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Invasion biology and risk assessment of the recently introduced Chinese mystery snail, *Bellamya (Cipangopaludina) chinensis* (Gray, 1834), in the Rhine and Meuse River basins in Western Europe

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Editor's note:

This study was first presented at the 19th International Conference on Aquatic Invasive Species held in Winnipeg, Canada, April 10–14, 2016 (<http://www.icaais.org/html/previous19.html>). This conference has provided a venue for the exchange of information on various aspects of aquatic invasive species since its inception in 1990. The conference continues to provide an opportunity for dialog between academia, industry and environmental regulators.

Abstract

The Chinese mystery snail, *Bellamya (Cipangopaludina) chinensis*, was recorded for the first time in 2007 in the Netherlands. By 2016, twelve water bodies (mostly riverine ecosystems) had been colonized by this freshwater snail. These records were the first known introductions of this alien species in the European Union (EU). Insight into the invasiveness and (potential) risks of ecological, socio-economic and public health effects of *B. chinensis* in Europe is urgently needed due to multiple introductions, permanent establishment and continuing secondary spread. A field survey was carried out to determine dispersal rate, habitat conditions and population characteristics of *B. chinensis* in the floodplain Eijsder Beemden along the Meuse River. The natural dispersal rate in this area was 0.1 km/yr and the average population density was 0.33 individuals/m². This species has colonized several floodplain lakes that are hydrologically connected to the Meuse River. New introductions and colonization of the main channels of large rivers are expected to accelerate the dispersal of this species through water flow and shipping vectors. A risk assessment of *B. chinensis* was performed using the Harmonia⁺ protocol. Evidence of deliberate and unintentional introductions led to a high score for introduction risk. Risk of establishment was also assessed as high. The risk assessment resulted in a medium score for spread risk due to dispersal by human action. The assessed impact on plant targets or animal targets was very low. A medium risk was assigned to impacts on environmental targets. Risk of impacts on human targets received a low score. The overall invasion risk was classified as high and environmental impact was medium, resulting in a medium overall risk score. Regulation of *B. chinensis* trade and an increase in public awareness about its impact are required to prevent new introductions and further spread of this species in Europe. Moreover, there is an urgent need for research concerning the effects of *B. chinensis* on native biodiversity and ecosystem functioning, and cost-effective management of this species (e.g., eradication, population control and containment measures).

Key words: *Bellamya (Cipangopaludina) chinensis*, Gastropoda, Harmonia⁺, invasiveness, risk analysis, Viviparidae

Introduction

The first record of the Chinese mystery snail, *Bellamya (Cipangopaludina) chinensis* (Gray, 1834) (Viviparidae), was made in 2007 in the Netherlands in a floodplain lake (Piters 2007; Supplementary material Table S1). By 2016, this freshwater snail had established populations at twelve sites in the Rhine and Meuse River basins in Western Europe. These were the first records of this alien freshwater snail in the European Union (EU). The Rhine and Meuse River basins are hot spots for introductions of alien species and act as an important European invasion corridors (Arbačiauskas et al. 2008; Leuven et al. 2009; Panov et al. 2009).

The nomenclature of *B. chinensis* is widely debated resulting in two commonly used genus names: *Bellamya* and *Cipangopaludina*. We follow Smith (2000) who advocates the placement of the Chinese mystery snail into the subfamily Bellamyinae due to the absence of folding of the gill filament characteristic of the genus *Cipangopaludina*. Smith (2000) suggests the provisional use of *Cipangopaludina* as a subgenus of *Bellamya*, representing the larger Bellamyinae with unbanded shells and a native distribution in Asia.

Bellamya chinensis is native to China, Taiwan, Korea and Japan (Chiu et al. 2002; Global Invasive Species Database 2011; Lu et al. 2014). The introduction of this species into the United States of America (USA) occurred at the end of the 19th century for the Asian food market (Jokinen 1982; Karatayev et al. 2009). Since then, *B. chinensis* has spread rapidly across North America and is currently present in Canada (Quebec, Ontario, British Columbia, Nova Scotia, New Brunswick and Newfoundland; McAlpine et al. 2016) and 32 states of the USA (including Hawaii and Alaska; Jokinen 1982; Karatayev et al. 2009; Global Invasive Species Database 2011).

Risk assessments are a common policy instrument to identify species likely to become invasive and cause significant adverse impacts (Verbrugge et al. 2012). Risk assessments for alien species generally take into account four main stages of invasion: entry, establishment, spread and impacts. Therefore, information is needed on all four stages of invasion to properly assess the invasion risk of *B. chinensis*.

The primary introduction pathways of *B. chinensis* are the aquarium and ornamental trade and imports for Chinese food markets (Karatayev et al. 2009; Strecker et al. 2011). Hobbyists and other consumers likely increase the secondary spread of *B. chinensis* (Strecker et al. 2011; Soes et al. 2016), indicated by its scattered distribution pattern (Kroiss 2005). The dispersal vectors that facilitate the secondary spread of *B. chinensis* are unknown. Due to the ability of

B. chinensis to attach to boat hulls and to survive prolonged air exposure, recreational boats could be a potential vector for dispersal via transport over land and through waterway networks (Havel 2011; Havel et al. 2014). This vector is supported by the higher chance of occurrence of *B. chinensis* with decreasing distance from a boat launch (Solomon et al. 2010). Waterfowl and aquatic mammals (e.g., otters and muskrats) can also act as dispersal vectors for *B. chinensis* (Claudi and Leach 2000).

