided about the IMH for each interaction type, ecological level and outcome of interaction with $\chi2$ values for comparison of the distribution of the three categories to an equal distribution. χ^2 tests were only conducted for comparisons with more than five studies. Binomial tests comparing the proportion of supporting versus questioning studies were only conducted when χ^2 tests were significant ($p< 0.05$).

	n	Supported	Undecided	Questioned	χ^2	Binomial test
Total	150	63.5%	15.5%	21.0%	**<0.001**	**<0.001**
A → B	58	77.1%	9.8%	13.1%	**<0.001**	**<0.001**
A ↔ B	34	51.4%	5.7%	43.0%	**0.002**	0.723
Multi Spp.	58	64.0%	17.6%	18.4%	**<0.001**	**<0.001**
Individual	89	58.7%	20.6%	20.7%	**<0.001**	**<0.001**
Resource	26	72.3%	27.7%	0.0%	**<0.001**	**<0.001**
Survival/Growth/Reproduction	50	55.3%	19.8%	24.9%	**0.002**	**0.011**
Dispersal	12	88.9%	8.9%	2.2%	**<0.001**	**<0.001**
Impact	6	41.1%	0.0%	58.9%	-	-
Population	87	70.9%	16.1%	13.0%	**<0.001**	**<0.001**
Abundance/Density/Biomass	75	65.5%	19.7%	14.7%	**<0.001**	**<0.001**
Dispersal	3	92.5%	7.5%	0.0%	-	-
Impact	12	97.6%	0.0%	2.4%	**<0.001**	**<0.001**
Community	21	71.3%	8.9%	19.8%	**<0.001**	**0.001**
Composition	4	58.8%	41.2%	0.0%	-	-

Table 1. continued

	n	Supported	Undecided	Questioned	χ^2	Binomial test
Richness	10	87.3%	6.9%	5.9%	**0.002**	**0.011**
Diversity	1	100%	0.0%	0.0%	-	-
Impact	7	98.3%	0.0%	1.7%	-	-
Ecosystem	3	100.0%	0.0%	0.0%	-	-

Table 6. Weighted evidence from empirical tests supporting, questioning or being undecided about the IMH for non-native species coevolution, direct and indirect effects, habitats and taxonomic groups with χ^2 values for comparison of the distribution of the three categories to an equal distribution. Binomial tests comparing the proportion of supporting versus questioning studies were only conducted when χ^2 tests were significant ($p < 0.05$).

	n	Supported	Undecided	Questioned	χ^2	Binomial test
With coevolution	33	31.1%	48.2%	20.7%	0.148	-
Without coevolution	43	57.7%	28.8%	13.5%	**0.001**	**<0.001**
Unknown	75	68.3%	9.1%	22.6%	**<0.001**	**<0.001**
Direct effect	87	76.7%	12.6%	10.8%	**<0.001**	**<0.001**
Indirect effect	56	67.3%	19.8%	12.8%	**<0.001**	**<0.001**
Unknown	23	41.0%	16.1%	42.9%	0.269	-
Terrestrial	94	67.1%	20.3%	12.6%	**<0.001**	**<0.001**
Freshwater	32	70.1%	11.8%	18.1%	**<0.001**	**0.002**
Marine	24	77.6%	3.4%	18.9%	**<0.001**	**0.002**
Plants and Algae	89	55.1%	20.2%	24.78	**<0.001**	**<0.001**
Invertebrates	83	69.1%	10.5%	20.4%	**<0.001**	**<0.001**
Vertebrates	51	39.4%	15.6%	45.0%	**0.024**	0.647

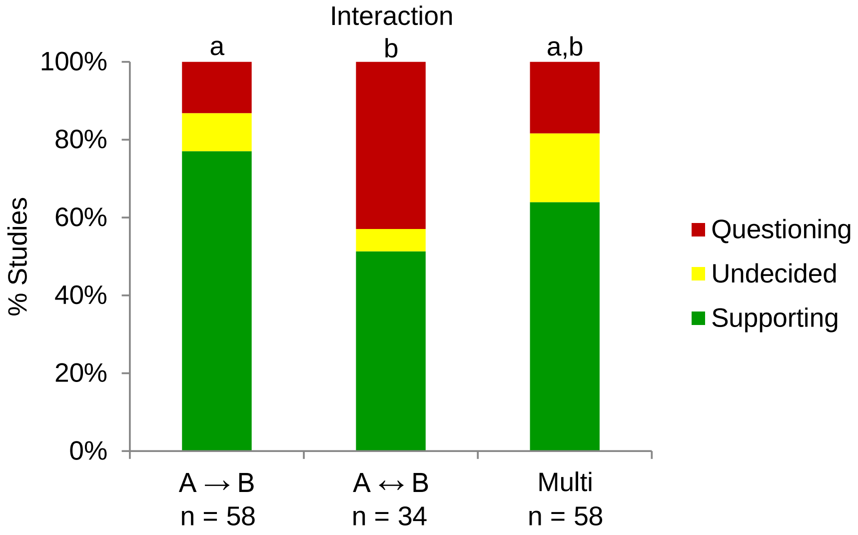

Figure 1. Weighted data on level of empirical support for the different types of interactions. Letters above bars indicate significant differences (U-tests, $p < 0.05$).

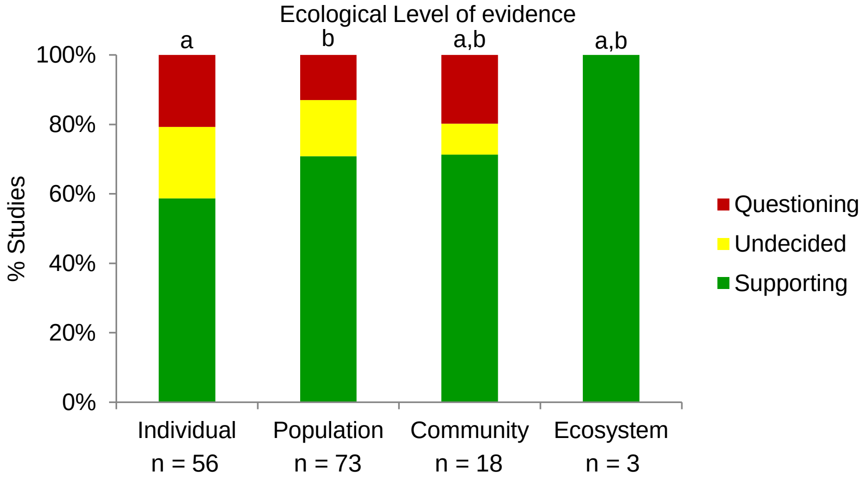

Figure 2. Weighted data on level of empirical support for the ecological level of evidences. Letters above bars indicate significant differences (U-tests, $p < 0.05$).

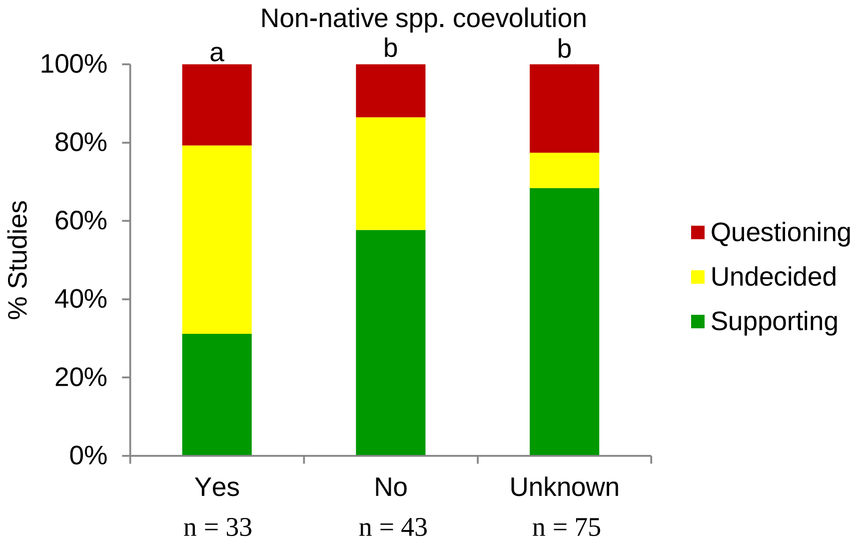

Figure 3. Weighted data on level of empirical support for non-native species coevolution. Letters above bars indicate significant differences (U-tests, p < 0.05).

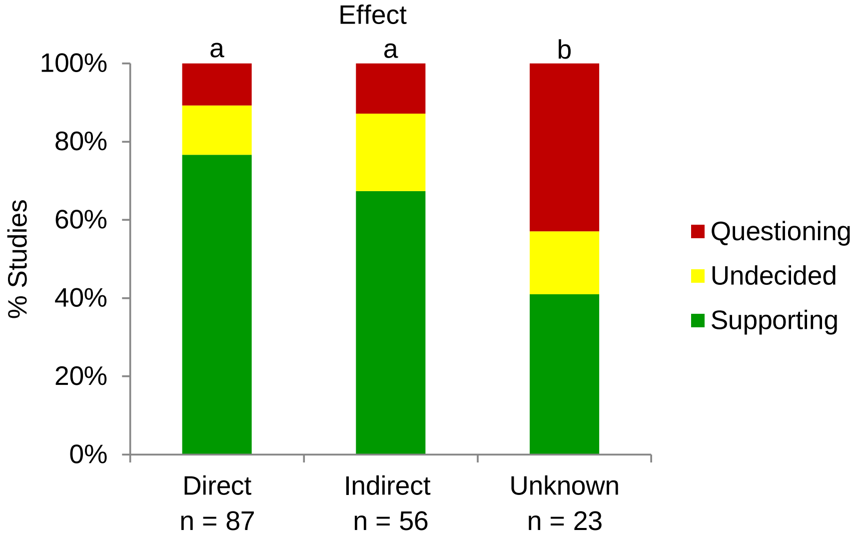

Figure 4. Weighted data on level of empirical support for the different types of effects. Letters above bars indicate significant differences (U-tests, p < 0.05).

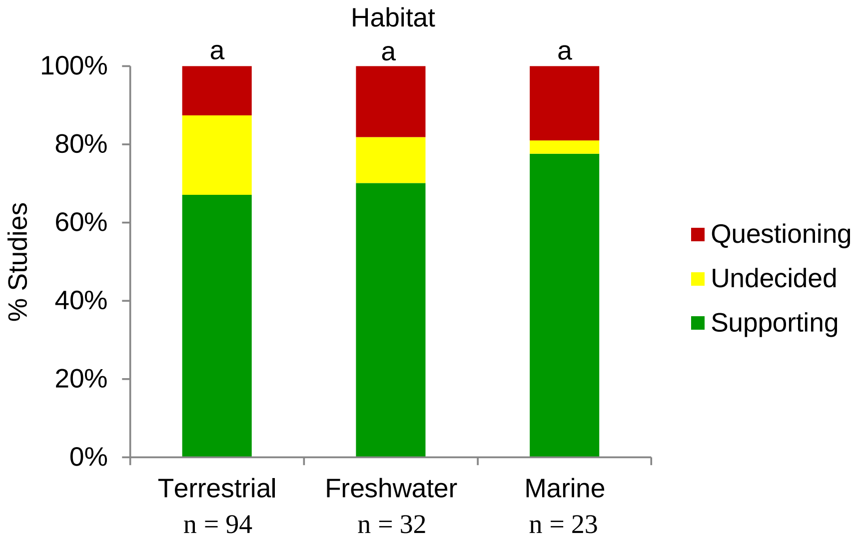

Figure 5. Weighted data on level of empirical support for the different habitats. Letters above bars indicate significant differences (U-tests, $p < 0.05$).

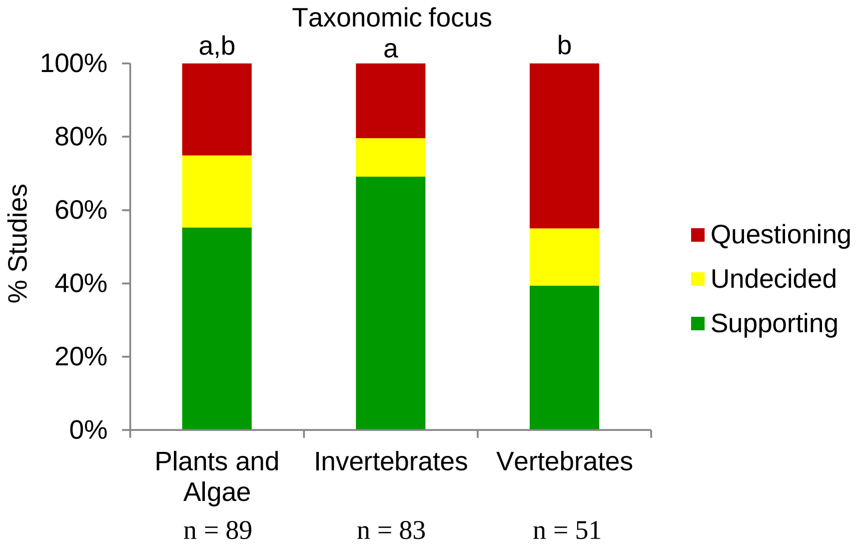

Figure 6. Weighted data on level of empirical support for the different taxonomic groups. Letters above bars indicate significant differences (U-tests, $p < 0.05$).

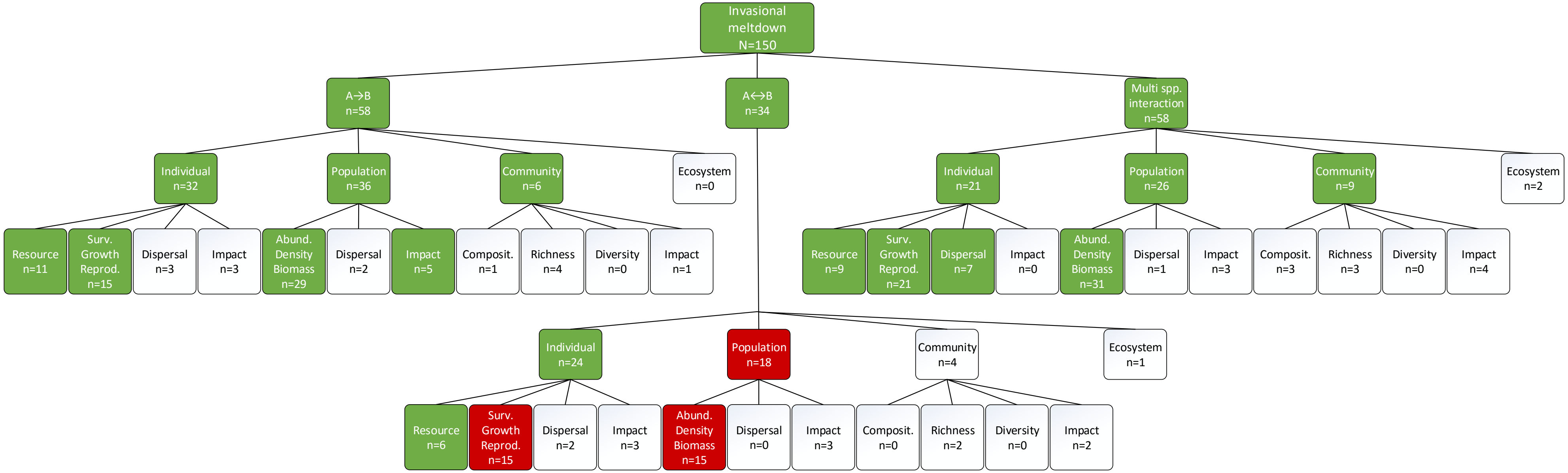

Figure 7. Schematic illustration of the hierarchy of hypotheses for the IMH. The HoH is structured according to three criteria: (1) type of interaction; (2) ecological level of evidence; and (3) outcome of interaction. Color codes indicate levels of empirical support, as follows: green boxes, n≥5 and >50% of weighted evidence supporting the sub-hypothesis; red boxes, n≥5 and >50% of weighted evidence questioning the sub-hypothesis; white boxes, all other cases (all n<5, so no comparisons were made here).

DISCUSSION

The HoH approach allowed us to identify differences in the number of studies investigating different sub-hypotheses of the IMH as well as differences in the levels of its support. Analyzing the general results and the majority of sub-hypotheses tested, there was an evident dominance of studies supporting the IMH. This result was independent of whether unweighted or weighted data were used. It is in contrast to Jackson's results (2015), however the two studies are not straightforward to compare. Jackson's analysis was more specific, not covering all aspects of the IMH, and thus based on a smaller dataset (n = 57 vs 150 here). He assessed (1) how non-natives influence one another by retrieving data on non-natives' performance when living together and separately from each other and (2) how non-natives' interactions affect one another's ecological impact on ecosystems. In addition, different criteria to search papers were used, and the analyses were done in a different way.

Despite the high level of support we found overall for the IMH, the majority of studies were not designed to test the IMH, as many studies even classified as "experimental" were designed to test different questions. Supporting information comes mainly from secondary results. This pattern may be the result of a short period since the IMH appeared in the literature and so, with the increasing attention given, we should expect more evidence provided by more specifically designed sampling and/or experiments. The shift towards replicated experimental designs is paramount to infer causal relationship between species interactions and the increasing rate of invasions and/or the synergistic impact upon the native community (Oksanen 2001).

When we divided the IMH into sub-hypotheses regarding the type of interaction between the interacting non-natives, many studies looking at A↔B interactions found questioning evidence. This pattern arose most likely because there is a large amount of evidence for competing species, possibly since for many decades competition was considered the main force structuring communities (Elton 1946; Diamond 1975; Ricklefs 1987; Gotelli and McCabe 2002; Richardson et al. 2000). Additionally, competition is a more conspicuous interaction whereas positive interactions often occur in an indirect manner (Bertness and Callaway 1994). On the other hand, there is a large body of evidence suggesting that mutualistic interactions are happening frequently among non-natives (reviewed by Richardson et al. 2000). It is possible that in the present global change scenario, positive interactions will increase in importance to the biota as posited by the stress-gradient hypothesis (Bertness and Callaway 1994, Kawai and Tokeshi 2007). The variety of A↔B interactions found in our study (e.g. mycorrhizal associations, plant-pollinator interactions, ant-scale insects exchanging resource for protection, seed dispersal) supports the view of the high importance that facilitation attains to communities in invasion scenarios.

Although highlighted by Simberloff and Von Holle (1999) and later by Simberloff (2006) that the IMH is a community-level phenomenon, there is currently scarce available evidence at this ecological level. Indeed, out of 150 investigations, our survey found only 21 carried out at the community level. The ultimate community outcome of the IMH would be the accelerating rate of invasion resulting from species introductions and facilitations (Simberloff 2006, Von Holle 2011). Despite the importance that invasion rates assume in empirical tests of the IMH, nearly none of the papers in our survey provided information on rates of invasion. One exception was the study by Wonham and Pachepsky (2006), who found an exponential increase in invasion records even with decreasing invasion success, therefore questioning the IMH.

In addition to IM, there are many other important aspects of invasion biology at the community level (Shea and Chesson 2002, Simberloff 2004). Community-level phenomena are, in Lawton's (1999) words, "orders of magnitude more complicated" than population dynamics. Thus, data at individual and population levels can be more easily collected (together representing 88% of the IMH studies analyzed here). Our results show that individual-level evidence is not always in accordance with population-level evidence for the IMH (see A↔B interaction of Fig. 1), so generalizations across ecological levels should be done carefully, and community-level evidence should be pursued (Simberloff 2004).

It has been hypothesized that coevolution might be important for IM (DeVanna et al. 2011), although this idea was not present in the original formulation by Simberloff and Von Holle (1999; see also Simberloff 2006 and Von Holle 2011). The importance of co-evolution remains a current question (Jackson 2015). In our analysis, studies on coevolved non-native species showed lower support for the IMH than studies on non-native species that did not coevolve (Fig. 3). This might surprise, but positive interactions seem to occur as frequently or infrequently as negative ones (Lortie and Callaway 2009), hence co-evolution does not automatically favor an IM scenario. The level of eco-evolutionary experience (sensu Saul et al. 2013, Saul and Jeschke 2015) (i.e. adaptations accumulated during evolution to biotic interactions in a species native range) that the non-native species have with each other is likely more important to the outcome of the new interaction. A high level of eco-evolutionary experience will favor the IMH only when previous interactions with archetypes of interaction partners were positive. In this sense, if we know the eco-evolutionary experience that a non-native species acquired in its native range, we might be able to predict the outcome of that invasion and the potential of IM.

