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Massive occurrence of the invasive alga *Hydrodictyon reticulatum* (L.) Bory in a Brazilian lotic system and variables explaining its biomass in microhabitat scale

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ABSTRACT

Massive occurrence of the invasive alga *Hydrodictyon reticulatum* (L.) Bory in a Brazilian lotic system and variables explaining its biomass in microhabitat scale

Invasive species are a common driver of biotic homogenization. This is especially concerning for aquatic environments in which new invasive species are recorded every year. *Hydrodictyon reticulatum* (L.) Bory is one of the most effective invasive algae, producing blooms in the Northern Hemisphere and in New Zealand. Here we report the invasive occurrence and analyse the environmental correlates of a massive growth of this species in an artificial channel in subtropical Brazil. We found that the biomass of *Hydrilla verticillata* (L.f.) Royle, which is used by *H. reticulatum* as a growing substrate, explained the differences in the biomass of *H. reticulatum* among the sites. In addition, increases in regional temperature may be the main driver of the algal occurrence. Given the potential risks of *H. reticulatum*, we recommend that a monitoring plan should be established.

Key words: Water net, green algae, bloom, channel, subtropical.

RESUMO

Ocorrência massiva da alga invasora* Hydrodictyon reticulatum *(L.) Bory em um ambiente lótico brasileiro e as variáveis explicando a sua biomassa na escala de microhabitat

Espécies invasoras são uma das principais causas da homogeneização biótica. Isso é especialmente preocupante nos ambientes aquáticos, nos quais são registradas novas espécies invasoras a cada ano. Hydrodictyon reticulatum *(L.) Bory é uma das algas invasoras mais importantes causando blooms no Hemisfério Norte e na Nova Zelândia. Aqui, nós relatamos a ocorrência desta invasora e analisamos as correlações ambientais do crescimento massivo desta espécie em um canal artificial, na região subtropical brasileira. Nós encontramos que a biomassa de* Hydrilla verticillata *(L.f.) Royle, a qual foi usada por* H. reticulatum *como um substrato para o seu crescimento, explicou as diferenças na biomassa de* H. reticulatum *entre os locais amostrados. Em adição, o aumento da temperatura regionalmente pode ser o principal fator responsável pela ocorrência desta alga. Tendo em conta os potenciais riscos de* H. reticulatum*, recomendamos que um plano de monitoramento deve ser estabelecido.*

Palavras chave: *Rede de água, algas verdes, floração, canal, subtropical.*

INTRODUCTION

Biological invasions can threaten biodiversity in various ways, ranging from genetic alterations to broad ecosystem disturbances (Wittenberg & Cock, 2001). Additionally, unlike many other environmental impacts, the effects of biological invasions are increasing with time because the eradication of established populations becomes more difficult with increasing time since establishment (IUCN, 2000).

The introduction of invasive species has increased in recent years, following intensive and large-scale environmental disturbances (Mack *et al.*, 2000). The increase in invasiveness is also correlated with global climate changes (Mooney & Hobbs, 2000). The scenario is even more dramatic in freshwater environments (Dudgeon *et al.*, 2006), which are often highly heterogeneous and harbour a number of microhabitats with specific characteristics (Wetzel, 2001). Freshwater ecosystems hold approximately 6% of all known species on Earth (Dudgeon *et al.*, 2006). Therefore, knowledge of invasive species is necessary for the effective design of plans to conserve and manage biodiversity.

Recent studies have evaluated the ecological consequences of invasions by exotic or allochthonous fish species (Latini & Petrere, 2004; Casal, 2006), aquatic plants (Hussner *et al.*, 2010; Strayer, 2010; Zhang *et al.*, 2010), and invertebrates (Boltovskoy & Cataldo, 1999; Darrigran *et al.*, 2000). However, the impacts of invasive algae are usually poorly known. The occurrence of massive algal growths (blooms) can displace native species and is a relevant conservation issue with consequences for agriculture, forestry, and aquaculture activities, and includes the potential mortality of fish and other aquatic organisms (Pimentel *et al.*, 2005). Algal blooms can also cause toxicity, aesthetic problems and restrict recreational and hydroelectric power generation uses of water (Smith *et al.*, 2003).

