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Effects of 254 nm UV irradiation on the mobility and survival of larvae of the invasive fouling mussel *Limnoperna fortunei*

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In order to investigate the feasibility of using ultraviolet (UV) irradiation to prevent the invasive Asian mussel, *Limnoperna fortunei,* from colonizing components of the cooling systems of industrial and power plants, the mobility and mortality of its larvae were assessed after exposure to different doses of UVC (λ = 254 nm) in laboratory conditions. Total (100%) mortality was achieved with a dose of 149 mJ cm^{-2} at 23 °C and 103 mJ cm^{-2} at 25.8 °C. Immediately after exposure, larvae were alive but had reduced mobility. The proportion of active larvae increased after 24 h, but fell again at 48 and 72 h to levels similar to those immediately after exposure. The highest mortality rates were always recorded at the last observation, 72 h after exposure. These results indicate that the larvae of *L. fortunei* are highly sensitive to UVC, suggesting that UV irradiation has the potential to control fouling by this mussel when the water is relatively clear. However, application of UV-based technologies in plants that use cooling water from water bodies with high loads of suspended solids (eg the Paraná-Uruguay basin, with *ca* 160 mg l^{-1} of suspended solids and absorbance values around 0.255) is unlikely to be effective without prior filtration of the water.

Keywords: macrofouling; *Limnoperna fortunei*; UV: ultraviolet irradiation; larvae; golden mussel

Introduction

Limnoperna fortunei Dunker (1857), the golden mussel, is a bivalve mollusc native to the freshwaters of Southeast Asia. It was unintentionally introduced to Hong Kong in 1965 (Morton 1979) and to Argentina and Japan around 1990 (Pastorino et al. 1993; Kimura 1994). In South America, it was first detected in the Río de la Plata estuary, from where it swiftly spread northwards at up to 240 km per year (Boltovskoy et al. 2006). By 2006, it was present as far north as the States of São Paulo and Minas Gerais in Brazil (Boltovskoy et al. 2006; Oliveira et al. 2006). At present, *L. fortunei* is one of the most common macro-invertebrate species in the Paraná-Uruguay basin, with reported population densities of over 200,000 mussels m^{-2} (Sylvester et al. 2007). *L. fortunei* attaches to any hard surface, as well as to other substrata like plant roots.

Shortly after its arrival, the growth of *L. fortunei* beds in raw cooling water conduits became a problem for many industrial and power plants due to clogging of water sieves, filters, pipes, valves, heat exchangers and condensers (Boltovskoy et al. 2006). Facilities are colonized by the planktonic larvae of the mussel, which attach to the inner surface of metal pipes and other components building massive beds. In Asia and South America, many nuclear and hydroelectric power plants, distilleries, refineries and other industrial installations have experienced clogging, pressure loss and temporary shutoffs due to fouling (Morton 1975; Matsui et al. 2001, 2002; Cataldo et al. 2003).

A variety of methods have been proposed to control the adverse effects of this fouling mollusc. Many of these are based on the experience gained in Europe and North America from the invasive zebra mussel *Dreissena polymorpha* (Pallas 1771), but their effectiveness for *L. fortunei* is limited by marked differences in the tolerance limits of the two species (Cataldo et al. 2003; Karatayev et al. 2007). Surveys on the control methods of fouling by byssate mussels include analyses of attachment strength to antifouling materials and coatings (Ohkawa et al. 1999; Matsui et al. 2001; Nagaya et al. 2001; Carl et al. 2012; Vucko et al. 2013), manipulation of flow rate (Matsui et al. 2002), use of toxicants (Morton et al. 1976; Cataldo et al. 2003; Liu et al. 2012; Calazans et al. 2013; Montresor et al. 2013), desiccation, anoxia, and thermal shock (Montalto & Ezcurra de Drago 2003; Perepelizin & Boltovskoy 2011a, 2011b, 2011c).

While many of these methods proved effective in controlling mussel fouling, it is clear that there is not a single optimum strategy. Options depend on many factors, including plant-specific technical issues, local

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climate, the characteristics of the raw cooling water used, and the mussel recruitment pattern. One alternative that has not been explored so far is the use of ultraviolet (UV) irradiation, widely employed in the water industry for disinfection, sterilization, and antifouling purposes.