Another challenge when studying species interactions is that the outcome of interactions might vary along the network of interactions and across ecological levels. As one example of the difficulties in analyzing network interactions, consider an indirect facilitation where one non-native species A reduces the population of an enemy B of another non-native species C (see Fig. 2 in Ricciardi 2001, Nuñez et al. 2008). In this case, if the enemy B that was reduced was also non-native, and the study only looked at the direct interaction, the IMH would be questioned. However, the outcome could be an increase of the population of species C which is also non-native, giving support to the IMH. Unfortunately indirect effects are often difficult to observe and measure because it requires a more complete look at the possibly interacting species. Indirect effects might yield also different interpretations at different ecological levels. Diet analysis and an enclosure experiment showed that a crayfish invasive to European waters consumed a non-native macrophyte, but on the other hand the macrophyte increased in biomass when the crayfish was present because the crayfish excluded macrophyte competitors (Chucholl 2013). In this case, a negative interaction was present at the individual level (measured as the presence of the non-native macrophyte in stomach contents), but a stronger positive outcome was observed at the population level (measured as the increase in biomass of the same non-native macrophyte).

Our results on habitats showed a predominance of studies in terrestrial systems. This bias does not seem to be restricted to the IMH, as it follows the pattern for general invasion biology (Lowry et al. 2013). A better representation of aquatic habitats would be important, as they are known to host a large number of non-native species and are under severe threat from invasion, particularly freshwater systems (Dudgeon et al. 2006, Gallardo et al. 2015, Havel et al. 2015). We also found a taxonomic bias in the studies, as the majority focused on plants and terrestrial insects (Pyšek et al. 2008). Studies on vertebrates showed lower support for the IMH. We suspect this is due to the high number of consumptive and competitive interactions detected and to the fact that these interactions are more conspicuous than facilitative ones, which is particularly important when studying large mobile organisms such as vertebrates (Bertness and Callaway 1999).

Only few studies with truly strong supporting evidence for the IMH were found (considering study weights, Supplementary material Appendix 2 Fig. A1). One such study is Stanley et al. (2013) who studied a network of beneficial direct and indirect interactions between Argentine ants, scale insects and boneseed which are invasive in New Zealand, and the negative effect on the native invertebrate community. The combined findings of O'Dowd et al. (2003) and Green et al. (2011) showed that the mutualism between the introduced yellow crazy ant and honeydew-secreting scale insects lead to a population burst of both species. In addition, the high abundance of the yellow crazy ant leads to a severe decrease of local populations of the native red land crab as the invasive ant kills the native crab by spraying formic acid over their eyes and mouthparts. By extirpating the local population of the native crab, the invasive ant creates an enemy-free space for the invasive giant African snail. The native crab is also responsible for regulating seedling abundance and litter breakdown, and so, the invasive snail also benefits from increased resource. Evaluating the synergistic effect among non-native crayfishes, Jackson et al. (2014) identified both additive and synergistic effects of crayfish on the ecosystem. This study has received the highest weight in our study. Although a few such studies exist, future research on the IMH should focus on multi-species interactions at the community or ecosystem level, ultimately linking interactions to the increasing number of non-native species. This task is yet to be done.

A next step would also be to link the HoH approach with formal meta-analytical tools. The challenge here is that formal meta-analysis can only be applied to research results that are either given in the same effect size or be transformed to the same effect size (Borenstein et al. 2009). Thus, formal meta-analyses are typically applied for a given, rather narrow research question or specific hypothesis where most research studies report similar types of effect sizes (research studies reporting other types of effect sizes are excluded). Yet for broad hypotheses such as the IMH, there is often a plethora of different research studies, which address different aspects of a broad hypothesis and thus report different types of effect sizes. Even if these *can* be transferred into each other, it is not always clear if they *should* be transferred, as effect sizes can often be expected to genuinely vary among sub-hypotheses. We also need a discussion on how different studies applying different research methods should be weighted: How can we best measure and weight differences among research studies?

In conclusion, the IMH appears to be supported along a large array of habitats and organisms, but strong cases of evidence are still rare. The HoH presented here can be continually updated, and thus the progress in research on the IMH monitored. Indeed, an extended HoH for the IMH is planned to be included in an online portal jointly with HoHs for other hypotheses. In this way, all researchers, managers and other interested people can access continually updated information about major hypotheses in invasion biology and other disciplines.

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Chucholl, C. 2013. Feeding ecology and ecological impact of an alien ‘warm water’ omnivore in cold lakes. - Limnologica 43: 219-229.

DeVanna, K. M. et al. 2011. An alternative hypothesis to the invasional meltdown in the Laurentian Great Lakes region: General facilitation by Dreissena. - J. Great Lakes Res. 37: 632-641.

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Dudgeon, D. et al. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. - Biol. Rev. 81: 163-182.

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Gallardo, B. et al. 2015. Global ecological impacts on invasive species in aquatic ecosystems. - Global Change Biol. doi: 10.1111/gcb.13004

Gotelli, N. J. and McCabe, D. J. 2002. Species co-occurrence: a meta-analysis of Diamond’s assembly rules model. - Ecology 83: 2091-2096.

Green, P. T. et al. 2011. Invasional meltdown: Invader-invader mutualism facilitates a secondary invasion. - Ecology 92: 1758-1768.

Halpern, B. S. et al. 2007. Incorporating positive interactions in aquatic restoration and conservation. - Front. Ecol. Environ. 5: 153-160.

Havel, J. E. et al. 2015. Aquatic invasive species: challenges for the future. - Hydrobiologia 750: 147-170.

Heger, T. and Jeschke, J. M. 2014. The enemy release hypothesis as a hierarchy of hypotheses. - Oikos 123: 741-750.

Heger, T. et al. 2013. Conceptual frameworks and methods for advancing invasion ecology. - AMBIO 42: 527-540.

Jackson, M. C. 2015. Interactions among multiple invasive animals. - Ecology 96: 2035-2041.

Jackson, M. C. et al. 2014. Niche differentiation among invasive crayfish and their impacts on ecosystem structure and functioning. - Freshw. Biol. 59: 1123-1135.

Jeschke, J. M. et al. 2012. Support for major hypotheses in invasion biology is uneven and declining. - Neobiota 14: 1-20.

Kawai, T. and Tokeshi, M. 2007. Testing the facilitation-competition paradigm under the stress-gradient hypothesis: decoupling multiple stress factors. - P. Roy. Soc. B-Biol. Sci. 274: 2503-2508

Lawton, J. H. 1999. Are there general laws in ecology? - Oikos 84: 177-192.

Lortie, C. J. 2014. Formalized synthesis opportunities for ecology: systematic reviews and meta-analyses. - Oikos 123: 897-902.

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Nuñez, M. A. et al. 2008. Enemy release or invasional meltdown? Deer preference for exotic and native trees on Isla Victoria, Argentina. - Austral Ecol. 33: 317-323.

O'Dowd, D. J. et al. 2003. Invasional 'meltdown' on oceanic island. - Ecol. Lett. 6: 812-817

Oksanen, L. 2001. Logic of experiments in ecology: is pseudoreplication a pseudoissue. - Oikos 94: 27-38.

Pyšek, P. et al. 2008. Geographical and taxonomic biases in invasion ecology. - Trends Ecol. Evol. 23: 237-244.

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Stanley, M. C. et al. 2013. Invasive interactions: can Argentine ants indirectly increase the reproductive output of a weed? - Arthropod-Plant Inte. 7: 59-67.

Von Holle, B. 2011. Invasional meltdown. - In: Simberloff, D. and Rejmánek, M. (eds.), Encyclopedia of Biological Invasion. University of California Press, pp. 360-364.

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SUPPLEMENTARY MATERIAL

Appendix 1

Studies included in the analysis, together with their level of empirical support for each sub-hypotheses of the invasional meltdown hypothesis (supporting, undecided, or questioning).

1. Sub-hypothesis "A→B“

1.1. Individual level

1.1.1. Resource

Supported

González-Bernal, E. et al. 2012. Cane toads on cowpats: commercial livestock production facilitates toad invasion in tropical Australia. -PLoS ONE 7: e49351.

Glen, A. S. et al. 2012. Ecology of brushtail possums in a New Zealand dryland ecosystem. -New Zeal. J. Ecol. 36: 29-37.

Kobak, J. et al. 2009. Preferences of the Ponto-Caspian amphipod *Dikerogammarus haemobaphes* for living zebra mussels. -J. Zool. 279: 229-235.

Hayden, T. A. and Miner, J. G. 2009 Rapid dispersal and establishment of a benthic Ponto-Caspian goby in Lake Erie: diel vertical migration of early juvenile round goby. -Biol. Invasions 11: 1767-1776.

Schüttler, E. et al. 2008. Diet of the American mink *Mustela vison* and its potencial impact on the native fauna of Navarino Island, Cape Horn Biosphere Reserve, Chile. -Rev. Chil. Hist. Nat. 81: 585-598.

Kobak, J. and Żytkowicz, J. 2007. Preferences of invasive Ponto-Caspian and native European gammarids for zebra mussel (*Dreissena polymorpha*, Bivalvia) shell habitat. -Hydrobiol. 589: 43-54.

Naoko, Y. et al. 2003. Seed and seedling demography of invasive and native trees of subtropical Pacific islands. -J. Veg. Sci. 14: 15-24.

Kobak, J. et al. 2013. The impact of zebra mussel (*Dreissena polymorpha*) periostracum and biofilm cues on habitat selection by a Ponto-Caspian amphipod *Dikerogammarus haemobaphes*. -Hydrobiol. 702: 215-226.

Hossain, M. M. et al. 2013. Examination of the effects of largemouth bass (*Micropterus salmoides*) and bluegill (*Lepomis macrochirus*) on the ecosystem attributes of lake Kawahara-oike, Nagasaki, Japan. -Ecol. Inform. 18: 149-161.

Ma, Z. et al. 2013. Effects on invasive cordgrass on presence of marsh grassbird in an area where it is not native. -Conserv. Biol. 28: 150-158.

Undecided

Pierre, K. J. L. et al. 2010. Strong feeding preferences of an exotic generalist herbivore for an exotic forb: a case of invasional antagonism. -Biol. Invasions 12: 3025-3031.

1.1.2. Survival, growth and Reproduction

Supported

Reiskind, M. H. et al. 2010. Invasive leaf resources alleviate density dependence in the invasive mosquito, Aedes albopictus. -Biol. Invasions 12: 2319-2328.

Relva, M. A. et al. 2010. Introduced deer reduce native plant cover and facilitate invasion of non-native tree species: evidence for invasional meltdown. -Biol. Invasions 12: 303-311.

Grosholz, E. D. 2005. Recent biological invasion may hasten invasional meltdown by accelerating historical introductions. -PNAS 102: 1088-1091.

Adams, M. J. et al. 2003. Indirect facilitation of an anuran invasion by non-native fishes. -Ecol. Lett. 6: 343-351.

Thouvenot, L. et al. 2013. Strategies of the invasive macrophyte *Ludwigia grandiflora* in its introduced range: competition, facilitation or coexistence with native and exotic species? -Aquat. Bot. 107: 8-16.

Ruffino, L. et al. 2013. Anthropogenic subsidies mitigate environmental variablility for insular rodents. -Oecologia 172: 737-749.

Flory, S. L. and Bauer, J. T. 2014. Experimental evidence for indirect facilitation among invasive plants. -J. Ecol. 102: 12-18.

Ma, Z. et al. 2013. Effects on invasive cordgrass on presence of marsh grassbird in an area where it is not native. -Conserv. Biol. 28: 150-158.

Undecided

Miller, T. E. X. et al. 2010. Experimental test of biotic resistance to an invasive herbivore provided by potential plant mutualists. -Biol. Invasions 12: 3563-3577.

Molina-Montenegro, M. A. et al. 2008. Positive interactions among plant species for pollinator service: assessing the 'magnet species' concept with invasive species. -Oikos 117: 1833-1839.

Tecco, P. A. et al. 2007. Facilitation and interference underlying the association between the woody invaders *Pyracantha angustifolia* and *Ligustrum lucidum*. -Appl. Veg. Sci. 10: 211-218.

Questioned

Stricker, K. B. and Stiling, P. 2012. Herbivory by an introduced Asian weevil negatively affects population growth of an invasive Brazilian shrub in Florida. -Ecology 93: 1902-1911.

Griffen, B. D. et al. 2011. Reduced fecundity by one invader in the presence of another: a potential mechanism leading to species replacement. -J. Exp. Mar. Biol. Ecol. 406: 6-13.

Tsai, C. et al. 2010. Interactions between two introduced species: *Zostera japonica* (dwarf eelgrass) facilitates itself and reduces condition of *Ruditapes philippinarum* (Manila clam) on intertidal flats. -Mar. Biol. 157: 1929-1936.

Harvey, B. C. et al. 2004. An emergent multiple predator effect may enhance biotic resistance in a stream fish assemblage. -Ecology 85: 127-133.

1.1.3. Dispersal

Supported

Davis, N. E. et al. 2010. Facilitative interactions between an exotic mammal and native and exotic plants: hog deer (*Axis porcinus*) as seed dispersers in south-eastern Australia. -Biol. Invasions 12: 1079-1092.

Bartuszevige, A. M. and Gorchov, D. L. 2006. Avian seed dispersal of an invasive shrub. -Biol. Invasions 8: 1013-1022.

Kelehear, C. et al. 2013. Invasive parasite in multiple invasive hosts: the arrival of a new host revives a stalled prior parasite invasion. -Oikos 122: 1317-1324.

1.1.4.Impact

Supported

Junker, R. R. et al. 2011. Hawaiian ant-flower networks: nectar-thieving ants prefer undefended native over introduced plants with floral defenses. -Ecol. Monogr. 81: 295-311.

Levin, P. S. et al. 2002. Community-wide effects of nonindigenous species on temperate rocky reefs. -Ecology 83: 3182-3193.

Questioned

Newsom, M. J. and Williams, S. L. 2014. Predation and functional responses of *Carcinus maenas* and *Cancer magister* in the presence of the introduced Cephalaspidean *Philine orientalis*. -Estuar. Coast. 37: 1284-1294.

1.2. Population level

1.2.1. Abundance, density and biomass

Supported

Villaseñor-Parada, C. and Neill, P. E. 2011. Distribución espacial de epifitos en el talo de la macroalga introducida *Codium fragile* subsp. *tomentosoides* en el submareal de Caldera. -Rev. Biol. Mar. Oceanogr. 46: 257-262.

Reiskind, M. H. et al. 2010. Invasive leaf resources alleviate density dependence in the invasive mosquito, Aedes albopictus. -Biol. Invasions 12: 2319-2328.

Watanabe, S. et al. 2010. Contrasting patterns of spread in interacting invasive species: *Membranipora membranacea* and *Codium fragile* off Nova Scotia. -Biol. Invasions 12: 2329-2342.

Boets, P. et al. 2010. Combining data-driven methods and lab studies to analyse the ecology of *Dikerogammarus villosus*. -Ecol. Inform. 5, 133-139.

Relva, M. A. et al. 2010. Introduced deer reduce native plant cover and facilitate invasion of non-native tree species: evidence for invasional meltdown. -Biol. Invasions 12: 303-311.

Isaac-Renton, M. et al. 2010. Effects of introduced Canada geese (*Branta canadensis*) on native plant communities of the Southern Gulf Islands, British Columbia. -Ecoscience 17: 394-399.

Oliver, M. et al. 2009. Do rabbits eat voles? Apparent competition, habitat heterogeneity and large-scale coexistence under mink predation. -Ecol. Lett. 12: 1201-1209.

Jäger, H. et al. 2009. Destruction without extinction: long-term impacts of an invasive tree species on Galápagos highland vegetation. -J. Ecol. 97: 1252-1263.

Siemann, E. et al. 2009. Experimental test of the impacts of feral hogs on forest dynamics and processes in the southeastern US. -Forest Ecol. Manag. 258: 546-553.

Preisser, E. L. et al. 2008. Range expansion and population dynamics of co-occuring invasive herbivores. -Biol. Invasions 10: 201-213.

Locke, A. et al. 2007. Invasion of the southern Gulf of St. Lawrence by clubbed tunicate (*Styela clava* Herdman): potential mechanisms for invasions of Prince Edward Island estuaries. -J. Exp. Mar. Biol. Ecol. 342: 69-77.

Von Holle, B. et al. 2006. Facilitations between the introduced nitrogen-fixing tree, *Robinia pseudoacacia*, and nonnative plant species in the glacial outwash upland ecosystem of Cape Cod, MA. -Biodivers. Conserv. 15: 2197-2215.

Tecco, P. A. et al. 2006. Positive interaction between invasive plants: the influence of *Pyracantha angustifolia* on the recruitment of native and exotic woody species. -Austral Ecol. 31: 293-300.

Heneghan, L. et al. 2007. Interactions of an introduced shrub and introduced earthworms in an Illinois urban woodland: impact on leaf litter decomposition. -Pedobiologia 50: 543-551.

Grosholz, E. D. 2005. Recent biological invasion may hasten invasional meltdown by accelerating historical introductions. -PNAS 102: 1088-1091.

Devin, S. et al. 2003 Ecological traits of the amphipod invader *Dikerogammarus villosus* on a mesohabitat scale. -Archiv. Hydrobiol. 158: 43-56.

Adams, M. J. et al. 2003. Indirect facilitation of an anuran invasion by non-native fishes. -Ecol. Lett. 6: 343-351.

Levin, P. S. et al. 2002. Community-wide effects of nonindigenous species on temperate rocky reefs. -Ecology 83: 3182-3193.

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Flory, S. L. and Bauer, J. T. 2014. Experimental evidence for indirect facilitation among invasive plants. -J. Ecol. 102: 12-18.

Ma, Z. et al. 2014. Effects on invasive cordgrass on presence of marsh grassbird in an area where it is not native. -Conserv. Biol. 28: 150-158.

Green, D. S. and Crowe, T. P. 2014. Context- and density-dependent effects of introduced oysters on biodiversity. -Biol. Invasions 16: 1145-1163.

Undecided

D’Antonio, C. M. et al. 2011. Long-term impacts of invasive grasses and subsequent fire in seasonally dry Hawaiian woodlands. -Ecol. Appl. 21: 1617-1628.

Pierre, K. J. L. et al. 2010. Strong feeding preferences of an exotic generalist herbivore for an exotic forb: a case of invasional antagonism. -Biol. Invasions 12: 3025-3031.

Maezo, M. J. et al. 2010. Potential and realized interactions between two aquatic invasive species: Eurasian watermilfoil (*Myriophyllum spicatum*) and rusty crayfish (*Orconectes rusticus*). -Can. J. Fish. Aquat. Sci. 67: 684-700.

Questioned

Melero, Y. et al. 2012. Evaluating the effect of American mink, an alien invasive species, on the abundance of native community: is coexistence possible? -Biodivers. Conserv. 21: 1795-1809.

Griffen, B. D. et al. 2011. Reduced fecundity by one invader in the presence of another: a potential mechanism leading to species replacement. -J. Exp. Mar. Biol. Ecol. 406: 6-13.

Schüttler, E. et al. 2010. Abundance and habitat preferences of the southernmost population of mink: implications for managing a recent island invasion. -Biodivers. Conserv. 19: 725-743.

Collin, S. B. and Johnson, L. E. 2014 Invasive species contribute to biotic resistance: negative effect of caprellid amphipods on an invasive tunicate. -Biol. Invasions 16: 2209-2219.

1.2.2. Dispersal

Supported

Rowles, A. D. and O’Dowd, D. J. 2009. New mutualism for old: indirect disruption and direct facilitation of seed dispersal following Argentine ant invasion. -Oecologia 158: 709-716.

Undecided

Maezo, M. J. et al. 2010. Potential and realized interactions between two aquatic invasive species: Eurasian watermilfoil (*Myriophyllum spicatum*) and rusty crayfish (*Orconectes rusticus*). -Can. J. Fish. Aquat. Sci. 67: 684-700.

