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The role of hydraulic conditions of coagulation and flocculation on the damage of cyanobacteria

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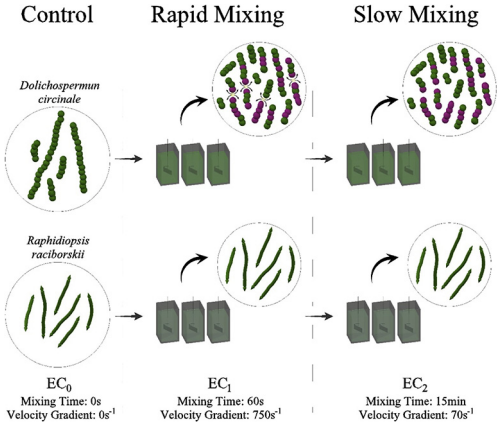
HIGHLIGHTS

- Hydraulic conditions suggested by technical standards can damage filamentous cyanobacteria.
- *D. circinale* intact cells and trichome length are substantially reduced after rapid mixing.
- Damage to *R. raciborskii* was not significant during rapid mixing.
- Unlike rapid mixing, slow mixing didn't compromise organisms significantly.
- Water treatment should operate according to the organisms present in the water.`

GRAPHICAL ABSTRACT



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ABSTRACT

Limited information exists on the damage of harmful cyanobacteria cells, such as *Raphidiopsis raciborskii* and *Dolichospermum circinale*, caused by the hydraulic conditions at water treatment plants especially when it comes to the mechanical stresses imposed by coagulation and flocculation. To close this gap, this study evaluated the impacts of rapid and slow-mixing on *R. raciborskii* and *D. circinale* cells and trichomes. The hydraulic conditions used during the experiment were selected based on AWWA, which are widely applied in the absence of specific treatability tests. Cellular integrity was evaluated by the Erythrosine B staining method and logistic regression was used to study the association between organism integrity and hydraulic conditions (i.e., velocity gradient and mixing time). Wilcoxon rank-sum test was used to verify if there was a significant reduction of the trichome length and cell integrity. Rapid-mixing (velocity gradient of 750 s^{-1} for 60 s) reduced the odds of finding intact *D. circinale* to <50%, whereas the odds of finding intact *R. raciborskii* cells did not significantly decrease. The odds of finding intact cells of *R. raciborskii* were 124 times greater than *D. circinale*. Rapid-mixing also reduced the length of *D. circinale* trichomes by approximately 50% but did not significantly decrease *R. raciborskii* trichomes. Slow-mixing did not significantly affect organisms or trichomes of either species. The results indicate that AWWA recommendations for coagulation may cause damage to *D. circinale* but not to *R. raciborskii*, suggesting that the operation of water treatment plants could be adjusted according to the dominant cyanobacterium present in the reservoir to avoid cell rupture and metabolite release.

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1. Introduction

Cyanobacterial blooms severely impact the performance of water treatment plants (WTPs) (Pestana et al., 2019; Barros et al., 2017; Li et al., 2016; Lopes et al., 2015). The presence of these organisms, above certain concentrations, in raw water may lead to several problems, such as the release of toxic secondary metabolites, taste & odor compounds, and disinfection by-product precursors (Li et al., 2019; Lin et al., 2018; He et al., 2016; Pestana et al., 2016; Xie et al., 2016; Daly et al., 2007).

The unit operations that compose a WTP, especially coagulation, can lead to cyanobacterial lysis (Liu et al., 2018; Pestana et al., 2019; Mucci et al., 2017). In general, coagulation can be understood as the destabilization of inorganic and organic particles through chemicals action (pre-oxidants, coagulants, and auxiliary polymers) and intense agitation during a short period. This step can be followed by the formation of flocs in the flocculation step, enabling the removal of colloids and suspended particles in subsequent treatment steps (Hussain et al., 2019; Liu et al., 2018).

Two common, toxigenic cyanobacterial species, *R. raciborskii* and *D. circinale*, are cosmopolitan organisms that can form persistent blooms in the northeastern region of Brazil (Barros et al., 2017; Lopes et al., 2015). Under certain environmental conditions, they are capable of producing powerful toxins (e.g., saxitoxins and cylinderspermopsins) and taste and odor compounds (2-methylisoborneol (MIB) and geosmin). Although *R. raciborskii* and *D. circinale* are both filamentous, they present different morphological characteristics (Genuário et al., 2019; Komárek and Johansen, 2015), suggesting they might behave differently during treatment processes, such as coagulation and flocculation.

Studies indicate that different species of cyanobacteria react differently to the chemical and physical stresses of water treatment. While dosages of polymeric aluminum ferric chloride (PAFC) >10 mg L^{-1} induced additional release of cylinderspermopsin from *R. raciborskii*, *Microcystis aeruginosa* was not affected by dosing up to 30 mg L^{-1} (Li et al., 2018). Lin et al. (2018) observed that pre-oxidation with NaOCl (associated or not with ClO_2) disrupted cells of *M. aeruginosa* and *R. raciborskii* at different levels. According to Zamyadi et al. (2012), only a CT (concentration × time) of chlorine >31 mg min L^{-1} was able to compromise the integrity of *M. aeruginosa* cells in >99%, whereas a CT of 8 mg min L^{-1} was enough to similarly impact cells of *D. circinale*, *R. raciborskii*, and *Aphanizomenon issatsckenka*. Although many studies have tried to describe the effect of water treatment processes on cyanobacteria, especially the addition of coagulants, oxidants, and other chemicals (Jian et al., 2019; Wang et al., 2018; Lin et al., 2017), the effect of mechanical stress was not observed separately, making it difficult to identify what caused the cell damage.

In addition to cell wall damage, filamentous cyanobacteria may also have the multicellularity of their trichomes compromised by water treatment steps. Pestana et al. (2019) observed that trichomes of different filamentous genera would break in different scales at various stages of direct filtration and conventional WTPs. The authors pointed out that genera of filamentous cyanobacteria with >30 cells (*Planktothrix, Geitlerinema*, and *Dolichospermum*) were more susceptible to treatment stresses (loss of cell integrity and/or trichome truncation) than those with trichomes with <12 cells (*Pseudanabaena* and *Planktolyngbya*).