The aim of this work was to assess the feasibility of using UV (254 nm) irradiation for precluding *L. fortunei* veliger larvae from colonizing components of the cooling system of industrial and power plants.

Materials and methods

Larvae of *L. fortunei* used in the experiments were collected along the coast of the Rio de la Plata estuary off Buenos Aires (34°36′S, 58°20′W) during periods of peak larval production (Boltovskoy et al. 2009, 2013): 1–9 November 2010 (spring) and 31 January – 7 February 2011 (summer), using short tows with a 37 μm mesh plankton net. Larvae were transferred from the net bucket to 2 l plastic vessels and immediately transported to the laboratory. All experiments were performed within 24 h of collection.

In the laboratory, larvae were irradiated with different doses of monochromatic UV short wavelength (254 nm, UVC) light, known to have strong biocidal effects (Chalker-Scott et al. 1994; Wright et al. 1997; Mackie & Claudi 2010). Veligers and early straight-hinged larvae 100–150 μm in size (Cataldo et al. 2005) (10–11 per exposure) were isolated from the plankton sample, transferred with a micropipette to a Petri dish (60 mm diameter, 15 mm deep), and the volume of the dish was filled to 5 ml with distilled water. Distilled water accounted for 10–15% of the final volume in the Petri dish; the resulting dilution of the total dissolved solids in the experimental chambers was thus well below the normal range of variation of salinity in water bodies colonized by the mussel (eg 32–115 μS cm^{-1} for the Paraná river; Depetris & Paolini 1991).

The UV light source used was a low-pressure mercury quartz lamp (Spectroline model 11SC-1, New York, USA) delivering 3.8–7.4 (average 5.9) mJ cm^{-2} (1 mJ = 1 mW s^{-1}) at 2.5 cm. The lamp was located 2.5 cm above the surface of the liquid at the center of the Petri dish. On the basis of preliminary trials, which showed that veligers were more vulnerable to UV at higher temperatures, four doses of UV irradiation were defined at each of the two water temperatures at which larvae were collected and exposed: 23 ± 0.5 °C (spring), and 25.8 ± 1.2 °C (summer). For animals collected in spring, doses used were 88, 113, 130, and 149 mJ cm^{-2}; whereas for those collected in summer, the doses were: 63, 75, 88, and 103 mJ cm^{-2}. Different doses were achieved by changing the exposure time (20–90 s) (Morita et al. 2002), which was calibrated at the beginning of each batch of 5 replicates using an ammonium iron (III) oxalate trihydrate (Sigma-Aldrich, St Louis, MO, USA) chemical actinometer (Montalti et al. 2006).

Simultaneously with each pair of UV tests, controls were performed under identical experimental conditions but without UV exposure (four controls in total: at 23 °C one for 88 and 113 mJ cm^{-2}, and one for 130 and 149 mJ cm^{-2}; at 25 °C one for 63 and 88 mJ cm^{-2}, and one for 75 and 103 mJ cm^{-2}). Each treatment was performed in quintuplicate and controls in triplicate. Throughout the experiments, the exposure and control chambers were maintained at constant *in situ* collection temperature.

Larvae were considered dead when their valves were fully open and they had no signs of visceral or ciliary activity (Lewis & Whitby 1995). In order to estimate the sublethal effects of UV exposure, a larva which had its velum extended or actively swimming (mobile larva), was differentiated from one that was motionless and had its velum retracted (stationary larva) (Chalker-Scott et al. 1994). In order to account for delayed mortality (Chalker-Scott et al. 1994; Lewis & Whitby 1995), all chambers (experimental and control) were checked 5 min, 24, 48, and 72 h after UV exposure. Beyond this time frame, estimates proved unreliable because of increasing mortality in the controls, probably in part due to extensive bacterial growth.

Data were checked for normality and homogeneity (Levene's test), and repeated measures ANOVA was used to compare differences in mortality and mobility between treatments at different post-exposure times using non-transformed data. Controls were included in the mortality assays only.

