Vol. 32: 13–19, 2023
https://doi.org/10.3354/ab00758

AQUATIC BIOLOGY
Aquat Biol

Published March 23

NOTE

Microplastic contamination reduces productivity in a widespread freshwater photosymbiosis

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ABSTRACT: Microplastic (plastic particles <5 mm in size) contamination is ubiquitous in nature and known to interact with organisms ranging from microbes to mammals. Notably, recent studies have shown that microplastics may interfere with photosymbiosis, an ecologically important association that has suffered pronounced recent declines in the face of contemporary climate change. However, limited findings thus far have largely focussed on select marine associations. Whether freshwater photosymbioses may also be affected remains poorly understood. Here, I aimed to help bridge this gap by asking whether microplastic contamination impacts several traits (growth rate, symbiont density, metabolic rate and feeding rate) in a common, widespread freshwater photosymbiosis, the *Paramecium bursaria–Chlorella* spp. association. To address how productivity, an important ecosystem service provided by photosymbiosis globally, could be affected, I also measured changes in photosymbiotic net productivity (net photosynthesis rate). To do so, I exposed the symbiosis to microplastics (microbeads extracted from commercial face wash) under laboratory conditions. My key result was that, compared with non-contaminated control cultures, the contaminated symbiosis demonstrated lower net productivity. This response raises concern for primary production rates in freshwater ecosystems contaminated with microplastics, adding to an established story of widespread degradation associated with microplastic pollution globally.

KEY WORDS: Microplastics · Symbiosis · Mutualism · Photosymbiosis · Productivity · Photosynthesis · Freshwater

1. INTRODUCTION

Microplastics (plastic particles <5 mm in size) are ubiquitous in nature and interact with organisms ranging from microbes to mammals (Kettner et al. 2019). Much research has focussed on marine contamination; impacts on freshwater ecosystems are comparatively poorly understood (Wagner et al. 2014, Issac & Kandasubramanian 2021). Furthermore, elucidating how impacts on individual species may translate into ecological impacts at the community and ecosystem levels remains an ongoing mission (Bucci et al. 2020, Nava & Leoni 2021). Accordingly, interest is growing regarding the impacts of microplastics on symbiosis, an important association globally (Huang et al. 2021).

Though still rarely reported (Huang et al. 2021), limited research has documented a range of impacts of microplastic contamination on photosymbiosis, an ecologically critical association responsible for approximately half of marine photosynthesis that has suffered pronounced recent declines in the face of contemporary climate change (Baker et al. 2008, Bailly et al. 2014). Such impacts include modified physiology, energetics, growth, health, feeding behaviour, photosynthetic performance, energy expend-

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Publisher: Inter-Research · www.int-res.com

iture, skeletal calcification, bleaching and necrosis (Huang et al. 2021). However, findings have thus far largely been confined to corals, with inconsistent and often contradictory results, suggesting that further work is required (Soares et al. 2020, Huang et al. 2021). Crucially, despite the omnipresence of photosymbioses in freshwater (Zagata et al. 2016), research on the impacts of microplastics on freshwater photosymbioses appears to be limited. A notable recent study investigated a plant–microbiome interaction (O'Brien et al. 2022), but research on microbial freshwater photosymbioses, which can be widespread in nature (Minter et al. 2018), is scarce. Furthermore, the study by O'Brien et al (2022) did not report on impacts on primary production.

Here, I aimed to help bridge this gap by asking whether microplastic contamination impacts a common freshwater microbial photosymbiosis, the *Paramecium bursaria–Chlorella* spp. association. This association was chosen as it is a microbial example of a 'classic' symbiosis that is both widespread and tractable (Minter et al. 2018) and could therefore be a useful model to understand the impacts of microplastics on photosymbiosis. Each heterotrophic ciliate *P. bursaria* cell contains *Chlorella* spp. algal symbionts, with the partners typically thought to be engaged in a mutualistic interaction whereby the symbionts provide the host with photosynthates while hosts, in return, provide nitrogenous compounds derived from their bacterivory (i.e. consumption of prey) (Lowe et al. 2016, Minter et al. 2018). *P. bursaria* is also known to modify symbiont density (i.e. the number of symbionts within the host cell) to maximise its own fitness in response to environmental change, which can be suggestive of distortion of the mutualism towards parasitism (Lowe et al. 2016). This means that host bacterivory, symbiosis metabolism, and symbiont density are likely to be informative for the maintenance of this mutualism and could shed light on the impacts of microplastics on microbial photosymbiosis.

