

Environmental factors affecting the distribution of Spongillidae (Porifera) in an artificial channel, in north-western Italy

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ABSTRACT

Environmental factors affecting the distribution of Spongillidae (Porifera) in an artificial channel, in north-western Italy.

The hydrographic network of the upper Po Valley (NW Italy) is characterized by the presence of numerous hydroelectric plants, often powered by artificial channels. These man-made water courses, which range from a few hundred meters to several kilometers in length, are used to convey water from the rivers to the electricity production plant. Power canals are usually characterized by concrete banks and bottoms, a geometric profile, and the absence of riparian vegetation so that they host peculiar invertebrate communities, often extremely well adapted to life in a completely artificial habitat. Sponges are an often little considered component of the freshwater macroinvertebrate fauna, whose functions and role within communities can be of considerable interest. These sessile, filter-feeding organisms can colonize artificial channels, but little is known about the local environmental characteristics that influence their distribution. In this study, we investigated the distribution of a freshwater sponge family Spongillidae, in a 4.5 km long artificial channel in Rocca Grimalda (Alessandria, NW Italy). A total of 863 specimens were localized and measured, and selected environmental characteristics were recorded. Results suggested that the distribution of sponges depends mainly on the lighting rather than the type of substrate or other local factors, so that this taxon is well-suited to colonizing artificial environments with good water quality conditions.

KEY WORDS: man-made water courses; microhabitat distribution; power canals; Spongillids.

RESUMEN

Factores ambientales que afectan a la distribución de Spongillidae (Porifera) en un canal artificial del noroeste de Italia.

La red hidrográfica del alto Po (noroeste de Italia) se caracteriza por la presencia de numerosas centrales hidroeléctricas, a menudo alimentadas mediante el uso de canales artificiales. Los canales hidreléctricos, que varían de unos cientos de metros a varios kilómetros, se utilizan para transportar el agua desde los ríos hasta las centrales y suelen caracterizarse por orillas y fondos de hormigón, un perfil geométrico y la ausencia de vegetación ribereña. Estos canales albergan comunidades de invertebrados peculiares, a menudo muy adaptados a la vida en un hábitat completamente artificial. Las esponjas son un componente muy poco considerado de la fauna de macroinvertebrados de agua dulce, cuyas funciones y papel dentro de las comunidades pueden ser de considerable interés. Estos organismos pueden colonizar diferentes hábitats, incluido canales artificiales, pero se conoce poco sobre las características ambientales locales que pueden influir en su abundancia y distribución. En este estudio analizamos la distribución de una familia de esponjas de agua dulce, en un canal artificial de 4.5 km de longitud en Rocca Grimalda (Alessandria, al noroeste de Italia). Se localizaron y midieron 863 ejemplares y se registraron las mas importantes características ambientales. Se observó que la distribución de las esponjas depende principalmente de la iluminación más que del tipo de sustrato u otros factores locales, por lo que esta especie es muy adecuada para colonizar entornos artificiales con buenas condiciones de calidad del agua.

PALABRAS CLAVE: cursos de agua artificiales; distribución del microhábitat; canales hidroeléctricos; esponjas de agua dulce.

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INTRODUCTION

The complex hydrographic network of north-western Italy is characterized by several natural and semi-natural rivers, streams, and creeks, but also by numerous strongly altered stretches and completely artificial canals (Rossetti *et al.*, 2009; Castaldini *et al.*, 2019). In this area, human activities often lead to the depletion of freshwater biota and environmental degradation. However, biological communities have shown great plasticity and adaptation abilities. Macroenthic communities, in particular, can thrive and spread using multiple strategies. For instance, certain taxa demonstrate a remarkable physiological capacity to adapt to morphological and chemical alterations of streams and rivers (Bo *et al.*, 2006; Bo & Fenoglio, 2011; Laini *et al.*, 2019), whereas others exhibit significant resilience to hydrological fluctuations (Mencio & Boix, 2018).

In freshwater studies, sponge assemblages are a group of neglected organisms (Manconi & Pronzato, 2016; Lo Giudice & Rizzo, 2023) and artificial channels are often completely unstudied (Clifford & Heffernan, 2018).