The waters where *L. fortunei* lives are lakes, reservoirs, and rivers that are characterized by variable, but often high, turbidity especially in its invasive range in South America. The concentration and type of suspended solids strongly affects UV penetration and effectiveness (Lewis & Whitby 1995; Gregg et al. 2009). In order to account for this variability and provide a more realistic assessment of the feasibility of using UV-based treatments in water bodies of medium and low transmittance levels, both in the Paraná-Uruguay watershed and elsewhere, a complementary set of measurements was carried out to determine the decay of UV irradiation as a function of the suspended solids load. For this purpose, coastal water samples from the Río de la Plata were oven-dried at 60 °C until all water was eliminated (constant weight). The resulting residue (seston) was subsequently resuspended in distilled water at 50, 100, 150, 200, and 250 mg (dry seston) l^{-1}. These levels of suspended solids encompassed the range of values normally observed in the basin (typical values for the Paraná river range around 160 mg l^{-1}) (Milliman & Syvitski 1992; Depetris & Kempe 1993). Triplicate measurements of UVC absorbance (254 nm) were performed with a

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Beckman DU-65 spectrophotometer using 1 cm quartz cells with distilled water as a blank. With these absorbance values, the irradiance needed to eliminate all *L. fortunei* larvae as a function of suspended solids in the water and distance from the UV source was calculated.

Results

Veliger larvae of *L. fortunei* are highly sensitive to UVC irradiation, and survival and vitality were significantly affected by increasing the UV dosage (ANOVA F-values for mortality, spring: 33.78, summer: 36.33; mobility: spring: 106.46, summer: 149.25; $p < 0.0001$ in all cases). At both temperatures, total mortality was reached at the highest doses assayed: 149 mJ cm^{-2} at 23 °C (Figure 1A) and 103 mJ cm^{-2} at 25.8 °C (Figure 1B). In all experiments, animals were alive immediately after ending the exposure, but died progressively during the following 72 h, with highest mortality rates occurring at the end of the observational period, even at the highest doses used (ANOVA F-values for mortality, spring: 40.85, summer: 53.46; mobility, spring: 21.22, summer: 17.25; $p < 0.0001$ in all cases) (Figure 1).

Unlike mortality, mobility was affected immediately after exposure. Organisms recovered partially after 24 h, but at 48 and 72 h their activity dropped to levels similar to those immediately after exposure (Figure 2A and B). Both mortality and mobility were more affected by irradiation at higher temperatures (Figures 1 and 2).

Tests of absorbance of 254 nm light (absorbance = $-\log_{10} (I_1/I_0)$, where I_0 is the intensity of the light received by the substance tested and I_1 is the intensity of the light that has passed through) indicate that decay of UV light is linearly associated with the amount of suspended solids (Figure 3). At the lowest concentration

