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Consistent separation of resting stages and vegetative cells of spring bloom dinoflagellates in the Baltic Sea benefits coherent data acquisition

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Commentary

This commentary refers to the article "Resolving species-specific abundances of three cold-water dinoflagellates using a simple staining technique" (Lipsewers 2023). It aims to explain which vegetative cells and resting stages might be included in the so-called spring dino-group, as well as to show the percentage relative contributions of resting stages of *Gymnodinium corollarium* along with the spring bloom in and in different sub-basins of the Baltic Sea. During sample analysis for Lipsewers 2023, resting stages of *Gymnodinium corollarium* were identified in addition to its vegetative cells (referred to henceforth as Gc RS and Gc cells, respectively). Gc cells and Gc RS look very similar, especially in samples preserved with Lugol's solution using 125-fold magnification. Nevertheless, with larger magnifications and the required expertise, they are distinguishable based on the distinct walls of Gc RS and cells (Fig. 1). At least some of the microscopists using the traditional monitoring protocol include Gc RS in the dino-group. In case of lower counting accuracy, summarising Gc RS and cells lead to an overestimation of its biomass compared to those species with only vegetative cells enumerated.

Lipsewers (2023) presented the relative species-specific abundances of three dinoflagellates in different bloom-phases and sub-basins. As Gc RS contributed to the grouped biomass derived from the original counts (traditional monitoring protocol), which was previously determined for each sample (method described in Lipsewers and Spilling 2018) and considered for further analyses, Gc cells and RS were summarised.

If not specified here, the material and methods section in Lipsewers 2023 is valid for this commentary. Note the number of samples (n) differs between the different bloom-phases (see definition in Spilling *et al.* 2019) and most sub-basins (Lipsewers 2023). All statistical tests were considered as successful by the software, meaning that the data fit the assumptions met by the t-tests (both paired and unpaired) so there are no error biases. The use of the Gc cells/Gc RS-index was inspired by Wasmund *et al.* (2017). This index ranges from zero to one (zero indicates absolute dominance of Gc RS; one indicates absolute dominance of Gc cells). The equation is: Gc cells/Gc RS-index = Gc cells % / (Gc cells % + Gc RS %). The indices and the average values considered for the contribution of the vegetative cells and resting stages in different bloom-phases, sub-basins, and

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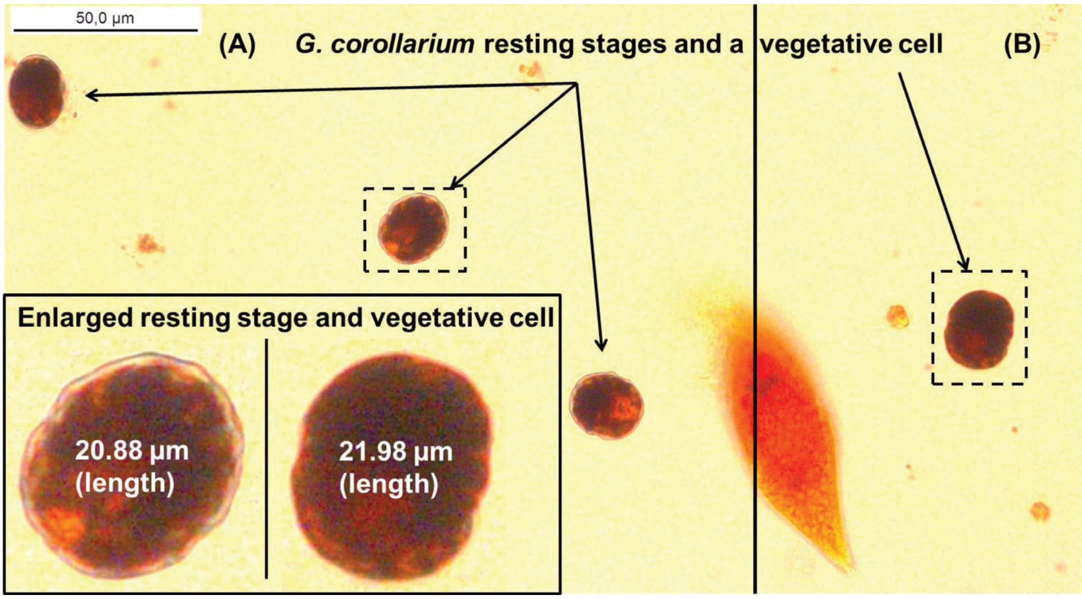

Fig. 1. Three resting stages (panel A) and a vegetative cell (panel B) of *Gymnodinium corollarium* in a sample preserved with neutral Lugol's solution collected in 2014 (Baltic Proper, post-bloom conditions). Note the different appearances of the walls comparing a vegetative cell to a resting stage. The enlarged images represent the original ones in the dashed frames.

the whole dataset are based on the percentage relative contributions to the dino-group. These contributions were calculated using the relative abundances of both Gc RS and Gc cells (e.g., 10 Gc RS amongst 100 counted units of the entire dino-group (vegetative cells of all three species and Gc RS combined) = 10% Gc RS).