1.2.3. Impact

Supported

Lang, A. C. and Buschbaum, C. 2010. Facilitative effects of introduced Pacific oysters on native macroalgae are limited by a secondary invader, the seaweed *Sargassum muticum*. -J. Sea Res. 63: 119-128.

Oliver, M. et al. 2009. Do rabbits eat voles? Apparent competition, habitat heterogeneity and large-scale coexistence under mink predation. -Ecol. Lett. 12: 1201-1209.

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Rand, T. A. and Louda, S. M. 2004. Exotic weed invasion increases the susceptibility of native plants to attack by a biocontrol herbivore. -Ecology 85: 1548-1554.

Questioned

Moorman, M. C. et al. 2009. Implications of beaver *Castor Canadensis* and trout introductions on native fish in the Cape Horn Biosphere Reserve, Chile. -Trans. Am. Fish. Soc. 138: 306-313.

1.3. Community level

1.3.1. Composition

Supported

Levin, P. S. et al. 2002. Community-wide effects of nonindigenous species on temperate rocky reefs. -Ecology 83: 3182-3193.

1.3.2. Richness

Supported

Von Holle, B. et al. 2006. Facilitations between the introduced nitrogen-fixing tree, Robinia pseudoacacia, and nonnative plant species in the glacial outwash upland ecosystem of Cape Cod, MA. -Biodivers. Conserv. 15: 2197-2215.

Tecco, P. A. et al. 2006. Positive interaction between invasive plants: the influence of *Pyracantha angustifolia* on the recruitment of native and exotic woody species. -Austral Ecol. 31: 293-300.

Molinari, N. A. and D'Antonio, C. M. 2014. Structural, compositional and trait differences between native- and non-native dominated grassland patches. -Funct. Ecol. 28: 745-754.

Undecided

D'Antonio, C. M. et al. 2011. Long-term impacts of invasive grasses and subsequent fire in seasonally dry Hawaiian woodlands. -Ecol. Appl. 21: 1617-1628.

1.3.4. Impact

Supported

Lang, A. C. and Buschbaum, C. 2010. Facilitative effects of introduced Pacific oysters on native macroalgae are limited by a secondary invader, the seaweed *Sargassum muticum*. -J. Sea Res. 63: 119-128.

2. Sub-hypothesis "A↔B“

2.1. Individual level

2.1.1. Resource

Supported

Padrón, B. et al. 2009 Impact of alien plant invaders on pollination networks in two archipelagos. -PLoS ONE 4: e6275.

López-Darias, M. and Nogales, M. 2008. Effects of the invasive Barbary ground squirrel (*Atlantoxerus getulus*) on seed dispersal systems of insular xeric environments. -J. Arid Environ. 72: 926-939.

Lafleur, N. E. et al. 2007. Invasive fruits, novel foods, and choice: an investigation of European starling and American robin frugivory. -Wilson J. Ornithol. 119: 429-438.

Helms, K. R. and Vinson, B. 2002. Widespread association of the invasive ant *Solenopsis invicta* with an invasive mealybug. -Ecology 83: 2425-2438.

Chucholl, C. 2013. Feeding ecology and ecological impact of an alien 'warm-water' omnivore in cold lakes. -Limnologica 43: 219-229.

Undecided

Savini, D. and Occhipinti-Ambrogi, A. 2006. Consumption rates and prey preference of the invasive gastropod *Rapana venosa* in the Northern Adriatic Sea. -Helgoland Mar. Res. 60: 153-159.

2.1.2. Survival, growth and reproduction

Supported

Zhou, A. et al. 2012. Does mutualism drive the invasion of two alien species? The case of *Solenopsis invicta* and *Phenacoccus solenopsis*. -PLoS ONE 7: e41856.

Lach, L. et al. 2010. Contrasting effects of an invasive ant on a native and an invasive plant. -Biol. Invasions 12: 3123-3133.

López-Darias, M. and Nogales, M. 2008. Effects of the invasive Barbary ground squirrel (*Atlantoxerus getulus*) on seed dispersal systems of insular xeric environments. -J. Arid Environ. 72: 926-939.

Simpson, S. R. et al. 2005. Broom and honeybees in Australia: an alien liaison. -Plant Biol. 7: 541-548.

Barthell, J. F. et al. 2001. Promotion of seed set in yellow star-thistle by honey bees: evidence of an invasive mutualism. -Ecol. Appl. 11: 1870-1883.

Ackerman, J. D. et al. 2014. Biotic resistance and invasional meltdown: consequences of acquired interspecific interactions for an invasive orchid, *Spathoglottis plicata* in Puerto Rico. -Biol. Invasions 16: 2435-2447.

Undecided

Wundrow, E. J. et al. 2012. Facilitation and competition among invasive plants: a field experiment with alligatorweed and water hyacinth. -PLoS ONE 7: e48444.

Questioned

Rauschert, E. S. J. and Shea, K. 2012. Invasional interference due to similar inter- and intraspecific competition between invaders may affect management. -Ecol. Appl. 22: 1413-1420.

Platvoet, D. et al. 2009. Invader-invader interactions in relation to environmental heterogeneity leads to zonation of two invasive amphipods, *Dikerogammarus villosus* (Sowinski) and *Gammarus tigrinus* Sexton:

amphipod pilot species project (AMPIS) report 6. -Biol. Invasions 11: 2085-2093.

Preisser, E. L. and Elkinton, J. S. 2008. Exploitative competition between invasive herbivores benefits a native host plant. -Ecology 89: 2671-2677.

Griffen, B. D. et al. 2008. Inhibition between invasives: a newly introduced predator moderates the impacts of a previously established invasive predator. -J. Anim. Ecol. 77: 32-40.

Mony, C. et al. 2007. Competition between two invasive Hydrocharitaceae (*Hydrilla verticillata* (L.f.) (Royle) and *Egeria densa* (Planch)) as influenced by sediment fertility and season. -Aquat. Bot. 86: 236-242.

Belote, R. T. and Weltzin, J. F. 2006. Interactions between two co-dominant, invasive plants in the understory of a temperate deciduous forest. -Biol. Invasions 8: 1629-1641.

Lohrer, A. M. and Whitlatch, R. B. 2002. Interactions among aliens: apparent replacement of one exotic species by another. -Ecology 83: 719-732.

Rice, E. S. and Silverman, J. 2013. Propagule pressure and climate contribute to the displacement of *Linepithema humile* by *Pachycondyla chinensis*. -PLoS ONE 8: e56281.

2.1.3. Dispersal

Supported

Zhou, A. et al. 2012. Does mutualism drive the invasion of two alien species? The case of *Solenopsis invicta* and *Phenacoccus solenopsis*. -PLoS ONE 7: e41856.

Padrón, B. et al. 2011. Integration of invasive *Opuntia* spp. by native and alien seed dispersers in the Mediterranean area and the Canary Islands. -Biol. Invasions 13: 831-844.

2.1.4. Impact

Questioned

Porter-Whitaker, A. E. et al. 2012. Multiple predator effects and native prey responses to two non-native Everglades cichlids. -Ecol. Freshw. Fish 21: 375-385.

Young, K. A. et al. 2009. The diversity of juvenile salmonids does not affect their competitive impact on a native galaxiid. -Biol. Invasions 11: 1955-1961.

Preisser, E. L. and Elkinton, J. S. 2008. Exploitative competition between invasive herbivores benefits a native host plant. -Ecology 89: 2671-2677.

2.2. Population level

2.2.1. Abundance, density and biomass

Supported

Zhou, A. et al. 2012. Does mutualism drive the invasion of two alien species? The case of *Solenopsis invicta* and *Phenacoccus solenopsis*. -PLoS ONE 7: e41856.

Abbot, K. L. and Green, P. T. 2007. Collapse of ant-scale mutualism in a rainforest on Christmas Island. -Oikos 116: 1238-1246.

Helms, K. R. and Vinson, S. B. 2003. Apparent facilitation of an invasive mealybug by an invasive ant. -Insect. Soc. 50: 403-404.

Chucholl, C. 2013. Feeding ecology and ecological impact of an alien ‘warm-water’ omnivore in cold lakes. -Limnologica 43: 219-229.

Undecided

Wundrow, E. J. et al. 2012. Facilitation and competition among invasive plants: a field experiment with alligatorweed and water hyacinth. -PLoS ONE 7: e48444.

Questioned

Rius, M. et al. 2011. Long-term coexistence of non-indigenous species in aquaculture facilities. Mar. Pollut. Bull. 62: 2395-2403.

Platvoet, D. et al. 2009. Invader-invader interactions in relation to environmental heterogeneity leads to zonation of two invasive amphipods, *Dikerogammarus villosus* (Sowinski) and *Gammarus tigrinus* Sexton: amphipod pilot species project (AMPIS) report 6. -Biol. Invasions 11: 2085-2093.

Preisser, E. L. and Elkinton, J. S. 2008. Exploitative competition between invasive herbivores benefits a native host plant. -Ecology 89: 2671-2677.

Griffen, B. D. et al. 2008. Inhibition between invasives: a newly introduced predator moderates the impacts of a previously established invasive predator. -J. Anim. Ecol. 77: 32-40.

Mony, C. et al. 2007. Competition between two invasive Hydrocharitaceae (*Hydrilla verticillata* (L.f.) (Royle) and *Egeria densa* (Planch)) as influenced by sediment fertility and season. -Aquat. Bot. 86: 236-242.

Belote, R. T. and Weltzin, J. F. 2006. Interactions between two co-dominant, invasive plants in the understory of a temperate deciduous forest. -Biol. Invasions 8: 1629-1641.

Ricciardi, A. and Whoriskey, F. G. 2004. Exotic species replacement: shifting dominance of dreissenid mussels in the Soulanges Canal, upper St. Lawrence River, Canada. -J. N. Am. Benthol. Soc. 23: 507-514.

Lohrer, A. M. and Whitlatch, R. B. 2002. Interactions among aliens: apparent replacement of one exotic species by another. -Ecology 83: 719-732.

Rice, E. S. and Silverman, J. 2013. Propagule pressure and climate contribute to the displacement of *Linepithema humile* by *Pachycondyla chinensis*. -PLoS ONE 8: e56281.

Hasegawa, K. et al. 2014. Replacement of nonnative rainbow trout by nonnative brown trout in the Chitose River system, Hokkaido, northern Japan. -Aquat. Invasions 9: 221-226.

2.2.3. Impact

Supported

Ross, D. J. et al. 2004. Interaction and impacts of two introduced species on a soft-sediment marine assemblage in SE Tasmania. -Mar. Biol. 144: 747-756.

Cameron, E. K. et al. 2013. Influence of two exotic earthworm species with different foraging strategies on abundance and composition of boreal microarthropods. -Soil Biol. Biochem. 57: 334-340.

Questioned

Lockwood, J. L. and Gilroy, J. J. 2004. The portability of foodweb dynamics: reassembling an Australian eucalypt-psyllid-bird association within California. -Global Ecol. Biogeogr. 13: 445-450.

2.3. Community level

2.3.2. Richness

Supported

Dickie, I. A. et al. 2010. Co-invasion by *Pinus* and its mycorrhizal fungi. -New Phytol. 187: 475-484.

Kuebbing, S. E. et al. 2014. Two co-occuring invasive woody shrubs alter soil properties and promote subdominant invasive species. -J. Appl. Ecol. 51: 124-133.

2.3.4. Impact

Supported

Cameron, E. K. et al. 2013. Influence of two exotic earthworm species with different foraging strategies on abundance and composition of boreal microarthropods. -Soil Biol. Biochem. 57: 334-340.

Questioned

Lockwood, J. L. and Gilroy, J. J. 2004. The portability of foodweb dynamics: reassembling an Australian eucalypt-psyllid-bird association within California. -Global Ecol. Biogeogr. 13: 445-450.

2.4. Ecosystem level

Supported

Kuebbing, S. E. et al. 2014. Two co-occuring invasive woody shrubs alter soil properties and promote subdominant invasive species. -J. Appl. Ecol. 51: 124-133.

3. Sub-hypothesis "Multi species interaction“

3.1. Individual level

3.1.1. Resource

Supported

Brandner, J. et al. 2013. Comparative feeding ecology of invasive Ponto-Caspian gobies. -Hydrobiol. 703: 113-131.

Spotswood, E. N. et al. 2012. An invasive tree alters the structure of seed dispersal networks between birds and plants in French Polynesia. -J. Biogeogr. 39: 2007-2020.

Miyake, M. and Miyashita, T. 2011. Identification of alien predators that should not be removed for controlling invasive crayfish threatening endangered odonates. -Aquat. Conserv.- Mar. Freshw. Ecosyst. 21: 292-298.

Sugiura, S. et al. 2008. Biological invasion into the nested assemblage of tree-beetle associations on the oceanic Ogasawara Islands. -Biol. Invasions 10: 1061-1071.

Nuñez, M. A. et al. 2008. Enemy release or invasional meltdown? Deer preference for exotic and native trees on Isla Victoria, Argentina. -Austral Ecol. 33: 317-323.

Bourgeois, K. et al. 2005. Invasional meltdown potential: facilitation between introduced plants and mammals on French Mediterranean islands. -Ecoscience 12: 248-256.

Mandon-Dalger, I. et al. 2004. Relationships between alien plants and an alien bird species on Reunion Islands. -J. Trop. Ecol. 20: 635-642.

Undecided

Shiels, A. B. and Drake, D. R. 2011. Are introduced rats (*Rattus rattus*) both seed predators and dispersers in Hawaii? -Biol. Invasions 13: 883-894.

Ness, J. H. et al. 2013. Reciprocally beneficial interactions between introduced plants and ants are induced by the presence of a third introduced species. -Oikos 122: 695-704.

3.1.2. Survival, growth and reproduction

Supported

Engelkes, T. and Mills, N. J. 2013. A fast-track for invasion: invasive plants promote the performance of an invasive herbivore. -Biol. Invasions 15: 101-111.

Beavon, M. A. ans Kelly, D. 2012. Invasional meltdown: pollination of the invasive liana *Passiflora tripartite* var. *mollissima* (Passifloraceae) in New Zealand. -New Zeal. J. Ecol 36: 100-107.

Green, P. T. et al. 2011. Invasional meltdown: invader-invader mutualism facilitates a secondary invasion. -Ecology 92: 1758-1768.

Abe, T. et al. 2011. Alien pollinator promotes invasive mutualism in an insular pollination system. -Biol. Invasions 13: 957-967.

Cushman, J. H. et al. 2011. Native herbivore and plant facilitation mediate the performance and distribution of an invasive exotic grass. -J. Ecol. 99: 524-531.

Linnebjerg, J. F. et al. 2009. Gut passage effect of the introduced red-whiskered bulbul (*Pycnonotus jocosus*) on germination of invasive plant species in Mauritius. -Austral Ecol. 34: 272-277.

Best, R. J. and Arcese, P. 2009. Exotic herbivores directly facilitate the exotic grasses they graze: mechanisms for an unexpected positive feedback between invaders. -Oecologia 159: 139-150.

Bourgeois, K. et al. 2005. Invasional meltdown potential: facilitation between introduced plants and mammals on French Mediterranean islands. -Ecoscience 12: 248-256.

Mandon-Dalger, I. et al. 2004. Relationships between alien plants and an alien bird species on Reunion Islands. -J. Trop. Ecol. 20: 635-642.

Stanley, M. C. et al. 2013. Invasive interactions: can Argentine ants indirectly increase the reproductive output of a weed. -Arthropod-Plant Interact. 7: 59-67.

Barrios-Garcia, M. N. and Simberloff, D. 2013. Linking the pattern to the mechanism: how an introduced mammal facilitates plant invasions. -Austral Ecol. 38: 884-890.

Undecided

Orchan, Y. et al. 2013. The complex interaction network among multiple invasive bird species in a cavity-nesting community. -Biol. Invasions 15: 429-445.

McCarville, M. T. et al. 2012. A nematode, fungus, and aphid interact via a shared host plant: implications for soybean management. -Entomol. Experimental. Appl. 143: 55-66.

Paynter, Q. et al. 2010. Disruption of an exotic mutualism can improve management of an invasive plant: varroa mite, honeybees and biological control of Scotch broom *Cytisus scoparius* in New Zealand. -J. Appl. Ecol. 47: 309-317.

Magnoli, S. M. et al. 2013. Responses to invasion and invader removal differ between native and exotic plant groups in a coastal dune. -Oecologia 173: 1521-1530.

Questioned

Kestrup, Å. and Ricciardi, A. 2009. Are interactions among Ponto-Caspian invaders driving amphipod species replacement in the St. Lawrence River? -J. Gt. Lakes Res. 35: 392-398.

Becerra, P. I. and Bustamante, R. O. 2008. The effect of herbivory on seedling survival of the invasive exotic species *Pinus radiata* and *Eucalyptus globulus* in a Mediterranean ecosystem of Central Chile. -Forest Ecol. Manag. 256: 1573-1578.

Bauer, C. R. et al. 2007. Predicting habitat use and trophic interactions of Eurasian ruffe, round gobies, and zebra mussels in nearshore areas of the Great Lakes. -Biol. Invasions 9: 667-678.

Kueffer, C. et al. 2007. Strong below-ground competition shapes tree regeneration in invasive *Cinnamomum verum* forests. -J. Ecol. 95: 273-282.

Griffiths, C. J. et al. 2013. Assessing the potencial to restore historic grazing ecosystems with tortoise ecological replacements. -Conserv. Biol. 27: 690-700.

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3.1.3. Dispersal

Supported

Spotswood, E. N. et al. 2012. An invasive tree alters the structure of seed dispersal networks between birds and plants in French Polynesia. -J. Biogeogr. 39: 2007-2020.

Best, R. J. and Arcese, P. 2009. Exotic herbivores directly facilitate the exotic grasses they graze: mechanisms for an unexpected positive feedback between invaders. -Oecologia 159: 139-150.

Bourgeois, K. et al. 2005. Invasional meltdown potential: facilitation between introduced plants and mammals on French Mediterranean islands. -Ecoscience 12: 248-256.

Mandon-Dalger, I. et al. 2004. Relationships between alien plants and an alien bird species on Reunion Islands. -J. Trop. Ecol. 20: 635-642.

Nuñez, M. A. et al. 2013. Exotic mammals disperse exotic fungi that promote invasion by exotic trees. -PLoS ONE 8: e66832.

Undecided

Shiels, A. B. and Drake, D. R. 2011. Are introduced rats (*Rattus rattus*) both seed predators and dispersers in Hawaii? -Biol. Invasions 13: 883-894.

Questioned

Calvino-Cancela, M. 2011. Seed dispersal of alien and native plants by vertebrate herbivores. -Biol. Invasions 13: 895-904.

3.2. Population level

3.2.1. Abundance, density and biomass

Supported

Green, P. T. et al. 2011. Invasional meltdown: invader-invader mutualism facilitates a secondary invasion. -Ecology 92: 1758-1768.

Helms, K. R. et al. 2011. Plant-based food resources, trophic interactions among alien species, and the abundance of an invasive ant. -Biol. Invasions 13: 67-79.

Heiman, K. W. and Micheli, F. 2010. Non-native ecosystem engineer alters estuarine communities. -Integr. Comp. Biol. 50: 226-236.

Davidson, T. M. et al. 2010. The composition and density of fauna utilizing burrow microhabitats created by a non-native burrowing crustacean (*Sphaeroma quoianum*). -Biol. Invasions 12: 1403-1413.

Belote, R. T. and Jones, R. H. 2009. Tree leaf litter composition and nonnative earthworms influence plant invasion in experimental forest floor mesocosms. -Biol. Invasions 11: 1045-1052.

Madritch, M. D. and Lindroth, R. L. 2009. Removal of invasive shrubs reduces exotic earthworm populations. -Biol. Invasions 11: 663-671.

Best, R. J. and Arcese, P. 2009. Exotic herbivores directly facilitate the exotic grasses they graze: mechanisms for an unexpected positive feedback between invaders. -Oecologia 159: 139-150.

Helms, K. R. and Vinson, S. B. 2008. Plant resources and colony growth in an invasive ant: the importance of honeydew-producing hemipteran in carbohydrate transfer across trophic levels. -Environm. Entomol. 37: 487-493.

Hazell, S. P. et al. 2008. The role of exotic planta in the invasion of Seychelles by the polyphagous insect *Aleurodicus disperses:* a phylogenetically controlled analysis. -Biol. Invasions 10: 169-175.

