Environmental DNA survey indicates arrival of quagga mussel in Ticino River basin

Camilla Capelli,[1*] Federica Mauri,[2] Elisa Pianta,[2] Federica Rotta,[1] Fabio Lepori[1]

[1]Institute of Earth Sciences; [2]Institute of Microbiology, University of Applied Sciences and Arts of Southern Switzerland, Campus Mendrisio, 6850 Mendrisio, Switzerland

ABSTRACT

The quagga mussel *Dreissena rostriformis bugensis* is an invasive species that has recently spread to western Europe, causing major impacts on aquatic ecosystems and human activities. We considered lakes Lugano and Maggiore (Ticino River basin, Italy and Switzerland) to be at high risk of colonization due to leisure boat traffic from already-colonized lakes. We performed an environmental DNA based survey to detect any quagga mussel invasions in these lakes as early as possible. The results of the survey confirmed the presence of this species in the lakes and support the use of molecular approaches for the early detection of invasive mussels.

INTRODUCTION

Biological invasions have increased during the last decades due to international trade and tourism (Hulme *et al*., 2009). The quagga mussel *Dreissena rostriformis bugensis* Andrusov, 1897, is a highly invasive bivalve native to the Dnieper-Bug basin within the Ponto-Caspian region that started spreading in North America since the late 1980s (Mills *et al*., 1993) and in western Europe since the mid-2000s (Heiler *et al*., 2013; Karatayev and Burlakova, 2022). Invasions by dreissenid mussels can have significant ecological and economic impacts because they alternative communities and clog water pipes and other infrastructure (Connelly *et al*., 2007; Karatayev *et al*., 2015). Compared to the closely related zebra mussel *Dreissena polymorpha* Pallas, 1771, which has already spread throughout Europe, the quagga mussel reproduces at lower water temperatures, grows faster, and has a higher assimilation rate (Karatayev *et al*., 2015). Moreover, this species can colonize softer substrates and has a higher tolerance to low oxygen concentrations, which allows it to colonize deeper waters down to 200 m (Nalepa *et al*., 2014; Karatayev *et al*., 2015).

Corresponding author: camilla.capelli@supsi.ch

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In Europe, after the first observation in the Rhine River in 2006, the quagga mussel was discovered in Lake Geneva in 2015 and in Lake Constance in 2016 (Haltiner *et al*., 2022). Soon after, this species spread to other Swiss northern perialpine lakes (Haltiner *et al*., 2022). Recently, it has also been reported from Lake Garda in the southern perialpine region (Salmaso *et al*., 2022). Boat transport is considered the most likely vector of inter-lake spread of quagga mussel (Haltiner *et al*., 2022; Salmaso *et al*., 2022). Adult mussels easily attach to boat hulls, whereas larvae can be transported in ballast waters (Dalton and Cottrell, 2013). Therefore, other European lakes subject to frequent transport of recreational boats could be at risk of colonization in the near future (De Ventura *et al*., 2016; Haltiner *et al*., 2022).

The management of invasive dreissenids can benefit from early detection. While full eradication or containment may be unfeasible, early detection would allow to apply damage control strategies to protect critical infrastructure and prevent spread to uninvaded waters (Hosler, 2011). However, invasive dreissenids in the early stages of colonization are inherently difficult to detect by using traditional monitoring (*e.g.*, artificial substrates, sediment grabs, plankton tows), due to low abundance, patchy distribution, and taxonomic identification difficulties (Wong and Gerstenberger, 2011; Haltiner *et al*., 2022). As a result, molecular surveys based on environmental DNA (eDNA) are increasingly used for the early detection of invasive species, because they provide a highly sensitive, cost-effective, and non-invasive approach (Morisette *et al*., 2021). These have been successfully applied for the surveillance of many freshwater invasive species, including the quagga mussel (De Ventura *et al*., 2017; Sepulveda *et al*., 2019; Blackman *et al*., 2020; Clusa *et al*., 2021).