Cyanobacteria are usually the group of most concern as invasive algal species, as they often produce toxins (Sivonen, 2000; Smith, 2001). Green algae, such as *Cladophora glomerata* (L.) Kützing and *Hydrodictyon reticulatum* (L.) Bory, have also been identified as important, especially in the Northern hemisphere (Whitton, 1970; Dodds & Gudder, 1992; Lembi, 2003; Higgins *et al.*, 2008). These two species can dominate natural aquatic ecosystems and can cause a number of problems. *Hydrodictyon reticulatum* is a green alga with a macro- or microscopic net of coenobia (John & Tsarenko, 2002). Each coenobium is formed by a large number of multinucleated cylindrical cells. Usually, three cells are linked by their tips to build a net of five-sided mesh (John & Tsarenko, 2002). Asexual propagation occurs by means of biflagellate zoospores, which form a new coenobium inside the wall of the mother cell. Daughter colonies may reach 1000 cells (Wells *et al.*, 1999). Sexual reproduction involves isogametes (Parmentier, 1998).

Occurrence records of *H. reticulatum* are often related to human-caused environmental changes. Its dispersal can also be influenced by humans. The oldest written text in which the species was mentioned reported a “water net” spreading on a dam built by emperor Wu-ti of the Han dynasty, between 140-87 BC (Minakata, 1904). Centuries later, due to its wide dispersion in Europe, Linnaeus described it in *Species Plantarum* under the name *Conferva reticulata* in 1753. Only in the following century was the present nomenclature combination proposed by Bory de Saint-Vicent in 1824, who was also working with European samples. In the last century, several records appeared of this species in the role of an invader of aquatic continental ecosystems worldwide: in Europe (Flory & Hawley, 1994; Parmentier, 1998; John *et al.*, 1998; Volodina & Gerb, 2013), North America (Dineen, 1953; Kimmel, 1981), Asia (Pocock, 1960), and Oceania (Coffey & Miller, 1988; Hawes *et al.*, 1991; Wells & Clayton, 2001). Apparently, it is rarer in the Southern hemisphere than in the Northern hemisphere (Pocock, 1960).

In South America, *H. reticulatum* has been recorded in recent decades in Argentina (Tell, 1985; Tracanna, 1985; Tracanna & Martínez De Marco, 1997) and Brazil (Sant’Anna, 1984; Menezes & Dias, 2001; Biolo *et al.*, 2009). This species usually occurs in low frequencies and low biomass composing the phytoplankton of lakes and higher-order rivers. Although easily distinguishable morphologically, the species is unknown by the general public and is seldom found in Brazilian herbariums (INCT, 2014). Here we report the first record of a massive growth of *H. reticulatum* in an artificial lotic environment in subtropical Brazil. We also analyse the environmental variables at the microhabitat scale correlated with its biomass to improve our knowledge about this important invasive alga.

MATERIALS AND METHODS

Study site

The algal bloom was recorded in the Piracema Channel, located in Foz do Iguaçu, Paraná, southern Brazil (25°26′7.20″S; 54°34′32.26″W). This is a channel 13.3 km long that receives a regulated flow of 12 m^3/s and is maintained by the hydroelectric plant of ITAIPU Binacional. The channel was built to connect downstream and upstream fish populations, allowing them to bypass the 120-metre-high dam. The stretch of the channel surveyed is 1620 m long and 12 m wide and is crossed by concrete obstacles to reduce the velocity of the water, which is determined by a declivity of 0.7%. The channel has a rocky bottom. During the study, the water flow had been interrupted due to low rainfall in the region. During this period, all the water flowing through the channel came from an urban stream (Brasília Creek), a tributary of the channel with a flow of 0.5 m^3/s. Brasília Creek is a second-order stream and drains an urban area of approximately 4 km^2, which receives charges of domestic effluents.