In the absence of specific operational parameters obtained by treatability tests, it is common to adopt the velocity gradient and mixing time recommended by technical organizations for the coagulation and flocculation steps. Although technical standards recommendations (Table 1) are generally chosen for the design and operation of the coagulation and flocculation units, little or no attention has been given to what happens to cyanobacteria under such conditions.

Despite the advances presented by Pestana et al. (2019) regarding trichome integrity in WTPs, specific knowledge about how velocity gradient and mixing time suggested by these technical standards impact the trichomes of *R. raciborskii* and *D. circinale* are still scarce and inconclusive. Thus, the main objective of this work was to evaluate the effects of velocity gradient and mixing time recommended by American Water Works Association (1998) for the coagulation and flocculation steps on *R. raciborskii* and *D. circinale* integrity.

Table 1
Velocity gradients and mixing times recommended by American Water Works Association (1998).

Parameter	Coagulation (rapid-mixing)	Flocculation (slow-mixing)
Velocity gradient (s^{-1})	600–1000	GT[a] = 24,000–84,000
Mixing times	10–60 s	20 min[a]

[a] Suggested.

2. Materials and methods

2.1. Cyanobacterial cultivation, cell density, and trichome length

Two cultivated species, *R. raciborskii* and *D. circinale* were used. They were cultured using ASM-1 medium (Gorham et al., 1964) with pH 8, non-axenic conditions, but with imperceptible bacterial contamination confirmed by conventional microscopic analysis. The species were kept under white light (470 nm) with an intensity of approximately 6.75 μmol m^{-2} s^{-1} (DIGITAL LUX TESTER YF-1065) with a photoperiod of 12:12 h (light/dark), the temperature of 24 ± 2 °C with aeration. The cultures were used at the age of 21 ± 2 days for further dilution at a concentration within the range of 10^5 to 10^6 cells mL^{-1}, similar to concentrations of blooms observed by Dugan et al. (2018), Lin et al. (2018) and Fan et al. (2013).

Cell density was measured using an inverted optical microscope (ZEISS, Model Vert.A1, Germany) with a magnification of 40× with a Sedgewick Rafter chamber with counts by bands or fields, according to the Poisson distribution and achieving a confidence interval of 95% ± 20% (APHA-AWWA-WEF, 2005). For quantification of the number of cells per trichome and determination of the length of the filaments, 30 organisms were selected randomly, using an optical microscope (Olympus Optical, Model: Cx-31, USA). Cell density values were expressed in cells mL^{-1} and lengths in μm.

2.2. Simulation of hydraulic conditions

Dechlorinated tap water was used as the matrix for the dilution of the cultured species to simulate as closely as possible the ionic strength of the raw water. Tap water presented turbidity equal to 0.4 NTU (Hach 2100P, USA), total hardness = 120 mg L^{-1} of $CaCO_3$, and free chlorine concentration below the limit of detection (<0.02 mg L^{-1}) of the N,N-diethyl-*p*-phenylenediamine (DPD) method. All analyses were performed following APHA-AWWA-WEF (2005).

The cyanobacterial suspension was prepared in a container and then distributed into jars of a jar tester (JAR.217-Ethik Technology) for simulation of the coagulation using rapid-mixing (targeted velocity gradient $G_R = 700–800\ s^{-1}$) followed by simulation of the flocculation step using slow-mixing (targeted velocity gradient $G_S = 50–90\ s^{-1}$). At the initial experimental condition - EC_0 ($T = 0$ s), at the end of coagulation simulation - EC_1 ($T = 60$ s), and at the end of flocculation simulation - EC_2 ($T = 14$ min), samples were withdrawn from the jars for organism integrity analyses. Twelve experiments (E1 to E12) were carried out, corresponding to two experiments in triplicates for each species. The mean of each triplicate was designated as SC_1 and SC_2 for the *R. raciborskii* experiments, and SC3 and SC_4 for the *D. circinale* experiments. To evaluate the trichome length, 30 organisms were analyzed for each of the twelve experimental samples (E1, E2, E5, E6, E9, and E10 for *R. raciborskii* and E3, E4, E7, E8, E11 and E12 for *D. circinale*).

2.3. Statistical analyses and organism integrity

To evaluate if the jar tester equipment maintained the same mixing conditions throughout the experiments, values of velocity gradient were measured during the coagulation simulation at times 20, 30, 40, 50, and 60 s and during flocculation at times 2 to 14 min.

Shapiro-Wilk test was used to analyze normality. Since the samples were non-parametric. Multiple pair-wise tests were made using a two-tailed Wilcoxon rank-sum test to identify differences between the samples. The samples were considered independent since each result did not influence the other. To reduce the chances of obtaining false-positive results when using multiple pair-wise tests, each test was adjusted by the Bonferroni method. The Bonferroni correction method is an adjustment made to the *p*-values when several comparisons are performed simultaneously (Giolo, 2017; Agresti, 2012). The same procedure was used to identify significant reductions in trichomes length.

At the significance level of 5%, the hypothesis of equality of mixing conditions in the experiments was accepted when $p > 0.05$. In the case of trichomes, the reductions in lengths were significant when $p < 0.05$ (dataset and *p*-values are provided in Supplementary Material I).

The membrane integrity of the *R. raciborskii* and *D. circinale* organisms was evaluated by a cell staining method using Erythrosin B ($C_{20}H_6I_4Na_2O_5$, Dynamica), which is a biological dye used in the food industry. Erythrosin B allows the identification of intact or damaged cyanobacterial cells by contrast. The dye enters the cell membrane that has suffered lysis, giving them a pink color while the intact cells remain green, even after the contrast addition. This color differentiation can be observed by optical microscopy (Calomeni and Rodgers, 2015; DiBartolomeis and Mone, 2004; Markelova et al., 2000).

Immediately after each experimental step (EC_i), 1 mL aliquot of the *R. raciborskii* and *D. circinale* suspensions were collected, transferred to a test tube and 1 mL of an erythrosine B aqueous solution (5% m/v) was added. After gentle homogenization, the resulting solution was kept for 15 min protected from the light and then examined under a microscope (Olympus Optical do Brazil Ltd., Model: Cx-31). The organisms with cell integrity compromised presented a pink color (Calomeni and Rodgers, 2015). Due to the morphological differences, *R. raciborskii* was considered compromised when trichomes were stained. On the other hand, *D. circinale* was considered compromised when cells were stained (Fig. 1). More micrographs of the intact and damaged cells can be found in the Supplementary Material II.