The presence of several human mediated dispersal vectors increases the likelihood of new introductions of *B. chinensis* in Europe. In a horizon-scanning exercise for the EU, Roy et al. (2015) classified *B. chinensis* as a high risk species, and emphasised the need for a detailed risk assessment. According to the New York invasiveness assessment *B. chinensis* is recognized as a potentially high risk species (Adams and Schwartzberg 2013). The management costs of invasive alien species (IAS) are high (Simberloff 2005; Pimentel et al. 2005; Williams et al. 2010; Oreska and Aldridge 2011; Kettunen et al. 2008). Therefore, it is urgent to assess the potential for invasiveness of *B. chinensis* in the Rhine and Meuse River basins. This paper aims to present relevant field and literature data on the entry, establishment, spread and impact of *B. chinensis* and to perform a risk assessment using this information. In order to achieve these goals a field survey was performed to 1) quantify the natural dispersal capacity of *B. chinensis*, and 2) determine the population characteristics of the largest known *B. chinensis* population in the Rhine and Meuse River basins. In addition, a risk inventory was conducted using information from an extensive literature review concerning the invasion biology and ecological, socio-economic and public health effects of this species. We then performed a risk assessment using the Harmonia⁺ protocol.

Methods

Field sampling

Field surveys were performed in the summer of 2016 focussing on floodplain lakes in the Eijsder Beemden (50°47'35.3"N; 5°41'49.1"E) along the impounded Meuse River, where the largest known population of *B. chinensis* in the Netherlands occurs (Soes et al. 2016; indicated by arrow in Figure 1A). At this site, conductivity (µs/cm), salinity (ppt) and temperature (°C) were measured using a YSI 30 meter (YSI incorporated, USA). Additionally, flow velocity (m/s) was measured using a TAD-micro flow velocity meter (probe: W16, Höntzsch GmbH-W, Germany). Snail density was only determined at Location 1