In this work, I tested the impacts of microplastic contamination on freshwater photosymbiosis by exposing *P. bursaria–Chlorella* spp. to microplastic particles (microbeads extracted from commercial face wash) under laboratory conditions. Following exposure, I assessed a number of traits likely to be important for symbiosis maintenance and ecosystem functioning (growth rate, symbiont density, metabolic rate and host heterotrophy [bacterivory rate]) and compared my findings with measurements in non-contaminated control cultures to investigate the impacts of microplastics on freshwater photosymbiosis. My predictions regarding the effects of the microbeads on the symbiosis were as follows: (1) photosymbiosis growth rate would be modified, suggestive of changing fitness; (2) host heterotrophy and/or symbiont productivity rates would change, potentially suggestive of altered host–symbiont dynamics (e.g. of degradation of mutualism towards parasitism and/or impacted metabolite trade); and (3) net productivity of the symbiosis would change, potentially flagging important concerns for global productivity rates should it decline (Bronstein 1994, Bailly et al. 2014, Lowe et al. 2016, Minter et al. 2018).

2. MATERIALS AND METHODS

2.1. Cultures and microplastics

The strain used in this study (HA1g; National BioResource Project) was originally isolated in Hirosaki, Japan, in 2010. Cultures were kept under a 12 h light:12 h dark cycle (~75 µmol photosynthetically active radiation m^{-2} s^{-1}) at 25°C in protozoan pellet-mineral water medium and were lightly shaken daily. A total of 3 contaminated (containing microbeads) and 3 control (not containing microbeads) cultures (i.e. N = 6 experimental units) were established at an initial *Paramecium bursaria* density of ~100 ml^{-1} and allowed to grow for 7 d. Measurements were taken on Day 0 and Day 7.

The microplastics used in this study were extracted from a commercial facewash containing microbeads that was readily available at the time of study planning. Microbeads were extracted by first observing them under the microscope, spinning down the suspended microbeads and washing in sterile mineral water repeatedly, then transferring them via micropipetting to a fresh mineral water stock. The 3 contaminated cultures were established using the same volume (100 µl) of the same single stock of microbeads to standardise the contamination level, that is, the density of microbeads (approximately 10^3 l^{-1}, similar to the highest microbead density detected across freshwater samples in one survey, therefore representing a naturally relevant level; Castañeda et al. 2014); the same volume of mineral water (without microbeads) was added to 3 control cultures. Although beyond the scope of my work, for the interested reader, the chemical and physical properties of microbeads extracted from commercial facewash have been studied elsewhere (Möhlenkamp et al. 2018). Microbeads in commercial facewash were chosen since, despite representing a subset of microplastic morphologies relevant

to the environment, they remain of major concern to both wildlife and humans (Sharma et al. 2022); indeed, a recent study investigated the impacts of microbeads originating from commercial facewash on freshwater snails (Wang et al. 2022).

2.2. Symbiont density

Symbiont density — the concentration of algal symbionts within the host cell — was estimated on Day 7 by using an established proxy (Minter et al. 2018): host fluorescence intensity. To acquire intensity measurements, each of the 6 cultures (i.e. 3 contaminated cultures and 3 control cultures) was photographed at 10× using fluorescence microscopy; these images were analysed for mean fluorescence intensity using the 'Mean Gray Value' parameter obtained with the 'Analyze Particles' base function in imageJ (Schneider et al. 2012).

2.3. Bacterivory rate

For bacterivory (the rate of host consumption of prey bacteria) estimates, the 6 cultures were plated on Luria-Bertani agar and the number of colony-forming units (CFUs) of bacteria per ml were recorded after 0 and 7 d. The number of bacteria consumed per host at each temperature was then estimated by calculating the reduction in CFUs ml^{-1} across the 1 wk period. Six additional control cultures (i.e. 3 containing microbeads, 3 not containing microbeads), established in the same way as outlined above but not containing *P. bursaria*, were used to adjust for bacterial growth: the 'control' mean change in CFUs ml^{-1} was subtracted from treatment estimates (this was done according to whether microbeads were present, i.e. the mean change in CFUs ml^{-1} in *P. bursaria*-free contaminated cultures was used to control for bacterial growth for the bacterivory estimates from the *P. bursaria*-containing contaminated cultures). CFUs consumed ml^{-1} were then divided by mean *P. bursaria* cell counts ml^{-1}.