In this stage, 100 organisms were randomly assessed in triplicate. Therefore, for each species, 900 organisms were evaluated for each of the four experimental conditions, totaling 3600 cell integrity analysis. Cell integrity was considered a categorical (dichotomous) variable (intact or not). Contingency tables (2×2) were prepared using the triplicate means (SC_1, SC_2, SC_3, and SC_4), comparing the experimental conditions. The compared scenarios are presented in Table 2. The association between integrity loss and experimental conditions was identified using Fisher exact test and quantified using *Odds Ratio* (OR) with confidence intervals (CI) of 95% and a significance level of 5% (Agresti, 2012). A transformation of the original data was necessary and was done by adding 1 to both "Yes" and "No" columns. This transformation was necessary because of observations equal to zero were causing indeterminations in the calculations (more detail in the Supplementary Material I) (Giolo, 2017). The tested hypotheses, as well as the interpretations of the OR and CI, are found in Table 2. Using the Estimated *Odds Ratio* ($\widehat{OR}$), calculated by logistic regression, it was possible to estimate how much the odds of finding intact organisms of one species is greater than for the other species. For that, the species of cyanobacteria ($X_{Species}$) and the experimental conditions (X_{EC1} and X_{EC2}) were considered covariates, making it possible to obtain the coefficients $\beta_{Species}$, β_{EC1} and β_{EC2}, respectively. These coefficients were considered statistically significant when $p < 0.05$.

To ensure the quality of fit of the proposed logistic regression model, the residues should comply simultaneously with (Agresti, 2012):

1. Graphical analysis of Pearson and Deviance residuals: The adjusted model should have randomly distributed residuals and should not be >3.0 or < 3.0 (Giolo, 2017);
2. Simulated envelope graph: Residuals (Pearson and Deviance) should be contained within the simulated envelope;
3. Chi-square likelihood ratio (QL) statistics and Pearson's chi-square statistic (QP) should have $p > 0.05$.

The $\widehat{OR}$ was estimated by applying exponential to the coefficient $\beta_{Species}$, β_{EC1}, and β_{EC2}. Their respective CI was calculated using Eq. (1). The interpretation of $\widehat{OR}$ and CI values are similar to the explanation presented in Table 2. All calculations were performed using RStudio software.

$$CI\left(\widehat{OR}\right) = \exp\left[\widehat{OR}_i \pm 1.96 \times EP\left(\widehat{OR}\right)\right] \quad (1)$$

where:

EP($\widehat{OR}$) = standard error associated with the estimated $\widehat{OR}$;
CI($\widehat{OR}$) = confidence interval (95%).

3. Results and discussion

3.1. Evaluation of equality of mixing conditions in the experiments

The mean velocity gradient of each experiment, indicated by the asterisks of each boxplot in Fig. 2, approximates the general mean of the

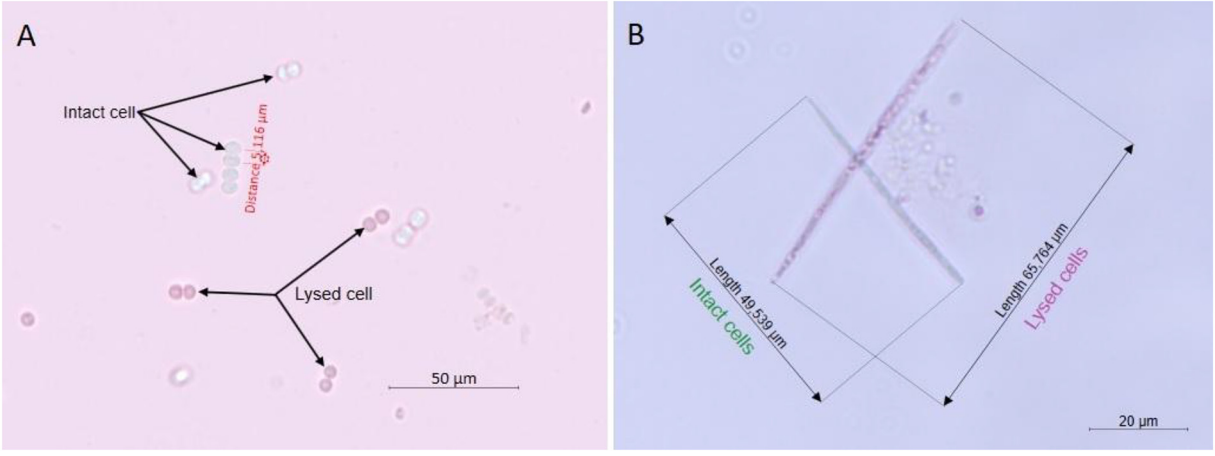

Fig. 1. *D. circinale* (A) and *R. raciborskii* (B) organisms intact and with compromised integrity (pink color). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
Comparison scenarios and the tested hypotheses H_1, H_2 and H_3.

Hypotheses	Compared scenarios	Description	Not rejected when Fisher's exact test is:	OR and CI	
				Values	Interpretations
H_1 H_1: $\pi_1 = \pi_0$ The proportion* of intact organisms after rapid-mixing (π_1) is equal to the proportion* of the initial conditions (π_0)	CE_1/CE_0	To verify if there is a significant difference in the number of intact organisms between the beginning of the experiment (CE_0) and after rapid-mixing (CE_1).	$p > 0.05$	OR > 100% and 100% ∉ CI	Increased chance of intact organisms
H_2 H_2: $\pi_2 = \pi_0$ The proportion* of intact organisms after rapid-mixing followed by slow-mixing (π_2) is equal to the proportion* of the initial conditions (π_0)	CE_2/CE_0	To verify if there is a significant difference in the number of intact organisms between the beginning of the experiment (CE_0) and after slow-mixing (CE_2). This hypothesis does not allow identifying which step impacted the most (rapid or slow-mixing).		OR < 100% and 100% ∉ CI	Reduced chances of intact organisms
H_3 H_2: $\pi_2 = \pi_1$ "The proportion* of intact organisms or not after slow-mixing (π_2) and equal after rapid-mixing (π_1)"	CE_2/CE_1	To verify if there is a significant difference in the number of intact organisms between after rapid-mixing (CE_1) and after slow-mixing (CE_2). This hypothesis allows identifying which step impacted the most (rapid or slow-mixing).		OR = 100% or 100% ∈ CI	Indicates that the odds are equal

(*) Number of intact organisms in 100 organisms.