Sponges (order Spongillida, Manconi & Pronzato, 2002) are a more important group than normally assumed, because they also increase invertebrate biodiversity and micro-spatial variability *e.g.*, acting as a shelter for different microorganisms (Manconi & Pronzato, 2008; Ruengsawang *et al.*, 2022). Freshwater Porifera (Spongillida) are usually associated with a wide variety of aquatic organisms including bacteria, algae, protists, hydrozoans, nematodes, rotifers, insects, and crustaceans (Skelton & Strand, 2013; Manconi & Pronzato, 2008, 2015; Ruengsawang *et al.*, 2022). Among the associated organisms, few are spongivorous, because sponges possess effective chemical and physical defenses.

Amidst the hosts of sponges, larvae of *Sisyra* spp. (Planipennia, Sisyridae) and caddisflies of the genus *Ceraclea* (Trichoptera, Leptoceridae) have been reported (Gugel, 2001; Skelton

& Strand, 2013; Ruengsawang *et al.*, 2022). The spongivorous caddisflies take advantage of the physical defenses of their prey by reinforcing their silken cases with spongy spicules (Skelton & Strand, 2013; Ruengsawang *et al.*, 2022). Larvae of Chironomidae (Insecta, Diptera), often found on and in sponges, could be both their inhabitants and predators. Moreover, freshwater sponges, Chironomidae and Culicidae (Insecta, Diptera) are highly adaptable to environmental alterations caused by climate change: this makes certain species such as *Aedes aegypti*, potential vectors for the global dissemination of tropical diseases (Trájer, 2021) and sponges their ideal hideaway. Larvae of *Hydropsyche* sp. and *Ecnomus tenellus* (Insecta, Trichoptera), both occasional sponge consumers, are reported to be more abundant in sites with sponges (Gugel, 2001; Gaino *et al.*, 2004; Ruengsawang *et al.*, 2022). Moreover, a study carried out in Poland also reported a rich association of Oligochaeta living on *S. lacustris* (Kahl & Konopacka, 1981). The species of Oligochaeta *Stylaria lacustris* and *Nais* sp. were occasionally found associated with sponges in the River Rhine, but no strong host specificity was observed (Gugel, 2001, Rota & Manconi, 2004). Bryozoans, as sessile filter-feeders, are the main competitors of sponges in space, but sponges often overgrow and eliminate them (Vohmann *et al.*, 2009). Freshwater mussels are competitors of sponges too; in order to complete their life cycle they need to find a suitable substrate to attach, thus competing with other sessile organisms (Molloy *et al.*, 1997). Sponges, however, can over-grow and kill the mussels by interacting with their normal feeding and respiration activity (Lauer & Spacie, 2000; Gaino, 2005).

For example, *Spongilla lacustris* (Linnaeus, 1759) is one of the most common freshwater sponge (Porifera, Spongillida, Spongillidae) species in Europe (Pronzato & Manconi, 2001). The Latin word “lacustris”, means “related or associated with lakes and ponds”, though this species can also be found in several lotic environments. Fur-



Figure 1. Map of the study site; location of the hydroelectric channel and its inlet and outlet. *Mapa del sitio de estudio, localización del canal hidroeléctrico con derivación y retorno.*

thermore, this sponge has also been occasionally reported in artificial habitats such as channels and reservoirs (Manconi & Pronzato, 2008). In freshwater and brackish ecosystems, *S. lacustris* often grows under logs, rocks, and cobbles, or on reeds, rocks and artificial substrates, showing a wide range of habitat preferences (e.g., shallow and clear waters), usually in shaded environments but also, if in symbiosis with green algae, in stretches more exposed to sunlight. This species reproduces both sexually and asexually, and become dormant during winter. The growth forms range from encrusting to digitate or branched, depending upon the habitat characteristics (Manconi & Pronzato, 1991; 2008). *Spongilla lacustris* is a filter feeding organism, which mainly consumes small floating particulate organic matter (FPOM) and dissolved matter, but this species often hosts zoochlorellae, green algae which strongly enhance their host growth and are responsible for their green color (Hall et al., 2021). It is known that when sponges are in poor light conditions, they are able to digest their host and turn pale again (Frost, 1980; Cleary et al., 2023). Moreover, populations of *Spongilla lacustris* and *Ephydatia muelleri* (Lieberkühn, 1856) are reported to be able to survive severe summer droughts in their habitats (Evans & Montagnes, 2019) by producing gemmules (Pronzato

& Manconi, 1995; De Santo & Fell, 1996; Manconi & Pronzato, 2015, 2016).