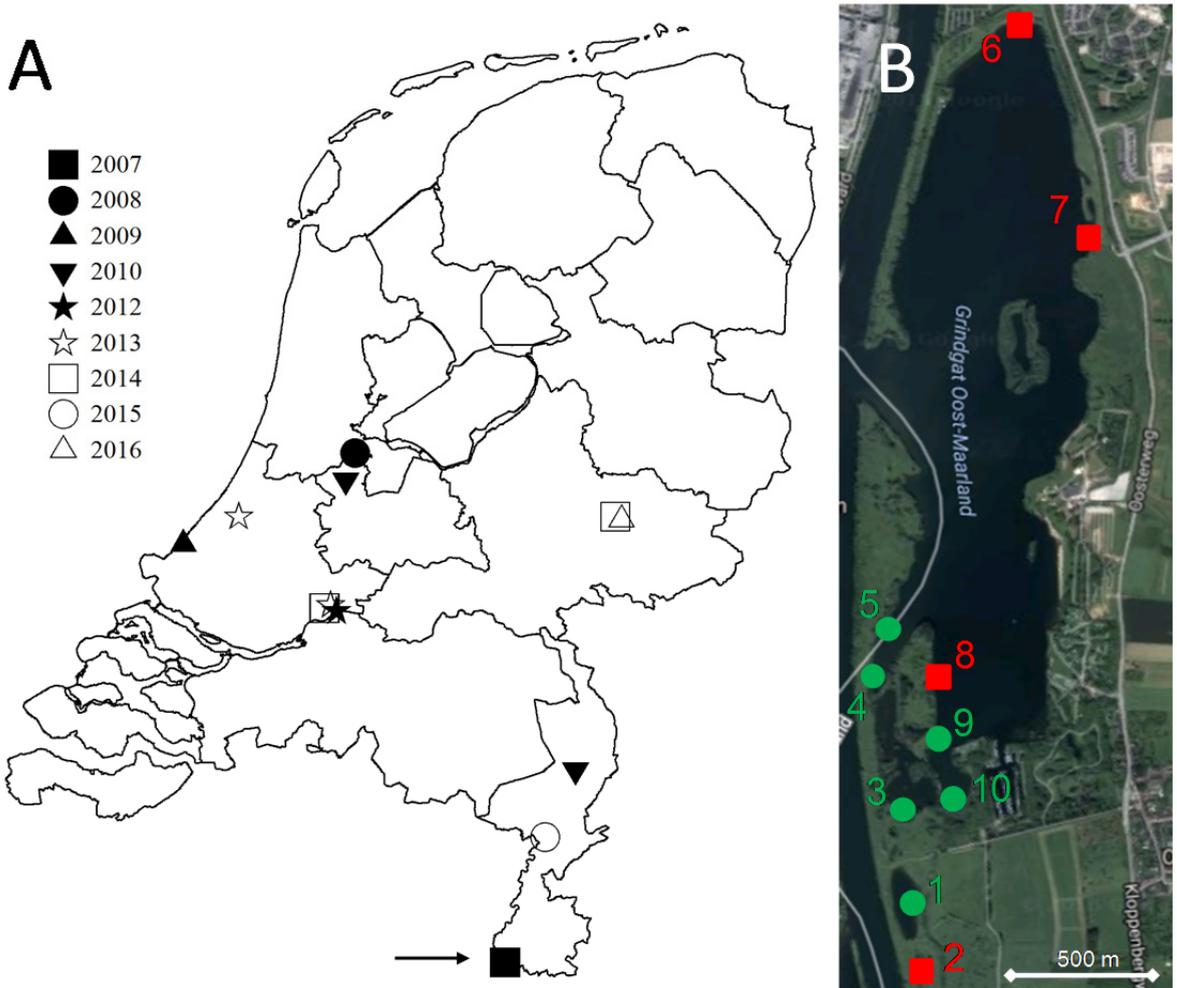


Figure 1. A) Distribution of *Bellamya chinensis* in the Netherlands. The arrow indicates the Eijsder Beemden location. Mapping was performed using the software program “STIPT” (Frigge 2014; Supplementary material Table S1); B) Distribution of *B. chinensis* in the Eijsder Beemden (Green circles: one or more living individuals; Red squares: no individuals found; numbers depict the different monitoring locations; Supplementary material Table S2).

(Figure 1B) by counting live individuals using an aquascope in three random $1 \times 1 \text{ m}^2$ plots and by taking three one metre long sediment scoops with a 0.5 metre wide pond net. Living individuals and empty shells were taken to the laboratory for weight and size measurements. The shells of all collected individuals were measured for length and width using a digital calliper. The gender and wet weight of collected living snails was determined. Wet weight measurements were performed using snails with a closed operculum and with their shell dried using a paper tissue. The fecundity of females was estimated after dissection by counting the total number of developing and released juveniles in the lab. After dissection of snails, their shell and operculum were

dried at $60 \text{ }^\circ\text{C}$ for 24 hours and subsequently weighed. We performed linear regression analyses of relations between 1) shell length and width, and 2) shell length and body (dry) weight, using Microsoft Excel.

Additionally, at ten locations in the Eijsder Beemden (Figure 2B) an assessment of the presence of *B. chinensis* was made using an aquascope. Subsequently, the date of first record of *B. chinensis* and distribution pattern over time in this floodplain area enabled calculations of natural dispersal rates of this species. The dispersal rate was expressed as the linear distance in kilometres between the initial record (2010) and most distant record divided by the time difference in years, yielding a maximum dispersal rate expressed as km/yr.

Risk inventory

In addition to the field surveys, a risk inventory was performed by searching available scientific literature and reviewing the current body of knowledge on the distribution, invasion biology and environmental impacts of *B. chinensis*. A risk inventory is a comprehensive process of data and information collection comprising a straight-forward accounting of everything involved in the risk assessment of interest. Literature data and information were collected on the life history traits (e.g., reproduction, dispersal and physiological tolerances), habitat, and on the ecological, socio-economic and public health impacts of *B. chinensis*. An additional search was performed to retrieve websites selling *B. chinensis*. Literature was searched using the Web of Science, Google Scholar and Google entering official and unofficial scientific species names (see Supplementary material Table S3 for a detailed description of the search strategy and results).

Risk classification using the Harmonia⁺ protocol

The internet-based Harmonia⁺ protocol was used as it incorporates environmental risks, impacts on human infrastructure, impact on ecosystem services and effects of climate change on risks (D'Hondt et al. 2015; Vanderhoeven et al. 2015). Moreover, this new version of the protocol was regarded as compliant with criteria of the EU regulation for risk assessments for listing IAS of EU concern (European Commission 2014). The protocol consists of 41 questions grouped in six categories: context, introduction, establishment, spread, impacts and future effect of climate change (Supplementary material Table S4). The impact categories concern: 1) environment, 2) plant cultivation, 3) domesticated animals, 4) public health, 5) human infrastructure, and 6) ecosystem services. A risk score and level of confidence (certainty score; Table 1) were assigned to each question. Depending on the question, different qualitative and quantitative scoring scales were applied, limiting comparisons between risk scores associated with different questions. Therefore, risk scores were standardised as low, medium and high according to Table 2. The Harmonia⁺ risk classification was based on the invasion score and the impact score. The invasion score was derived through calculating the geometric mean of the introduction, establishment and spread risk scores. The combined maximum impact of *B. chinensis* of the different subgroups was used as the impact score. For further details regarding the Harmonia⁺ protocol see D'Hondt et al. (2015). The Harmonia⁺ assessment was carried out and discussed by the authors during a workshop until consensus was reached.

Table 1. Cut-off values for levels of certainty used for the risk classification of *Bellamyia chinensis* using the Harmonia⁺ protocol (cut-off values were adopted from Schiphouwer et al. 2017).

Color code certainty	Certainty: classification	Certainty: quantitative score ranges
	Low	< 0.33
	Medium	0.33 ≤ CS ≤ 0.66
	High	> 0.66

Table 2. Cut-off values for risk scores used for the risk classification of *Bellamyia chinensis* using the Harmonia⁺ protocol (cut-off values were adopted from Schiphouwer et al. 2017).