12 experiments in both coagulation and flocculation steps. Furthermore, according to the Wilcoxon statistic, differences between the velocity gradients of the 12 experiments tested were not significant ($p > 0.05$), suggesting that results differences (Table 3) were not caused by mixing abnormality, but by the proposed experimental conditions themselves. Thus, the estimated values of G_R and G_S were 748.3 ± 31.1 s^{-1} (approximately 750 s^{-1} or 325 rpm) and 69.4 ± 10.4 s^{-1} (around 70 s^{-1} or 70 rpm), respectively. Therefore, the hydraulic conditions practiced here were aligned with the AWWA norm cited previously.

Li et al. (2018) investigated the fate of *R. raciborskii* during coagulation with several PAFC dosages (0–30 mg L^{-1}) with five different mixing conditions and mixing times. The results demonstrated that *R. raciborskii* cells were removed intact after coagulation. Further, fluorescence analysis demonstrated that *R. raciborskii* cells remained active after the PAFC-assisted coagulation.

Similar hydraulic conditions used in the current study (i.e., 325 rpm for rapid-mixing and 70 rpm for slow-mixing) were also used by Li et al. (2018) to represent the mixing conditions during coagulation and flocculation steps of water treatment. The results from Li et al. (2018) corroborate with the present study in which *R. raciborskii* was resistant to physical stress.

Lin et al. (2018) performed a bench-scale study with a jar-test to evaluate the effects of pre-oxidation using NaOCl and ClO_2. Their study applied oxidant dosages of 1 mg L^{-1} with mixing time of 1 min at 200 rpm (G = 350 s^{-1}) followed by charge neutralization using of Aluminum sulfate ($Al_2(SO_4)_3 \cdot 18H_2O$) on a suspension of

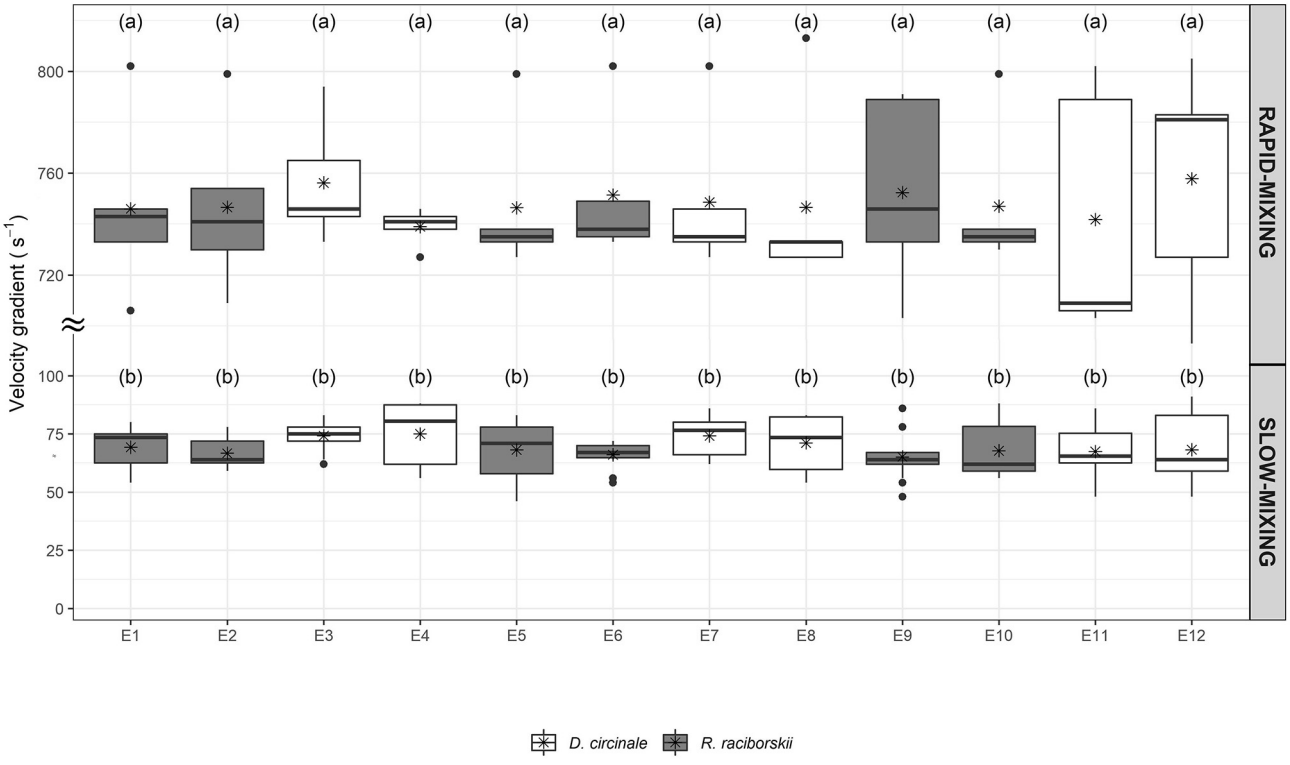

Fig. 2. Velocity gradients for rapid-mixing and slow-mixing. The dots represent outliers, the asterisks represent means and different letters mean significant statistical differences ($p < 0.05$).

Table 3
Mean and standard deviation (SD) of *R. raciborskii* and *D. circinale* intact organisms for each SCi after each experimental condition (H_1, H_2, and H_3) with their respective Fisher exact test *p* values, OR and 95% confidence interval. The significant values are presented in bold.