To date, the quagga mussel has not been observed in the southern Alpine Lake Lugano and Lake Maggiore (Ticino River basin, Italy and Switzerland). However, due to the quagga mussel's recent spread in Swiss northern perialpine lakes and in Lake Garda, and the frequent connection via overland transport of recreational boats (De Ventura *et al*., 2016; Salmaso *et al*., 2022), we considered these to be at high risk of invasion. In 2020, we performed an eDNA survey in lakes Lugano and Maggiore to attempt to detect any invasion by quagga mussel as early as possible. Here we present the results of this survey and discuss the status of quagga invasion in southern Alpine lakes.

METHODS

Lake Lugano and Lake Maggiore are two deep lakes located in the Ticino River basin, across the border between Switzerland and Italy. Lake Lugano is divided by a causeway into two basins, the north basin (max depth 288 m) and the south basin (max depth 95 m). Lake Maggiore is the second deepest lake in Italy, with a maximum depth of 372 m. The Swiss portion of the lake (hereafter, the Swiss basin) is shallower and makes up approximately 20% of the lake's surface. The two lakes are connected by the River Tresa, the outlet of Lake Lugano. These lakes lie in a temperate region and have different mixing regimes: Lake Maggiore is holo-oligomictic (Ambrosetti and Barbanti, 1999), the north basin of Lake Lugano is meromictic, and the south basin is holomictic (Barbieri and Mosello, 1992). Turnovers occur once a year in late winter or early spring. Both lakes experienced eutrophication in the 1960-70s. Subsequent restoration by phosphorus control re-established mesotrophic conditions in Lake Lugano (Lepori, 2019) and oligotrophic conditions in Lake Maggiore (Salmaso *et al*., 2014). The littorals of both Lake Lugano and the Swiss basin of Lake Maggiore are characterized by steep and rocky banks (except for an area of marsh habitat between the deltas of the rivers Ticino and Verzasca) already colonized by the invasive zebra mussel since the 1980s (Cianfanelli *et al*., 2010).

Water samples for eDNA analysis were collected in August 2020 in the littoral area of the north and south basin of Lake Lugano (BNINT and BSINT) and in the Swiss basin of Lake Maggiore (MAINT; Fig. 1). In each basin, water samples (2.5 L) were collected within 5-10 m from the shore at 1.5 m depth. Stations were selected every 1-5 km along the littoral, near natural or semi-natural shoreline. Samples from the same basin were then pooled to reduce the time and the cost of the analysis (Tab. 1). The pooled samples were filtered using a cross flow filtration capsule (VigiDNA®; Spygen, Le Bourget-du-Lac, France; 0.45 µm) until the filter became clogged. New DNA-free materials were used in all the steps to avoid cross-contamination and one liter of distilled water was filtered using the same sampling materials to serve as sampling and filtration control. The cartridges were stored at -20°C within 8 h. Environmental DNA was extracted from the crossflow filtration capsule following the protocol developed in the Eco-AlpsWater project (https://www.alpine-space.org/projects/eco-alpswater) with minor adaptions to extract DNA from frozen filters (https://dx.doi.org/10.17504/protocols.io.4r3l27o2xg1y/v1). A negative control, containing no sample, was extracted and processed alongside the samples to detect any cross-contamination.

The amplification of the COI gene of quagga mussel was performed through conventional PCR using the species-specific primers DRB1F and DRB1R, following the protocol described by Blackman *et al*. (2020), with minor changes (Tab. S1). The COI gene of *D. r. bugensis* was cloned in plasmid pUC57 (GenScript) and used as positive control. The PCR products (188 bp) were checked on 1% agarose gel electrophoresis and evaluated against positive and negative (no DNA) controls. The product of positive samples was sequenced with the same primers using a BigDye Terminator Cycle Sequencing technology (3500 Genetic Analyser; Applied Biosystems, Waltham, MA, USA). The sequences were analysed by Chromatogram Explorer (Heracle Biosoft, Mioveni, Romania) and checked against GenBank using conventional BLAST software (Altschul *et al*., 1990). Distinct sequences (one from Lake Maggiore and one from Lake Lugano) were deposited to the European Nucleotide Archive (ENA; Tab. 1) and species occurrence was updated in Info Species database (www.infospecies.ch).