Although sponges have been poorly studied in hydroelectric channels, they are key organisms due to their ability to serve as microhabitats and food sources for many other macroinvertebrate species (Manconi & Pronzato, 2015; Ruengsawang et al., 2022). Artificial canals are often considered to be environmentally homogeneous and devoid of biological relevance. However, they constitute a significant portion of the surface water network of a region and can, therefore, be a valuable subject for research on regional biodiversity. The aim of this paper is to evaluate the potential adaptability of freshwater sponges within artificial systems, addressing only the family Spongillidae, because of the inability to exactly identify each specimen without the analysis of skeletal spicules morphs in light microscopy slides.

MATERIALS AND METHODS

Field

This study was carried out in an artificial channel built for hydroelectric purposes during summer 2020. This region is characterized by hot

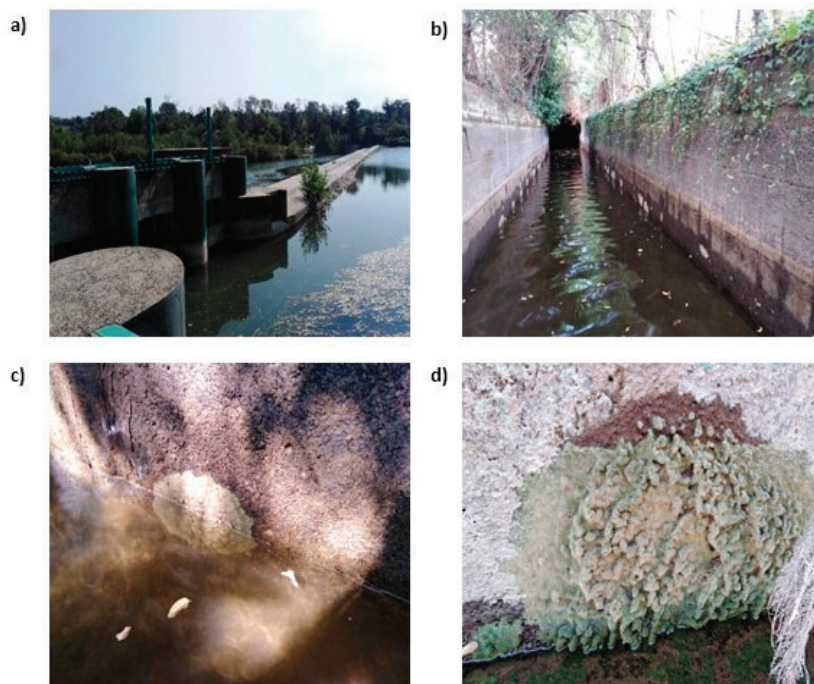


Figure 2. Photos of the channel inlet (a) and view of the artificial channel (b). In (c) and (d) a representation of the freshwater sponges surveyed in this artificial habitat. *Fotos (a) de la derivación del canal y (b) vista del canal. En (c) y (d), vista de las esponjas de agua dulce observadas en este hábitat artificial.*

and dry summers, typically sunny and with very few precipitations occurring during this season. The channel originates from the Orba stream (44°39'20" N 08°39'09" E – 160 AMSL) in Rocca Grimalda (North-Western Italy) and, after 4.5 km, it returns the water into the same stream (44°31'30" N 08°40'08" E – 142 AMSL; Fig. 1). The average width of the channel is approximate-

ly 3.5 m, while the medium water depth is 1.1 m, and a medium slope around 0.4%; as a whole, it has a rectangular cross-section and is mainly made up of both smooth and irregular concrete walls, bricks, and to a lesser extent aggregates of gravel deposits. No boulders or cobbles are present at the bottom, only locally there are small deposits of gravel and pebbles. The channel runs in a tunnel for 800 m long, while the remaining part flows out into the open air (Fig. 2 a and b) and is annually maintained and cleaned in its entirety (both in the tunnel and in the open air). From a chemical point of view, the water is good and does not present particular critical issues (Table 1).