Color code risk	Risk: classification	Risk: qualitative scores	Risk: quantitative score ranges
	Low	Inapplicable, no/very low, very low, low, neutral, no change	< 0.33
	Medium	Medium, moderately negative	0.33 ≤ RS ≤ 0.66
	High	High, optimal	> 0.66

Results

Field sampling

Currently, *B. chinensis* has been recorded at twelve sites in the Netherlands (Figure 1A; Supplementary material Table S1). The species was first identified at the Eijsder Beemden site (indicated by an arrow in Figure 1A) in 2007, in a small floodplain lake of the Meuse River (Location 1, Figure 1B). In 2010 the species was recorded at Location 3 (Figure 1B) During the field survey in 2016, the species was recorded at Location 5, 0.62 kilometres from the initial Location 3 in this floodplain lake (Figure 1B), suggesting a natural dispersal rate of 0.1 km/yr. *B. chinensis* occurred in the Eijsder Beemden on muddy bottom sediment (substrates) and small boulders. *B. chinensis* habitat was characterized during the sampling period by temperatures ranging from 19.0 to 21.4 °C, flow rates of 3 to a maximum of 8 cm/s, conductivity ranging from 140 to 437 μs/cm and salinity ranging from 0.1 to 0.2 ppt (Table 3).

The average density of *B. chinensis* ± SD in the Eijsder Beemden (Location 1, Figure 1B) was 0.33 ± 0.52 individuals/m² (range: 0–2 individuals/m²). Compared to reported habitat densities in other introduced areas, the density in the Eijsder Beemden was low (Table 4). Population size in the single pool surrounding Location 1 in the Eijsder Beemden (Location 1, Figure 1B) was estimated to be about 6600 individuals (Table 4).

Table 3. Abiotic habitat conditions of *Bellamyia chinensis* in its native range (Taiwan) and introduced areas (North America and the Netherlands).

Parameter	Introduced area		Native range Taiwan
	United States of America	The Netherlands	
pH	6.5–8.4 ^a	NA	4.0–9.0 ^d
Conductivity (µs/cm)	63.0–400 ^a	140–437 ^c	< 30–> 195 ^d
Calcium concentration (ppm)	5.0–97 ^a	NA	< 2–> 20 ^d
Magnesium concentration (ppm)	13–31 ^a	NA	NA
Sodium concentration (ppm)	2.0–49 ^a	NA	NA
Oxygen concentration (ppm)	7.0–11 ^a	NA	NA
Water temperature (°C)	0.0–30 ^b	19.0–21.4 ^c	NA
Salinity (ppt)	NA	0.1–0.2 ^c	NA
Flow rate (m/s)	NA	0.03–0.08 ^c	NA

^a: Jokinen (1982); ^b: Karatayev et al. (2009); ^c: this study; ^d: Chiu et al. (2002); NA: not available.

Table 4. Estimated densities and population size of *Bellamyia chinensis* at locations in its native range (Japan) and introduced areas (North America and the Netherlands).

Location	Density (individuals/m ²)	Number of individuals in water body (total surface area)	Reference
Introduced area			
Wild Plum Lake, Nebraska, USA	2.62–3.92	169,400–253,570 (6.47 ha)	Chaine et al. (2012)
Long Island, New York, USA	0.01–0.07	150–970 (1.46 ha)	McCann (2014)
Kidd Springs, Dallas, Tarrant County, USA	100	NA	Karatayev et al. (2009)
Otter Lake, Wisconsin, USA	38	NA	Solomon et al. (2010)
s-Gravenzande, The Netherlands	< 0.5	NA	Soes et al. (2011)
Eijsder Beemden, The Netherlands	0.33	6600 (2.00 ha)	This study
Native range			
Paddy fields, Takashima, Japan	0.25–30	NA	Nakanishi et al. (2014)

In total 40 empty shells without operculum and 9 living individuals were collected at the Eijsder Beemden. The length/width relation of all collected *B. chinensis* was linear (F-value = 447; *P*-value = < 0.001, $R^2 = 0.90$; Figure 2A). For the living individuals a linear relationship was found between length and wet weight of the snail including their shell inclusive of operculum (F-value = 1012; *P*-value = < 0.001, $R^2 = 0.99$) and dry weight of the body excluding the shell (F-value = 95.7; *P*-value = < 0.001, $R^2 = 0.93$; Figure 2B). The size frequency distribution was constructed using all collected individuals, living or dead (Figure 2C). All living individuals were found to be female and had on average 35.2 (SD = 29.1; range: 1–81) developing young.