Heiman, K. W. et al. 2008. Non-native habitat as home for non-native species: comparison of communities associated with invasive tubeworm and native oyster reefs. -Aquat. Biol. 2: 47-56.

Hughes, R. F. and Denslow, J. S. 2005. Invasion by a N_2-fixing tree alters function and structure in wet lowland forests of Hawaii. -Ecol. Appl. 15: 1615-1628.

Wonham MJ, O’Connor M, Harley CDG (2005) Positive effects of a dominant invader on introduced and native mudflat species. Marine Ecology Progress Series 289, 109-116.

Mandon-Dalger, I. et al. 2004. Relationships between alien plants and an alien bird species on Reunion Islands. -J. Trop. Ecol. 20: 635-642.

O’Dowd, D. J. et al. 2003. Invasional ‘meltdown’ on oceanic island. -Ecol. Lett. 6: 812-817.

Stanley, M. C. et al. 2013. Invasive interactions: can Argentine ants indirectly increase the reproductive output of a weed. -Arthropod-Plant Interact. 7: 59-67.

Lantschner, M. et al. 2013. Do exotic pine plantations favour the spread of invasive herbivorous mammals in Patagonia? -Austral Ecol. 38: 338-345.

Barrios-Garcia, M. N. and Simberloff, D. 2013. Linking the pattern to the mechanism: how an introduced mammal facilitates plant invasions. -Austral Ecol. 38: 884-890.

Lobe, J. W. et al. 2014. Removal of an invasive shrub (Chinese privet: *Ligustrum sinense* Lour) reduces exotic earthworm abundance and promotes recovery of native North American earthworms. -Appl. Soil Ecol. 83: 133-139.

Undecided

McCarville, M. T. et al. 2012. A nematode, fungus, and aphid interact via a shared host plant: implications for soybean management. -Entomol. Experimental. Appl. 143: 55-66.

Britton, J. R. et al. 2011. The introduced *Micropterus salmoides* in an equatorial lake: a paradoxal loser in an invasion meltdown scenario? -Biol. Invasions 12: 3439-3448.

Paynter, Q. et al. 2010. Disruption of an exotic mutualism can improve management of an invasive plant: varroa mite, honeybees and biological control of Scotch broom *Cytisus scoparius* in New Zealand. -J. Appl. Ecol. 47: 309-317.

Wilson, S. J. and Ricciardi, A. 2009. Epiphytic macroinvertebrate communities on Eurasian watermilfoil (*Myriophyllum spicatum*) and native milfoils *Myriophyllum sibericum* and *Myriophyllum alterniflorum* in eastern North America. -Can. J. Fish. Aquat. Sci. 66: 18-30.

Riper, L. C. V. and Larson, D. L. 2009. Role of invasive *Melilotus officinalis* in two native plant communities. -Plant Ecol. 200: 129-139.

Giantomasi, A. et al. 2008. Canopy effects of the invasive shrub *Pyracantha angustifolia* on seed bank composition, richness and density in a montane shrubland (Córdoba, Argentina). -Austral Ecol. 33: 68-77.

Vaz-Pinto, F. et al. 2013. Role of top-down and botton-up forces on the invisibility of intertidal macroalgal assemblages. -J. Sea Res. 76: 178-186.

Ness, J. H. et al. 2013. Reciprocally beneficial interactions between introduced plants and ants are induced by the presence of a third introduced species. -Oikos 122: 695-704.

Magnoli, S. M. et al. 2013. Responses to invasion and invader removal differ between native and exotic plant groups in a coastal dune. -Oecologia 173: 1521-1530.

Questioned

Kestrup, Å. and Ricciardi, A. 2009. Are interactions among Ponto-Caspian invaders driving amphipod species replacement in the St. Lawrence River? -J. Gt. Lakes Res. 35: 392-398.

Reinhart, K. O. et al. 2005. Effects of *Acer platanoides* invasion on understory plant communities and tree regeneration in the northern Rocky Mountains. -Ecography 28: 573-582.

Griffiths, C. J. et al. 2013. Assessing the potencial to restore historic grazing ecosystems with tortoise ecological replacements. -Conserv. Biol. 27: 690-700.

Russel, J. C. et al. 2014. Over-invasion by functionally equivalent invasive species. -Ecology 95: 2268-2276.

3.2.2. Dispersal

Supported

Green, P. T. et al. 2011. Invasional meltdown: invader-invader mutualism facilitates a secondary invasion. -Ecology 92: 1758-1768.

3.2.3. Impact

Supported

Blackburn, T. M. et al. 2005. Functional diversity of mammalian predators and extinction in islands birds. -Ecology 86: 2916-2923.

O’Dowd, D. J. et al. 2003. Invasional ‘meltdown’ on oceanic island. -Ecol. Lett. 6: 812-817.

Jackson, M. C. et al. 2014. Niche differentiation among invasive crayfish and their impacts on ecosystem structure and functioning. -Freshw. Biol. 59: 1123-1135.

3.3. Community level

3.3.1. Composition

Supported

Heiman, K. W. and Micheli, F. 2010. Non-native ecosystem engineer alters estuarine communities. -Integr. Comp. Biol. 50: 226-236.

Hughes, R. F. and Denslow, J. S. 2005. Invasion by a N_2-fixing tree alters function and structure in wet lowland forests of Hawaii. -Ecol. Appl. 15: 1615-1628.

Undecided

Neils, L. C. and Wootton, J. T. 2010. Treatment-based Markov chain models clarify mechanisms of invasion in an invaded grassland community. -Proc. R. Soc. B-Biol. Sci. 277: 539-547.

3.3.2. Richness

Supported

Davidson, T. M. et al. 2010. The composition and density of fauna utilizing burrow microhabitats created by a non-native burrowing crustacean (*Sphaeroma quoianum*). -Biol. Invasions 12: 1403-1413.

O'Dowd, D. J. et al. 2003. Invasional 'meltdown' on oceanic island. -Ecol. Lett. 6: 812-817.

Undecided

Giantomasi, A. et al. 2008. Canopy effects of the invasive shrub *Pyracantha angustifolia* on seed bank composition, richness and density in a montane shrubland (Córdoba, Argentina). -Austral Ecol. 33: 68-77.

3.3.4. Impact

Supported

Abe, T. et al. 2011. Alien pollinator promotes invasive mutualism in an insular pollination system. -Biol. Invasions 13: 957-967.

O'Dowd, D. J. et al. 2003. Invasional 'meltdown' on oceanic island. -Ecol. Lett. 6: 812-817.

Stanley, M. C. et al. 2013. Invasive interactions: can Argentine ants indirectly increase the reproductive output of a weed. -Arthropod-Plant Interact. 7: 59-67.

Jackson, M. C. et al. 2014. Niche differentiation among invasive crayfish and their impacts on ecosystem structure and functioning. -Freshw. Biol. 59: 1123-1135.

3.4. Ecosystem level

Supported

O'Dowd, D. J. et al. 2003. Invasional 'meltdown' on oceanic island. -Ecol. Lett. 6: 812-817.

Jackson, M. C. et al. 2014. Niche differentiation among invasive crayfish and their impacts on ecosystem structure and functioning. -Freshw. Biol. 59: 1123-1135.

Appendix 2

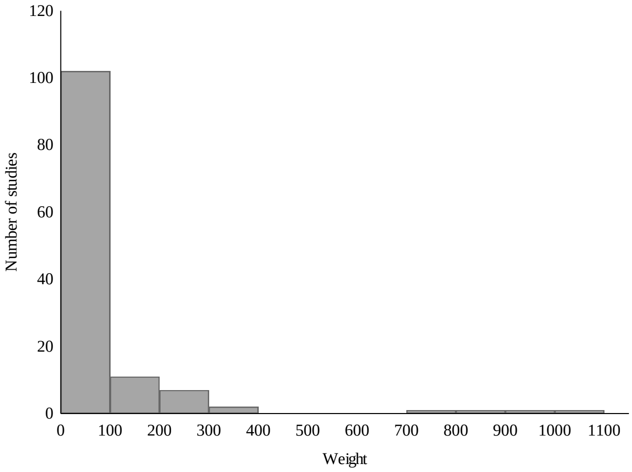

Figure A1. Number of studies according to weights.

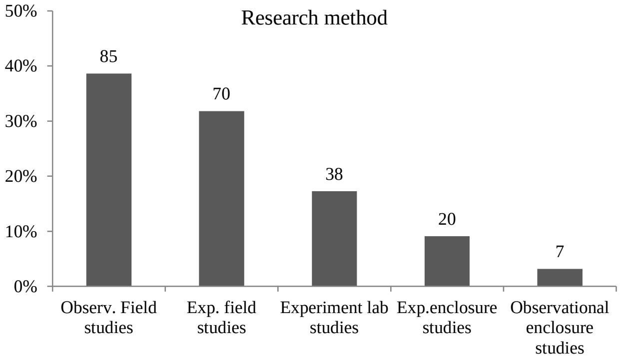

Figure A2. Evidence on the IMH acording to research method.

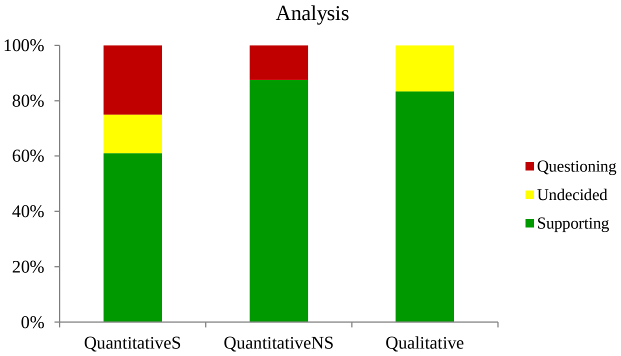

Figure A3. Unweighted data on level of empirical support for the different analysis.

Letters S represent studies with and NS without statistical analysis.

Table A7. Unweighted evidence from empirical tests supporting, questioning or being undecided about the invasional meltdown hypothesis for habitats and taxonomic focus with χ^2 values for comparison of the distribution of the three categories to an equal distribution.

	n	Supported	Undecided	Questioned	χ^2	Binomial test
Total	150	63.3%	13.3%	23.3%	**<0.001**	**<0.001**
A → B	58	74.1%	10.3%	15.5%	**<0.001**	**<0.001**
A ↔ B	34	52.9%	5.9%	41.2%	**0.002**	0.479
Multi Spp.	58	58.6%	20.7%	20.7%	**<0.001**	**0.001**
Individual	89	60.7%	13.5%	25.8%	**<0.001**	**<0.001**
Resource	26	84.6%	15.4%	0.0%	**<0.001**	**<0.001**
Survival/Growth/Reproduction	50	48.0%	16.0%	36.0%	**0.019**	0.354
Dispersal	12	83.3%	8.3%	8.3%	**0.001**	**0.006**
Impact	6	33.3%	0.0%	66.7%	0.135	-
Population	87	60.9%	14.9%	24.1%	**<0.001**	**<0.001**
Abundance/Density/Biomass	75	58.7%	17.3%	24.0%	**<0.001**	**<0.001**
Dispersal	3	66.7%	33.3%	0.0%	0.367	-
Impact	12	83.3%	0.0%	16.7%	**<0.001**	**0.020**
Community	21	71.4%	14.3%	14.3%	**0.001**	**0.004**
Composition	4	75.0%	25.0%	0.0%	0.173	-
Richness	10	70.0%	20.0%	10.0%	**0.044**	**0.033**
Diversity	1	100%	0.0%	0.0%	0.364	-
Impact	7	85.7%	0.0%	14.3%	**0.011**	**0.058**
Ecosystem	3	100.0%	0.0%	0.0%	**0.049**	0.083

Table A8. Unweighted evidence from empirical tests supporting, questioning or being undecided about the invasional meltdown hypothesis for non-native species coevolution, direct and indirect effects, habitats and taxonomic groups with χ^2 values for comparison of the distribution of the three categories to an equal distribution.

	n	Supported	Undecided	Questioned	χ^2	Binomial test
With coevolution	33	57.6%	15.2%	27.3%	**<0.001**	0.058
Without coevolution	43	60.5%	16.3%	23.3%	**<0.001**	**0.007**
Unknown	75	66.7%	12.0%	21.3%	**<0.001**	**<0.001**
Direct effect	87	72.4%	12.6%	14.9%	**<0.001**	**<0.001**
Indirect effect	56	55.4%	12.5%	32.1%	**<0.001**	0.063
Unknown	23	47.8%	32.1%	26.1%	0.336	-
Terrestrial	94	70.2%	14.9%	14.9%	**<0.001**	**<0.001**
Freshwater	32	46.9%	12.5%	40.6%	0.039	-
Marine	24	60.9%	8.7%	30.4%	**<0.001**	0.126
Plants and Algae	89	64.0%	21.3%	14.6%	**<0.001**	**<0.001**
Invertebrates	83	67.5%	10.8%	21.7%	**<0.001**	**<0.001**
Vertebrates	51	62.7%	7.8%	29.4%	**<0.001**	**0.013**

CHAPTER II

INVASIONAL MELTDOWN EXPERIMENTALLY TESTED: INSIGHTS ON THE SYNERGY OF NON-NATIVE SPECIES

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ABSTRACT

Positive interactions among non-natives can lead to increased establishment and invasion rates. Additionally it has the potential to increase non-natives negative impacts upon the native community in a synergistic way. Such effects are termed invasional meltdown. Given the scarcity of properly designed experiments and contrasting results on the invasional meltdown hypothesis we used mesocosms experiment to test the interactions among non-natives and measure their impact to the native biota. Our study was primarily designed to evaluate the individual, additive and synergistic effect of different non-native species upon the native community and ecosystem. We found direct and indirect positive effects among the non-native species with community and ecosystem parameters affected by their presence. The invasive golden mussel (*Limnoperna fortunei*) acts as a new resource to the invasive predator (*Astronotus crassipinnis*) which facilitates the invasive macrophyte (*Hydilla verticillata*) by generating numerous propagules, promoting the spread of the macrophyte. In addition, the population of the native prey (*Moenkhausia forestii*) was negatively affected by the invasive predator. Ecosystem impacts were detected through abiotic alterations. Although the synergism facet of the invasional meltdown definition was not met, we found evidence indicating the importance of one non-native species presence to the successful survival and establishment of other non-native species, what can be also considered an important evidence of an invasional meltdown scenario. The understanding of how multiple non-native species interact and are inserted in the native community is paramount for predicting negative impacts and for successful management.

Keywords: Paraná River floodplain, mutualism, interspecific interactions; indirect interactions; introduced species.

INTRODUCTION

The intense human mediated transport of species around the globe is leading to different interactions between native and non-native biota, as well as among non-native species. Indeed, much attention has been given to the outcome of interactions between non-native species and the recipient community, and the negative impact upon the native biota is well documented (Simberloff et al. 2013, Gallardo et al. 2015). These evidences date back from the publication of the classical book *The ecology of invasions by animals and plants* (Elton 1958). However, recently ecologists have given attention to facilitative interactions that are happening at least as frequent as detrimental ones (e.g. competition, predation, parasitism) in both non invaded (Stachowicz 2001, Bruno et al. 2003, Bronstein 2009) and invaded habitats (Simberloff and Von Holle 1999, Ricciardi 2001). It is crucial in the nowadays and future invasion scenario to understand how multiple invaders interact and the outcomes for the ecosystem, providing managers more precise information to subsidize conservation actions (Sutherland et al. 2008, Blois et al. 2013).

Positive interactions among non-natives can be problematic because it can lead to increased invasion rates. Additionally it has the potential to increase non-natives negative impacts upon the native community in a synergistic way. The term "Invasional Meltdown" (hereafter IM) has been used to describe "*the process by which a group of nonnative species facilitate one another's invasion in various ways, increasing the likelihood of survival and/or of ecological impact, and possibly the magnitude of impact*" (Simberloff and Von Holle 1999). Since its conception, the invasional meltdown hypothesis (hereafter IMH) has become frequently cited. However there is still controversy regarding the IMH support. A recent meta-analysis suggests that neutral and negative interactions are more common among non-natives and the impact upon the native biota is rarely synergistic (Jackson 2015). On the other hand, when using the hierarchy of hypothesis approach and comparing several invasion hypotheses, IMH was the one with highest level of support (Jeschke et al. 2012).

Different conclusion of studies evaluating the IMH may arise because, as many other invasion hypotheses, different aspects might be at test (Heger and Jeschke 2014). For example, the IMH definition encompass how a group of non-native species can aid each other in their establishment, how their positive interaction can accelerate the rate of invasions, and how non-native species interaction can have negative synergistic impact upon the native community (Simberloff and Von Holle 1999, Simberloff 2006, Von Holle 2011). For this last aspect, the sum of each species' individual impact should be poorer than their resulting synergic impact.

So far evidence for synergistic impact mostly involves neutral or even negative interaction between the non-natives. For instance, a previous study have shown that two marine predators consume different size of a same prey species (resource competition) and likely have a synergistic effect at a large spatial scale (Ross et al. 2004). In another study, the combined effect of a crayfish and a non-native snail lead a native snail's population nearly to extinction due to predatory and competitive relations. However, the non-native snail was also negatively affected by the crayfish species although in a lesser extent (Johnson et al. 2009). Jackson et al. (2014) conducted a mesocosm experiment and showed additive and synergistic impact of crayfish on the ecosystem, although with no clear benefit for each other. There is no doubt on the relevance of these studies to elucidate the combined detrimental effects of multiple species introduction, however the IMH posits that the synergistic effect would come from positive interactions among the non-natives (Simberloff and Von Holle 1999, Von Holle 2011).

The link between non-native species positive interaction and synergistic effect to native biota was evaluated through a mesocosm experiment (Meza-Lopez and Siemann 2015). They demonstrated that a non-native plant facilitated a non-native herbivore but their effect on native plants was additive. Perhaps the strongest example on how a combination of non-native species lead to synergistic impact comes from a combination of studies on Christmas Island. There, a network of interactions between non-native species leads to severe synergistic impact on population, community and ecosystem levels (O'Dowd et al. 2003, Abbott and Green 2007, Green et al. 2011, O'Loughlin and Green 2015).

Given the scarcity of evidence linking non-native species positive interactions and their synergistic impact we designed a mesocosm experiment to test the IMH in its full definition using a highly invaded dynamic freshwater ecosystem. We hypothesize that three non-native invasive species, a mussel, a predatory fish and a macrophyte act in synergy benefiting each other, impacting native species and altering the ecosystem dynamics of the recipient community.

METHODS

Study system

The Upper Paraná River floodplain (PRF) in southern Brazil is within an underrepresented category of high biodiversity (Brown and Lomolino, 1998), intense human alterations (Agostinho et al. 2004) and high invasion level on an aquatic environment (Vitule et al. 2012). This river system has been deeply modified by cascading reservoirs. These alterations have already been shown to be the main cause for the introduction of a large amount of non-native species in PRF (Vitule et al. 2012, Skóra et al. 2015). The PRF has been subject of systematic surveys of aquatic communities for nearly 30 years through the Brazilian Long-Term Ecological Studies

(PELD/CNPq, http://www.peld.uem.br) and previous studies (see http://www.nupelia.uem.br). This allowed the detection and monitoring of non-native species throughout this period. Among these non-natives, three species have raised awareness by their impact and possible interaction among them. The invasive golden mussel (*Limnoperna fortunei* Dunker 1857) is widely distributed along the PRF and tributaries with severe economic and environmental impacts (Boltovskoy and Correa 2015). Both Larvae and adults of this invasive bivalve have been reported as an important food item for some fish species (García and Protogino 2005, Paolucci et al. 2007) and adults if consumed might be able to survive gut passage and increase upstream dispersal. Among the fish species that might be able to do so is the invasive *Astronotus crassipinnis* (Heckel, 1840). Species of the *Astronotus* genus are voracious predators with indiscriminate feeding habits but with a diet largely composed of smaller fish (Robins 2015). We thus expect *A. crassipinnis* to have a significant impact upon native small sized fish. Native preys might be more susceptible to non-native predator than to native predator due to lack of eco-evolutionary experience with non-native *A. crassipinnis* (Carthey and Banks 2014, Saul and Jeschke 2015). A third important invader of the PRF is the submerged macrophyte *Hydrilla verticillata* (L.F.) Royle (1977) which has caught attention due to its severe negative ecological impacts (Sousa 2011). Additionally this species is known to host more attached mussels than the native macrophyte *Egeria najas* Planch. therefore facilitating mussels invasion (Michelan et al. 2014). Present evidence of these three non-native species invasion, impact and interaction makes them strong candidates to be part of an IM scenario.

