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Short Communication

Establishment of a new alien species in Lake Maggiore (Northern Italy): *Anodonta* (*Sinanodonta*) *woodiana* (Lea, 1834) (Bivalvia: Unionidae)

Lyudmila Kamburska, Rosaria Lauceri and Nicoletta Riccardi*
CNR - Istituto per lo Studio degli Ecosistemi, via Largo Tonolli 50, 28922 Verbania Pallanza (VB), Italy
E-mail: *l.kamburska@ise.cnr.it* *(LK),* *r.lauceri@ise.cnr.it* *(RL),* *n.riccardi@ise.cnr.it* *(NR)*
**Corresponding author*

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Abstract

This note is the first communication of the occurrence of the alien *Anodonta (Sinanodonta) woodiana* (Lea, 1834) (Bivalvia: Unionidae) in Lake Maggiore (Northern Italy). We have found empty shells of the bivalve first in August 2010, and since then, this species is colonizing rapidly the second deepest subalpine lake in Italy. Preliminary findings on morphometric features of *A. woodiana* in the Lake are provided.

Key words: *Anodonta woodiana*; Chinese pond mussel; invasive species; subalpine lake; Italy

Introduction

Freshwater mussels are one of the most threatened groups of organisms worldwide (e.g. Lydeard et al. 2004), but also include many of the most harmful alien species (Karatayev et al. 2007). Among the bivalves recently introduced in Italy, *Anodonta woodiana* (Lea, 1834) (Bivalvia: Unionidae) is the largest freshwater bivalve (valve length of up to about 30 cm) and the fastest spreading (Cianfanelli et al. 2007). Since the first report in 1996 (Manganelli et al. 1998), it has colonized eight Italian regions, mainly the hydrographic basins of the Po, Adige, Piave, Reno, Arno, and Tiber rivers (Manganelli et al. 1998; Bodon et al. 2005; Solustri and Nardi 2006). According to the same authors, this rapid spread suggests that there will be population explosions in many parts of northern and central Italy. In fact, the Chinese pond mussel *A. woodiana* was observed in the largest Italian Lake, Lake Garda for the first time in February 2009, during a survey carried out to investigate the distribution and dispersal of another alien species *Corbicula fluminalis* (Cappelletti et al. 2009). The presence of different length classes of *A. woodiana* along the south-eastern shore of Lake Garda allowed the authors to assume that the species is probably now present in the lake with a stable and naturalized population, but the low number of shells and lack of living specimens prevented evaluation of the consistency of the population, e.g. it was not possible to define population density (Cappelletti et al. 2009). Furthermore, specimens were also reported from southern Italy - the beach of Palo Laziale near Roma (Albano 2006) and even from a more southern locality: the River Calore Irpino (or Beneventano), where it joins the River Volturno in Campania, north-east of Naples (De Vico et al. 2007).

During the last 20 years, Lake Maggiore (the second largest subalpine lake in Italy) has been highly invaded and permanently colonized by various non-indigenous freshwater bivalves. Currently established invasive molluscs are *Dreissena polymorpha* (Pallas, 1771) observed in the Lake since the 1990s and the Asian clam *Corbicula fluminea* (O.F. Müller, 1774) recorded in 2010 (Kamburska et al. 2013). Empty shells of the bivalve *Anodonta woodiana* (Lea, 1834) were observed for the first time in August 2010. Similar to Lake Garda (Cappelletti et al. 2009), we found accidentally single live individuals of the Chinese pond mussel *A. woodiana* in October 2010 during a survey carried out to monitor the spread of *Corbicula* sp. in Lake Maggiore. The growing body of studies documenting the worldwide spreading of *A. woodiana,* assigned by some authors to the genus *Sinanodonta*,

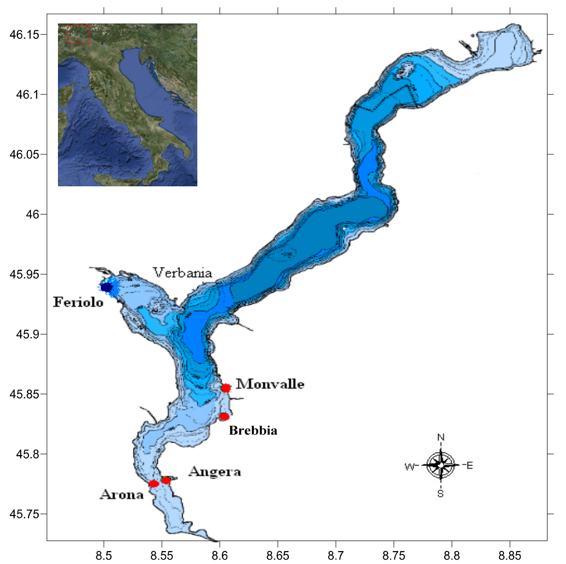

Figure 1. Study area and record locations of *Anodonta woodiana* in Lake Maggiore (see Appendix 1 for geographic coordinates); red circles –sites with *Anodonta woodiana*, blue –absent.