Species identification

The identification of the target species followed morphological criteria as given in John & Tsarenko (2002). Other *Hydrodictyon* species are uncommon, although in South America *H. majus*, described by Kühnemann (1957), also occurs. This taxon has already been recorded in Buenos Aires, Argentina. Some characteristics of our samples allow us to assign them to *H. reticulatum*, in particular, i) the closed tubular colonies of different sizes, ii) three connected cells forming a mesh with five or six sides, and iii) predominantly asexual reproduction, which generates daughter colonies.

Sampling

The algal bloom first occurred in January and February 2014 in a large stretch of the channel. We established ten linear transects perpendicular to the channel, each separated by 17 m. We sampled algae and environmental variables using a Surber sampler (0.08 m^2) at three random points along each transect for a total of 30 sampling sites. After sampling the algae were washed from the substrate, transferred to a filter (200 µm mesh), and the fresh samples were stored in glass vials. These samples were then analysed under a microscope at the laboratory and preserved in 4% formaldehyde.

Depth (m) and flow velocity (m/s) were measured in situ using a ruler and an acoustic Doppler velocimeter (SonTek FlowTracker Handheld ADV, SonTek, San Diego, California, U.S.A), respectively. Water temperature (°C), turbidity (NTU), specific conductivity (µS/cm), pH, redox potential (mV), dissolved oxygen (mg/L), and total dissolved solids were measured using a Horiba U-50 multiparameter water quality metre.

The biomass of *H. reticulatum* (response variable) was measured by weighing dry samples at 100 °C until they were of constant weight. Most samples of *H. reticulatum* were attached to the macrophyte *Hydrilla verticilata* (L.f.) Royle; the latter was separated before estimating the dry biomass of the former. The dry biomass of *Hydrilla verticilata* was also quantified and used as a predictor in further analysis.

Data analysis

We used a hierarchical partitioning analysis with 10 000 permutations (Chevan & Southerland, 1991; Mac Nally, 2000; 2002) to disentangle the independent and dependent contributions of environmental variables to explain the variation in biomass of *H. reticulatum*. Prior to analysis, the data were standardized (z-transformation, where the arithmetic mean equals zero and a standard deviation equals one) and checked for multicollinearity with variance inflation factors (VIF). Turbidity and total dissolved solids had VIF values > 2 and were excluded from further

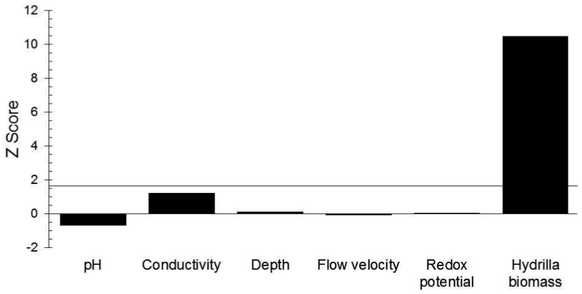

Figure 1. The results of hierarchical partitioning analysis showing the variables related to *Hydrodictyon reticulatum* biomass in Piracema Channel, a subtropical region of Brazil; z score values above 1.65 are significant. *Resultados da Análise de Partição Hierárquica mostrando as variáveis relacionadas a biomassa de* Hydrodictyon reticulatum *no Canal da Piracema, região subtropical do Brasil. Valores de "z score acima de 1.65 são significativos.*

analysis. Analyses were run using the "vegan" (Oksanen *et al.*, 2013), "car" (Fox & Weisberg, 2010) and "hier.part" (Walsh & Mac Nally, 2013) packages of the software R version 3.0.3 (R Core Team, 2012).