Risk inventory

Species description

B. chinensis can reach up to 70 mm in length and its lifespan ranges from four to five years (Jokinen 1982; Kroiss 2005; Prezant et al. 2006; Soes et al. 2011; Global Invasive Species Database 2011). This species is a facultative detritivore that filter-feeds and grazes on epiphytic diatoms, periphyton, and detritus (Cui et al. 2012; Olden et al. 2013). It mostly occurs on sandy to muddy substrates in standing and slowly flowing waters (Jokinen 1982; McCann 2014; Soes

et al. 2016). Adults are often found on lake bottoms or partially buried in mud or silt, juveniles are often found in crevices under rocks (Prezant et al. 2006). Male and female sexes are present. Males can be recognized by one shorter tentacle which functions as a penis (Global Invasive Species Database 2011). *B. chinensis* is viviparous and gives birth to fully grown living young (Jokinen 1982; Stephen et al. 2013). This species is possibly able to reproduce via parthenogenesis since this is a known reproduction strategy for Viviparidae species (Johnson 1992; Claudi and Leach 2000). Release of the young takes place between June and October (late spring to autumn), hereafter snails migrate to deeper waters for the winter season (Jokinen 1982). Reproduction is possible for females in their first year, is continuous throughout their entire life span and fecundity increases with body size (Stephen et al. 2013). The fecundity of a population in Wild Plum Lake (southeast Nebraska, USA) was estimated to average between 27.2 and 33.3 young per female per year (Stephen et al. 2013).

Ecological effects

Several ecological effects, both positive and negative, relating to *B. chinensis* have been reported. Snail species have a competitive advantage at a low trematode infection rate, as trematode infection lowers

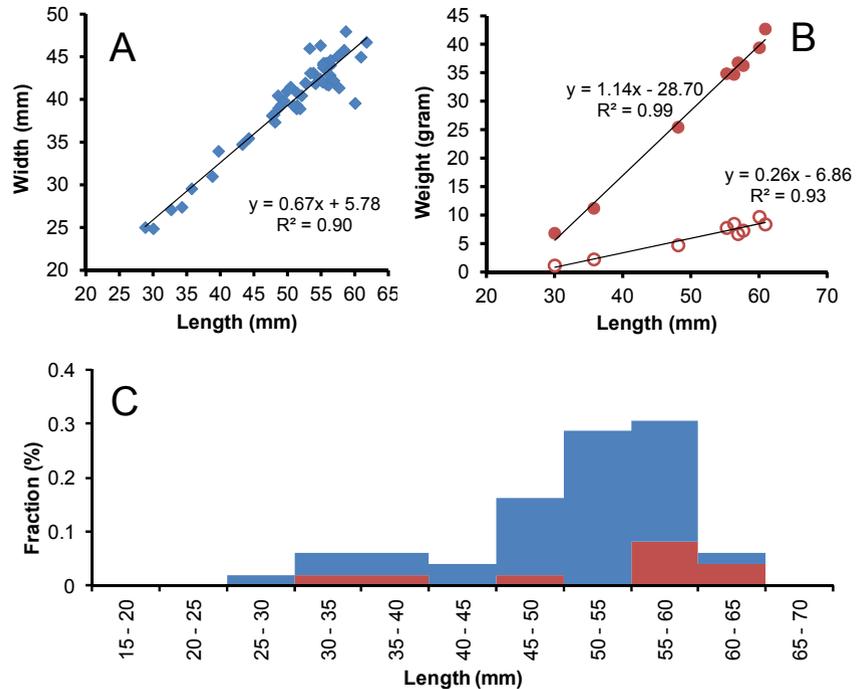


Figure 2. A) Relationship between the shell length and width of living and dead *Bellamya chinensis* (n = 49) collected at the Eijsder Beemden site in the Netherlands, B) relationship between shell length and body wet weight (solid red circles; n = 9) and shell length and body dry weight excluding the shell (open red circles; n = 9) for Eijsder Beemden *B. chinensis* snails that were living when sampled, and C) frequency distribution of all *B. chinensis* individuals (blue, n = 49) and live snails (red, n = 9) found in the Eijsder Beemden.

reproduction and survival. In its Asian native range it is often infected by trematode species (Bury et al. 2007), whereas in North America, *B. chinensis* infection by trematodes is rare (Harried et al. 2015) so it may experience a competitive advantage compared to native species. Experimental exposure of *B. chinensis* to the trematode *Sphaeriodiotrema pseudoglobulus* (McLaughlin et al., 1993) resulted in a significantly lower infection level compared to *Physa gyrina* (Say, 1821) and *Bithynia tentaculata* (Linnaeus, 1758), two snail species that co-occur with *B. chinensis* (Harried et al. 2015). When the infection rate increases, parasites may be transmitted from *B. chinensis* to predatory birds and mammals (Chao et al. 1993).

B. chinensis negatively affects the occurrence of some native snail species in North America (Solomon et al. 2010). In a mesocosm experiment, the abundance of *Lymnaea stagnalis* (Linnaeus, 1758) decreased by 32% after the addition of *B. chinensis* (Johnson et al. 2009). Following the addition of rusty crayfish, *Orconectes rusticus* (Girard, 1852), to the mesocosm the abundance of *L. stagnalis* decreased by 100% (Johnson et al. 2009). *O. rusticus* also reduced the abundance of *B. chinensis*, though the total biomass of *B. chinensis* was not altered (Johnson et al. 2009). *B. chinensis* can serve as a food source for predators. In mesocosm experiments performed in Washington State (USA), the native crayfish

Pacifastacus leniusculus (Dana, 1852) consumed more *B. chinensis* compared to the alien crayfish species *Procambarus clarkii* (Girard, 1852) and *Orconectes virilis* (Hagen, 1870) (Olden et al. 2009). All three of these crayfish species have been introduced to the Rhine and Meuse River basins (Kouba et al. 2014). Due to its thick shell, however, *B. chinensis* is better protected from crayfish attacks compared to native thin-shelled snail species (Johnson et al. 2009). Predation by waterfowl and rodents on adult and juvenile snails has been observed (Soes et al. 2016).