RESULTS AND DISCUSSION

The COI gene of quagga mussel was detected in pooled samples collected in the Swiss basin of Lake Maggiore and both basins of Lake Lugano through conventional PCR. All the samples showed an amplicon of ca. 200 bp, of the same size of positive control, while negative control showed no amplification (Fig. 2). The sequences of positive amplification were identical, and they were all attributed to *D. r. bugensis* (100% similarity). These results are the first to in-

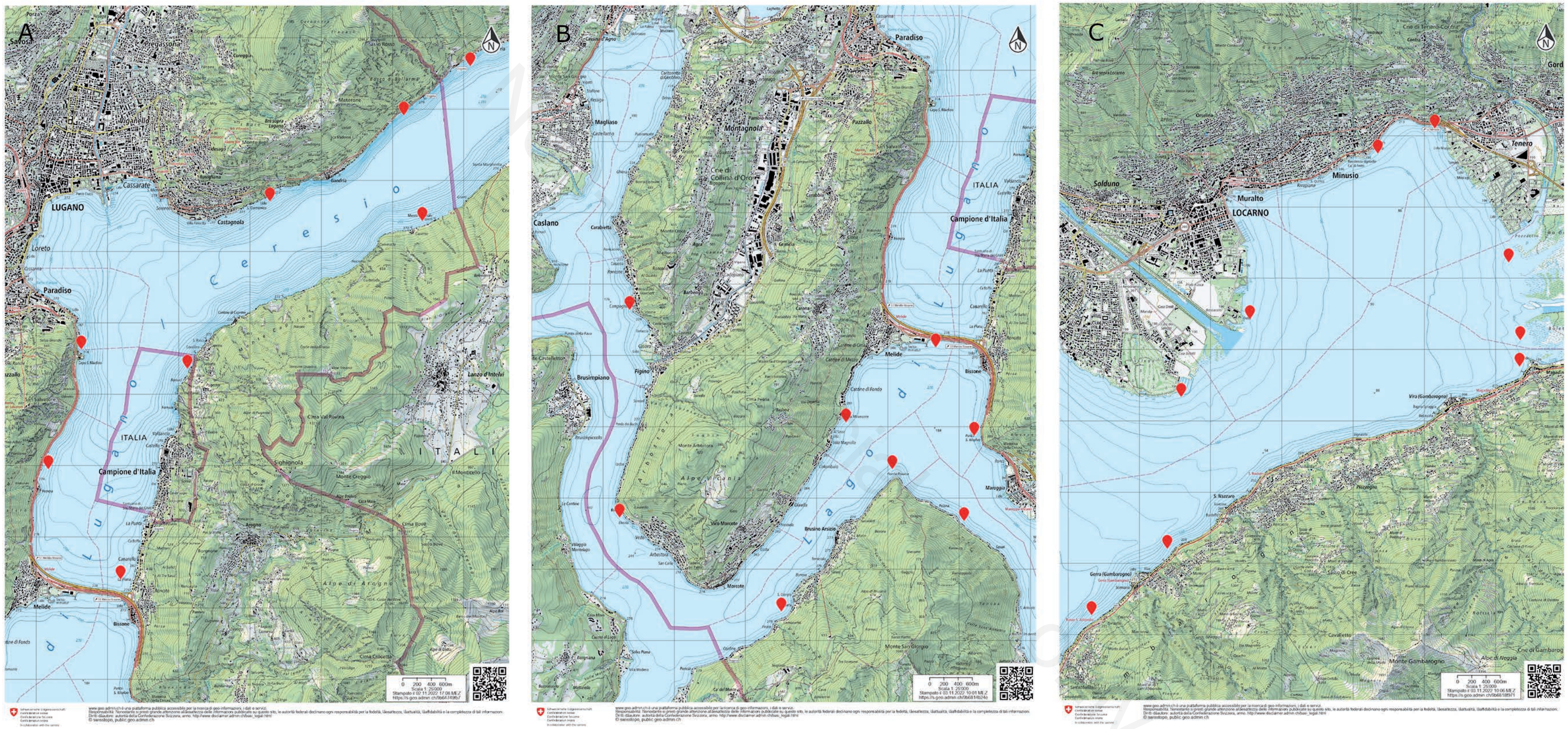

Fig. 1. Map showing the location of sampling sites in a) the north basin of Lake Lugano, b) the south basin of Lake Lugano, and c) the Swiss basin of Lake Maggiore. The single-point stations, from where the pooled samples were collected, are indicated with a red circle. Full details on samples are provided in Tab. 1.

dicate the presence of quagga mussel in Lake Maggiore and Lake Lugano.

Although the precise time of arrival of the quagga mussel cannot be determined from our data, we suggest that our survey detected low-density populations at early stages of colonization. The quagga mussel was not observed in either lakes during recent traditional surveys of benthic fauna (Lake Lugano: 2010-2012, Oikos, 2016; Lake Maggiore: 2017-2020, Beghi *et al*., 2019; Boggero *et al*., 2021). Moreover, the species was not detected by metagenomic surveys carried out in 2019 in the littoral or pelagic area of lakes Lugano and Maggiore (Kurmayer *et al*., 2021; Egeter *et al*., 2022). Therefore, it is likely that the quagga mussel invasion occurred after 2019. Alternatively, the quagga mussel could have arrived earlier but remained at low densities, preventing detection before this study. This scenario seems less likely because physical and chemical conditions (*e.g.*, temperature, dissolved oxygen, pH, calcium) in lakes Lugano and Maggiore are potentially suitable for a rapid growth of quagga mussel populations (Karatayev and Burlakova, 2022).