RESULTS

The peak dry biomass of *H. reticulatum* was 119.2 g/m^2. The predominant reproductive strategy was vegetative. We found coenobia in several developmental stages, from microscopic to 30 cm long, formed by cells varying in length from a few micrometres to almost 1 cm.

The means of the environmental variables measured were as follows: water temperature 23.8-24.7 °C ($\bar{x} = 24.3 \pm 0.3$); pH 6.0-7.1 ($\bar{x} = 6.6 \pm 0.2$); redox potential 175-350 mV ($\bar{x} = 296 \pm 47$); specific conductivity 94-104 μS/cm ($\bar{x} = 102 \pm 1$); depth 4-43 cm ($\bar{x} = 16 \pm 9$); flow velocity 0-34 cm/s ($\bar{x} = 6 \pm 7$); dissolved oxygen 6.8-7.8 mg/L ($\bar{x} = 7.1 \pm 0.4$), total dissolved solids 30-40 mg/L ($\bar{x} = 38 \pm 8$); turbidity 5-40 NTU ($\bar{x} = 10 \pm 13$). There was no variation in the light incidence because there was no riparian vegetation. *Hydrilla verticillata* was present in about a half of the samples, and the biomass in one reached 1400 g/m^2.

The set of predictor variables explained the variation in biomass of *H. reticulatum* ($z = 10.5$; $p < 0.05$) (Fig. 1). However, water quality was not significantly related to algal biomass. *Hydrilla verticillata* biomass was the single significant variable and accounted for 62.65% of *H. reticulatum* biomass variation, of which 50.75% was independent and 11.90% was dependent on other variables. In summary, a positive relationship was found between the biomass of *H. reticulatum* and that of *Hydrilla verticillata*.

DISCUSSION

This study provides the first record for the continent of *H. reticulatum* in a potentially harmful algal bloom. The highest dry biomass we found was 119.2 g/m^2, much higher than in New Zealand, which reached 35 g/m^2 in floating aggregates and 68 g/m^2 at the bottom of a disturbed lake (Wells & Clayton, 2001).

Vegetative growth through coenobium formation inside cellular walls is a key characteristic of this species (John & Tsarenko, 2002). This growth form also promotes rapid dispersal, making *Hydrodictyon* an efficient invader. Coenobia are carried by insects, aquatic birds, wind, or humans (e.g., shipping or aquarists; Wells *et al.*, 1994). Additionally, this species can grow quickly and increase its biomass by 30% per day (Wells *et al.*, 1994). This capability has been investigated in many studies. Algal blooms of this species have been recorded worldwide in recent decades: North America (Dineen, 1953), India (Rai & Chandra, 1989), the Czech Republic (Lelková *et al.*, 2004), England (Flory & Hawley, 1994; John *et al.*, 1998), and Taiwan (Chou *et al.*, 2006). Its native range is in the Northern hemisphere and blooms of the species have been frequently associated with disturbances in England (John & Tsarenko, 2002) and the Czech Republic (Lelková *et al.*, 2004), where it has caused a number of problems. Therefore, the present record is particularly relevant and indicates that the species should be monitored.

The most striking case of *H. reticulatum* invasion occurred in New Zealand (Coffey & Miller, 1988). After two years the species had spread a distance of 35 km from the introduction point and had caused serious problems (Hawes *et al.*, 1991). Some years later, the species had spread widely in North Island, impairing fisheries, navigation, aquatic sports, tourism, hydropower generation, and drinking water supply (Wells *et al.*, 1999; Wells & Clayton, 2001). An organization named the "Water Net Technical Committee" was created in 1991 to address the problem. It proposed a management plan that included mechanical removal, environmental amendments, and even use of algaecides to constrain the spread of *Hydrodictyon* (Wells *et al.*, 1999).