Considering the entire dataset (n = 62), Gc RS were detected in 38.7% of samples and contributed 5.76 ± 12.61 SD % (average ± standard deviation (SD)) to the dino-group, which was dominated by Gc cells (71.35 ± 24.32 SD %).

The average Gc cells/Gc RS-index decreased from the growth phase of the bloom (1.00 ± 0.00 SD %) to post-bloom (PB)-conditions (0.82 ± 0.22 SD %), indicating an increasing contribution of Gc RS as the bloom progressed. An unpaired Student's t-test revealed significant differences between the average indices for three comparisons of bloom-phases, supporting the increasing trend: 1) peak (P) vs. decline (D): p = 0.0161, T = 2.5021, degrees of freedom (df) = 44; 2) P vs. PB: p = 0.0023, T = 3.3512, df = 28; 3) D vs. PB: p = 0.0156, T = 2.5249, df = 40. According to Sundström *et al.* (2009), cultured Gc forms resting stages at nitrogen (N)-deplete conditions. The spring bloom usually depletes the inorganic N-pool in most sub-basins of the Baltic Sea (Tamminen and Andersen 2007), explaining the increasing proportion of Gc RS along with the bloom.

Regarding the sub-basins, the minimum average Gc cells/Gc RS-index was found in the Baltic Proper (BP, 0.82 ± 0.27 SD %) and the maximum (1.00 ± 0.00 SD %) was found in the Bothnian Bay (BB), Bothnian Sea (BS), and The Quark (Kv). Four of the comparisons of the average indices for different sub-basins revealed significant differences: 1) Archipelago Sea (ArS) vs. BS: p = 0.0237, T = –2.4598, df = 19; 2) Aland Sea (AS) vs. BS: p = 0.0310, T = –2.4435, df = 12; 3) Gulf of Finland (GOF) vs. BS: p = 0.0259, T = 2.3636, df = 26; 4) BP vs. BS: p = 0.0417, T = –2.1929, df = 18. The latter comparison was also done with a paired test, due to equal sample sizes for both sub-basins but this did not result in a significant difference (p = 0.0560, T = –2.1929, df = 9). Gc was originally isolated from the BP (Sundström *et al.* 2009) but it can become abundant in several sub-basins of the Baltic Sea during the spring bloom (Sundström *et al.* 2009, Lipsewers 2023).

Gc RS contributed the largest proportions to the dino-group at different stations (in 2014 and 2016) in the BP during PB-conditions (76.92% and 38.96%, respectively), indicating encysting Gc populations (Fig. 1). Excluding the Gc RS from the microscopy counts for these samples did not result in different dominance patterns. For example, Gc and *Biecheleria baltica* (Bb) cells contributed 85.71% and 14.29% (respectively) when including Gc RS, 78.57% and 21.43% (respectively) when excluding them, and *Apocalathium malmogiense* (Am) cells contributed 0% in both cases for the sample from 2016. So, Gc cells clearly dominated the dino-group at the mentioned sampling stations as shown for the whole dataset (see above).

For instance, the sampling strategy is discussed in detail in Lipsewers 2023. The presented Gc RS- and species-specific results are based on the work of one microscopist. Different microscopists count different life cycle stages as dino-group and it is highly likely that they even include different species, since the traditional monitoring protocol does not allow a detailed identification of vegetative cells and resting stages with highly similar shapes, sizes, and appearances. This affects the grouped biomass (dino-group) and produces scattered information on all of the included species. It is advisable to consistently separate the cells of Gc, Bb, and Am according to Lipsewers (2023) as well as Gc cells and RS as described here. This is particularly relevant for research focusing on, for example, the life cycle and population dynamics of Gc, modelling approaches, biogeochemistry as well as long-term trends. Especially considering that Gc is one of the major players during the Baltic Sea spring bloom (Lipsewers 2023), an adjusted strategy for monitoring and studying the plankton community is recommended.

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