Experimental design

To understand the role of simultaneous biological interactions among non-natives on the native and non-native community, we conducted a mesocosm mid-term experiment that simulated a simplified real scenario of what now inhabits the PRF. We assessed the individual and multiple effects, along with the interactive roles of non-native species. First, we constructed mesocosms representing a native community. The simplified native biota was constituted of phytoplankton and zooplankton collected with the river water, two small sized fish to act as secondary consumers (*Astyanax altiparanae* Garutti and Britski, 2000 and *Moenkhausia forestii* Benine, Mariguela and Oliveira, 2009), one submerged macrophyte (*E.najas*) and one fish species to act as top predator (*Hoplias malabaricus* (Bloch, 1794)). Secondly, additional mesocosms were created adding one, two or three non-native species to the same native community. The chosen non-native species were a submerged macrophyte (*H. verticillata*), a mussel (*L. fortunei*), and a predatory fish (*A. crassipinnis*). Upon adding one non-native species, the same abundance of the similar native species was removed to maintain equal densities among mesocosms. The mesocosms with two non-native species were assembled with all possible combinations of non-native species; and a last mesocosm was assembled adding all three non-natives. The experimental design consisted of eight treatments replicated five times (Fig. 1). Mesocosms were 1000 l round tanks filled with river water, continually aerated and monitored for biotic and abiotic alterations for 20 days. Number of replicas and experiment duration were limited by the number of thanks, logistical support and personnel available. Although several biotic and abiotic components were not present, we constructed a simplified but realistic interacting network of native and non-native species, which was enough for our objectives.

Experiment assembly

All species used in the experiment were collected from the PRF. Submerged macrophytes were collected during free dives and separated in order to get 700 g/m^2 in each trial (see Supplementary material Appendix 1 for detailed weighing method). In treatments with the non-native *H. verticillata*, we used 350 g/m^2 of each species. Densities were chosen according to a plausible representation of natural conditions (Pelicice et al 2005). After a thorough search and removal of attached mussels, macrophytes were divided and planted into four vases containing sand and ground soil to allow better establishment. At the end of the experiment, macrophytes' remaining loose fragments were counted and divided into three fragment size classes (class 1, < 15 cm; class 2, between 15 and 30 cm; and class 3, > 30 cm) to allow further evaluation of dispersal potential of fragments.

The non-native invasive golden mussel (*L. fortunei*) is an ecosystem engineer with no ecologically similar native species for the PRF. For this reason, it was considered a new additional component to the PRF community and its effects were measured without removing any native component of the mesocosms. Submerged trunks with attached individuals of *L. fortunei* were used reaching approximately 1500 ind/m^2 (see Supplementary material Appendix 1 for details).

Astyanax altiparanae (SL = 60 mm; SE ± 0.09) and *M. forestii* (SL = 31 mm; SE ± 0.02) were seine netted and acclimatized to laboratory conditions for at least two days before experiment. During this period fish were fed with commercial pellets to satiation. Ten adult individuals of each species were added to each treatment two hours before predators, to both allow them to find refuge and prevent early ambush predation. Density (10 ind/m^2) represented plausible values for the PRF (see Agostinho et al. 2007) and sufficient number to avoid predation of all individuals during the experiment (trial experiment information). Four six-holed bricks (9 x 14 x 19 cm) were added to each mesocosm to provide extra refuge for prey fish (Supplementary material Appendix 1 Fig. A4).

Native (*H. malabaricus*, SL = 218 mm; SE ± 0.15) and non-native (*A. crassipinnis* SL = 211; SE mm ± 0.09), predators used were seine netted or caught with rods. Prior to the experiment both species were separately acclimatized to experiments conditions for at least 2 days. This period was also important to separate healthy and similar sized specimens. They were fed to satiation until 24 hours prior to the experiment. We added two individuals of *H. malabaricus* when in purely native treatments, and one individual of *H. malabaricus* and one of *A. crassipinnis* in treatments where a non-native predator was required. Predators were used in the minimum density possible due to its size.

Every two days we assessed information on pH, water temperature and conductivity to monitor possible alterations during the experiment. On the last day we assessed information on abiotic factors such as total dissolved solids, water turbidity and dissolved oxygen. In addition, biotic factors such as chlorophyll-a concentration, phytoplankton and zooplankton density and richness, macrophyte integrity and biomass, prey fish survival, predator gut content, and fragmented mussel shell biomass (as a proxy for mussel predation) were measured (for detailed mesocosm assembly protocol see Supplementary material Appendix 1).

Statistics

For testing the hypothesis that invaders alter any of the community or ecosystem parameters, we calculated the difference between the native and invaded mesocosms (delta values; see Fig. 1). We then obtained delta value for each invasion scenario and each measured parameter within each replica (Fig.1). For detecting possible synergistic effects of the non-natives, the sum of individual species' impact should be lower than when the same species are invading together, if so the result should be, for example, $\Delta G > \Delta A + \Delta B + \Delta C$ (see Fig. 1 for description of used Δ). Analyses of variance were used to test for significant differences ($p<0.05$) between the different non-native species and levels of invasions using STATISTICA 12 (Dell 2014). When ANOVA assumptions were not in accordance, non-parametric Kruskall-Wallis test was used. Phytoplankton densities were compared using Sorensen and Bray-Curtis dissimilarity using PRIMER 6 and PERMANOVA+ (Clarke and Gorley 2006).

In addition, meta-analyses were used to summarize overall effect size difference considering the presence of any scenario of biological invasion and the control, and also to compare scenarios of invasion in the case of high heterogeneity in effect sizes between treatments (Borenstein et al. 2009). Effect sizes (Hedge's *g*) and summary meta-analyses were carried out in Metawin 2.1 (Rosemberg et al. 2000).

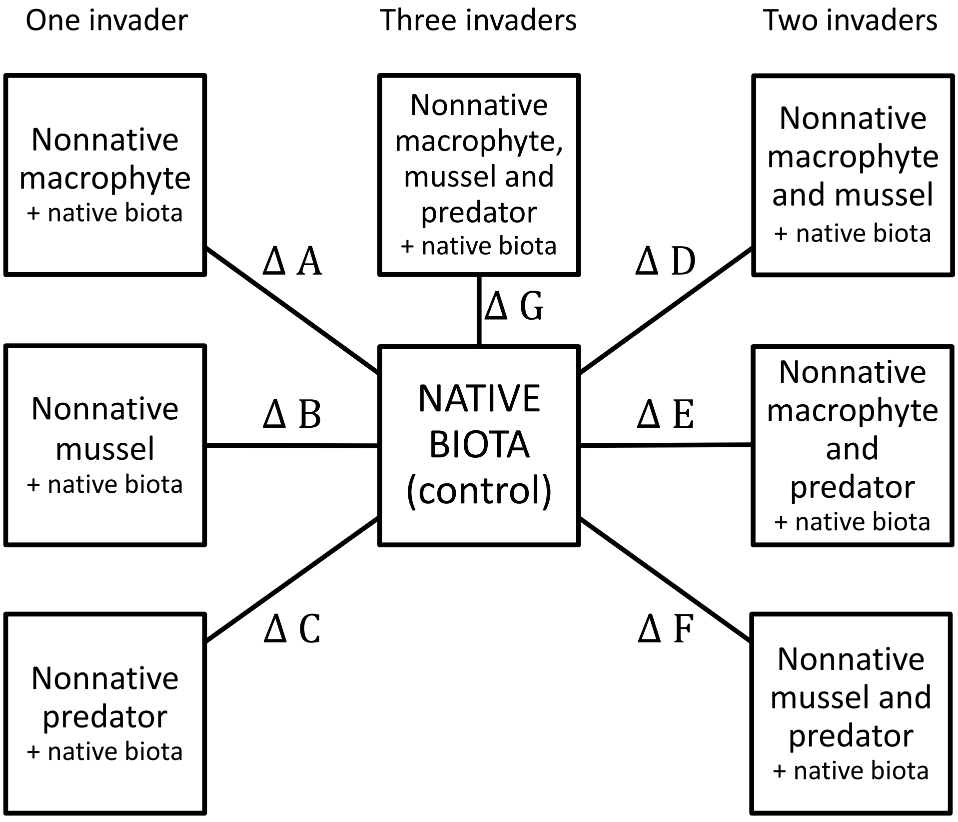

Figure11. Experimental design and different mesocosms (boxes) replicated five times. Center box represents the simplified native biota and the others represent each treatment with added single or combined non-native species. Delta was calculated to account for

alterations in measured parameters related to single, additive or synergistic effect of the chosen non-native species.

RESULTS

During the experiment, no difference in means of pH (7.0; SD 0.12) ($F_{7;32}$ = 1.961; p = 0.091), temperature (27.0; SD 0.5) ($F_{7;32}$ = 0.304; p = 0.946) and conductivity (54.7; SD 7.62) ($F_{7;32}$ = 0.272; p = 0.961) was found between treatments. Turbidity showed a slight increase for all treatments compared to the native ecosystem (Hedge's *g* = 0.787; Bootstrap CI = 0.737 to 0.845) but no significant difference between treatments was found ($F_{6;28}$ = 0.018; p < 0.001) (Fig. 2). The addition of non-native species also increased total dissolved solids (TDS) (Hedge's *g* = 3.098; Bootstrap CI = 1.591 to 4.989) and was significantly different among treatments (KW-H = 20.415; p = 0.002), but with no evidence for synergistic effects (Fig. 3). Analyzing separately, treatments with *L. fortunei* were significantly different from the control (Fig. 3). Meta-analysis results showed a significant decrease in dissolved oxygen concentrations in the scenarios of biological invasion in relation to the control (Hedge's *g* = -0.945; Bootstrap CI = -1.141 to -0.629). Although we found significant differences among treatments ($F_{6;28}$ = 3.004, p = 0.021), when analyzing each treatment separately, only treatments with *L. fortunei* differed significantly from the control (Fig. 4).

Similar to the majority of the abiotic factors, phytoplankton richness was significantly lower upon the addition of non-native species (Hedge's *g* = -3.545; Bootstrap CI = -5.135 to -2.343) but with no difference among treatments ($F_{6;28}$ = 0.595, p = 0.731). Phytoplankton densities were compared through Sorensen and Bray-Curtis dissimilarity and no difference were found among treatments ($F_{6;28}$ = 0.755, p = 0.610 and $F_{6;28}$ = 1.794, p = 0.136 respectively) and to the control mesocosms (Supplementary material Appendix 2 Fig. A6 and A7 respectively).

Accounting for the number of macrophyte fragments at the end of the experiment there was a significant difference among treatments for both *E. najas* and *H. verticillata* ($F_{3;16}$ = 4.915; p = 0.013 and $F_{3;16}$ = 9.628; p = 0.0007 respectively), but with no difference between macrophyte species ($F_{1;32}$ = 0.926; p = 0.343) (Fig. 5). A higher number of small fragments was found when *A. crassipinnis* was present (*E. najas*, $F_{1;18}$ = 15.398; p = 0.001 and *H. verticillata*, $F_{1;18}$ = 26.412; $p < 0.001$, see Supplementary material Appendix 2 Fig. A8).

The addition of non-native species caused no effect upon *A. altiparanae* survival ($F_{6;28}$ = 1.083; p = 0.395) (Hedge's *g* = 0.617; Bootstrap CI = -0.132 to 1.365), however the survival of *M. forestii* was negatively affected (Hedge's *g* = -1.604; Bootstrap CI = -2.374 to -1.119) but with no distinction of any treatment ($F_{6;28}$ = 0.436; p = 0.848) (Fig. 6).

Furthermore we compared the remaining fragmented shell of *L. fortunei* and found a significant higher biomass for treatments with *A. crassipinnis* ($F_{1;18}$ = 7.918; p = 0.011). Still among biotic factors, chlorophyll-a was the only measured parameter with no alterations between treatments and compared to the control ($F_{6;28}$ = 0.336; p = 0.911) (Hedge's *g* = -0.064; Bootstrap = CI -0.139 to 0.013). Analysis of all predators' gut content confirmed its effects over preys. Fish scales were found in 4% of analyzed *H. malabaricus* stomachs. Mussel shells were found in 20% and fish scales in 5% of *A. crassipinnis* stomachs.

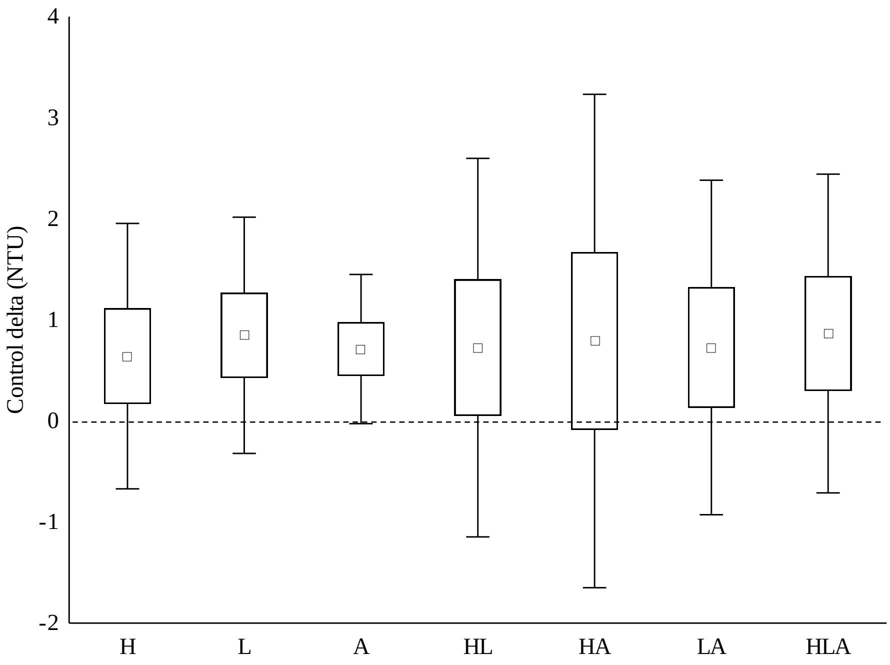

Figure 12. Water turbidity for each invasion scenario in relation to the native (control) mesocosms after 20 days. Dashed line indicates the control treatment. Letters below bars represent which non-native species were present. *Hydrilla verticillata* (H), *Limnoperna fortunei* (L) and *Astronotus crassipinnis*(A). Data are mean, standard error (box) and 95% confidence interval (whiskers).

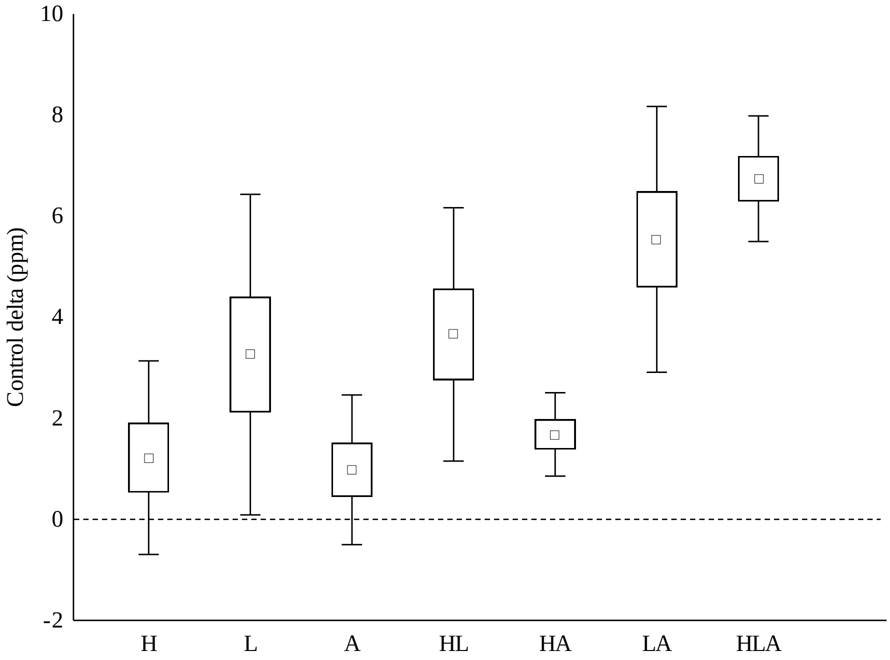

Figure 13. Total dissolved solids for each invasion scenario in relation to the native (control) mesocosms after 20 days. Dashed line indicates the control treatment. Letters below bars represent which non-native species were present. *Hydrilla verticillata* (H), *Limnoperna fortunei* (L) and *Astronotus crassipinnis* (A). Data are mean, standard error (box) and 95% confidence interval (whiskers).

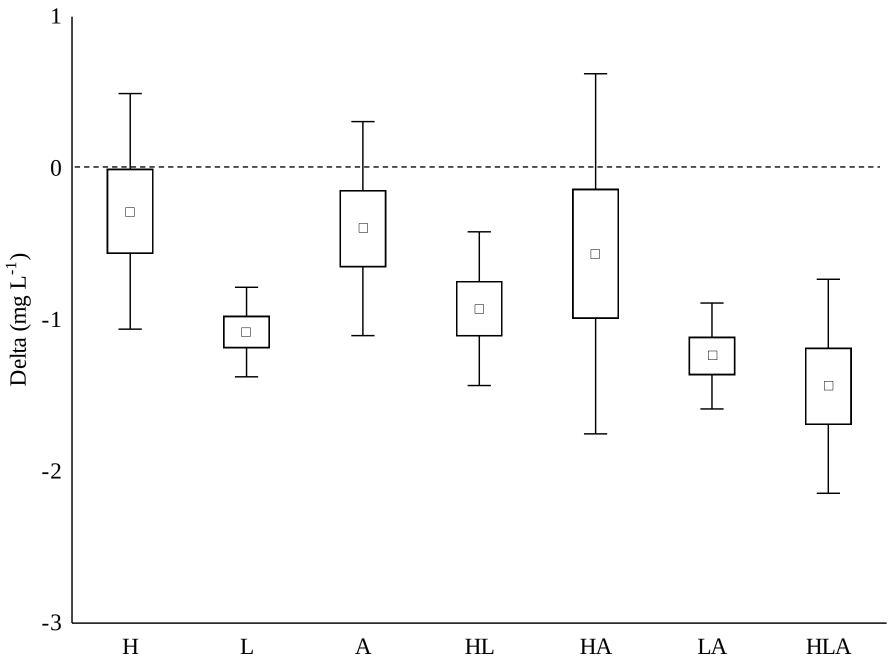

Figure 14. Dissolved oxygen concentrationsfor each invasion scenario in relation to the native (control) mesocosms after 20 days. Dashed line indicates the control treatment. Letters below bars represent which non-native species were present. *Hydrilla verticillata* (H), *Limnoperna fortunei* (L) and *Astronotus crassipinnis* (A). Data are mean, standard error (box) and 95% confidence interval (whiskers).