Fig. 2. COI gene of *D. r. bugensis* amplification (conventional PCR) from eDNA with species-specific primers (DRB1; Blackman *et al.*, 2020). Samples are coded as in Tab.1. "C+" positive and "C-" negative controls. "PM100" DNA ladder. The amplicon products (188 bp) are located at the bands 200 bp.

Assuming a low population density of quagga mussels at the time of the surveys, we suggest that the eDNA based approach used in the survey was instrumental in identifying the species, due to extra sensitivity in detecting scarce molecular "traces" left behind by mussels or free-swimming planktonic veliger larvae. Pooling samples within basins before the analysis may have also increased our ability to detect quagga's DNA, while maintaining the cost-effectiveness of the approach. However, we suggest that traditional surveys and further eDNA studies should be applied after the initial discovery to assess crucial population parameters and investigate the spatial spread of the colonization in detail.

The colonization of lakes Lugano and Maggiore by quagga mussel was expected and its future spread to other southern Alpine lakes (*e.g.*, Como, Iseo) seems very likely. The southern Alpine region attracts large numbers of tourists every year, especially from central and northern Europe. Inter-lake movements within the region are also prevalent. This movement of people facilitates the dispersal of alien species. For example, during the last decades, lakes south of the Alps already experienced invasions by the Asiatic clams *Corbicula fluminea* and *Sinanodonta woodiana* (Kamburska *et al*., 2013; Ciutti *et al*., 2011), the Ponto-Caspian amphipod *Dikerogammarus villosus* (Ciutti *et al*., 2011; Altermatt *et al*., 2019), and the North American bryozoan *Pectinatella magnifica* (Lepori *et al*., 2021). We suspect that the quagga mussel was introduced to lakes Lugano and Maggiore by recreational boats hauled overland. However, the assessment of the spread dynamics will require further studies (*e.g.*, shell size, spatial distribution, phylogenetic analysis).