After some years of continuous expansion, the *Hydrodictyon* population in New Zealand began to decrease, but the causes for that decrease are still unclear. Probable causes include herbivory, disease, or loss of genetic variability (Wells *et al.*, 1999). Similarly, the reasons for the successful invasion likewise remain unclear. There is a debate about which environmental factors were correlated with its growth. High calcium and pH values, along with eutrophication have appeared as possible key factors in other parts of the world (Hall & Cox, 1995). However, the growth of the species in New Zealand seems to be related with the opposite pattern of low calcium, neutral pH, and moderate levels of eutrophication (Hall & Cox, 1995; Hall & Payne, 1997). A previous study (Hall & Payne, 1997) suggested that the fast growth of the species is due to its low nitrogen and phosphorous requirements, or a low N:P ratio. A common pattern observed worldwide is that the bloom tends to occur in the summer (Hall & Payne, 1997; Whitton, 2000; Wells & Clayton, 2001). Another study (John & Tsarenko, 2002) emphasised that the spread of *Hydrodictyon* is probably related to lower flows and long-term higher temperatures in the summer, rather than eutrophication. Our results support this claim because the bloom occurred under air temperatures of 37 °C, which was the hottest summer in the last 50 years in the region. When the samples were collected, the water temperature was 24 °C, just one degree lower than that of the best growth condition for the species (Hawes & Smith, 1993). Our results support other studies that found that algal blooms occurred after large environmental alterations and climatic changes.

Despite its negative impact on human activities (Hall & Cox, 1995), some studies also identify positive effects of *H. reticulatum*, such as improving water transparency, suppressing cyanobacterial blooms (Flory & Hawley, 1994), sequestering heavy metals (Rai & Chandra, 1989), and providing shelter for gastropods, which are the main food resource of trout, thus indirectly improving fish stocks (Thomas, 1996). However, *H. reticulatum* is still a pest species in New Zealand (Champion *et al.*, 2013) and is included in the "Global Compendium of Weeds" (Randall, 2012). This is mostly because its negative impacts are not negligible, and include large pH and oxygen changes (Lelková & Poulíčková, 2004), alterations in the community of native macrophytes (Hawes *et al.*, 1991), pollution related to biomass decomposition (Volodina & Gerb, 2013), and decrease in phytoplankton richness and abundance (Lelková *et al.*, 2004).

We found that the biomass of the macrophyte *Hydrilla verticillata* was the only significant predictor of the biomass of *H. reticulatum*, which explained 62.65% of its variation. Some studies have reported an association of *H. reticulatum* with submerged macrophytes (Wells & Clayton, 2001; Volodina & Gerb, 2013). Because the sampled site is a lotic system, the branches and leaves of *Hydrilla verticillata* might have provided a substrate for the growth of *H. reticulatum*. In fact, substrate complexity is one of the environmental factors that have been pointed out as key to the occurrence of benthic algae (Murdock & Dodds, 2007). Previous studies in streams found that more complex substrates provide refuge against predation and water flow (Bergey & Weaver, 2004), which likely influenced this bloom.

Hydrilla verticillata has been recorded in this same channel since 2005, and represents a recent invasion event in Brazilian inland waters (Sousa *et al.*, 2011), where it has caused severe impacts. Additionally, we found large numbers of the "golden mussel" [*Limnoperna fortunei* (Dunker, 1856)] associated with *H. verticillata* and *H. reticulatum* foraging above plant colonies (pers. obs.). The golden mussel is also an invasive species recognized worldwide, having recently been introduced into Brazilian ecosystems (Darrigran *et al.*, 2000). We thus recorded an association of three invasive species that are probably dislocating native species from their habitats.

In summary, we record an algal bloom of *H. reticulatum* in an artificial channel in subtropical Brazil. The biomass of *H. reticulatum* was significantly related to the biomass of *Hydrilla verticillata*, which provided a growth substrate for the former. The growth is probably related to high summer temperatures in eutrophic systems. This is the first record of such massive growth of *H. reticulatum* in South America. The impact of *H. reticulatum* reported worldwide suggest that a monitoring plan should be established to track its population dynamics.

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