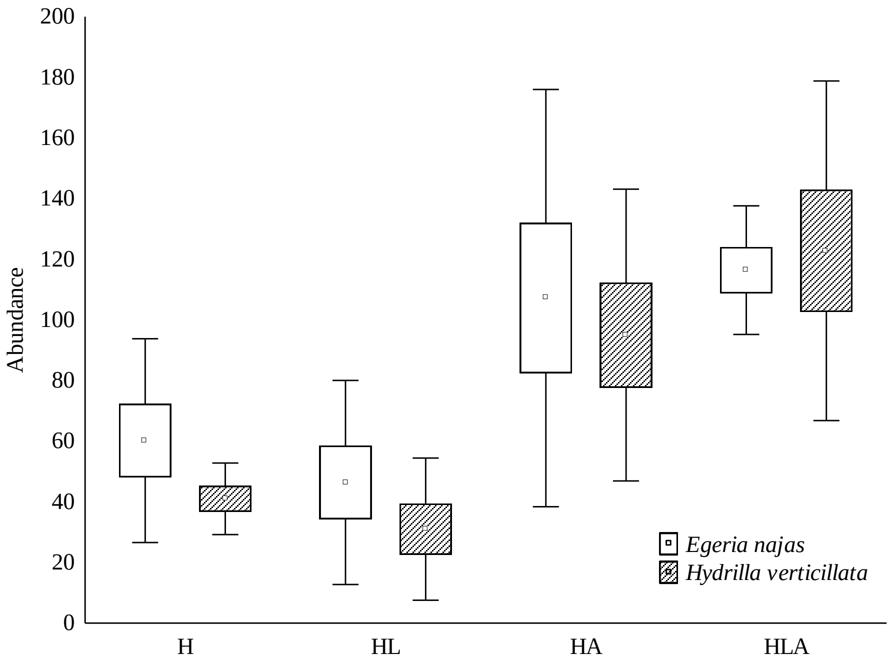

Figure 15. Native (*E. najas*) and non-native (*H. verticillata*) macrophytes abundance of size class 1 (< 15 cm) fragments after 20 days. Letters below bars represent which non-native species were present. *Hydrilla verticillata* (H), *Limnoperna fortunei*(L) and *Astronotus crassipinnis* (A).Only invasion scenarios with both macrophyte species present are shown. Data are mean, standard error (box) and 95% confidence interval (whiskers).

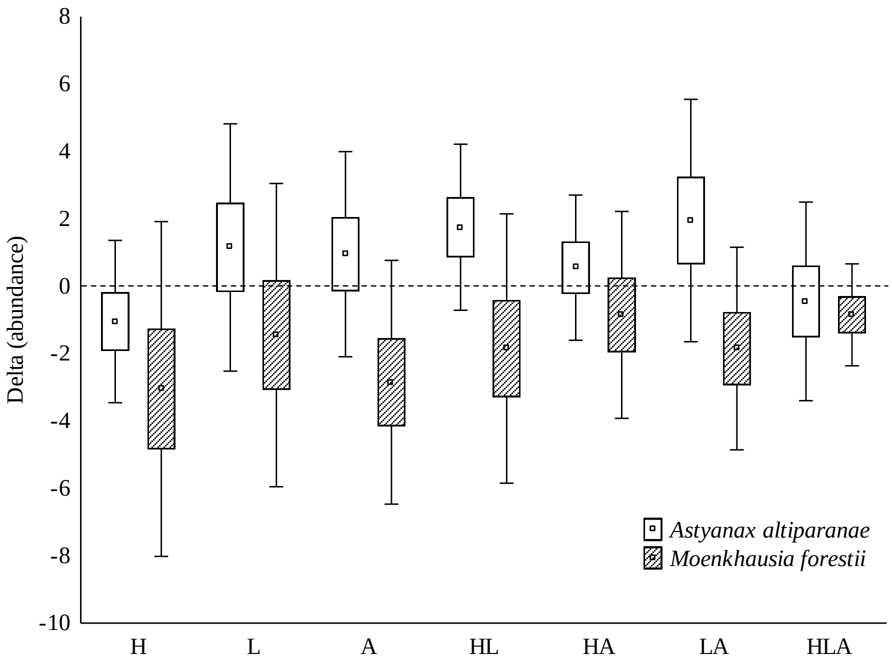

Figure 16. Native prey survival after 20 days. Dashed line indicates the control treatment. Letters below bars represent which non-native species were present. *Hydrilla verticillata* (H), *Limnoperna fortunei* (L) and *Astronotus crassipinnis* (A). Data are mean, standard error (box) and 95% confidence interval (whiskers).

DISCUSSION

Although we found no synergistic effect among the non-native species evaluated, several community and ecosystem parameters were affected by their presence. Contrary to the expected, water turbidity increased upon adding any single or combined non-native species. As a filter feeding species, *L. fortunei* was expected to decrease water turbidity (Cataldo et al. 2012). However, the Paraná River's water flow at the research station where the study took place is strongly affected by a river dam located upstream nearly 45 km, and so, water velocity, depth and transparency vary abruptly. As water turbidity was already very low when mesocosms were filled and during the experiment, filtering rate of mussels may have been very low. Mussels are also expected to decrease filtering rate in stagnate or low current waters (Ackerman 1999). Additionally mussels were attached to submerged tree branches and the addition of the branch itself and sediment stuck within individuals of mussels may have added extra sediment to the mesocosms. Another factor that might have caused a slight increase in water turbidity and TDS is the aggressive social behavior of *Astronotus* species (Beechng 1997, Golçalves-de-Freitas and Mariguela 2006). This species was observed disturbing and flipping over the vases where macrophytes were planted. Substrate manipulation by *Astronotus* species is a common behavior (Beechng 1997) and the constant disturbance of soil is likely to increase water turbidity especially inside the mesocosm with no flowing water. However, no single species caused significant alterations in water turbidity and significant alteration was observed only when all small effects were accounted in the meta-analysis.

Treatments with the invasive golden mussel and especially with the three non-natives added showed an increase of total dissolved solids.This increase might be related to mussel excretes. Additional inputs in substances such as ammonia, sulfates and phosphates have already been reported for *L. fortunei* (Boltovsky and Correa 2014). The extra availability of such substances to aquatic plants and algae can cause drastic changes in their abundance and biomass and consequently to the whole structure of the environment. The increased turbidity and total dissolved solids leading to a decrease in light penetration will negatively affect primary productivity. Additionally lower transparency is expected to decrease macrophyte species richness and biomass (Sousa et al. 2010, Schneider et al. 2015). Therefore the addition of these non-native species will potentially cause significant negative impact at the community and ecosystem levels.

Treatments that contained the invasive mussel showed low dissolved oxygen values, and the lowest was when all three non-natives were present. Increased organic matter as found in TDS results lead to low oxygen concentrations (Paerl et al. 1998). In addition, mussel influence on oxygen concentrations was expected since it was a new component to the mesocosms. The ecosystem effect of increased organic matter and oxygen depletion is expected to be worse in lagoons which are only connected to the main river during floods. Additionally, as the large number of dams has a strong influence on floods regime (Agostinho et al. 2004) the synergistic effect might be between the non-native species and habitat alteration, in this case both aggravating oxygen depletion that possibly affect native biota. Cases in which invasive species and ecosystem changes contribute to native species declines are not rare (Bauer 2012).

The lack of difference in phytoplankton abundance and the decrease of phytoplankton richness among treatments may be related to different adaptation and survival for different species to the mesocosm environment. Some species might have decreased their densities while other might have increased. Although richness decreased rapidly during the experiment it is possible that abundances would take longer than the experiment duration to be detected. A time delay should be expected for a top down effect starting from prey fish abundance decrease leading to zooplankton increase (Estes et al. 2011). Consequently phytoplankton decrease might take longer than 20 days to be detected (Wojciechowski and Padial 2015).

Despite the lack of difference in the phytoplankton abundance and chlorophyll-a among treatments, the introduction of non-native species caused a deep shift in the algae community. Native algae community was composed mainly by planktonic algae, e.g. *Fragilaria* and *Cosmarium* species, and perifitic algae, that must have been released from perifiton due to flushing (e.g. *Gomphonema affine* and *Eunotia* spp.). At the end of the experiment, there was a decrease in phytoplankton richness, possibly due to the water stagnation itself and the decrease in light incidence resulting from the increasing turbidity. Resultant community was composed by algae groups adapted to low light intensity, mobile and/or mixotroph, e.g. *Phormidium* sp. (a low light perifitic cyanobacterium), and *Trachelomonas* spp. (a mobile mixotroph algae).

We were also able to detect direct interactions between the non-native species evaluated. The non-native fish *A. crassipinnis* impact may not be only upon predating small native fishes. Species from the *Astronotus* genus, as other cichlids, are known to present a behavior of altering their surrounding habitat (e.g. digging nests) (Beeching 1997). During our experiment we observed that *A. crassipinnis* frequently manipulated plant stems and also flipped over the vases were macrophytes were planted. This behavior reflected in a higher abundance of small fragments of both native and non-native macrophytes. *Hydrilla verticillata* fragments can rapidly establish in a new location after being dispersed by the river water flow even after facing dessication, leading to an increased propagule pressure of the macrophyte at the landscape scale (Silveira et al. 2009, Sousa 2011, Ribas et al. manuscript in preparation). Although both species might be facilitated by *A. crassipinnis*' fragmentation and dispersal, *H. verticillata* is a superior competitor with strong effects upon both abiotic and biotic components (Sousa 2011).

Observations of higher mortality of small native fish species *M. forestii* may be the result of prey naivety towards new non-native predators, like *A. crassipinnis*, as shown for several predator-prey relations (Carthey and Banks 2014). Due to *A. crassipinnis* small mouth gape size its impact upon the native fish community is expected to be more prominent on small-sized species and juveniles of large species. This explains the lack of influence upon *A. altiparanae* mortality during the experiment. Nevertheless the population level impact upon *M. forestii* might indirectly affect other native community components not yet evaluated. A trophic cascade effect where the elimination of consumers by a predator leads to indirect effects to lower trophic levels has a strong potential for wide effects on multiple trophic levels (White et al. 2006). Another resource used by *A. crassipinnis* is the non-native mussel *L. fortunei*. In accordance to our prediction, the predation of *A. crassipinnis* upon the non-native mussel, as demonstrated by the higher mussel shell abundance found, may have facilitated the successful establishment of the fish species in the Paraná River basin serving as an additional food resource. Several native species are also relying on the large amount of available resource provided by the abundance of *L. fortunei* (García and Protogino 2005). Although it remains unclear, this new interaction can also have a beneficial facet to the mussel if some individuals are able to survive gut passage, increasing its dispersal rate upstream.

In a network of direct and indirect positive and negative interactions (Fig. 7), the invasive golden mussel acts as a new resource to the invasive predator *A.crassipinnis* which in a local scale can offer resistance to mussel invasion but possibly increases mussel dispersion in a large spatial scale. *A. crassipinnis* also facilitate and increase rate of dispersal of the invasive macrophyte (*H.verticillata*) by fragmenting small pieces of the plant that are carried and possibly reaching novel areas to establishment. The invasive macrophyte *H. verticillata* also facilitates the establishment of *L. fortunei* by providing a higher quality substrate than native macrophytes (Michelan et al. 2014). The population of native prey *M. forestii* is negatively affected by the non-native predator *A. crassipinnis* and also is negatively affected by the presence of the non-native macrophyte which provides a lower habitat quality as refuge for this species (Ribeiro et al. manuscript in preparation). In addition, ecosystem alteration might happen to this network of interactions as water turbidity, TDS and oxygen levels are affected.

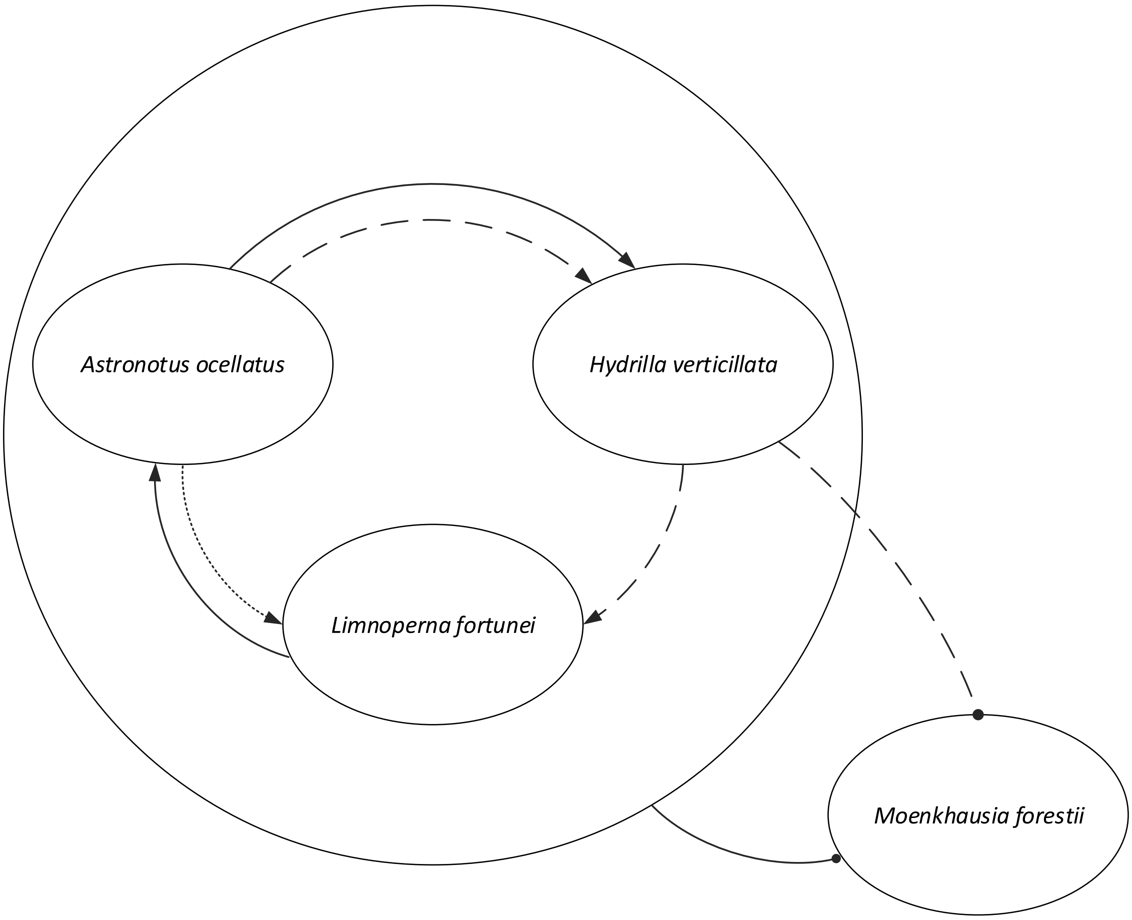

Figure 17. Network of positive interactions between three non-native species inhabiting the Paraná River floodplain leading to a negative effect upon a native fish species. Lines ending in arrows indicate positive effects and lines ending in solid circles indicate negative effects. Solid lines indicate evidence provided by our present study, dashed lines indicate evidence provided by published evidence and dotted lines indicate partially supported evidence by our study and published evidence.

After the detection of a new non-native species it is highly important to evaluate its impact and promptly implement management and eradication measures when necessary (Simberloff et al. 2013, Simberloff and Vitule 2014). However, many actions in this sense fail due to the high monetary cost of management actions and to the complexity of interaction networks to which the new species become involved (Genovesi 2011). Unwanted side effects to native species or even broad ecosystem alterations may result due to incorrect management actions (Pitcairn 2011). In this sense, understanding how non-native species are inserted in the native community as well as how they interact with previously introduced non-native species is paramount for a successful management or eradication plan (Genovesi 2011).

Although we were able to understand several aspects of non-native species interactions in the Paraná River basin we need to take care in drawing conclusion based solely in information provided by a closed mesocosm system. On the other hand, mesocosms experiments offer the advantage of manipulating the addition of non-native species without ethical concerns of introducing invasive species in the field experiments (Bauer 2012). Additionally, this experiment's importance is based on the first attempt to understand the effects of non-native species interactions and their synergistic effect in this highly diverse Neotropical freshwater system. The synergy facet of the IM definition was not met but we found evidence indicating the importance of one non-native species presence to the successful survival and establishment of other non-native species. With these evidences the IMH cannot be completely ruled out. If the positive interactions found lead to a cumulative increase of non-native species establishment it still conforms to the IMH (Chapter III).

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SUPPLEMENTARY MATERIAL

Appendix 1

Assembly procedures

The experiment was conducted in the NUPELIA (UEM) research station located in the city of Porto Rico, state of Paraná. A covered area fitting 32 mesocosms housing up to 1000 liters was used (Fig. A1). As the experiment design required 40 mesocosms we assembled five separate replicas, each containing all the eight different treatments. The whole experiment took 42 consecutive days (from 01/29/2014 to 03/12/2014). The first replica was assembled on January 29th, the second replica on February 3rd, the third replica on February 7th, the fourth replica on February 12th and the fifth replica on February 20th. Before filling mesocosms with river water, all of them were washed using salt water and ethanol 70° avoid any fungi influence. As water used was pumped directly from the river we left water running for 20 minutes prior to mesocosm filling to avoid using water stuck in pipes.

Every mesocosm was covered with a 2.5cm mesh to prevent fish to escape (Fig. A2). To avoid the influence of sunlight on the growth of macrophytes, phytoplankton and chlorophyll and on water temperature, curtains were used to close the lateral side of the experiment area during the early hours of the day when direct sunlight affected the mesocosms (Fig. A2). Duration of direct sunlight varied between three and four hours (between 6:30 and 10:30 maximum). To minimize light incidence difference among mesocosms we also used fluorescent light for 12 hours (from 6:30 to 18:30).

To assure equal amounts of macrophytes in every mesocosm, selected plants were agitated 50 times to remove as much water as possible and then we weighted (0,01g) to achieve 700g in each mesocosm. We previously evaluated how many times it would be necessary to agitate the macrophytes to remove as much water as possible

(Fig. A3). The exact location where macrophytes were put inside each mesocosm was determined by light incidence. Prior to the experiment we measured light incidence in each mesocosm using a photometer at four distinct daylight hours (7:30, 10:30, 13:30, 16:30 and 19:30). Macrophytes were located where the average indirect daylight incidence was higher (Fig. A4).

For mussel abundance standardization we used a 10 x 10 cm square to count the number of individuals (Fig. A5). In a previous evaluation, we counted 10 times different areas and found on average 150 mussels in the used square area. During assembly we selected a total of 10 squares totalizing 1500 mussels for each mesocosm. All fishes were held in 300 l aquariums with fungicide and were fed once a day before adding to the mesocosms.

Figure A18. Experiment location and layout.

Figure A19. Curtains closed during direct sunlight hours and mesocosms protected with 2.5 cm mesh.

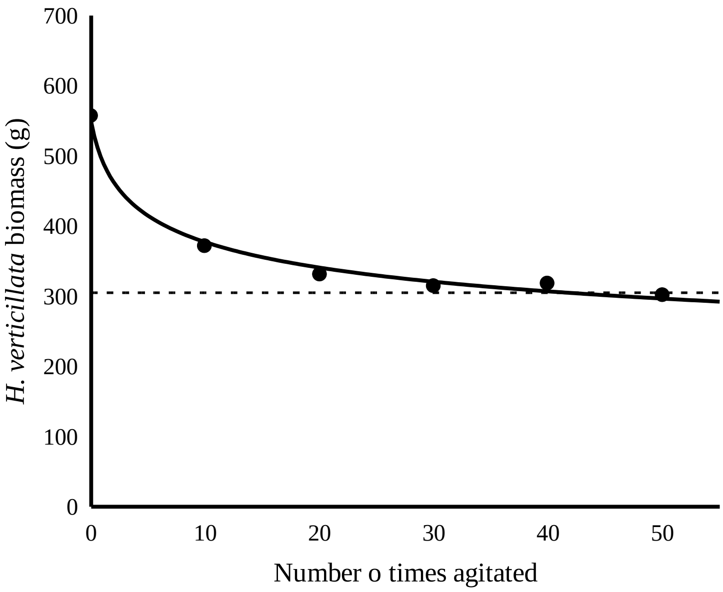

Figure A20. Evaluation of how many times it was necessary to agitate macrophytes in order to remove water and standardize weights added to mesocosms.

Figure A4. Mesocosm layout of a trial containing all three non-native species.

Figure A5. Flexible square used for mussel counting.

Experiment measurements

Abiotic factors measurement such as pH, water temperature and conductivity were conducted in alternate days (days 1, 3, 5, 7, 9, 11, 13, 15, 17, 19), always at 16:00 hours. Turbidity, total dissolved solids and dissolved oxygen were measured at the final day of the experiment at 15:00 hours, before disassembly. For pH measurements we used an Adwa model AD11 device. For water temperature, conductivity, total dissolved solids and dissolved oxygen we used a multiparameter device Extech model DO700. For turbidity we used the Policontrol model AP2000 device.