confirms this freshwater mussel as one of the most potential invasive alien species (IAS), though not yet among the 100 worst IAS (http://www.europe-aliens.org). Apparently, the specific physiological predisposition, associated with cholinesterase enzyme activity, to tolerate a broad range of unfavorable environmental conditions (Corsi et al. 2007) in concert with being a broad host generalist (Douda et al. 2012), enables *A. woodiana*'s dispersal from slowly running rivers to eutrophic ponds (Welter Schultes 2010). In fact, from its native habitat in East and South- East Asia, Primorye and the Amur Basin in eastern Russia (Zhadin 1952; Graf 2007), the bivalve has already successfully invaded Europe (Sárkány-Kiss et al. 2000; Lodde et al. 2005; Cianfanelli et al. 2007; Ciutti et al. 2011; Mienis 2011), the USA (Benson 2012), Central America and Indonesia (Watters 1997).

This study aimed to complement the invasion history of *A. woodiana* in Europe, in particular in Italy, by providing first evidence for its spreading in Lake Maggiore.

Materials and methods

Study area

Lake Maggiore (lat 45°57'N; long 8°32'E) lying at an altitude of 194 meters above the sea level, is the second deepest (maximum depth 370 m) and largest (area 212.5 km^2; volume, 37.5 km^3) subalpine lake in Italy. Phosphorus limited, the lake was brought back to oligotrophy by the middle 1980s and 1990s, after eutrophication in the 1960s - late 1970s (Consiglio Nazionale delle Ricerche- Istituto per lo Studio degli Ecosistemi Sede di Verbania 2011). The studied area is situated along the southwest - southeastern shore of Lake Maggiore (Figure 1). Geographical positions of five sampling sites are presented in Appendix 1.

Materials and methods

At each sampling location we inspected an area of 50 meters along the shoreline by 20 meters offshore, with water depth between 0.20 and 0.75 m. The bivalves were collected by hand or by sieving the sediments through a 2 mm^2 mesh size net within 0.5 m^2 squares. Density of *Anodonta woodiana* and of the native mussel species of family Unionidae per square meter was calculated for March 2012 at the Brebbia site by inspecting an area of 9 m^2 (Figure 1, Appendix 1). Morphometric parameters of 18 collected specimens were measured in the laboratory using a digital caliper to the nearest 0.01 mm. Specimen body weight (W, g) was measured to 0.01 g accuracy. The biometric parameters under consideration are total length (L), wing/top ventral edge height (h) and width (w) (Figure 2).

All sampled specimens were classified in four size groups in respect to their shell length (L) (Afanasjev et al. 2001; Spyra et al. 2012): *i)* up to 5 cm (young), *ii)* from 5 to 10 cm (small), *iii)* from 10 to 15 cm (medium), and *iv)* above 15 cm (large) (Table 1). Based on the same criteria, we attempt to estimate also the age structure of the individuals (Table 1) as suggested by some foregoing studies (Dudgeon and Morton 1983; Spyra et al. 2012). The counting of growth annual rings visible on the shell was not used because it is of limited use for age estimation of unionids (e.g. Neves and Moyer 1988).

Results

In August 2010, single empty shells of *A. woodiana* were found for the first time in the southern part of the lake (Arona location, Figure 1). Live mussels were found initially in October 2010. Afterward specimens were recorded also in March and August 2011, and in March 2012. *A. woodiana* occurred at Monvalle, Brebbia,

Figure 2. *Anodonta woodiana*: W – width, L – total length, h – wing/top ventral edge height shell measurements (mm). Photographs by Lyudmila Kamburska.

Table 1. Morphometric measurements of *Anodonta woodiana* collected in Lake Maggiore during March 2012.