During a temporary and total channel dry-out, sponge distribution was examined and analyzed along the entire system focusing on 30 transects, each extending for 10 m, spaced at 150 m apart, for a total of 4650 m of sampled area. For each sponge found in the channel (Fig. 2 c and d), its position was noted (specified as bottom, right side, or left side), and its height (compared to the bottom level). The specimens were also measured

Table 1. Main chemical and microbiological parameters of artificial channel water (mean \pm SD). *Principales parámetros químicos y microbiológicos del agua (media \pm DE).*

Variable	Mean	SD
pH	8.24	0.75
Conductivity (μ S/cm)	259.25	48.40
NH ₃ (mg/L)	0.20	0.21
N-NO ₂ (mg/L)	0.08	0.02
N-NO ₃ (mg/L)	<0.5	nc
COD (mg/L)	11.50	1.73
BOD5 (mg/L)	5.50	0.71
P tot (mg/L)	0.75	0.21
DissolvedOx (mg/L)	94.73	1.01
<i>E.coli</i> (cfu/mL)	551.75	424.89

for their maximum width (863 measurements), obtaining a total of 22 average width measures because in eight of the transects no sponges were found. Furthermore, a perpendicular photo was taken to evaluate the degree of shading in every transect, and the material constituting the channel substrata was evaluated, selecting four main categories, i.e., 1) smooth concrete, 2) irregular concrete, 3) brick, 4) gravel aggregate deposits. Pictures taken in the field, analyzed using the image processing software ImageJ (Schneider et al., 2012), were used to estimate vegetation coverage percentage, assessing the area above the transects that was covered by tree branches, leaves and vegetation in general.

Data analysis

We used Cleveland dot plots to identify outliers, and removed the abundance value of one sponge. Since our data were not normally distributed, differences between number of sponges observed and each environmental variable were tested with Kruskal-Wallis tests (H). Correlations between number and average dimensions of sponges and coverage percentage have been tested using Spearman's correlation tests (ρ).

All analyses were performed in R (R core Team 2019) and plots were drawn using the packages ggplot2 (Wickham, 2016) and ggpvr (Kassambara, 2017).

RESULTS

In total, we found, located and measured 863 Spongillidae specimens in 300 m of examined channel. The average width of the sponges was 16.27 cm (± 19.39 SD). No statistical significances were found when looking at the number of sponges observed in the four categories of substrates (Kruskal-Wallis test, $H = 6.37$; $df = 3$; $p = 0.095$; Fig. 3a).

When looking at the sponges' position along the channel, non-significant differences were found among sponges' number observed on the bottom, right or left bank (Kruskal-Wallis test, $H = 1.42$; $df = 2$; $p = 0.49$; Fig. 3b).

Coverage percentage (Spearman correlation test (ρ) = 0.194; $p = 0.068$; Fig. 4a) and coverage

category (Kruskal-Wallis test, $H = 5.95$; $df = 3$; $p = 0.11$; Fig. 4b) showed no significant results..

When looking at sponge average width, a statistical significance was found between this value and substrate type (Kruskal-Wallis test, $H = 14.406$; $df = 3$; $p = 0.0024$; Fig. 5a) while vegetation coverage percentage (Spearman correlation test, (ρ) = 0.146; $p = 0.170$; Fig. 5b) showed no statistically significant correlation with this parameter. These results highlight that the observed environmental conditions can influence this family's growth, even if a strong statistical correlation has not been found.

DISCUSSION AND CONCLUSIONS

Results concerning the distribution of Spongillidae in an artificial channel revealed that this family exhibited considerable ubiquity. No correlation was found between the sponges' abundance and substrate types, thus, this population proved to have no preferences regarding the artificial channel's constituent material. Furthermore, the sponges' topographic distribution along the channel was found to be of no statistical significance in explaining the number of sponges observed. Therefore, it is possible to hypothesize that Spongillidae are able to equally colonize the channel bed (where, before drying, water was certainly present) and the two channel banks, where water level fluctuations could have been more pronounced. This result confirms that this family could be able to survive even if not completely covered by water, making it more resistant in the case of sudden droughts in preserved natural habitats as well (Manconi & Pronzato, 2016). Specifically, in the investigated artificial channel, flowing water was present all year round, and 2-3 days planned droughts occur only during hydroelectric plant maintenance. In this context, the literature reports that sponges in general are able to survive temporary environmental challenges (Harrison, 1974) producing gemmules, whose cells are in a state of metabolic arrest called diapause, controlled by endogenous factors (Loomis, 2010) and also by dormancy (Calheira et al., 2020; Paix et al., 2024). As a result, freshwater sponges have a particular life cycle, where diapause and quiescence enable these organisms to survive in