Final procedures

Chlorophyll-a was samples at the end of the experiment were taken before the water was disturbed by mesocosm disassembly. We retrieved one liter of water for each mesocosm and maintained at 5° C for later laboratorial analysis. Samples were protected from light incidence at full time. In the lab…

For phytoplankton sampling at the end on the experiment, the water in each mesocosm was agitated to homogenize water stratification, providing a good sample of the entire mesocosm. Samples from each mesocosm were filtered in mesh with 15 µm of aperture and the phytoplankton retained was washed with distilled water. Samples were stored in amber flasks and fixed with acetic lugol. Phytoplankton density (cells.mL-1) was measured according to Utermöhl (1958) technique by random fields using an inverted microscope Olympus IX70. All the species present in at least 75 fields were counted, or at least 100 cells of the most abundant individual, reducing the error count to 20% (Lund et al., 1958). The dilution conversion was calculated from the formula: ((VCS * N)/VQ)/VF, where VCS refers to volume of concentrated sample, N to number of cells quantified, VQ to volume quantified and VF to filtered volume.

Zooplankton sampling was also conducted after water homogenization at the end of the experiment. We then filtered 60 liters of water in a 40 micrometers net. Samples were fixed in formaldehyde at 4% for later laboratorial analysis. In the lab…

Predator and prey fishes were measured (standard length) only at the end of the experiment to avoid mortality during the experiment due to stress. Due to similarity between prey species from the Paraná River basin, some individuals were anesthetized using benzocaine, fixed in formaldehyde 10% and stored in ethanol 70° for posterior species identity confirmation. Identification was conducted by fish specialists from the Universidade Estadual de Maringá (UEM). The majority of preys used were released back to the river. Predators were anesthetized in benzocaine and immediately after their death, qualitative gut content analysis were conducted.

Appendix 2

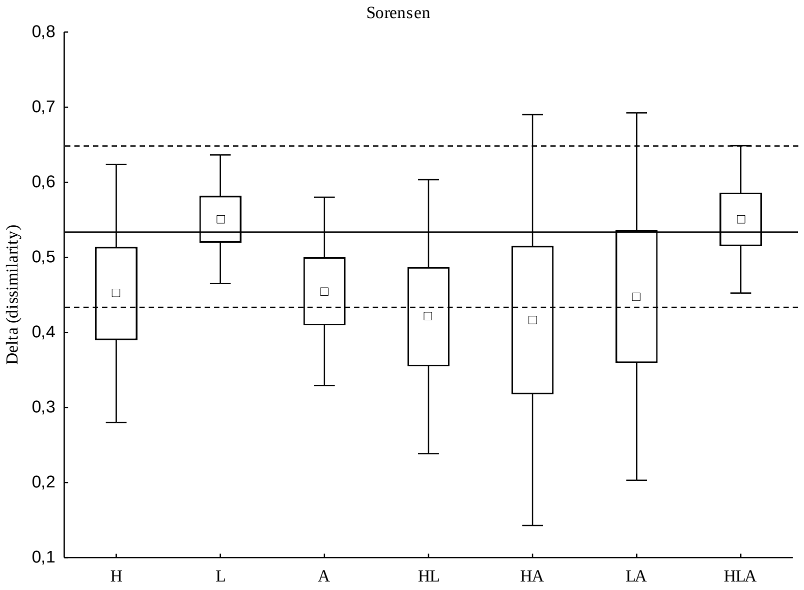

Figure A6. Delta of phytoplankton densities compared through Sorensen dissimilarity index for each invasion scenario in relation to the native (control) mesocosms. Solid line represent the average Sorensen dissimilarity index within control mesocosms. Dashed lines represent control’s minimum and maximum values of Sorensen dissimilarity index. Letters below bars represent which non-native species were present. *Hydrilla verticillata* (H), *Limnoperna fortunei* (L) and *Astronotus crassipinnis*(A).

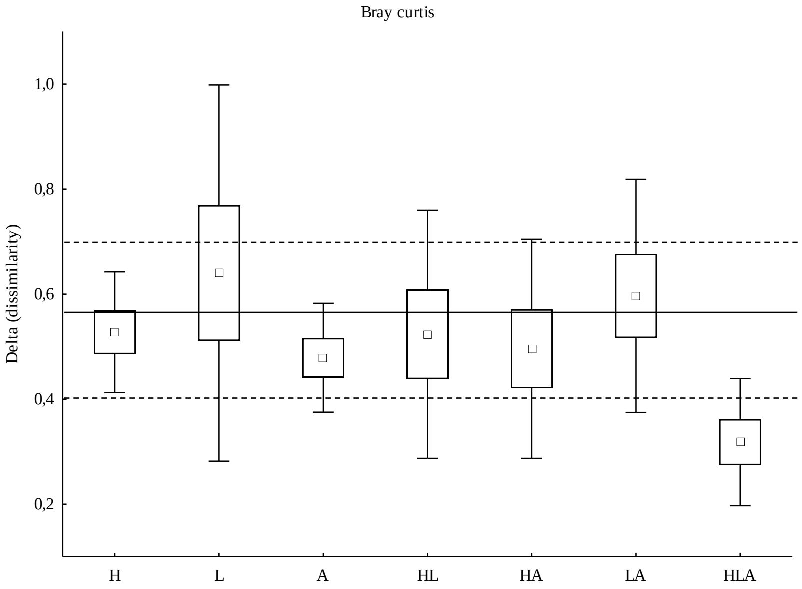

Figure A7. Delta of phytoplankton densities compared through Bray-curtis dissimilarity index for each invasion scenario in relation to the native (control) mesocosms. Solid line represent the average Bray-curtis dissimilarity index within control mesocosms. Dashed lines represent control's minimum and maximum values of Bray-curtis dissimilarity index. Letters below bars represent which non-native species were present. *Hydrilla verticillata* (H), *Limnoperna fortunei* (L) and *Astronotus crassipinnis*(A).

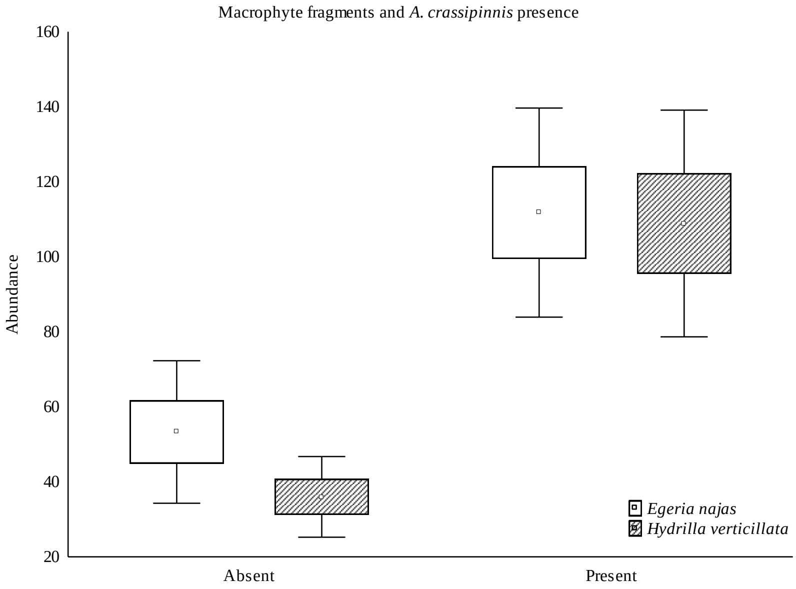

Figure A8. Influence of *A. crassipinnis* presence on native (*E. najas*) and non-native (*H. verticillata*) macrophytes abundance of size class 1 (< 15 cm). Data are mean, standard error (box) and 95% confidence interval (whiskers).

CHAPTER III

INVASIONAL MELTDOWN: MAIN CONCEPTS, MISCONCEPTIONS, HIGH RISK SCENARIOS AND FRAMEWORK FOR HYPOTHESIS TESTING AND SYNTHESIS

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ABSTRACT

Available evidence for the original invasional meltdown hypothesis (IMH) covers a range of distinct aspects. Failing to differentiate which aspect is evaluated impedes further synthesis and development. We therefore highlight IMH misconceptions, differentiate main aspects of invasional meltdown (positive interactions, increasing rates of invasion, synergistic impact), providing examples and ways to test them, identify high-risk scenarios for conservation, highlight important neglected aspects, and finally provide a framework for testing and synthesis. Interaction between two or more non-native species is how invasional meltdown operates. Specialization level and eco-evolutionary experience of both native and non-native species is important for risk assessment. The outcome of interactions among non-natives is also of major importance and evidence at the community level is necessary for full support of the hypothesis. The increasing rate of invasions is the ultimate outcome to the community, but finding evidence linking an increasing rate to species interactions is difficult, especially because colonization and propagule pressure are confounding factors. Scenarios with non-native ecosystem engineers are likely to lead to an increasing rate of invasion. Evidence of synergistic impact is often demonstrated through resource competition, so species in high trophic levels are of major concern in invasional meltdown. By disentangling main concepts and linking them to important causal factors, we propose a new framework for a more practical and accurate way to test the IMH and to synthesize existing information.

Keywords: facilitation; mutualism; interspecific interactions; indirect interactions; introduced species; conceptual framework.

INTRODUCTION

Species interactions play a key role in structuring communities (Hildrew et al. 1984; Spiller and Schoener 1998; Jackson et al. 2001; Filotas et al. 2010), and competition has long been considered the main structuring force (Elton 1946; Diamond 1975; Ricklefs 1987; Richardson et al. 2000; Gotelli and McCabe 2002). Many classic ecological hypotheses and theories – e.g. natural selection (Darwin 1859), biotic resistance (Elton 1958), niche segregation (Hutchinson 1959; Leibold 1995), the dynamic equilibrium model of island biogeography (MacArthur and Wilson 1963; 1967), and metapopulation dynamics (Levins 1969; Hanski 1998) – rely mainly on negative interactions, such as interference or resource competition, to explain ecological processes, especially at the community level.

Positive interactions (i.e. interactions that benefit at least one of the interacting species and do not harm either) are historically less studied than other types of interactions such as competition or predation (Stachowicz 2001; Bruno et al. 2003; Bronstein 2009), but studies of both types of interactions are being cited equivalently (Lortie and Callaway 2009), indicating that they currently attain the same degree of interest in ecology. For instance, interactions such as mutualism, facilitation, and commensalism began to emerge as important ecological forces only in the late 1980s (Bronstein 2009), and a few years later invasion biologists also began to consider their role in biological invasions (Lockwood et al. 2007). In fact, we now know that, in many situations, positive interactions play the most important role in regulating ecosystem structure and function, and this knowledge helps managers to implement restoration and conservation strategies (Halpern et al. 2007; Simberloff and Vitule 2014; Zwiener et al. 2013).

The relative lack of interest in positive interactions was even more apparent when non-native species were investigated (Simberloff and Von Holle 1999; Ricciardi 2001). Concerned about the undervaluation of positive interactions in invasion biology, Simberloff and Von Holle (1999) reviewed published literature on interactions between non-native species and quantified how often positive interactions were reported in comparison to negative ones. They showed that positive interactions among non-native species can cause synergies that disrupt natural communities and lead to an "invasional meltdown" (hereafter IM). They defined IM as "*the process by which a group of nonindigenous species facilitate one another's invasion in various ways, increasing the likelihood of survival and/or of ecological impact, and possibly the magnitude of impact*" (Simberloff and Von Holle 1999). Egler (1947) had previously observed that non-native livestock in Hawaii had facilitated the spread of unpalatable non-native plants, and Beverton (1992) had similarly stated that it is not uncommon for two or more threats to operate together, causing more intensive damage than the sum of their independent effects. Despite the existence of older similar ideas, Simberloff and Von Holle's (1999) paper was so influential that only four years after its publication, it was the 35th most cited paper in the field of biological invasions (Pyšek et al. 2006); since the publication of that paper, IM has been a major hypothesis in the field (Jeschke et al. 2012). We found that between its publication and December 2015, it was cited 834 times according to the Scopus database and 1243 times according to Google Scholar. IM has even influenced the sciences of conservation and climate change to the extent that it was considered, in a horizon-scan assessment, to be one of the main biodiversity threats to the UK in the next 50 years (Sutherland et al. 2008) and a topic of major concern in climate change scenarios (see Fig. 3 in Blois et al. 2013).

However, the original definition of IM has been interpreted in misleading fashion, and little strong evidence that fully supports the hypothesis was available when Simberloff (2006) revisited the idea. Part of the problem may be because Simberloff and Von Holle's (1999) definition may lead readers to choose different interpretations. Broad definitions of ecological hypotheses are common, and this drives researchers to test different aspects of the same hypothesis, sometimes reaching different conclusions (Heger et al. 2013; Heger and Jeschke 2014).

In light of the relevance of the invasional meltdown hypothesis (hereafter IMH), misleading interpretations, and the need to understand how different aspects of the hypothesis are connected and tested, our aims here are i) to highlight and provide evidence against misconceptions of the IMH, ii) to disentangle main IMH concepts and provide examples and ways to test them, ii) to identify high risk scenarios to provide managers information to identify potential IM, iii) to call attention to the link between propagule pressure and the IMH, and iv) to provide a framework for IMH testing and synthesis. To do so we extensively reviewed publications citing Simberloff and Von Holle (1999) and other literature known to us.

INVASIONAL MELTDOWN MISCONCEPTIONS

DeVanna (2011) incorporates ideas that are not part of the original definition by Simberloff and VonHolle (1999) and Simberloff (2006), listing three criteria: i) the resulting balance of all facilitative interactions happening in a community should account for the effects of the non-native species on both native and non-native species; ii) the facilitated species and the invader must have coevolved; iii) there has to be a second tier of interactions involving species not engaged in the original facilitation.

With respect to the first criterion, it does not matter if the resulting balance of the interaction in a community is not negative for the overall native community. If it is positive for additional non-native species and negative for only one native species it should be considered a negative effect. Somebody who should not be losing is losing. In addition, quantifying impact is hard, so it is difficult and impractical to quantify how much a species is being negatively or positively affected in comparison to other species (Parker et al. 1999). Even if there is a neutral outcome from the net interactions, there can be structural changes at another level, for example loss in β-diversity (Dornelas et al. 2014).

As for the second criterion, Simberloff and Von Holle (1999) give explicit examples (e.g. the ant-scale insect case in California and the *Zosterops-Myrica* case in Hawaii) of species from different continents that together may lead to meltdown. In fact there appears to be as much evidence with non-coevolved species as with co-evolved species for the IMH today (Chapter I). The case of chikungunya virus (Enserink 2014) also demonstrates that coevolution is not needed for facilitation to occur. However, coevolutionary genetic change can alter species interactions in ways that generate new synergistic interactions. Genetic alteration of chikungunya allowed it to replicate efficiently in a new vector, *A. albopictus*, facilitating spread of the virus worldwide (Enserink 2014).

With respect to the third criterion, perhaps DeVanna et al. (2011) referred to a nuclear meltdown, the original referent for the meltdown metaphor. However, for an IM, although a second tier of interactions would make the IM more expansive, consequential, or widespread, it is not obligatory. If evidence for the IMH could be put in a graded scale, identifying second tiers would constitute evidence for a greater community-wide impact. For example, consider the *Morella* (*Myrica*) *faya* case in Hawaii. The Japanese white-eye (*Zosterops japonica*), its main seed disperser (Vitousek and Walker 1989), and non-native earthworms in great density beneath it (Aplet 1990) cause *M. faya* to be more common and widespread than it otherwise would be (and *M. faya* probably also aids the earthworms and possibly the white-eye). In any event, *M. faya*, a nitrogen-fixer in an area lacking native nitrogen-fixers, then facilitates the invasion of other non-native plants that would have been precluded by the nutrient-poor soil (Vitousek 1990), a second-tier effect.

It also does not matter which species reaches the new ecosystem first, as claimed by DeVanna et al. (2011). One introduced species can be present for a long time, but in low density until a second species arrives, and the second species could facilitate the first and make it much more common (e.g. Grosholz 2005). In general, there is no specific time requirement for IM. These cases may explain the lag time manifested by some species until they suddenly "explode" in numbers, density, and/or spread across the new invading area (Crooks 2005; Simberloff and Vitule 2014) or may even contribute to the "invasion debt" of impacts that will occur in the future owing to previous invasions (Essl et al. 2011).

MAIN ASPECTS OF INVASIONAL MELTDOWN

The original definition of IM, the attempt to clarify the concept by Simberloff (2006), and empirical evidence to date suggest that there are three main aspects of the IMH: i) positive interactions among non-natives, ii) increasing rates of invasion, and iii) synergistic impact of non-natives. Although these need not be independent, separating them will allow future empirical test to be more specific with regard to which aspect is being tested. Failure to specify which aspect is evaluated in different studies impedes synthesis and development of the hypothesis. This separation will also help us to understand why different reviews of the IMH have reached different conclusions (e.g. Jeschke et al. 2012; Jackson 2015).

1. SPECIES FACILITATION AND ECOLOGICAL LEVEL

The interaction between two or more non-native species is how the IMH starts to operate. Simberloff (2006) differentiated between a simple facilitation (i.e. only one species receives a benefit while the other is neutrally or even negatively affected) and mutual facilitation. The latter would suggest greater net community impact than the former (Simberloff 2006) (Fig. 4). We identify a third type of interaction where indirect effects play a role. In a three-species scenario, one species might negatively affect another's enemy (e.g. Levin et al. 2002; Relva et al. 2010) or positively affect its facilitator (e.g. Helms et al. 2011), while not affecting the other species directly (see Fig. 2, Ricciardi 2001). This rationale can be extended to a network of interactions with several non-native species, yielding a meltdown likely with even greater impact. An IM could be occurring through indirect facilitation (*sensu* Ricciardi 2001; White et al. 2006), as with commensal, exploitative, and amensal relations among three or more populations of different species that turn out to be beneficial to at least one pair of introduced species. This net effect might then have consequences at the community level, such as changes in species richness, evenness, composition, turnover, beta-diversity, trophic structure, etc. However, indirect effects are difficult to detect, so they are relatively poorly represented in the literature (White et al. 2006). Less diverse communities may be the best ones in which to begin investigating the IMH, as predictions regarding them are easier to generate and to test (Strauss 2013).

It is important to pay attention to Simberloff's (2006) claim that identifying these interactions between populations does not constitute by itself a full IM. Information on these interactions can show researchers where there is a higher probability that an IM, a community-level phenomenon, is taking place. So, it is only the first step for this complex, community-level phenomenon. We therefore need to elucidate how to link these different types of interactions between individuals to population impacts and of population interactions to community-level effects.

A large body of evidence demonstrates how simple facilitations and mutualisms benefit the survival, growth, or reproduction of one or more non-native species. For instance, the survival and weight of an introduced herbivore was demonstrated to be greater in the presence of non-native plants than when only natives were present (Engelkes and Mills 2013). The authors suggested that the presence of non-native plants can accelerate the invasion process and range expansion of the herbivore.

Another common interaction is pollination. Widely introduced honeybees are important pollinators and can increase the reproductive output of non-native plants (Barthell et al. 2001; Beavon and Kelly 2012). In these cases evidence is provided only for the individual level and effects are speculated to occur at the population level. The question of how these would lead to an increasing number of non-native species (one community-level impact) was not answered. Adding to these examples of facilitating survival growth and reproduction, individual-level evidence can also include resource and habitat provision, dispersal of individuals, and impact of individuals. All these effects can happen through simple facilitation, mutualism, and multispecies interactions.

On the population level, evidence can include increase in abundance, density, and biomass as well as increased population dispersal and impact (e.g. Belote and Jones 2009; Helms et al. 2011; Ma et al. 2014). Population-level dispersal differs from individual-level dispersal by subsuming not only how individuals disperse but also how populations spread; relevant evidence often consists of increased abundance in new locations with a plausible explanation linking this increased abundance to other non-native species (e.g. Green et al. 2011).

Community-level studies constitute the most complete examples of IM. The invasion of the yellow crazy ant, honeydew-secreting scale insects, and the giant African snail on Christmas Island is perhaps the best example so far (O’Dowd et al. 2003; Abbott and Green 2007; Green et al. 2011). This non-native triad facilitates each other through direct and indirect interactions and negatively affects the dominant native herbivore (the Christmas Island red crab), which substantially changes understory plant composition. At the ecosystem level, some studies report changes caused by non-native species interactions (O’Dowd et al. 2003; Jackson et al. 2014; Kuebbing et al. 2014). Ecosystem changes might increase invasibility (Hughes and Denslow 2005), and if an increase in the number of non-native species ensues, this would constitute a situation in which IM likely has a huge impact.