№ of sp	Length (L, mm)	Wing height (h, mm)	Width (w, mm)	Body Weight (W, g)	Ratio L/W	size group/ estimated age in years (yrs)
1	104.73	64.25	40.42	120.55	0.87	medium/4-5 yrs
2	136.87	79.37	48.28	229.34	0.60	medium /7-8 yrs
3	123.65	70.74	43.93	181.49	0.68	medium /7 yrs
4	124.3	76.01	46.26	195.59	0.64	medium /7 yrs
5	121.64	79.64	48.49	194.23	0.63	medium/7 yrs
6	107.04	69.59	48.51	140.25	0.76	medium/4-5 yrs
7	161.18	101.08	52.98	374.41	0.43	large/≥ 8 yrs
8	151.79	84.68	54.94	318.38	0.48	large/≥8 yrs
9	166.55	95.55	56.9	400.12	0.42	large/≥ 8 yrs
10	106.6	67.82	41.92	136.33	0.78	medium/4-5 yrs
11	155.95	92.81	55.06	355.15	0.44	large/≥8 yrs
12	154.47	88.57	52.8	241.08	0.64	large/≥8 yrs
13	92.46	61.28	29.34	64.82	1.43	small/3-4 yrs
14	157.68	96.7	50.12	327.65	0.48	large/≥8 yrs
15	162.54	94.74	53.26	359.34	0.45	large/≥ 8 yrs
16	161.46	91.61	56.3	383.2	0.42	large/≥8 yrs
17	155.61	100.95	58.3	359.34	0.43	large/≥8 yrs
18	148.09	84.47	49.98	294.83	0.50	medium /7-8 yrs
mean±std	138.47 ±24.1	83.33 ±12.8	49.32 ± 7.1	259.78 ±105.4	0.62 ±0.3	
Min.	92.46	61.28	29.34	64.82	0.41	
Max.	166.55	101.08	58.3	400.12	1.43	

Angera and Arona locations, all of them at the south-east and south-west shores, while not yet documented at Feriolo (the central-west side of the lake) (Figure 1, Appendix 1).

The morphometric parameters of the 18 specimens collected at the Brebbia site during March 2012 deliver preliminary indications of the *A. woodiana* population status in the Lake. The length of the shells ranged from 92.46 to 166.55 mm (Table 1). The individual body weight ranged from 64.82 to a maximum of 400.12 g. The wing height (h) fluctuated between 61.28 and 101.08 mm. The ratio of length to body weight varied from 0.41 to 1.43 (L/W). Regarding size groups/age of the sampled individuals, young specimens (<3 years) were missing. Large specimens were 9 (at approximately ≥8 years old), 8 medium size (4-5 years and around 7 years), and only one small (3-4 years) (Table 1). The estimated density of *A. woodiana* at the sampling site was 2.0±1.6 ind.m^{-2}. During the same inspection, the density of native unionid species was also assessed. *Unio mancus* (Lamarck, 1819) was present at a density of 8.2±8.5 ind^{-2}, while no *Anodonta* spp. specimen was found in the inspected area. Only by a further inspection in an area of approximately 50 m^2 inside the reeds belt, two *Anodonta cygnea*

(Linnaeus, 1758) and three *Anodonta anatina* (Linnaeus, 1758) were found.

Discussion

The density and biomass of *Anodonta woodiana* in a novel environment such as Lake Maggiore, together with the growth rate and maximum size of the species, certainly differs from those in its native habitats. This could depend on several factors, for example, water velocity and temperature, substrate type, macrophytes presence (Kraszewski and Zdanowski 2007). Our preliminary observations deliver the initial idea of the mussel population status in the lake, since we have not yet estimated the population density in the whole lake. The amount of large-sized individuals aged more than 8 years may well suggest the entrance of *A. woodiana* in the lake at least 10 years ago. The introduction of the species could be linked to the invasion by the roach *Rutilus rutilus* (Linnaeus, 1758) during the late 1990s (Volta and Jepsen 2008). In effect, accidental transport by alien fishes hosting the parasitizing mussel larvae (glochidia) could be the main vector of *A. woodiana* introduction, and a possible pathway of spread in Italy appears to be fish stocking (Gherardi et al. 2008). This hypothesis may lack substance as the presence of *A. woodiana* is limited to the southern basin of the lake, despite roach diffusion throughout the lake. Douda et al. (2012) claimed that host availability does not constitute a major limit for *A. woodiana* to conquest most aquatic habitats in Central Europe. However, we cannot absolutely exclude its expansion also in the northern basin-because although it is characterized by steep and rocky banks, soft substrates suitable for *A. woodiana* do occur at greater depths. In our study *A. woodiana* was always collected very close to the shoreline, at 0.7 meters maximum depth, while deeper areas, which may be colonized, are still to be explored. For instance *A. woodiana* was found between 0.30 and 6 meters depth in France (Adam 2010). Kraszewski and Zdanowski (2007) found aggregations, the largest of which occurred at depths from 1.5 to 2.5 meters, in the heated lakes of the Konin system, Poland. In addition, it is known that temperature influences gametogenesis, incubation, and larval development in temperate regions (Araujo and Ramos 2000). Kraszewski (2007) suggested that water temperature may play a pivotal role in the variation of *A. woodiana* abundance and biomass and may limit its distribution as the species is sensitive to low water temperature. According to Kraszewski and Zdanowski (2007) a mean shell length of 160 mm and a maximum shell length of 241 mm were recorded in the warmest zones of the Konin heated lake system, while in zones of moderate water temperature it did not exceed 125 mm. In the case of Lake Maggiore where the water temperature ranges from about 6 to 27°C (e.g. Ambrosetti et al. 2006) we found maximum shell length of 165–170 mm and mean length of 138.5 mm. The role of water temperature is most likely to be manifested by the failure of gametes to mature (Galbraith and Vaughn 2009), nonetheless physical interaction with fish is necessary for the stimulation of large releases of larvae of the freshwater bivalve family Unionidae (Haag and Warren-Junior 2003). However, details concerning environmental tolerance and the role of temperature and other habitat factors on *A. woodiana* survival and reproduction remain still unclear. Although the spatial niche occupied by the species can be expected to vary depending on environmental conditions, more extensive and intensive sampling efforts are needed to establish the actual distribution and the depth range of the population in Lake Maggiore.