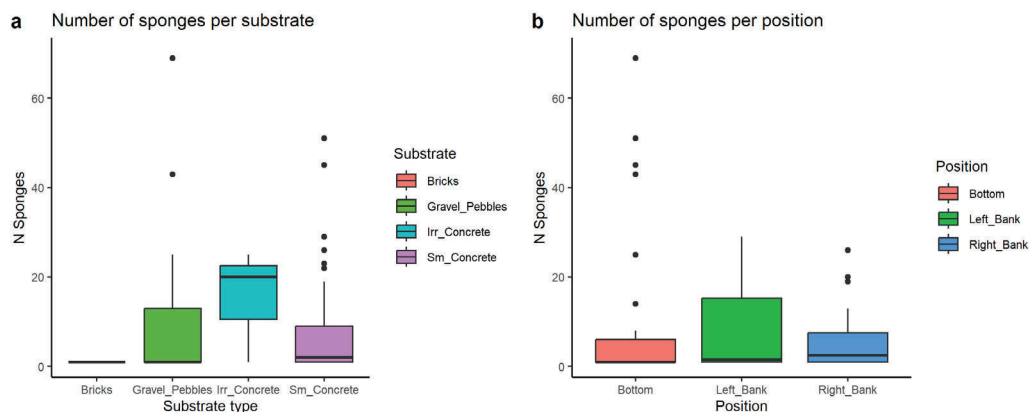


Figure 3. Boxplots assessing the number of sponges found on each substrate (a) and in each position (b). *Diagramas de caja que evalúan el número de esponjas encontradas en cada sustrato (a) y en cada posición (b).*

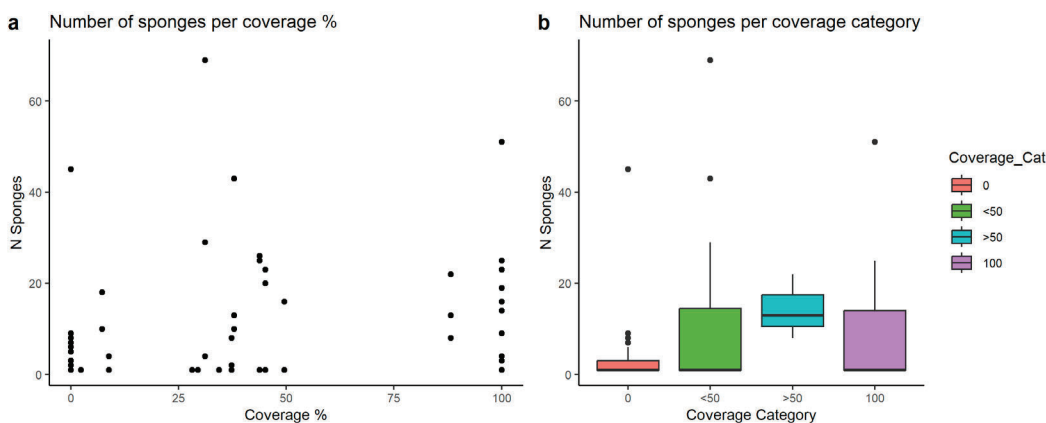


Figure 4. Graph assessing the correlation between number of sponges and vegetation coverage percentage (a) and boxplot assessing the number of sponges per coverage category (b). *Gráfico que evalúa la correlación entre el número de esponjas y la porcentaje de cobertura vegetal (a) y diagrama de caja que evalúa el número de esponjas y la categoría de cobertura (b).*