2.4. Growth rate

Cell density was estimated manually using microscopy, whereby each of the 6 experimental cultures was sampled on Day 0 and Day 7 and counted following fixation with 3% formaldehyde in a 96-well plate (i.e. 6 measurements in total). Growth rate was estimated by using the formula for specific growth rate ($\mu\ d^{-1}$), $\mu = \ln(N_1/N_0)/\Delta T$, where ln is natural logarithm, N_1 is the final cell density (on Day 7), N_0 is the initial cell density (on Day 0) and T is time (in days).

2.5. Metabolism

To characterise metabolic responses, I measured net photosynthesis (NP) (via oxygen evolution at different light intensities) and respiration *(R)* (via oxygen evolution in the dark) in 1 ml aliquots of each experimental culture in both the contaminated and non-contaminated conditions prior to the end of the experiment (i.e. 6 measurements in total). Oxygen evolution measurements were conducted using a Clark-type oxygen electrode (Hansatech; Chlorolab). *R* was estimated as the rate of change of oxygen concentration (i.e. via uptake by respiring organisms) in the dark. NP was measured at increasing light intensities in intervals of 50 $\mu mol\ m^{-2}\ s^{-1}$ (photosynthetic photon flux density; PFD) up to 200 PFD, and then in intervals of 100 PFD up to 1000 PDF, and finally at 1200, 1500 and 1800 PDF. This yielded a photosynthesis–irradiance curve at each assay temperature; these curves were fitted to a photoinhibition model using non-linear least squares regression (following the methodology and statistical methods of Padfield et al. 2016). The maximum oxygen evolution in the light (i.e. at the optimum light intensity) was taken as the maximum NP (P_{max}). I used P_{max} to control for any potential interactions between light intensity and any presence of undetected microplastic contamination (e.g. due to shading) in measuring NP. Gross photosynthesis (GP) was then estimated as:

$$GP = P_{max} + R \text{ (absolute)} \quad (1)$$

The metabolic rates were controlled for population size to enable meaningful comparison between treatments by dividing by mean holobiont cell density (estimated manually using the same methods described for the growth rate measurements above).

2.6. Statistical procedures

Each measured trait (holobiont intensity, bacterivory rate, growth rate, *R*, GP and NP) was analysed using a general linear model (GLM) in R statistical software v.4.2.1 (R Core Team 2022) created for each of the traits. In each model, the trait was the dependent variable and the presence/absence of microbeads was the explanatory variable (n = 3 contami-

nated replicates were compared with n = 3 controls for each measured trait; N = 6 experimental units in total). Model assumptions were tested with the diagnostic plots available using the 'plot' function in R. The quantile–quantile plot indicated that the normality of residuals assumption was met; the (1) residuals vs. fitted, (2) scale-location and (3) residuals vs. leverage diagnostic plots were also viewed to check that GLM regression assumptions were met. No data transformations were used based on these test results. Each model was compared with a null model created for each trait using ANOVA to uncover whether microplastic presence/absence significantly impacted the measured traits. A significance level of 0.05 (5%) was used.

3. RESULTS

Holobiont intensity, bacterivory rate and specific growth rate did not differ significantly between contaminated and non-contaminated cultures ($F_{4,5} = 0.024$, p = 0.886, Fig. 1a; $F_{4,5} = 0.057$, p = 0.823, Fig. 1b; and $F_{4,5} = 0.143$, p = 0.725, Fig. 1c, respectively).

Rates of R ($F_{4,5} = 0.012$, p = 0.917; Fig. 2a) and GP ($F_{4,5} = 6.514$, p = 0.063; Fig. 2b) did not differ significantly between contaminated and non-contaminated cultures, but NP was significantly lower in contaminated cultures ($F_{4,5} = 25.796$, p = 0.007; Fig. 2c). Notably, GP was numerically lower with microplastic contamination (Fig. 2b).