Experiments (species)	Mean of triplicates	Intact organisms (SD)			Hypothesis [comparative scenarios] (Fisher's exact test *p*-value)	OR [CI (95%)] (%)
		EC_0	EC_1	EC_2		
E1, E5 and E9 (*R. raciborskii*)	SC_1	100 (0.00)	99 (1.15)	98 (2.00)	H_1 [EC_1/EC_0] (1.000) H_2[EC_2/EC_0] (0.621) H_3 [EC_2/EC_1] (1.000)	49.7 [0.8–968.2] 32.8 [0.6–416.8] 66.1 [5.4–590.3]
E2, E6 and E10 (*R. raciborskii*)	SC_2	100 (0.00)	100 (0.00)	100 (0.00)	H_1 (EC_1/EC_0) 1.000 H_2(EC_2/EC_0) 1.000 H_3 (EC_2/EC_1) 1.000	100.0 [1.3–7921.8] 100.0 [1.3–7921.8] 100.0 [1.3–7921.8]
E3, E7 and E11 (*D. circinale*)	SC_3	71 (10.69)	54 (28.99)	53 (16.86)	**H_1 (EC_1/EC_0) 0.021** **H_2(EC_2/EC_0) 0.014** H_3 (EC_2/EC_1) 1.000	**48.9 [26.2–90.3]** **47.1 [25.2–86.7]** 96.2 [53.4–172.9]
E4, E8, E12 (*D. circinale*)	SC_4	85 (1.52)	55 (0.70)	62 (25.65)	**H_1 (EC_1/EC_0) <0.001** **H_2(EC_2/EC_0) <0.001** H_3 (EC_2/EC_1) 0.394	**22.8 [10.9–45.7]** **30.2 [14.4–61.1]** 132.5 [73.1–241]

R. raciborskii. The authors observed that the pre-oxidation-assisted coagulation caused cellular damage on *R. raciborskii* with a 59% reduction of cell density from 88,000 to 36,250 cells mL^{-1}. The difference in the results between Lin et al. (2018) and our experiment might be attributed to the chemicals stress during peroxidation and charge neutralization steps.

3.2. Impact of experimental conditions on the organism's integrity

The mean of the triplicates for experiments using *R. raciborskii* (SC_1 and SC_2) and *D. circinale* (SC_3 and SC_4) are presented in Table 3. *R. raciborskii* and *D. circinale* were not equally affected by the mechanical stress of the mixing conditions (i.e., velocity gradient and mixing time). There was no association between different experimental conditions and the organism integrity in both SC_1 and SC_2 ($p > 0.05$) for the species *R. raciborskii*, in all hypotheses tested (Table 3). This absence of association indicates that the proportion of intact organisms of *R. raciborskii* at the beginning of each experiment (CE_0) did not differ significantly from that found after rapid-mixing (CE_1) and after slow-mixing (CE_2). The observed differences in the amounts of organisms at SC_1 were caused by randomness and not by the combination of the velocity gradient and the mixing time during the process. Additionally, in all comparative scenarios for *R. raciborskii*, the CI of OR presented a value of 100%, making it possible to state that, after rapid-mixing (CE_1) and slow-mixing (CE_2), the odds of finding intact organisms were equal to the initial condition CE_0. Thus, the hypotheses H_1, H_2, and H_3 (Table 2) are not rejected and this suggests that the lower limit of the velocity gradient recommended by AWWA does not seem to affect this species.

According to Zarantonello et al. (2018) *R. raciborskii* forms chained filaments or trichomes, varying from about 50 to 300 μm in length. Its structure is composed of two bilayered membranes: the inner or plasma membrane and the outer membrane that encloses the periplasmic space with a thin peptidoglycan layer. It can also produce a thick-walled, cylindrical, spore-like structure known as akinetes. Hamilton et al. (2005) also observed that *R. raciborskii* cell walls were thick and conspicuous These membrane characteristics may be responsible for the high resilience to the mechanical stress observed.

There was evidence of association ($p < 0.05$) between the mixing conditions and *D. circinale* integrity (Table 3). The proportions of intact organisms in CE_1 and CE_2 are significantly different from that in CE_0, in both experiments (SC_3 and SC_4).

Rapid-mixing (scenario CE_1/CE_0) compromised *D. circinale* reducing the number of intact organisms after CE_1 and therefore, H_1 was rejected in both experiments (SC_3 and SC_4). After rapid-mixing followed by slow-mixing (scenario $CE_2 \times CE_0$), there was also a significant reduction in the number of intact organisms, in both experiments, the reason why H_2 was rejected as well. In contrast, the number of intact organisms after CE_1 and CE_2, in both replicates, was not significantly different (Table 3) and thus, there was not enough evidence ($p > 0.05$) to associate changes in the integrity of *D. circinale* and slow-mixing conditions (Table 3), causing H_3 to be not rejected. Since H_1 and H_2 were rejected and H_3 was not, we can deduce that damage to *D. circinale* was due only to rapid-mixing conditions (750 s^{-1} and 60 s). These results imply that the velocity gradient close to the lower limit recommended by American Water Works Association (1998) for coagulation, led to integrity loss of *D. circinale*, while recommended values for flocculation did not. In that case, the odds of finding intact organisms reduced to <50% compared to the initial condition CE_0 (Table 3). Moisander et al. (2002) showed that cyanobacteria of the genus, *Dolichospermum*, are more susceptible to physical stress than another filamentous cyanobacterium (i.e., *Nodularia* sp.).

The literature contains very limited information about the cell wall composition of *D. circinale*. However, as a proxy to *D. circinale* cell wall composition, we examined the cell wall composition of *Anabaena cylindrica*. Dunn and Wolk (1970) evaluated that the *Anabaena cylindrica* cell wall contained amino compounds (65%), lipid (3%), and

Table 4
Estimation of model parameters.