The effect of quagga mussel after the introduction in a new environment depends on water mixing rates, lake morphology, and turnover rates (Karatayev *et al*., 2015). Based on patterns observed in North America (Karatayev *et al*., 2021) and north of the Alps (Haltiner *et al*., 2022), deep stratified lakes are especially vulnerable to quagga mussel colonization, due to the ability of this species to re-

Tab. 1. Description of the samples collected in August 2020 in the north basin (NB) and in the south basin (SB) of Lake Lugano and in the Swiss basin (SW) of Lake Maggiore.

Water body	Sample ID	Sampling strategy	Water volume (L)	Genbank number
NB Lake Lugano	BNINT	Pooled sample (8 stations)	19	OX249797
SB Lake Lugano	BSINT	Pooled sample (8 stations)	20	-
SW Lake Maggiore	MAINT	Pooled sample (9 stations)	12	OX249798

produce in the cold profundal zone (Haltiner *et al*., 2022). In these lakes, quagga mussel populations can dominate over zebra mussel significantly faster than in shallow lakes (Karatayev *et al*., 2021). Consistent with this pattern, in Lake Constance, quagga mussels replaced zebra mussels in less than three years in the littoral zone (Haltiner *et al*., 2022). Therefore, we suggest that a similar effect will take place in the deep lakes Maggiore and Lugano. The quagga mussel also impacts the native planktonic and benthic communities (Karatayev *et al*., 2015). The high filtration rates of quagga mussels can reduce the abundance of primary producers, which may result in cascading effects on nutrient availability, water clarity, macrophyte abundance, and zooplankton (Higgins *et al*., 2011). However, due to thermal stratification and greater water volume, in deep lakes, the effects of mussel grazing on primary production may be smaller than in shallow lakes (Karatayev *et al*., 2021). In addition, invasions by dreissenid mussels have been associated with increases in harmful cyanobacteria blooms due to selective filtering (Vanderploeg *et al*., 2013), an effect which may have important implications for water quality. Concerning the benthic community, impacts may be stronger in the profundal (*versus* littoral) area, where the quagga mussel can outcompete native invertebrates for space and food resources, causing population declines (Karatayev *et al*., 2015). However, the ecological effects of quagga mussel in the southern perialpine lakes may differ from the effects observed in other regions. New research is therefore needed to understand the impact of its arrival in this new context.

The management of the quagga mussel and other invasive species is particularly challenging in transboundary lakes, which are subject to different national laws and regulations. We suggest that an internationally coordinated program for the management of quagga mussel would facilitate the sharing of knowledge and solutions and would help preventing further invasions in southwestern Europe. Surveys of this species based on eDNA can play an important role in helping water managers detect invasions and plan mitigation actions as early as possible.

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REFERENCES

Altermatt F, Alther R, Fišer C, Švara V, 2019. Amphipoda (Flohkrebse) der Schweiz: Checkliste, Bestimmung und atlas. Fauna Helvetica 32. Info Fauna CSCF, Neuchâtel: 392 pp.

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ, 1990. Basic local alignment search tool. J Mol Biol 215:403-410.

Ambrosetti W, Barbanti L, 1999. Deep water warming in lakes: an indicator of climatic change. J Limnol 58:1-9.

Barbieri A, Mosello R, 1992. Chemistry and trophic evolution of Lake Lugano in relation to nutrient budget. Aquat Sci 54:219-237.

Beghi A, Gariboldi L, Boggero A, Riccardi N, Genoni P, 2019. SPecie Alloctone invasive nel bacino del Lago Maggiore (SPAM). Programma triennale 2016-2018. Commissione Internazionale per la Protezione delle Acque Italo-Svizzere.

Blackman R, Bennucci M, Donnelly R, Hänfling B, Harper LR, Sellers G, Lawson-Handley L, 2020. Simple, sensitive and species-specific assays for detecting quagga and zebra mussels (*Dreissena rostriformis bugensis* and *D. polymorpha*) using environmental DNA. Manag Biol Invasions 11:218-236.