4. DISCUSSION

My key finding is that while symbiont density, bacterivory rate and growth rate all appeared unaffected compared with non-contaminated control cultures, the contaminated symbiosis demonstrated a lower NP. Photosymbiosis is known to play a major role in global productivity (Johnson 2011, Bailly et al. 2014); therefore, this decline in photosymbiotic productivity observed in response to contamination with microplastics warrants further research.

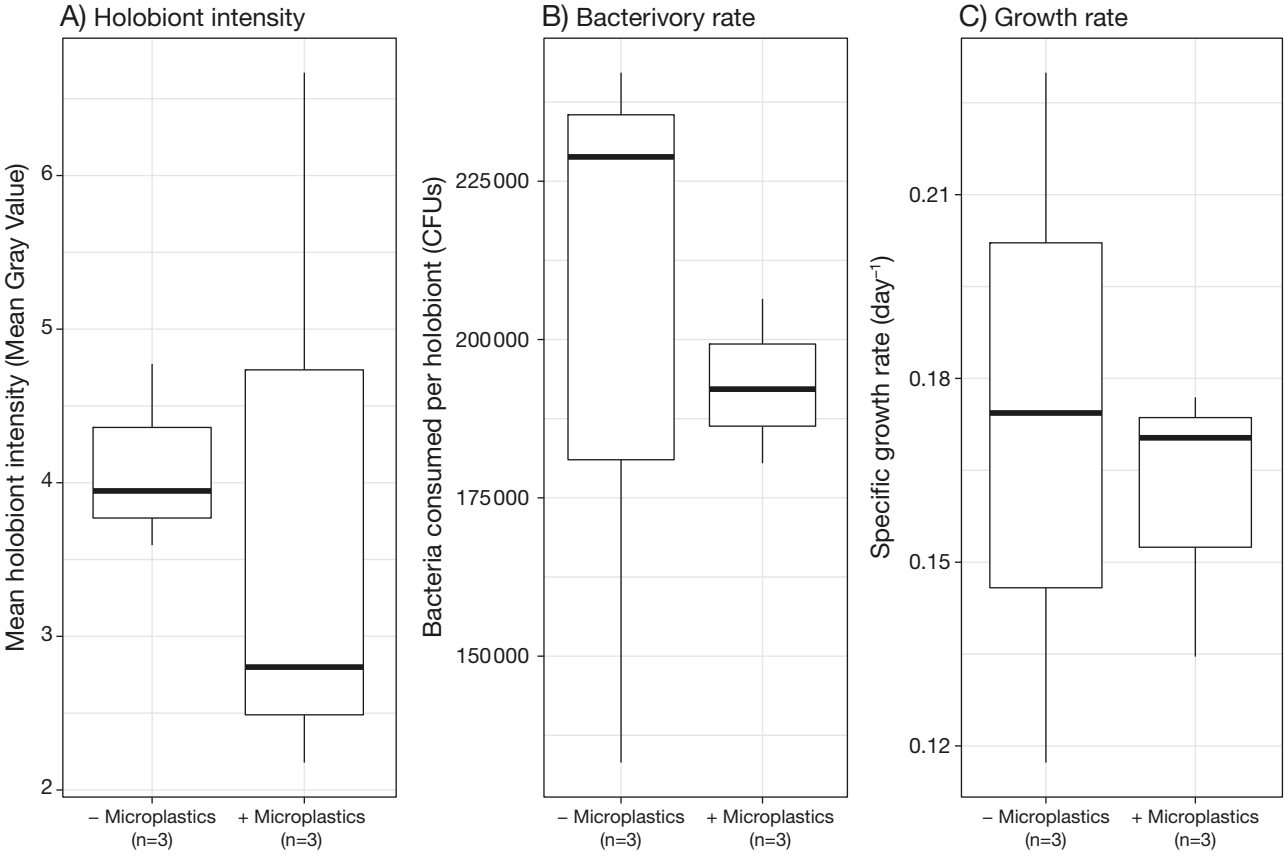

Fig. 1. Various traits of the *Paramecium bursaria–Chlorella* spp. photosymbiosis: (A) holobiont fluorescence intensity (Mean Gray Value as analysed in ImageJ), a proxy for symbiont density within hosts; (B) holobiont bacterivory rate (colony-forming units [CFUs] of bacteria consumed); and (C) holobiont specific growth rate (d^{-1}). Boxplots show median, interquartile range, and minimum and maximum values