The filtration rate of *B. chinensis* is comparable to several highly invasive freshwater bivalves, such as *Dreissena polymorpha* (Pallas, 1771), *Dreissena rostriformis bugensis* Andrusov, 1897 and *Limnoperna fortunei* (Dunker, 1857) (Olden et al. 2013). This high filtration rate can give *B. chinensis* a competitive advantage. The microbial community alters slightly when *B. chinensis* is present, probably due to the snail's excretion products and feeding behaviour (Olden et al. 2013). This alteration can change the composition, and decrease the variability, of the microbial community. Additionally, the N:P ratio of a system may increase if *B. chinensis* is introduced, possibly due to the low P excretion of this species compared to native snails in North America (Johnson et al. 2009). Changes in N:P ratio can significantly affect algal community structure in natural systems.

Table 5. Risk classification of *Bellamyia chinensis* in the Netherlands and the certainty of the risk scores calculated according to the Harmonia⁺ protocol (risk classification and colour coding are explained in Table 1). The detailed risk assessment can be found in Supplementary material Table S4.

Risk category	Risk classification	Risk score	Certainty	Certainty score ³
Introduction ¹	High	1.00	High	0.67
Establishment ¹	High	1.00	High	1.00
Spread ¹	Medium	0.50	Medium	0.50
Impacts: environment targets ¹	Medium	0.50	Medium	0.50
Impacts: plant targets ¹	Low	0.00	High	1.00
Impacts: animal targets ¹	Low	0.00	Low	0.33
Impacts: human health ¹	Low	0.25	Low	0.33
Impacts: other targets ¹	Low	0.00	High	1.00
Invasion score ²	High	0.79		
Impact score	Medium	0.50		
Risk score (invasion x impact)	Medium	0.40		

¹: maximum risk score for each risk category; ²: the geometric mean of the introduction, establishment and spread scores; ³: arithmetic mean of each category

Socio-economic effects

The availability of *B. chinensis* on the Chinese food market and their culture as “escargot” by some American entrepreneurs exerts a positive influence on economy (Claudi and Leach 2000). The internet search yielded one website selling Chinese mystery snails in the USA. However, *B. chinensis* may clog water intake pipes which subsequently have to be cleaned (Kipp et al. 2016). At high densities, fishing nets can become clogged with *B. chinensis* shells reducing the catch of fisherman (Global Invasive Species Database 2011). Additionally, high densities can result in shores covered with dead or decaying snails and shells, which can be a nuisance (Bury et al. 2007). We observed numerous decaying dead individuals along the shores at the Eijsder Beemden site. Since this area is a popular recreational area during summer, an increase in *B. chinensis* density may cause a decline in recreational value.

Public health effects

In its native range, *B. chinensis* is an intermediate host for the trematode *Echinostoma gotoi* Ando and Ozaki, 1923 that can infect humans (Chao et al. 1993). Infection occurs when raw or under cooked snails are consumed (Graczyk and Fried 1998). Once infected, echinostomiasis can develop which can result in diarrhoea, abdominal pain and anorexia (Graczyk and Fried 1998), however, a treatment for this infectious disease is available (Chen 1991). Despite *B. chinensis* being an important source for transmission of endemic infection diseases in Taiwan (Chen 1991), so far no infections have been reported for the USA (Bury et al. 2007), though, there are several recipes available on websites that use *B. chinensis* as a food ingredient (Invasivore.org

2011; JCC 2012), increasing the chance of infections. To prevent infections, the recipes stress the importance of thoroughly cooking the snails before consumption (Invasivore.org 2011).

Risk classification using the Harmonia⁺ protocol

Natural dispersion capability was scored as low, due to the low dispersal rate observed at the Eijsder Beemden. Since the first observation of *B. chinensis* nine years ago, this species has been reported at twelve locations, showing that potentially more than 10 introductions can occur in a decade, resulting in a high risk score for intentional introductions. *B. chinensis* tolerates a wide range of climatic and physical conditions and has already established viable populations in the Netherlands. Therefore, the risk of establishment was scored as high. The risk of natural spread of *B. chinensis* was scored as low due to the low dispersal rate calculated from records from Eijsder Beemden (< 1 km/yr). Since records of new locations with viable populations of *B. chinensis* in the Netherlands are increasing, the risk of dispersal by human action was scored as medium. The impacts on plant targets or animal targets were scored inapplicable and very low, respectively. Impacts on environmental targets relate to *B. chinensis*' host function for parasites (Chao et al. 1993; Harried et al. 2015), competition with other species (Johnson et al. 2009; Solomon et al. 2010; Olden et al. 2013) and effects on biotic and abiotic conditions (Johnson et al. 2009; Olden et al. 2013). Since the severity of impact is unclear, a medium risk score was assigned. Human target impact was scored as low since no transmissions of infectious diseases have been reported from North America (Bury et al. 2007), although *B. chinensis* is known to be an important

source of endemic infectious diseases in Asia (Chen 1991). The overall invasion risk of this species was classified as high (Table 5; Supplementary material Table S4). The environmental impact of *B. chinensis* was classified as medium, resulting in an overall risk score of medium (Table 5).