Coefficients	Estimation	Default error	*p*-Value
β_0 (Intercept)	1.28	0.17	<0.0001
$\beta_{Species}$	4.82	0.56	<0.0001
β_{EC1}	−1.10	0.22	<0.0001
β_{EC2}	−0,99	0.22	<0.0001

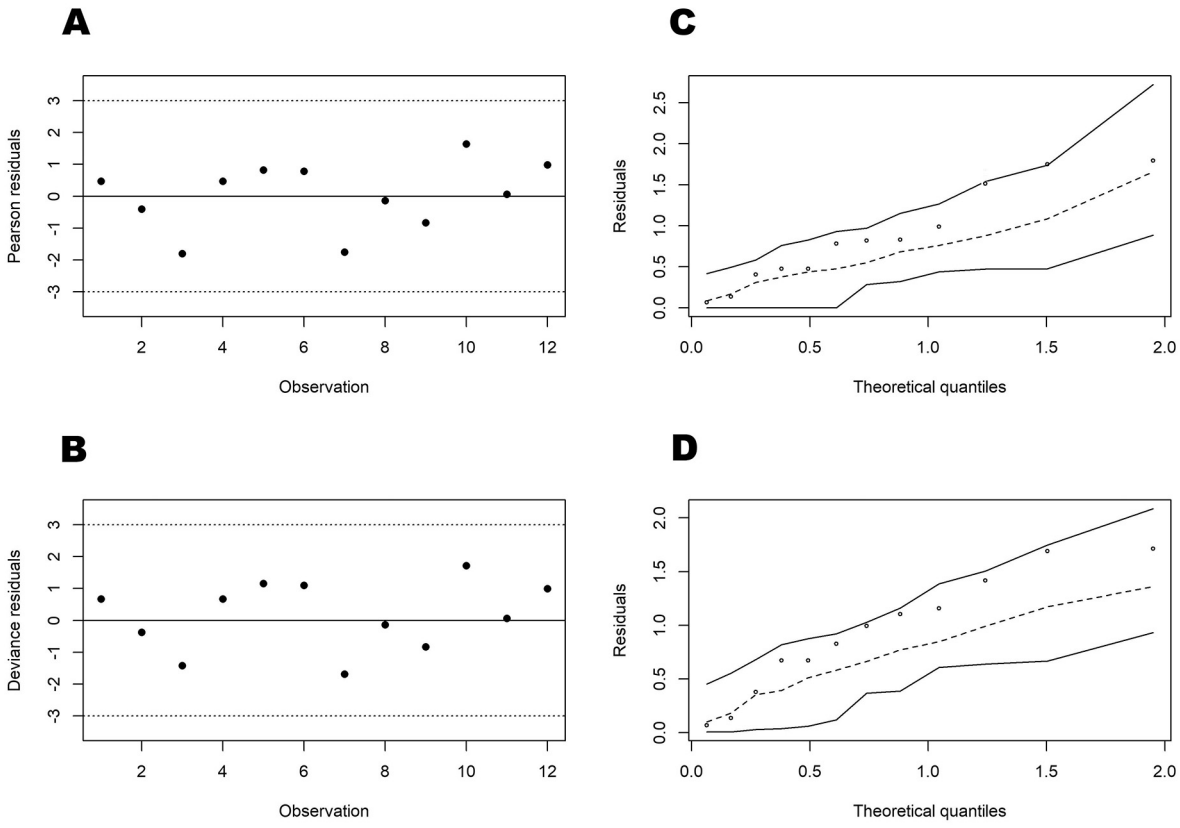

Fig. 3. Graphical analysis of residue models.

polysaccharides (18%). The polysaccharides consisted mainly of mannose, with smaller amounts of glucose, galactose, fucose, and xylose. Considering *D. circinale* and *Anabaena cylindrica* have similar membrane composition, this relatively low amount of polysaccharides may explain why *D. circinale* is more shear stress-sensitive than *R. raciborskii* (Woitzik et al., 1988).

Studies have shown that using a high-velocity gradient increased the coagulation performance and could reduce the consumption of chemicals (Byun et al., 2005), increasing turbidity removal and improving filterability (Lin et al., 2013). Ebie and Azuma (2002) also observed an improvement of coagulation with the increase of the velocity gradient from 450 s^{-1} to 1000 s^{-1}. On the other hand, even for purely inorganic suspensions, prolonged exposure to intense mixing may compromise the structure of the flock formed during flocculation. This fact can be attributed to the high shear caused by intense velocity gradients, that may compromise the performance of these steps (Watanabe, 2017; Lin et al., 2013; Jarvis et al., 2005). Similarly, the intense mixing conditions provided by coagulation in this work may have caused enough shear stress to compromise the structure of *D. circinale* and possibly causing cellular lysis.

Although *R. raciborskii* and *D. circinale* are filamentous species, they differed in some morphological characteristics. *D. circinale* strain used in the experiments presented trichome with spherical cells (diameter: 5.40 ± 0.20 μm), usually isodiametric. The *R. raciborskii* strain presented cylindrical cells (length: 2.80 ± 1.00 μm). The filaments of *D. circinale* were slightly curved or flexuous with a flexible aspect. The trichomes of *R. raciborskii* were straight, narrowing to the ends in some trichomes and did not show any flexibility. These morphological characteristics are consistent with Komárek and Johansen (2015) and may justify their different sensibility. As trichomes of *D. circinale* appeared to be more flexible, the intense mixing conditions may have flexed the trichomes and, combined with the high shear, may have caused the significant integrity loss observed. Since the trichomes of *R. raciborskii* seemed less flexible than those of *D. circinale*, twisting did not look like an issue.

The odds of finding intact organisms in the two species.

The parameters of the logistic model are found in Table 4, in which all estimated coefficients were found to be significant ($p < 0.05$). The model was considered well-adjusted since it met simultaneously the necessary conditions for the Pearson residuals (Fig. 3A), Deviance residual (Fig. 3B), their respective simulated envelope graphs (Fig. 3C and D), and statistics $Q_L = 13.10$ ($p = 0.11$) and $Q_P = 12.57$ ($p = 0.13$).

According to Table 4, the chances of intact organisms, as estimated by the logistic model, have a high dependence on the cyanobacterial species ($\beta_{Species} = 4{,}82$) and low dependence on the experimental conditions of rapid-mixing ($\beta_2 = -1{,}10$) and slow-mixing ($\beta_2 = -0{,}99$), but with rapid-mixing being more important than slow-mixing.

At any experimental condition, the odds of finding intact *R. raciborskii* were approximately 124 times greater than the *D. circinale*, reiterating that *R. raciborskii* was more resilient to the effects of rapid-mixing than the *D. circinale*. The estimated coefficients emphasized the greater relevance of cyanobacteria species in comparison to the experimental conditions, highlighting the importance of raw water cyanobacteria composition to the WTP operational conditions and design parameters.

Although it is known that when velocity gradient is increased, lower mixing time is needed (Lin et al., 2013; Byun et al., 2005) decreasing the hydraulic detention time and consequently the cost of WTPs, a lower velocity gradient should be pursued when high concentrations of cyanobacteria are present, even if it means increasing mixing time. In this context, the use of enhanced coagulation can be an efficient measure to compensate for these milder conditions. Using this technic, Vadasarukkai and Gagnon (2015) observed that it was possible to reduce the velocity gradient (from 750 s^{-1} to 300 s^{-1}) and energy consumption without compromising treated water quality in terms of turbidity and removal of dissolved organic matter.