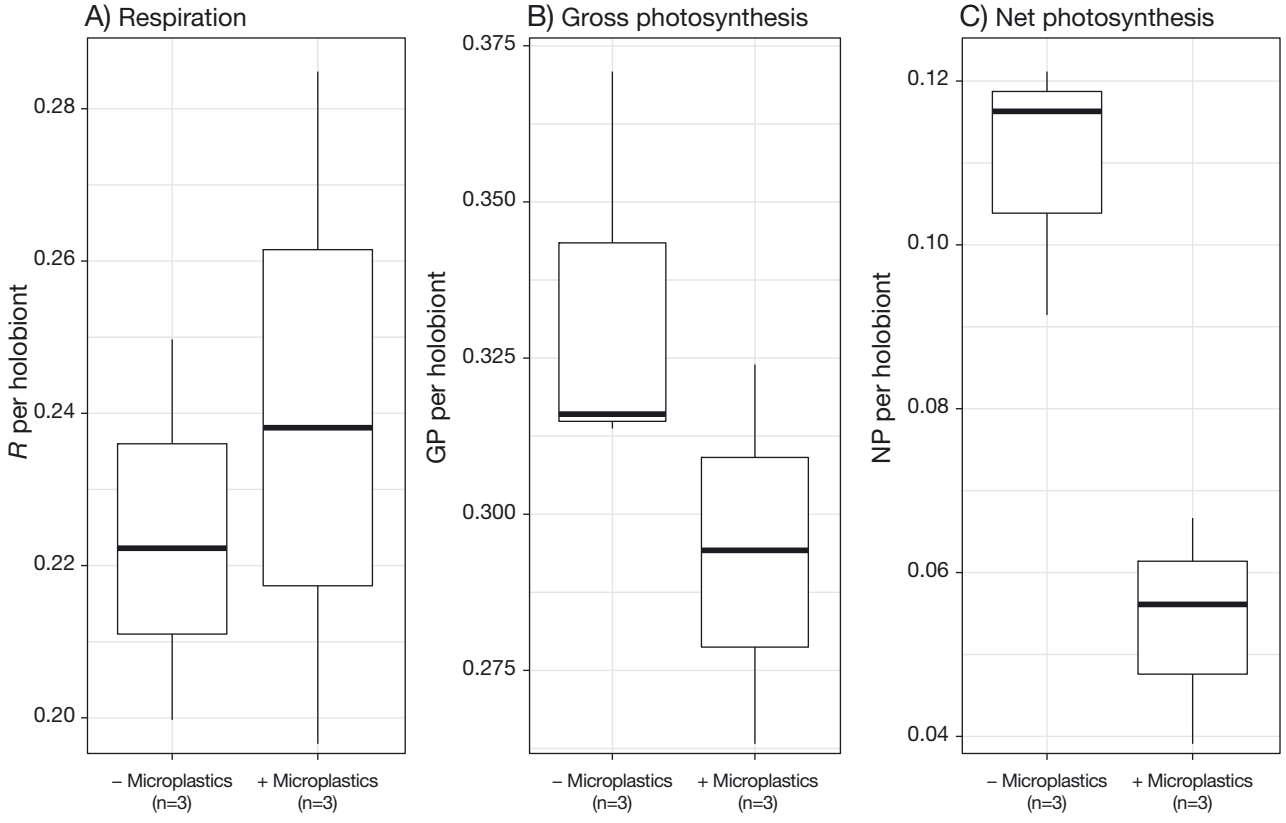

Fig. 2. (A) Respiration *(R)*, (B) gross photosynthesis (GP) and (C) net photosynthesis (NP) per *Paramecium bursaria–Chlorella* spp. holobiont cell analysed by the presence of microplastic contamination. All rates are presented in micromoles of oxygen gas evolved per hour and were measured using a Clark-type oxygen electrode (see Section 2.5). Boxplots show median, interquartile range, and minimum and maximum values

There is currently limited research with which to draw parallels with this study. Research on the impact of microplastic contamination on photosymbiotic productivity in general is scarce: despite species-specific impacts discovered in coral photosymbioses (Huang et al. 2021), research in freshwater photosymbioses appears to be limited. Notably, a recent study investigated duckweed–microbiome interactions in response to stressors including microplastics originating from tyres, but the study did not investigate metabolism or productivity (O'Brien et al. 2022). The finding that NP declines with a numeric reduction in GP in response to microplastic exposure in the *Paramecium bursaria* association studied is alarming, as photosymbiosis is known to play a major role in global photosynthesis rates (Johnson 2011, Bailly et al. 2014). The impact of ubiquitous microplastic pollution on widespread freshwater photosymbiosis, therefore, deserves urgent research attention.