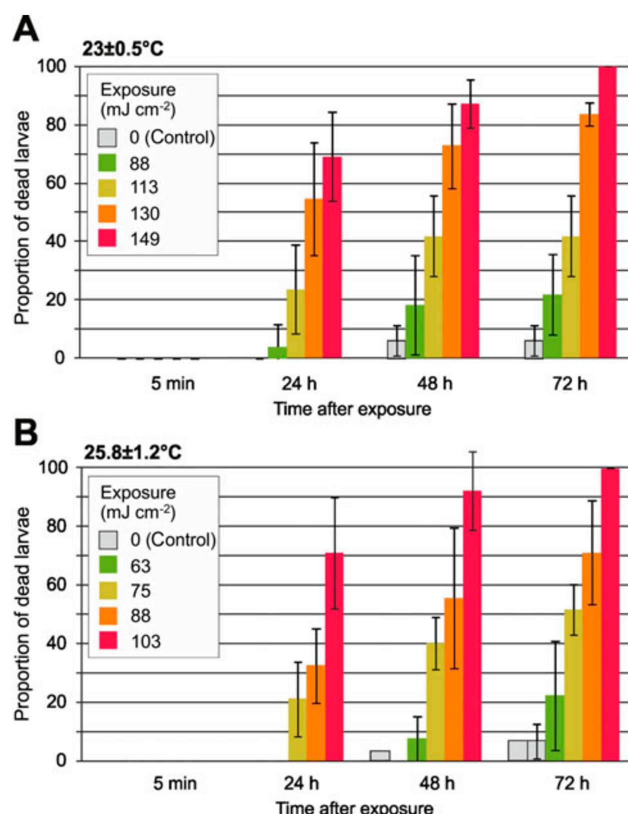

Figure 1. Mortality of *L. fortunei* larvae exposed to 254 nm light at the doses indicated 5 min to 72 h after exposure, at two different temperatures. Error bars denote 1 SD (controls are based on 3 replicates, exposures 5 replicates, in all cases with *ca* 10 larvae per replicate). The first control bar refers to exposures at 88 and 113 mJ cm^{-2} (23 °C), and 63 and 88 mJ cm^{-2} (25 °C), the second control bar is for exposures at 130 and 149 mJ cm^{-2} (23 °C), and 75 and 103 mJ cm^{-2} (25 °C). Absence of error bars denotes SD = 0.

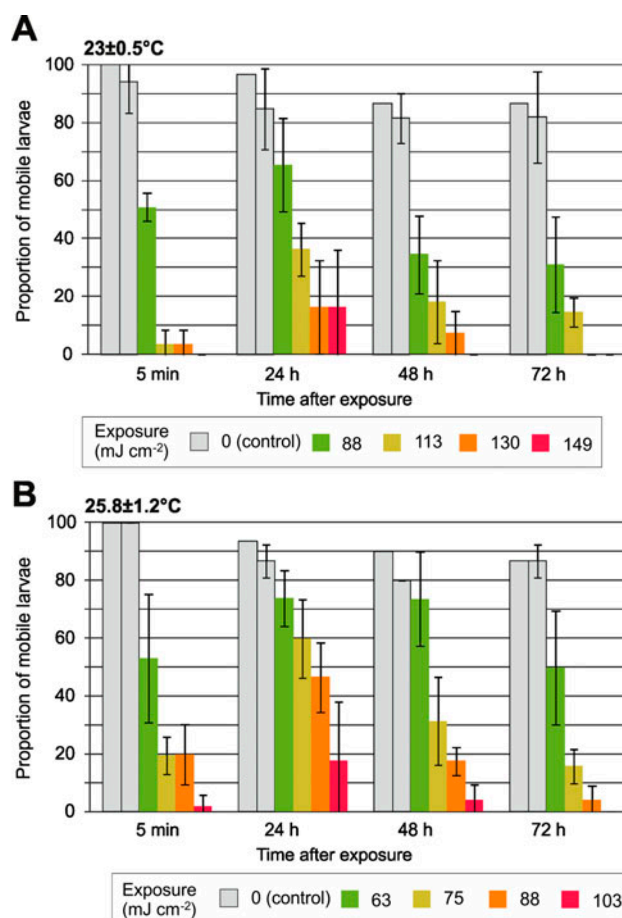

Figure 2. Mobility of *L. fortunei* larvae exposed to 254 nm light at the doses indicated 5 min to 72 h after exposure, at two different temperatures. Error bars denote 1 SD (controls are based on 3 replicates, exposures 5 replicates, in all cases with *ca* 10 larvae per replicate). The first control bar refers to exposures at 88 and 113 mJ cm^{-2} (23 °C), and 63 and 88 mJ cm^{-2} (25 °C), the second control bar is for exposures at 130 and 149 mJ cm^{-2} (23 °C), and 75 and 103 mJ cm^{-2} (25 °C). Absence of error bars denotes SD = 0.

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tested (50 mg l^{-1}), ~ 20% of the UV light emitted is absorbed at 1 cm from the source (absorbance ~ 0.1), whereas at 250 mg l^{-1} almost 60% of the irradiation disappears at 1 cm from the source (absorbance around 0.4 cm^{-1}). Based on these values, the intensities needed at the UV source in order to cover distances of up to 10 cm with doses sufficient to kill all larvae at 23 °C are 149 mJ cm^{-2}, and at 25.8 °C 103 mJ cm^{-2} (Figure 4). Obviously, higher suspended solid loads, associated with higher absorbance values, require higher doses, with up to *ca* 1,000 J cm^{-2} to yield a lethal dose at 10 cm from the UV source in waters with 250 mg l^{-1} of suspended solids.