Boggero A, Zaupa S, Gariboldi L, Garzoli L, Paganelli D, Arianna O, Fontaneto D, 2021. Ricerca ed innovazione sul Lago Maggiore: indicatori di qualità nel continuum acqua-rive. Campagna 2020. Commissione Internazionale per la Protezione delle Acque Italo-Svizzere: 33 pp.

Cianfanelli S, Lori E, Bodon M, 2010. *Dreissena polymorpha*: current status of knowledge about the distribution in Italy, p. 93-100. In: G van der Velde, S Rajagopal, A bij de Vaate (eds.), The Zebra Mussel in Europe. Kerkwerve; Backhuys Publishers.

Ciutti F, Beltrami ME, Confortini I, Cianfanelli S, Cappelletti C, 2011. Non-indigenous invertebrates, fish and macrophytes in Lake Garda (Italy). J Limnol 70:315-320.

Clusa L, Garcia-Vazquez E, Fernández S, Meyer A, Machado-Schiaffino G, 2021. Nuisance species in lake Constance revealed through eDNA. Biol Invasions 235:1619-1636.

Connelly NA, O'Neill CR, Knuth BA, Brown TL, 2007. Economic impacts of zebra mussels on drinking water treatment and electric power generation facilities. Environ Manag 40:105-112.

Dalton LB, Cottrell S, 2013. Quagga and zebra mussel risk via veliger transfer by overland hauled boats. Manag Biol Invasions 4:129-133.

De Ventura L, Weissert N, Tobias R, Kopp K, Jokela J, 2016. Overland transport of recreational boats as a spreading vector of zebra mussel *Dreissena polymorpha*. Biol Invasions 18:1451-1466.

De Ventura L, Kopp K, Seppälä K, Jokela J, 2017. Tracing the quagga mussel invasion along the Rhine River system using eDNA markers: Early detection and surveillance of invasive zebra and quagga mussels. Manag Biol Invasions 8:101-112

Egeter B, Veríssimo J, Lopes-Lima M, Chaves C, Pinto J, Riccardi N, Beja P, Fonseca NA, 2022. Speeding up the detection of invasive bivalve species using environmental DNA: A Nanopore and Illumina sequencing comparison. Mol Ecol Res 22:2232-2247.

Haltiner L, Zhang H, Anneville O, de Ventura L, Deweber T, Hesselschwerdt J, et al., 2022. The distribution and spread of quagga mussels in perialpine lakes north of the Alps. Aquat Invasions 17:153-173.

Havel JE, Kovalenko KE, Thomaz SM, Amalfitano S, Kats LB,

2015. Aquatic invasive species: challenges for the future. Hydrobiologia 750:147-170.

Heiler KCM, Bij de Vaate A, Ekschmitt K, Oheimb PV von, Albrecht C, Wilke T, 2013. Reconstruction of the early invasion history of the quagga mussel (*Dreissena rostriformis bugensis*) in Western Europe. Aquat Invasions 8: 53-57.

Higgins SN, Vander Zanden MJ, Joppa LN, Vadeboncoeur Y, 2011. The effect of dreissenid invasions on chlorophyll and the chlorophyll: total phosphorus ratio in north-temperate lakes. Can J Fish Aquat Sci 68:319-329.

Hosler DM, 2011. Early detection of dreissenid species: zebra/quagga mussels in water systems. Aquat Invasions 6:217-222.

Hulme PE, 2009. Trade, transport and trouble: Managing invasive species pathways in an era of globalization. J Appl Ecol 46:10-18.

Kamburska L, Lauceri R, Riccardi N, 2013. Establishment of a new alien species in Lake Maggiore (Northern Italy): *Anodonta* (*Sinanodonta*) *woodiana* (Lea, 1834) (Bivalvia: Unionidae). Aquat Invasions 8:111-116.

Karatayev AY, Burlakova LE, Padilla DK, 2015. Zebra versus quagga mussels: a review of their spread, population dynamics, and ecosystem impacts. Hydrobiologia 746:97-112.

Karatayev AY, Karatayev VA, Burlakova LE, Mehler K, Rowe MD, Elgin AK, Nalepa TF, 2021. Lake morphometry determines *Dreissena* invasion dynamics. Biol Invasions 23:2489-2514.