Since the photosymbiosis is founded on metabolite trade of host heterotrophic compounds and symbiont photosynthates (Lowe et al. 2016, Minter et al. 2018), my observation that growth rate (a common proxy for fitness) and bacterivory rate remain apparently unaffected while NP declines (with a numeric decline in GP) may appear paradoxical, since symbiont-provided photosynthates may be limiting. This lack of apparent impact on fitness could be the result of rapid compensation by hosts and/or symbionts. Notably, such compensatory mechanisms did not include regulation of symbiont density in this study — shown previously to be modified by the host to maximise its fitness in response to abiotic change (Lowe et al. 2016) — nor did they invoke a change in bacterivory rate, since both symbiont density and bacterivory rate remained the same. This opens up the possibility that there are other compensatory mechanisms utilised by the photosymbiosis that can offset reductions in productivity caused by microplastic contamination; these mechanisms should be explored in further studies.

Notably, although growth rate was unaffected in this short-term experiment, declines in algal productivity are likely to change the 'value' of symbionts to their hosts; i.e. the per capita provision of photosyn-

thates (Lowe et al. 2016). Symbioses are known to be dynamic and context-dependent, and changes in symbiont productivity in photosymbiosis therefore theoretically risk an evolutionary trajectory towards symbiosis breakdown (Bronstein 1994, Dean et al. 2016). Accordingly, the decline in NP observed in this experiment may be concerning from a symbiosis-maintenance perspective for naturally occurring photosymbioses over evolutionary timescales. This concern may be especially realistic if (1) the photosymbiosis is unable to compensate for declining symbiont productivity (Lowe et al. 2016), for example, through modifications to symbiont load or bacterivory rate, and (2) microbial symbiont evolution has the capacity to occur strikingly fast (Chakravarti & van Oppen 2018), meaning that some evolutionary timescales relevant to photosymbiosis could be as short as a few years.

Furthermore, bacterivory rate per se in the photosymbiosis is likely to be ecologically important, as the association is widespread in nature (Minter et al. 2018), and photosynthetic mixotrophs can dominate bacterivory in aquatic ecosystems (Unrein et al. 2007). Interestingly, I observed no impact of microbeads on bacterivory rate in this experiment, implying that microbial photosymbioses may not modify feeding behaviour in response to microplastic contamination, in contrast with some research in coral symbioses (Huang et al. 2021). However, the many morphologies and size distributions of microplastics in natural freshwater environments (Castañeda et al. 2014) means that further work could usefully test a wide range of different microplastic exposures to explore whether feeding impacts are specific to the characteristics of the microplastics.

Strengths of this work include the fact that the focal symbiosis in this study, The *P. bursaria*–*Chlorella* spp. association, is widespread and abundant in freshwater ecosystems while being tractable in the laboratory and is therefore a useful study organism for the impacts of microplastics on photosymbiosis (Minter et al. 2018). While the laboratory environment is clearly not reflective of the multifactorial complexity of real-world ecosystems, these results serve as a pilot study of the potential impacts of microplastic pollution on widespread freshwater photosymbiosis. Further work could bridge the gap between these laboratory findings and the real world; for example, using semi-natural mesocosms. In addition, a clear weakness of this study is the low replication and limited microplastic exposure concentrations employed, something which could be rectified with further research.

In summary, this experiment has shown that significant reductions in productivity of a widespread photosymbiosis occur in response to exposure to microbeads relevant to the real world. This finding adds to an emerging picture of degradation of photosynthetic potential in photosymbiosis and a widespread image of the negative impacts of ubiquitous microplastics in nature (Wagner et al. 2014, Kettner et al. 2019, Soares et al. 2020, Huang et al. 2021).

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Editorial responsibility: Christine Paetzold, Oldendorf/Luhe, Germany
Reviewed by: 2 anonymous referees

Submitted: July 20, 2022
Accepted: February 10, 2023
Proofs received from author(s): March 3, 2023