3.3. Impact of experimental conditions on trichomes length

The values of trichome length mean did not present normal distribution ($p < 0.05$) by the Shapiro-Wilk test. A significant ($p < 0.05$) reduction of trichome length of *D. circinale* was observed only in the experiments E_3 and E_7 (Fig. 4) when organisms had an initial mean length above 25 μm. Trichome reduction was not significant ($p > 0.05$) for trichome with length below 25 μm. This suggests that larger organisms showed greater susceptibility to the effect of hydraulic stress (i.e., velocity gradient and mixing time). The differences between the lengths after rapid-mixing (EC_1) and after slow-mixing (EC_2) were not significant ($p > 0.05$), suggesting that the reduction effects occurred at the rapid-mixing step. Even in the experiments in which *R. raciborskii* demonstrated the highest trichome length, approximately 100 μm (E_9 and E_{10}), a significant reduction in trichome length ($p > 0.05$) was not observed (Fig. 5). In sum, the rapid-mixing conditions significantly affected both trichome length and organism integrity of *D. circinale*, but not those of *R. raciborskii*. Additionally, slow-mixing conditions did not significantly affect either species despite the longer exposure time (14 min).

The same observation was presented in a study by Pestana et al. (2019) when evaluating the effects of water treatment on different filamentous cyanobacteria genera. Because of physical and chemical stress during the full and pilot-scale treatment unit operations, *Dolichospermum* sp. was more susceptible to trichome reduction (from 63 to 73%) than *Cylindrospermopsis/Raphidiopsis* sp. (from 25 to 26% of trichome lysis). Pestana et al. (2019) justified this behavior explaining that *Dolichospermum* sp. does not show a rigid sheath and only a slight mucilaginous layer.

The reduction may occur until the trichome reaches a minimum size in which the effects of the hydraulic conditions are no longer important. Similar behavior may be also observed with flocks when they reach a minimum size for a given velocity gradient (Jarvis et al., 2005; Watanabe, 2017). This fact may explain why larger trichomes (≈25 μm) of *D. circinale* reduced while smaller ones (<25 μm) did not, as also observed by Pestana et al. (2019).

4. Conclusions

The hydraulic conditions suggested by AWWA for coagulation were associated with organism damage and trichome reductions of *D. circinale*, even with the velocity gradient close to the minimum limit (750 s^{-1}). Further, the mixing time used (60 s) may have intensified the damages during rapid-mixing. Under the same rapid-mixing conditions (750 s^{-1} during 60 s), both *R. raciborskii* organisms and trichome were not significantly affected, showing more resilience to hydraulic stresses than *D. circinale*. There are indications that *D. circinale* organisms with smaller trichomes (<25 μm) were not significantly reduced when exposed to rapid-mixing conditions. The hydraulic conditions suggested by AWWA for flocculation could be safely applied to both species with a small risk of trichome reduction, organism damage, and probable release of intracellular material, including toxic and unpleasant taste and odor metabolites. The results demonstrate the