Very recently two new alien littoral fishes, *Rhodeus amarus* (Bloch, 1782) and *Gymnocephalus cernuus* (Linnaeus, 1758), have been found in Lake Maggiore (Volta P. personal unpublished data), which could be associated with *A. woodiana* introduction and/or diffusion by hosting the mussel glochidia (e.g. Kiss 1990; Blažek and Gelnar 2006). In particular, the bitterling fish (*R. amarus*) is able to avoid parasitism by native European unionids but is a suitable host of *A. woodiana* (Reichard et al. 2006, 2012). Genetic analysis and DNA microsatellite markers are needed as the population genetic studies could help to infer important aspects of the invasion process of the species, like the route(s) of invasion, the time and number of colonization events, and variability of populations of *A. woodiana* in native and invaded areas (Popa et al. 2011). The same authors described eight polymorphic microsatellite loci as important molecular markers that may solve several issues regarding the invasive process of this species, like determining the source populations of invasive species, ways of introduction, initial size of invasive population, sustainability and variability of populations of *A. woodiana*.

Whatever the vector/ pathway responsible for *A. woodiana* introduction in Lake Maggiore, this new invader seems to be successfully established in the southern lake basin. An increase of population density and spread to other lake areas can be expected to produce an impact on native unionids. The last are represented by two *Anodonta* species (*A. cygnea* and *A. anatina*) and one *Unio* species, previously reported as *U. mancus*, but actually belonging to *U. elongatulus* (Pfeiffer, 1825) according to latest genetic identification (Prie et al. 2012). Similar to most bivalve populations of European water bodies, their distribution and population density has never been assessed over the whole lake. Only a limited set of quantitative data is available for *U. mancus* (*U. elongatulus*), whose population density at Brebbia location (Figure 1) was about 47 ind.m^{-2} (Ravera et al. 2007) in 2003–2005, when neither *A. woodiana* nor C. *fluminea* had been recorded. Recent estimates of *U. mancus* density at the same site (8 ind.m^{-2} in March 2012) suggest a dramatic decline following alien bivalve establishment. Even though these data clearly indicate that native unionid populations are declining, the colonization by alien bivalve species can be only hypothesized to be one of the possible reasons. Despite the indication that *A. woodiana* can potentially threaten native unionids, there are no comprehensive data documenting effects on native communities by *A. woodiana* invasion (Douda et al. 2012), though the first signs of native unionids impairment by *A. woodiana* have been observed in Italy (summarised in Cianfanelli et al. 2007). The possibility for the co-existence of the 3 invasive bivalves (*A. woodiana*, *Corbicula* sp., *D. polymorpha*) and its ecological significance for the lake fauna is still a crucial question to answer. Further studies should be addressed to evaluate if the competition with alien bivalves can actually account for native mussel decline, and to determine the contribution of other stressors (e.g. drought, parasites) to the competition outcome.

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The following supplementary material is available for this article:

Appendix 1. Records of *Anodonta woodiana* from Lake Maggiore (Northern Italy).

This material is available as part of online article from:
http://www.aquaticinvasions.net/2013/Supplements/AI_2013_Kamburska_etal_Supplement.pdf