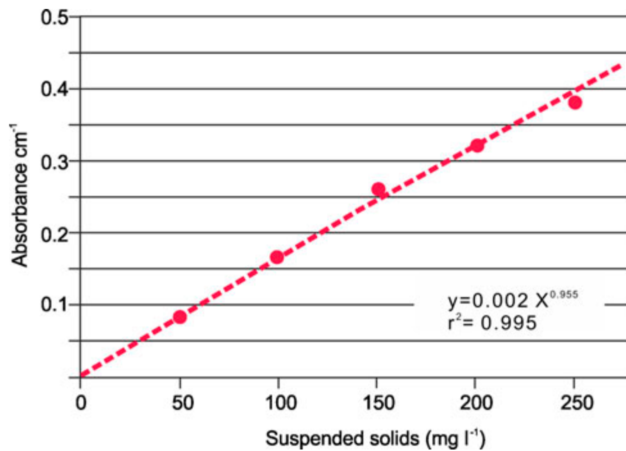

Figure 3 Relationship between absorbance of UV (254 nm) light and amount of suspended solids for coastal waters of the Río de la Plata estuary.

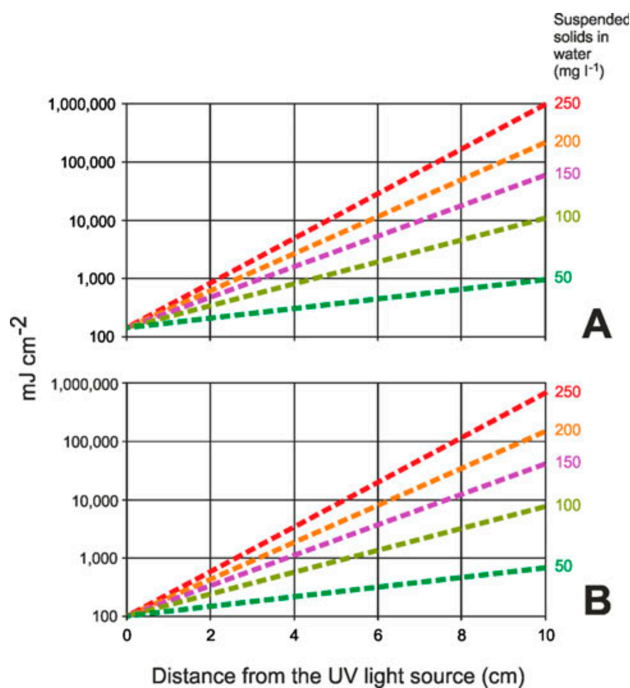

Figure 4. Emission strength needed to achieve 149 (A, treatment at 23 °C) or 103 (B, treatment at 25.8 °C) mJ cm^{-2} at 0–10 cm from the source in waters with 50–250 mg of suspended solids l^{-1} (notice that Y-scale is logarithmic).

Discussion

Despite the fact that comparison of these results with previous data is complicated by the different methods used, in general, the lethal doses found were similar to those reported earlier for several aquatic invertebrates (Buchholz 1998; Gregg et al. 2009), and within the range of those found effective for mussel larvae. Wright et al. (1997) reported 100% mortality in dreissenid veligers exposed to 254 nm light at doses from 100 mJ cm^{-2}. Chalker-Scott et al. (1994) recorded 100% mortality of zebra mussel veligers after exposure to a dose of 350 mJ cm^{-2} of 280–320 nm light. Some surveys, however, found much lower mortality rates even at very high UV doses, but interpretation of these results is hindered by methodological problems, such as absorbance of the media used and consideration of latent mortality (eg Lewis & Whitby 1995).

Pinheiro dos Santos (2011) performed trials on the effects of UV light on *L. fortunei* larvae and concluded that for 100% mortality the dose needed was above 781 mJ cm^{-2}. However, experimental conditions in this study were poorly defined: the absorbance of the ambient water used was not assessed, experimental doses were calculated on the basis of the lamp manufacturer's specifications rather than measured, delayed mortality was not checked, and irradiated experimental larvae were concentrated with a plankton net to estimate the proportions of dead individuals without prior assessment of the impact of these manipulations on larval integrity, which hampers comparisons with the present results.