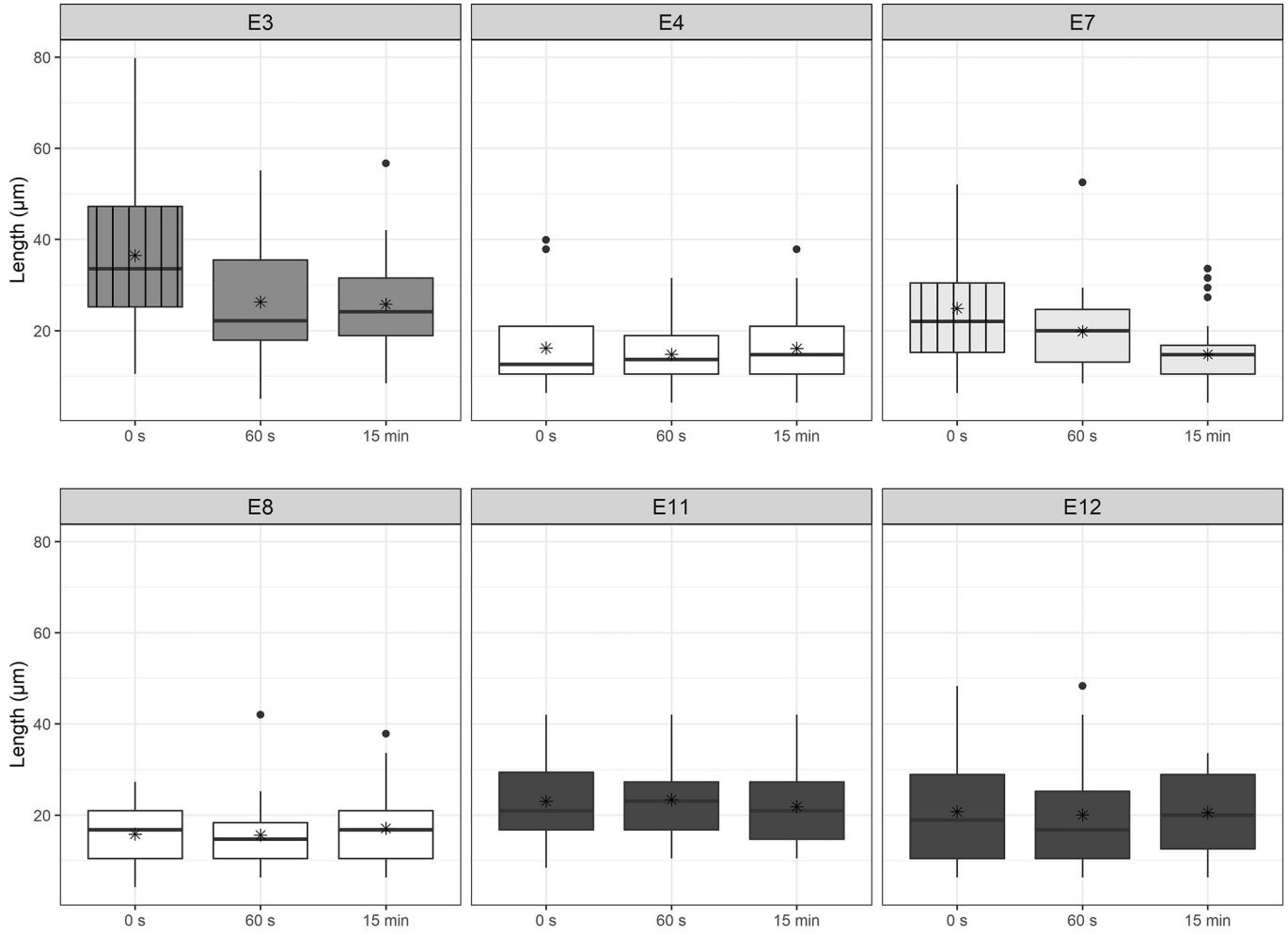

Fig. 4. Length reduction of *D. circinale* trichome due to rapid-mixing (750 s^{-1} for 60 s) and slow-mixing (70 s^{-1} for 14 min). The points represent the outliers, the asterisks represent means. Experiments with different colors represent significantly different lengths ($p < 0.05$) and boxes within one experiment with different textures are significantly different ($p < 0.05$).

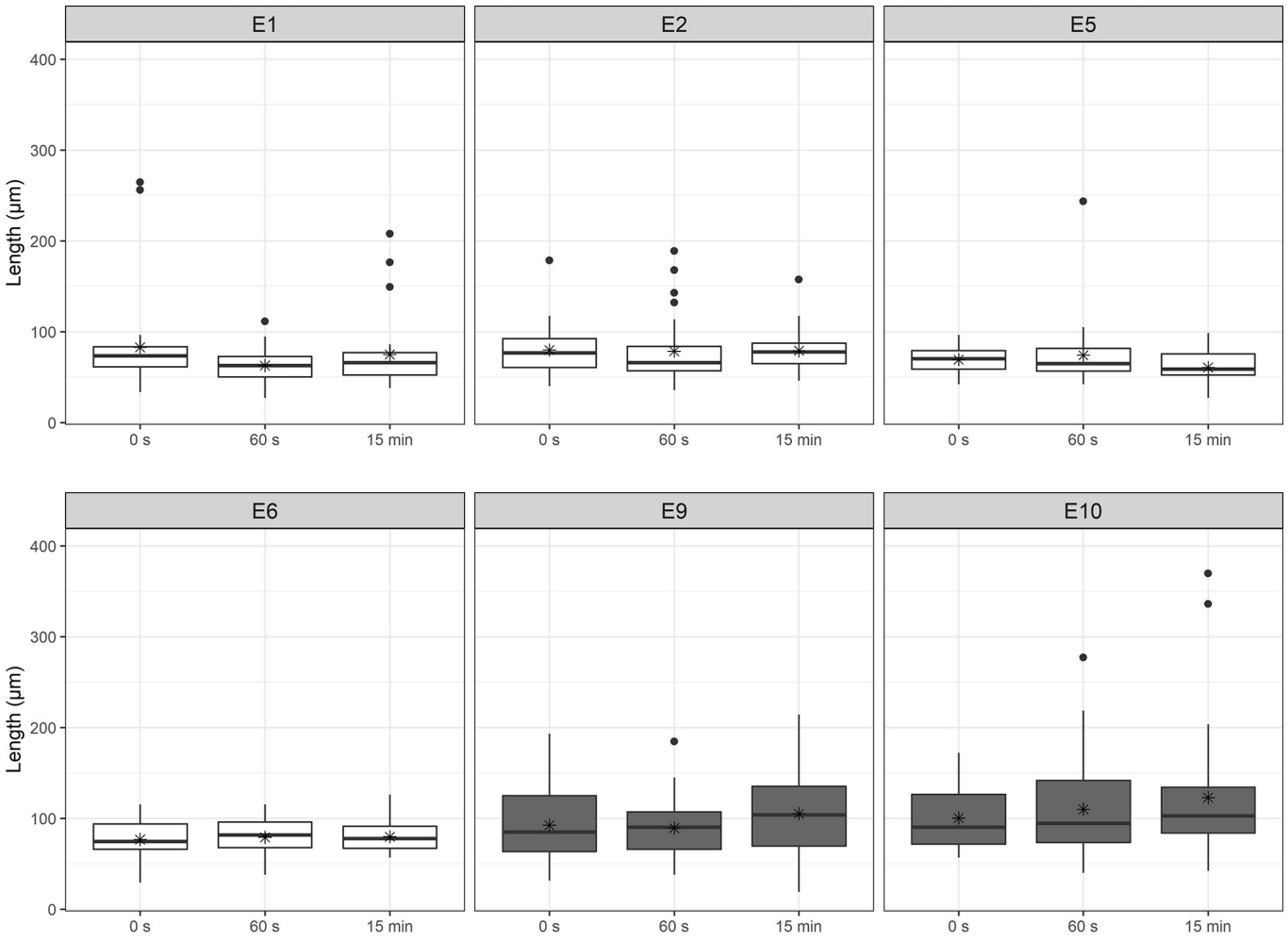

Fig. 5. Length reduction of *R. raciborskii* trichome due to the effects of rapid-mixing (750 s^{-1} for 60 s) and slow-mixing (70 s^{-1} for 14 min). The points represent the outliers, the asterisks represent means. Experiments with different colors represent significantly different lengths ($p < 0.05$).

importance of an optimal combination of velocity gradient and mixing time during water treatment operations. They also suggest that water treatment plants should be designed to be flexible enough that operators could adjust rapid-mixing conditions according to the dominant cyanobacteria species. Furthermore, organisms more sensitive to these hydraulic conditions, such as *D. circinale*, could be used as indicators to guide coagulation procedures.

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CRediT authorship contribution statement

Allan Clemente: Investigation, Formal analysis, Writing - original draft, Supervision.**Alan Wilson:**Validation, Writing - review & editing.**Samylla Oliveira:**Formal analysis, Data curation, Investigation.**Indira Menezes:**Formal analysis, Investigation.**Amanda Gois:** Formal analysis, Investigation.**Jose Capelo-Neto:**Methodology, Conceptualization, Funding acquisition, Project administration, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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