Obviously, investigation of community-level phenomena benefit greatly from studies at other ecological levels, particularly those of populations and ecosystems (Fig. 1). In other words, it is necessary to gather basic information from many specific empirical studies at different organizational levels to understand fully the meltdown phenomenon and to assess its prevalence. Population or ecosystem-level studies in isolation are weaker in capturing the essence of the IM phenomenon. Population-level studies nevertheless have much to contribute, not least because data can be collected more easily at this level (Fig. 1). In fact, we must gather as much information as possible at the population-level for many different invaded ecosystems because community-level phenomena are context-dependent (Simberloff 2004; Simberloff et al. 2013). It is also important to recognize that contingency is more easily understood and managed at the population than at the community level (Lawton 1999). Ecosystem-level information is much harder to collect (Odum and Barret 2005), which makes the confirmation of meltdown more difficult at this level (Fig. 1).

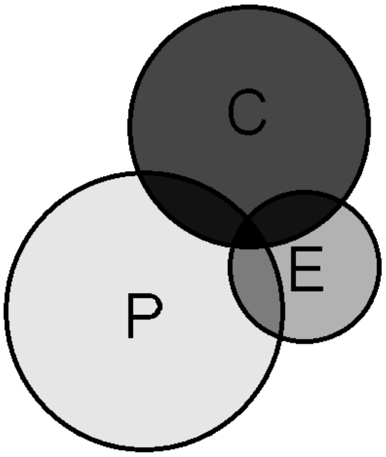
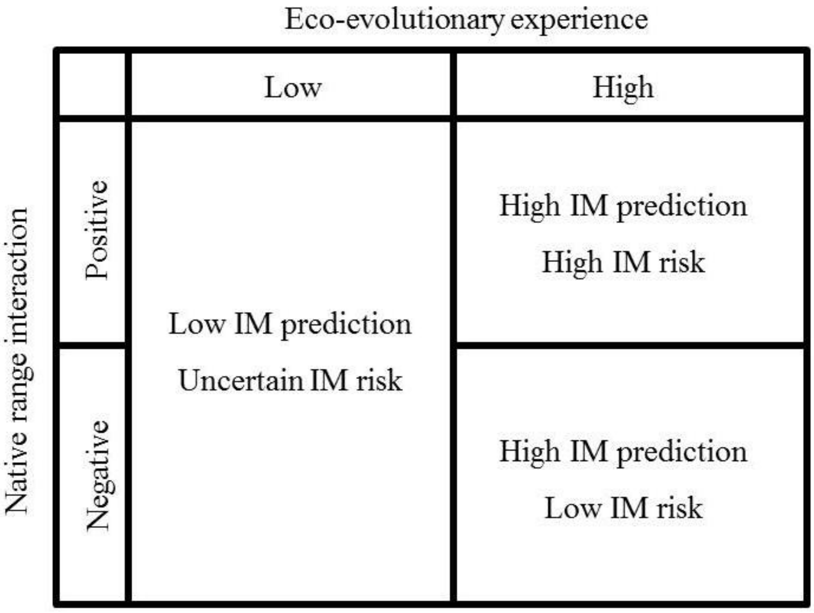


Figure 1. Each circle represents an ecological level. P is population, C is community and E is ecosystem. Circle sizes are proportional to the ease of data collection, with the largest being the easiest and the smallest the hardest. Shading represents the likely severity of the total impact of invasional meltdown, with darker the most severe and lighter the least severe. Shading in circle junctions represents the power of hypothesis-testing for each combination of ecological level studies. Studies combining the community level and any other level are stronger than the combination of population and ecosystem levels. The most powerful way to test the invasional meltdown hypothesis would be to combine all three levels (central area); on the other hand, it is the most difficult combination for which such information can be collected (smallest area).

1a. **High risk scenarios for conservation**

Whether or not an invader establishes facilitative interactions when arriving in a new environment might be the decisive factor in success or failure of the invasion (Nuñez et al. 2009). Generalist species able to form several new mutualist interactions are more prone to participate in an IM process. For example, honey bees are generalist pollen and nectar explorers, so they are expected to be involved in many mutualisms with non-native plants that have a generalized reproductive biology (Barthell et al. 2001; Olesen et al. 2002; Beavon and Kelly 2012).

Together with information on the specialization level of a species, we can use the degree of eco-evolutionary experience (*sensu* Saul and Jeschke 2015) an invading species has with co-occurring non-natives to predict the outcome of a new interaction. Let's say that the non-native species A coevolved with species B in its native range, but A now co-occurs with another species C that has similar traits to species B (i.e. high degree of eco-evolutionary experience), in this case one might expect that they will interact in a similar manner and predict that the risk of IM is substantial (top right box of Fig. 2) (Saul et al. 2013; Saul and Jeschke 2015). On the other hand, if one non-native species faces another species whose traits are similar to the species with which it had negative interactions in its native range (according to its eco-evolutionary experience), then we can predict a low IM risk (low right box in Fig. 2). This does not mean that co-evolution is required for IM (see above – IM misconceptions). Comparisons between interacting biota in the native range of a species versus its invaded range can therefore provide useful information on the probability of new positive or negative associations (Fig. 2). For the honey bee example, comparisons would include acquiring information on the morphological traits of flowers.



		Eco-evolutionary experience	
		Low	High
Native range interaction	Positive	Low IM prediction Uncertain IM risk	High IM prediction High IM risk
	Negative		High IM prediction Low IM risk

Figure 2. Information on the degree of eco-evolutionary experience that a non-native species has with the community it invades guides managers in predicting an invasional meltdown scenario. A high degree of eco-evolutionary experience suggests a higher risk of invasional meltdown. Depending on the interaction (positive or negative), the risk of IM will be higher or lower, respectively.

Both specialization level and degree of eco-evolutionary experience can aid risk assessors to identify high-risk species in the light of the IMH. Risk assessments are important for prioritization of control and management actions, as it is crucial to act early in the invasion process (Rejmánek and Pitcairn 2002; Lonsdale 2011; Westbrooks and Eplee 2011). In the first assessment of specialization level, if the evaluated species falls into the high-risk category (upper left box of Fig. 3a), it should be ranked as a top priority for management and eradication, and possibly for stronger actions to avoid intentional introduction of that species. However, if the species falls into the low-risk category of specialization level (lower right box of Fig. 3a), information on its eco-evolutionary experience with the new community should be assessed. If it falls into the high-risk category of eco-evolutionary experience (lower right box of Fig. 3b), appropriated prevention and management actions should be taken whether or not specialization level is high.

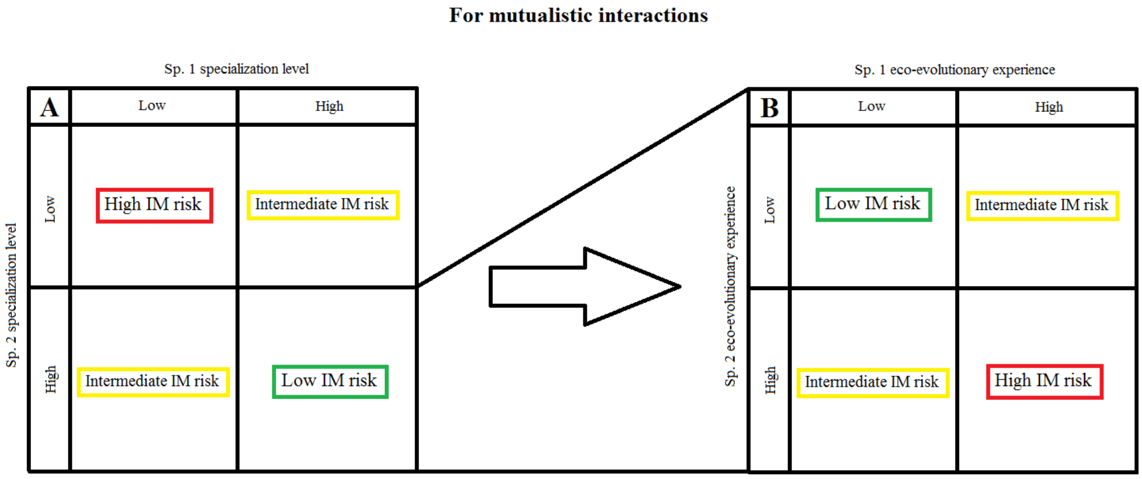


Figure 3. Risk assessment chart for invasional meltdown in light of interaction specialization level and eco-evolutionary experience. If two interacting non-native species have low specialization levels for a specific trait, the probability that they will engage in a mutualistic interaction is high, so the probability that they will participate in an invasional meltdown is high. However if both species are highly specialized, the chance of mutualism is low, but it continues to be low only if neither possesses eco-evolutionary experience with a species with the other's traits.

2. INCREASING RATE OF INVASIONS

Nowadays most ecosystems worldwide are invaded by multiple non-native species. If each non-native species can potentially facilitate further invasions, the rate of invasions will accelerate, resulting in an exponential increase in non-native species richness (Von Holle 2011). This result would be the opposite of that predicted by the biotic resistance hypothesis, which envisions the rate of establishment of new species decreasing with increasing species richness, ultimately reaching an asymptote (see Fig.

1 in Ricciardi 2001). Identifying species interaction that lead to an increasing invasion rate is crucial, as ecosystems might have a tipping point of alterations driven by species invasion at which its resilience will be strongly diminished, compromising any conservation action. For example, the colonization of aquatic ecosystems by the free-floating macrophyte *Eichhornia crassipes* reduces the under-water light what may cause a regime shift (Scheffer et al. 2003). Thus, this invasive species may be the cause of a tipping point in aquatic ecosystems state and identifying potential facilitators of its success is key to assess its impacts.

Species records since the early 1800s until the end of the 1990s in the Great Lakes accord well with the IM model of cumulative number of non-native species (Ricciardi 2001). However, the author lacked data to explain whether this pattern was due to an IM or other factors, such as increased awareness of non-native species or increased shipping activity in the region, resulting in higher propagule pressure (Ricciardi 2001). Additionally, it is possible that exponential increases in non-native species richness arise with constant introduction rates and success, and independently of species interactions (Wonham and Pachepsky 2006). Tecco et al. (2006) found higher non-native species richness under the canopy of a non-native shrub than under natives. Possibly a long period of monitoring of non-native species would similarly reveal an increasing rate of invasion.

For the IMH, an increasing rate of invasions is the ultimate outcome to the community dynamics (Simberloff 2006; Von Holle 2011). However, evidence linking species interactions directly to the rate of invasions is nonexistent (Fig. 4). Researchers attempting to fill this lacuna should take care to account for effects of increased propagule [and colonization] pressure. Perhaps a good way to initiate community-level investigations would be to evaluate how species interactions would affect non-native species richness, composition, turnover, trophic structure, and changes in beta diversity.

2a. **High risk scenarios for conservation**

Because ecosystem engineers (organisms that modulate availability of resources for other species by changing ecosystem components; Jones et al. 1994) can potentially alter habitat and disrupt ecosystem processes, they might well indirectly facilitate several species. The construction of a river dam by beavers transform a lotic to lentic environment, thus it can facilitate the survival and establishment of common widespread fish species adapted to low water flow that would otherwise not be able to survive a strong river flow. Where there is an established ecological engineer we expect greater probabilities of meltdown and larger effects of meltdowns. This is because ecological engineers disrupt native communities and can facilitate establishment of many other non-natives (i.e. stronger "starts" for meltdown in the establishment phase of invasions).

3. SYNERGISM

The ecological consequences of an invasion to the native biota are often higher impact owing to non-native species interaction compared to single species invasion, and this impact is an important aspect of the IMH. In the same manner as explained previously, the impact to native species can occur at the individual, population, community or ecosystem level (Parker et al. 1999). However, the IMH differentiates additive effects (i.e. the summed impact of two or more non-native species) from synergistic effects. Synergistic effects entail the exacerbation of each other's impact when two species invade, so the resulting impact to the native biota is higher than the sum of their individual impacts (Simberloff and Von Holle 1999; Von Holle 2011).

Using field sampling to compare the isolated and combined impact of two functionally similar non-native woody shrubs, Kuebbing et al. (2014) found both additive and synergistic impact on ecosystem variables. Similarly, using a mesocosm experiment, Jackson et al. (2014) also found additive and synergistic impacts of crayfish on an ecosystem, although in this case apparently no species benefited by another's presence. In fact, some cases of synergistic impact upon the native biota can occur through negative interactions, with the presence of one non-native detrimental to the other. For example Ross et al. (2004) demonstrated using a field experiment that the combined impact of two marine predators (Pacific seastar and European green crab) upon native bivalves can be synergistic even though it arises through resource competition. Additionally, Johnson et al. (2009) found synergistic effects of non-native crayfish and snails to the point of leading a native snail to extinction in a mesocosm experiment, although the crayfish also negatively affected non-native snail abundance, possibly by predation. We emphasize that it is important to evaluate if the effect of a non-native species interaction is positive for a least one of the species before asserting that an IM is underway (Fig. 4). The difficulty in detecting positive interactions between non-natives in the cases above rests on the fact that the positive impact might be occurring through indirect effects that are difficult to detect (White et al. 2006). The link between synergy and facilitation was well established by a mesocosm experiment involving a non-native plant and a non-native herbivore although only additive effects were found (Meza-Lopez and Siemann 2015).

3a. **High risk scenarios for conservation**

As synergy in the IMH is related to the impact of non-native species on the native community, non-native predators are the species most prone to lead to an IM in this sense. Predation is a particularly severe effect (at least at the individual level), because by definition it implies individuals of one species killing those of the other in the short term (Begon et al. 2007; Clout and Russel 2011). Two or more predators can exert strong predation pressure upon different life stages of a prey species or at complementary parts of the prey species' spatial distribution, and in such cases their combined effect is likely to be more than additive (e.g. Ross et al. 2004) and additionally to benefit a third species previously competing with that shared prey species. Most synergistic impact assessments have not considered this third benefited species, but it is probably not difficult to find some species benefiting from the decline of any species. We reinforce the need to detect this third, benefited species, because the synergistic impact of two predators is most likely result from resource competition between them, and therefore will not be the product of a positive interaction. In this circumstance, if only the competition between two non-natives, but not their indirect facilitation on a third invasive species was detected, IM would be ignored.

PROPAGULE PRESSURE: UNDERREPRESENTED IMPORTANCE TO THE IMH

The discipline of invasion biology is rife with hypotheses trying to explain the success or failure of non-native species (e.g. Catford et al. 2009; Lowry et al. 2013; Jeschke 2014). Definitions and mechanisms cited by different authors overlap, though often with conflicting explanations. A similar situation obtains for the IMH. An analysis of 29 hypotheses of invasion ecology showed that six of them consider mutualist relationships as an important factor contributing to invasion success (Catford et al. 2009). Our intention here is not to review extensively how these hypotheses contribute to an evaluation of the IMH. However propagule pressure seems to be an important and underestimated factor linked to the IMH.

Propagule pressure is one of the most important key factors determining invasion success (Lockwood et al. 2005; Duncan 2011; Wittmann et al. 2014). As explained previously, interpreting increasing rates of invasion as due to IM and not simply to propagule pressure is difficult. However, with increasing colonization pressure (high numbers of introduced species in a single location; *sensu* Lockwood et al. 2009), the greater the likelihood that a species prone to form mutualistic associations will arrive. Also the chance of introducing a pair of mutualists will be higher, diminishing the probability of a failed introduction owing to missed mutualism (Nuñez et al 2009). Increased propagule pressure will strengthen the chances of generalist mutualists to find individuals of a suitable other species. With more individuals interacting the greater the probability of invasion success and of increased rates of population growth. This scenario is opposed to the global competition hypothesis, which predicts that higher numbers of introduced species means a greater probability of finding a competitor (Catford et al. 2009). This scenario is also missing in the classical Island Biogeography Hypothesis (MacArthur and Wilson 1967), which predicts an increase in extinction rates with the number of species owning to competition, but it ignores the possibility of an increase in mutualists. Additionally, ecosystems with high propagule and/or colonization pressure might be expected to increases in invasibility because arriving and established species alter the system, making it more susceptible to invasion.

CONCLUDING REMARKS AND THE WAY FORWARD

Based on the first definition by Simberloff and Von Holle (1999), published evidence so far, misconceptions of the hypothesis, and important neglected aspects of invasions, we propose a new, synthetic conceptual framework that provides a more practical and accurate way to apply the meltdown concept and to test the IMH. Our framework should help guide future research, data collection, and interpretation of results regarding the IMH.

Our framework incorporates the notion that, if either synergistic impact or increased invasion rate is being evaluated, it is likely to be occurring through positive interactions among non-native species. Such interactions lead to synergistic impacts on the native biota and allow an increasing number of arriving non-native species to establish populations (Fig. 4). It is also possible that with the increasing number of non-native species establishing, invaders produce greater synergistic impacts (Parker et al. 1999). As detailed in each respective section above, differences in species interaction type and ecological level of the evidence will lead to support for predictions of different levels of impact of IM. Evidence in support of the IMH should not be restricted to a dichotomous “black” and “white” scale, but could fruitfully be viewed as falling in a “gray” gradient from darker to lighter. With this framework future studies can clearly differentiate which aspect of the IMH is being tested. Also, synthesis on the subject will allow differentiation of the various aspects of the IMH, and in so doing will enable selection of further research targets.

In view of the multiple invasions occurring in many ecosystems and the increasing rate of invasion, we must understand the importance and consequences of IM fully. If it is demonstrated conclusively to be verisimilitudinous in many cases, the IM scenario shows that research on single non-native species or non-native species effects on native species will often not suffice to support effective management strategies where many invaders are present (cf. Kuebbing et al. 2013). The understanding of biotic interactions, especially when they arise from introduction of non-native species, is of high priority for the future of ecology and conservation.

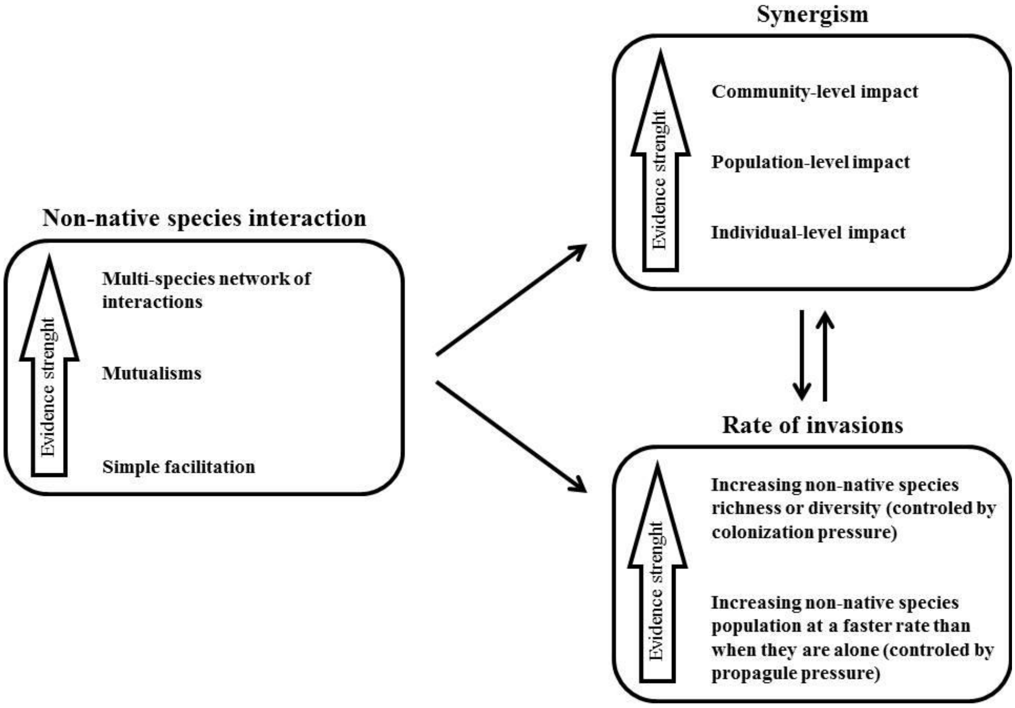


Figure 4. Invasional meltdown hypothesis framework integrating the three main aspects involved. Inside each box, information on evidence of increasing impact is provided. Arrows indicate the direction of influence.

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