In common with these findings, death of irradiated organisms occurred a few days after exposure, but mobility was affected immediately (Chalker-Scott et al. 1994; Wright et al. 1997). In the present study, up to 40% of the larvae that were immobile immediately after exposure showed signs of recovery 24 h later, yet after 2–3 days most of these animals were dead. Wright et al. (1997) noticed the same phenomenon with *D. polymorpha* larvae: stunned individuals immediately after exposure would even settle and develop a bysssus 24 h later, yet after a week they were all dead. The fact that the UV doses that immobilize *L. fortunei* larvae are considerably lower than those needed to kill them is important because stunning the larvae to prevent movement and settlement allows them to be swept through the sensitive areas, which may be more cost-effective than killing them (Chalker-Scott et al. 1994).

UV treatments aimed at eliminating pathogens and fouling organisms have been used for decades (Lakretz et al. 2010). Among the major advantages of UV are the

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absence of toxic by-products or changes in the properties of the water treated, lack of corrosion or damage to structural components of the facility, no chemicals are required, and it has no overdose-related risks. Also, it may be comparatively inexpensive, easy to operate, and environmentally safe (Chalker-Scott et al. 1994; Cloete et al. 1998). In the particular case of mussel veligers, the penetration of the irradiation is facilitated by the transparency of the shells at this age (the protection conferred to juvenile and adult mussels by their pigmented shell makes UV treatments much less effective, Chalker-Scott et al. 1994). However, a major constraint for the efficacy of UV treatments is the transmittance of the water. Turbid waters hinder penetration of the UV light, as suspended solids, most organic components and certain inorganic salts absorb UV irradiation (Cloete et al. 1998), thus higher capacity lamps, slower water velocities or lower water thickness, or all of the above are required. In the case of industrial plants that use water from the Paraná and Uruguay rivers, this constraint is particularly important because of their very high turbidity.

For waters with *ca* 160 mg l^{-1} of suspended solids (absorbance: 0.255), a common value for these floodplain rivers (Milliman & Syvitski 1992; Depetris & Kempe 1993), in order to deliver at least 103 mJ cm^{-2} (the 100% mortality dose at 23 °C) to the bottom layer of a 10 cm-deep water column, the lamp must yield 40,700 mJ cm^{-2}. For a dose of 149 mJ cm^{-2} under the same conditions, the UV source must deliver 59,000 mJ cm^{-2}. At these absorbance values, even reducing the flow rate and the thickness of the water layer makes UV treatments economically non-viable in most cases. One possible alternative is filtering the water before applying the UV treatment. Small-pore self-cleaning filters in conjunction with UV reactors have proven effective in some applications, such as ballast water treatments (Gregg et al. 2009; Mackie & Claudi 2010). However, the volumes of water that can be processed are moderate (usually < 1,000 m^3 h^{-1}; Gregg et al. 2009) compared with the requirements of most industrial open-circuit cooling systems.

Another disadvantage of UV treatments is that, as opposed to methods aimed at settled mussels, which can be implemented a few times per year (Perepelizin & Boltovskoy 2011b), they must be applied throughout the entire reproductive period of the animal. In the case of *L. fortunei,* this is an important drawback because the mussel produces larvae for up to 6–9 months a year (Boltovskoy et al. 2009, 2013).

On the other hand, UV treatments may prove effective for plants using raw cooling water from clear lakes and reservoirs, such as most of those located in central Argentina. Several of these water bodies have been colonized by L. *fortunei* over a decade ago, and represent a major nuisance for hydraulic and nuclear power plants (Boltovskoy et al. 2009). The transparency of these water bodies is *ca* 10 times higher than that of the Paraná-Uruguay system and production of larvae spans a considerably shorter period (Boltovskoy et al. 2009), which may allow for efficient use of UV-based technologies.

Conclusion

The doses needed to kill or neutralize *L. fortunei* veligers in water used for cooling purposes are reasonably low (up to 100–150 mJ cm^{-2}, depending on temperature) to render this antifouling method effective. However, highly turbid waters and large cooling water flows may pose unsurmountable difficulties requiring economically unviable installations, both in terms of initial investment and in terms of operational energy expenditures. Nevertheless, UV treatments for controlling macrofouling by *L. fortunei* may be an excellent option for facilities drawing moderate volumes of cooling water from clear water bodies.

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