Karatayev AY, Burlakova LE, 2022. What we know and don't know about the invasive zebra (*Dreissena polymorpha*) and quagga (*Dreissena rostriformis bugensis*) mussels. Hydrobiologia Online ahead of print.

Kurmayer R, Elersek T, Domaizon I, Capelli C, Schaumburg J, Mischke U, et al., 2021. Perspectives in eDNA monitoring in alpine waters – for stakeholders and decision-makers. Eco-AlpsWater project. Ljubljana, National Institute of Biology: 52 pp

Lepori F, 2019. Il risanamento del Lago di Lugano: tendenze pluridecennali dei carichi e delle concentrazioni di fosforo. Bollettino Società ticinese Scienze naturali 107:13-19.

Lepori F, Capelli C, Foresti D, Kuzmic M, Togni M, Veronesi M, 2021. Prima segnalazione del briozoo alloctono *Pectinatella magnifica* a sud delle Alpi. Bollettino Società ticinese Scienze naturali 109:121-123.

Mills EL, Dermott RM, Roseman EF, Dustin D, Mellina E, Corm DB, Spidle AP, 1993. Colonization, ecology, and population structure of the "quagga" mussel (Bivalvia: Dreissenidae) in the lower Great Lakes. Can J Fish Aquat Sci 50:2305-2314.

Morisette J, Burgiel S, Brantley K, Daniel WM, Darling J, Davis J, et al., 2021. Strategic considerations for invasive species managers in the utilization of environmental DNA (eDNA): steps for incorporating this powerful surveillance tool. Manag Biol Invasions 12:747-775.

Nalepa TF, Fanslow DL, Pothoven SA, 2010. Recent changes in density, biomass, recruitment, size structure, and nutritional state of *Dreissena* populations in southern Lake Michigan. J Great Lakes Res 36:5-19.

Nalepa TF, Fanslow DL, Lang GA, Mabrey K, Rowe M, 2014. Lake-wide benthic surveys in Lake Michigan in 1994-95, 2000, 2005, and 2010: Abundances of the amphipod *Diporeia* spp. and abundances and biomass of the mussels *Dreissena polymorpha* and *Dreissena rostriformis bugensis*. NOAA Technical Memorandum GLERL 164:1-21.

Oikos 2000, 2016. Studio di valutazione del potenziale di rivitalizzazione e di pubblica fruizione delle rive del lago Ceresio. Programma 2013-2015. Commissione Internazionale per la Protezione delle Acque Italo-Svizzere: 64 pp.

Salmaso N, Buzzi F, Cerasino L, Garibaldi L, Leoni B, Morabito G, Rogora M, Simona M, 2014. Influence of atmospheric modes of variability on the limnological characteristics of large lakes south of the Alps: a new emerging paradigm. Hydrobiologia 731:31-48.

Salmaso N, Ciutti F, Cappelletti C, Pindo M, Boscaini A, 2022. First record of quagga mussel, *Dreissena bugensis* Andrusov, 1897, in Italy: morphological and genetic evidence in Lake Garda. BioInvasions Rec 11:1031-1044.

Sepulveda AJ, Amberg JJ, Hanson E, 2019. Using environmental DNA to extend the window of early detection for dreissenid mussels. Manag Biol Invasions 10:342.

Vanderploeg HA, Wilson AE, Johengen TH, Dyble Bressie J, Sarnelle O, Liebig JR, Robinson SD, Horst GP, 2013. Role of selective grazing by dreissenid mussels in promoting toxic *Microcystis* blooms and other changes in phytoplankton composition in the Great Lakes, p. 509-523. In: TF Nalepa, and DW Schlosser (eds.), Quagga and Zebra mussels: biology, impacts, and control. Boca Raton, CRC Press.

Wong WH, Gerstenberger S, 2011. Quagga mussels in the western United States: Monitoring and management. Aquat Invasions 6:125.