Discussion

The risk inventory resulted in an overview of all available information concerning the entry, establishment, spread and potential impacts of *B. chinensis* in the Netherlands. Currently, *B. chinensis* has been established at several locations in the Rhine and Meuse River basins in the Netherlands (e.g., Table S1 and S2). Recently, the species has been also recorded in Belgium (Van den Neucker et al. 2017). The presence of several active introduction pathways and dispersal vectors increases the likelihood of new introductions and secondary spread of *B. chinensis* in Europe. The species may experience a competitive advantage compared to native species due to the lower infection rate by parasites, higher filtration rate and lower predation. However, the ecological risks associated with extremely high population densities are largely unknown (Bury et al. 2007). Moreover, information on animal and human impacts remains scarce. Therefore, future research should focus on gaining data and mechanistic understanding of the species' dispersal processes, ecosystem alterations and impacts on human health and domesticated animals.

The risk assessment using the Harmonia⁺ protocol resulted in a high risk score for intentional introductions. The risk of establishment is high as *B. chinensis* tolerates a wide range of climatic and physical conditions. Assessment by the authors resulted in a high invasion score and a medium impact score, yielding an overall risk score of medium. Due to data deficiency, this medium risk score should be considered preliminary and must be applied with caution. Re-evaluation of the risk scores of *B. chinensis* is recommended when more data become available. According to the New York invasiveness assessment *B. chinensis* received a very high invasiveness rank (Adams and Schwartzberg 2013). *B. chinensis* scored 83 out of 100 points, based on four risk categories, i.e., 1) the ecological impact, 2) biological characteristics and dispersal ability, 3) ecological amplitude and distribution, and 4) difficulty of control. However, comparisons between the Harmonia⁺ protocol and the New York invasiveness assessment are problematic as different risk categories are applied in each. Inherent differences in risk scores produced by various risk assessment protocols can be explained by

differences in cut-off values and criteria for risk categories, assessor variability and context dependency (Verbrugge et al. 2012).

Risk assessments for newly introduced alien species are often difficult to complete as a lot of data and information is required to perform scientifically sound risk assessments. Often this data and information requirement is not met for a newly introduced alien species. When enough data and information becomes available, it is often too late to counteract the effects of, or eradicate the introduced species. Extensive data requirements are complicated by the need for rapid action to prevent the spread and impacts of invasive species, the need for public and political support, and the need to justify the allocation of financial budgets. These conflicting requirements pose an important dilemma in the management of biological invasions. The Harmonia⁺ protocol requires assessments of confidence level of the assigned risk scores. This allows assessors to perform risk assessments with limited data enabling a timely response when the confidence level is regarded as sufficient, or to undertake additional field surveys, experimental studies or modelling approaches to reduce major uncertainties.

The scattered and increasing nature of *B. chinensis* records and the low natural spread of this species (< 1 km/yr) suggest that records are the result of new introduction events. The increase in recorded locations can also be related to the increased awareness of the presence of a new alien viviparid species in the Netherlands. *B. chinensis* continues to expand its spatial distribution at the Eijsder Beemden location. Here, the species has colonized a floodplain lake that is permanently connected to the Meuse River. In the event that *B. chinensis* is able to colonize the main stream of the river, it can be expected that the downstream dispersal rate strongly increases due to water flow and shipping vectors, as has been shown for many other alien mollusc species after their initial colonization of the Rhine and Meuse River basins (e.g., Leuven et al. 2009; Marescaux et al. 2012; Matthews et al. 2014).

The densities of *B. chinensis* were determined in the shallow parts of the floodplain lake in the Eijsder Beemden (up to a depth of 1.5 m). The species is known to colonize deeper water (Jokinen 1982). However, no surveys were made in the deeper parts of Eijsder Beemden. Therefore, a determination of density could not be made for these deeper portions. The size distribution of the *B. chinensis* population in the Eijsder Beemden was skewed towards larger individuals. It is likely that juveniles were missed during sampling as they normally hide in crevices between rocks or bury themselves in the sediment

(Prezant et al. 2006). In the future an effort has to be made to characterize the (potential) environmental conditions required for the establishment of viable populations of *B. chinensis* in Europe as this will allow habitat suitability predictions of non-colonized ecosystems and the delineation of endangered areas. The temperature and conductivity of *B. chinensis* habitat in the Eijsder Beemden were in the range of suitable conditions reported for its native habitat (Taiwan) and other introduced regions (USA). The density of snails found at the Eijsder Beemden location was low compared to the highest reported density in other invaded regions (100 individuals/m²; USA, Karatayev et al. 2009). In the Japanese native range of *B. chinensis*, densities in rice paddy fields ranged from 0.25 to 30 individuals/m² (Nakanishi et al. 2014).