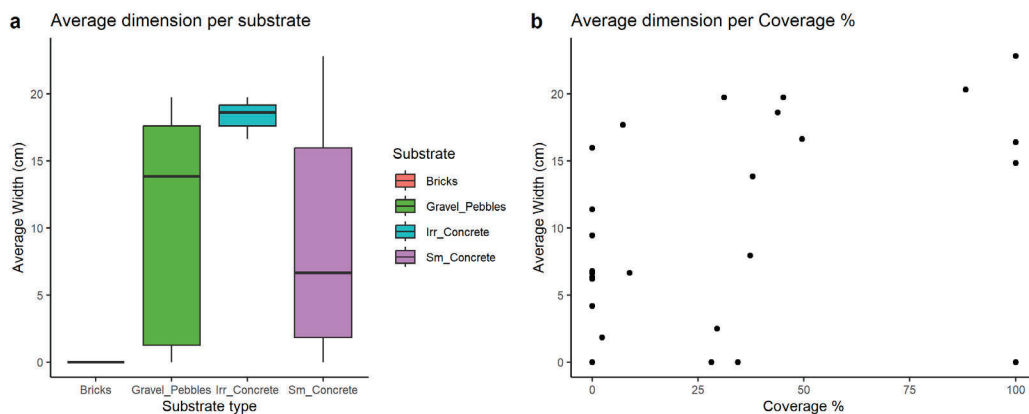


Figure 5. Boxplot assessing the average width of sponges in correlation with type of substrate (a) and graph assessing the correlation between average width of sponges and vegetation coverage percentage (b). *Diagrama de caja que evalúa la anchura media de las esponjas en correlación con el tipo de sustrato (a) y gráfico que evalúa la correlación entre la anchura media de las esponjas y el porcentaje de cobertura vegetal (b).*

unfavorable environmental conditions (Rasmont, 1954; Pronzato & Manconi, 1995).

Results also showed that this family prefers habitats in which a certain shading effect of vegetation coverage is present, due to fewer temperature changes and thus, increased moisture. In fact, the number of sponges was higher in those transects where vegetation covered at least 30% of the channel (Fig. 4), being more abundant and in wider clusters in shaded sites. This result highlights the fact, supported by literature, that Spongillidae have two living forms, namely symbiotic, where the association with zoochlorellae, diatoms and cryptophytes gives them a characteristic green color (Jensen & Pedersen, 1994; Trautman & Hinde, 2001), and aposymbiotic, where the pale or hash-brown color is determined by the lack of association with green algae (Frost & Williamson, 1980). The usual sponges' preference for shaded habitats, here, can be justified by the fact that in the investigated artificial channel, tunnels were present for 800 m long, providing a suitable and extended shaded area. Moreover, these areas were more humid and cooler with respect to the transects in the open, hotter areas that were exposed to the sunlight at different percentages. All these factors could have influenced the observed taxon, adapting it to an artificial habitat, exploiting the transects where life and growth were possible without necessarily undergoing diapause or quiescence.

When looking at the single sponges' dimensions, data highlight the fact that the average width was statistically correlated with two of the observed environmental variables (i.e., substrate type and coverage percentage of the vegetation). This result implies that larger colonies of this family are mainly located on irregular concrete, preferring rougher, irregular substrates which allow *in situ* persistence during floods, avoiding gemmules displacement, rather than smooth ones in an artificial environment. Furthermore, the sponges located in shady tracts of the channel appeared to be of larger dimensions when compared to the ones in stretches more exposed to sunlight. In accordance with previous results, Spongillidae showed a preference for irregular, moist, and shaded habitats, not only in natural conditions as reported by literature, but also in artificial envi-

ronments such as canals for hydroelectric purposes. However, this family is also able to develop in full sunlight (see Fig. 4b), because of the symbiosis with zoochlorellae, diatoms or cryptophytes which photosynthesize and improve the sponges' growth in sunny habitats.

In conclusion, our results show that Spongillidae are able to spread and thrive in a wide range of habitat conditions: from natural to artificial ones, on different substrates and in varying illumination conditions and also during drying phases. The potential adaptation to particular habitats makes this freshwater family highly adaptable and ubiquitous, as long as the environmental conditions (especially water quality) are good.

This work, though small, has given interesting insights regarding the distribution of freshwater Spongillidae in a completely artificial habitat. Since there is a lack of data and information regarding this particular family in Italy, our work can be a useful starting point for future studies addressing these topics. Further work is certainly needed; new molecular techniques such as DNA metabarcoding and metabolomics could be helpful to better understand the species' symbionts and metabolites involved in environmental stress responses.

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AUTHOR CONTRIBUTIONS

L. G.: Investigation, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **S. F.:** Writing – review & editing, Supervision, Validation. **M. M.:** Writing – original draft, Supervision. **T. B.:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project Administration, Writing – original draft, Writing – review & editing.

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