Owing to a high introduction and establishment risk of *B. chinensis*, it is important to prevent further spread of this species in Europe. The greatest risk for dispersal of *B. chinensis* results from human-mediated activities as our field study indicated that the current dispersal rate of *B. chinensis* is low. The aquarium trade is an especially important pathway for *B. chinensis* and many other aquatic invasive species (Padilla and Williams 2004; Karatayev et al. 2009; Strecker et al. 2011; Soes et al. 2016; Matthews et al. 2017). The species was sold at a garden centre in the Netherlands and found for sale online from one website in the USA. Better regulation of the international aquarium trade and public education to prevent deliberate and unintentional release into nature by hobbyists and other consumers is necessary to combat this important introduction pathway (Verbrugge et al. 2014). Legislative regulation has been implemented in order to prevent the introduction and spread of invasive species (e.g., USA: Clean Boating Act 2008). Teaching people to drain, clean, dry and check their boats, trailer and equipment could prevent overland transport of *B. chinensis* via recreational boats (Havel 2011). An additional control measure to prevent upstream spread of *B. chinensis* may be the application of culverts with high flow velocities as *B. chinensis* is dislodged and flushed away at speeds near 4.5 m/s (Rivera 2008). High flow culverts, or other means of accelerating water current, can probably be used to prevent the upstream dispersal of *B. chinensis* populations at sites with interconnected lakes or water ways. However, these measures can also have negative impacts on non-target species.

Several eradication and control measures for established populations of *B. chinensis* have recently been assessed. Laboratory experiments on the effect of chemical treatments with rotenone and copper

sulphate turned out to be ineffective, likely due to their large size, thick shell and operculum (Haak et al. 2014). The application of copper sulphate in Oregon (USA) by the Oregon Department of Fish and Wildlife did not result in eradication of the entire population (Freeman 2010), although this treatment decreased snail density and thus could serve as a population control measure. However, the use of copper sulphate and rotenone in aquatic ecosystems is prohibited in the Netherlands (EU monitor 2017; CTGB 2017). For application of copper sulphate and rotenone in aquatic ecosystems in the Netherlands an exemption from the Dutch law on crop protection and biocides is required (De Hoop et al. 2015). Experimental drawdowns resulting in air exposure of *B. chinensis* have proven to be an ineffective control measure as this species can withstand long periods of desiccation by closing its operculum and its burrowing behaviour (Havel 2011; Unstad et al. 2013; Havel et al. 2014). In Missouri (USA) manual removal was performed using volunteer snorkelers and scuba divers (Hanstein 2012), resulting in the removal of a large amount of mainly adult snails. As the juveniles are small and hide in crevices between rocks or bury themselves in sediment, full eradication by manual removal is not possible and recurrent removal will be required.

Considering the high invasion risk of *B. chinensis*, its limited capacity for secondary spread and low number of colonized water systems in the Netherlands, rapid eradication and containment measures by competent authorities may still be considered. This also applies in case the species is recorded during early detection activities or regular monitoring elsewhere in Europe. Early detection and rapid eradication and containment on a European level is recommended, especially since the species has recently been recorded in Belgium (Van den Neucker et al. 2017). This highlights the need for an allocated budget at a national or international scale to fund rapid actions. If eradication becomes too expensive or impractical due to high abundance and recurrent removal actions, it is important to monitor the spread and establishment of *B. chinensis*. It is also relevant to study whether dispersal of this species is facilitated by high river discharge conditions and inundation of floodplains. To prevent new introductions and secondary spread it is vital to increase the awareness of consumers, aquarium hobbyists, water gardeners, recreational boaters and recreational fishermen of the risk that the species poses and the way that it is introduced and spread. Additionally, a national or EU ban on the trade in living *B. chinensis* and replacement by harmless species to reduce the socio-economic impact of this measure can be considered

in order to limit new introductions. The currently available eradication and containment measures did not result in full eradication of the species, thus there is a need to undertake research to identify cost-effective management measures. As *B. chinensis* is currently present in the Netherlands in close proximity to other EU member states (i.e., Belgium and Germany), regional cooperation should be stimulated to perform early detection protocols and to prevent further spread. Furthermore, an extensive risk assessment on introduction, potential spread, establishment and impacts of the species in the entire EU is recommended. Such an assessment requires additional data, field surveys and monitoring and subsequently an analysis of cost-effectiveness of available measures for prevention, eradication, population control, containment and mitigation of impacts. When deemed potentially invasive for several EU member states, *B. chinensis* may be considered for listing as an IAS of Union concern.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Overview of all *Bellamyia chinensis* populations in the Netherlands.

Table S2. Overview of monitored locations within the Eijsder Beemden in the Netherlands.

Table S3. Overview of literature search strategy to acquire relevant scientific information regarding the invasion biology and environmental risks of *Bellamyia chinensis*.

Table S4. Consensus risk scores of *Bellamyia chinensis* in the Netherlands with the Harmonia⁺ protocol.

This material is available as part of online article from:

http://www.aquaticinvasions.net/2017/Supplements/AI_2017_Collas_etal_Supplement.xls