Actions taken to accommodate the 2nd revision comments of reviewer #1 on "Arctic (Svalbard Islands) Active and Exported Diatom Stocks and Cell Health Status" by Susana Agustí et al. https://doi.org/10.5194/bg-2018-459-RC1, 2018

Reviewer #1.- Revised MS has been improved to describe the Author's logic more clearly along the line of (1) nutrient depletion leads unhealthy status of diatom --> (2) unhealthy/senescent diatom sinks down faster than healthy diatom --> (3) diatoms reaching the aphotic zone die and lyse soon --> (4) downward export of unhealthy/senescent diatom increase the progression of the surface pCO2 unsaturation. Results from the experiments fully support (2) and (3), but not (1), even though many previous studies suggest it. I would like to request Authors (i) to state insignificant correlation between nutrient concentrations and %living biomass in the photic zone, and, (ii) if possible, to cite the previous studies on the mechanisms driving diatom senescence other than nutrient depletion. My other concern is about (4), as rapid lysis of senescent diatoms (3) might result in supply of suspended/dissolved organic carbon and their remineralization within the subsurface waters which reduces air sea disequilibrium in the surface through processes such as upwelling, mixing and vertical diffusion (Bates and Mathis, 2009). I suggest Authors (iii) to explain the contradicting effects of (2) and (3) for the surface pCO2. There are many errors in writing. I recommend (iv) to ask an English proofreading service before re-submission. I hope my comments would help Authors to enhance the value of their findings.

Authors: We thank the reviewer for the useful comments. We implemented the revised version with the suggestions of the reviewer.

Action: We added different paragraphs and corrections as indicated below::

(i) p. 5 Lines 26-27: *"The percentage of living cells was not significantly correlated with the concentration of NO_3 + NO_2 (2-tail test, r = -0.54, P = 0.17) or $Si(OH)_4$ (2-tail test, r =-0.69, P = 0.06)."*

(ii) p. 2 Lines 23- 25, we added the paragraph: "*Alou-Font et al. (2016) observed large variability in the health status of phytoplankton in the Canadian Arctic, influenced by the light and temperature conditions, but not by nitrate concentration —typically thought to be the main yield-limiting nutrient*."

In p. 8, we added the following paragraph: *"Cell abundances and health state observed were consistent with previous studies. In the Canadian Arctic, living cells in open-water and ice-covered stations represented the 57.3 ± 5.8% and 48.0 ± 3.9% (± SE), respectively (Alou–Font et al., 2016), similar to proportions in our study. The percentage of living cells was higher during the bloom periods than periods before and after (Alou-Font et al. 2016). This trend appeared to be driven by light and low nutrient concentrations (Alou–Font et al., 2016)."*

(iii) There is no contradiction because dead cells sink below the photic layer. Also, because phytoplankton cell mortality and lysis imply several steps of cell degradation (e.g. Segovia et al. 2003), the release of the carbon will follow the degradative process. If dead cells sink below the photic layer most of the carbon will be released below the photic layer. In p.8 Lines 25-29, we added the following paragraph: "*Acute silicic acid limitation, is identified, therefore, as the event leading to loss of the capacity to actively regulate buoyancy that characterizes diatom cells (Smayda, 1970), and rapid sinking of the bloom. The potential for rapid sedimentation is enhanced, due to the higher silica quotas for polar diatoms (Lomas et al. 2019) compared to lower-latitude diatoms (Brzezinski 1985)."*

(iv) English native coauthors revised the language.

Reviewer #1. Specific comments
p. 4, l. 26: Write the station ID (#3?) whose sample was used in the sinking experiment.
p. 5, l. 7: NO3 should be NO3 + NO2.
p. 6, l. 2 – 4: "At station #4, the community sampled was more diverse at the aphotic than at the photic layer (Fig. 4) indicating high sinking despite the low biomass." Why does high sinking rate contribute to higher diversity in the aphotic zone than that in the photic zone?
p. 6, l. 17 – 16: "Initially, only 6.7 % of the cells of the Fragilariopsis sp. and Thalaisiosira sp. colonies dominating the community tested were dead." It seems there is no stations where these two species were dominant in Fig. 4. Again, at which station was the sinking experiment conducted?
p. 6, l. 29 – 30: "6.3 days for the largest Thalaisiosira sp. cells" "6.3 days" would be "5.3 days".
p. 7, l. 18: "..., although diatom sinking was still low. " as it confirmed at this station?

Authors: We corrected all the specific comments in the revised manuscript.

Actions taken to accommodate the 2nd revision of reviewer #2 on "Arctic (Svalbard Islands) Active and Exported Diatom Stocks and Cell Health Status" by Susana Agustí et al. https://doi.org/10.5194/bg-2018-459-RC1, 2018

Reviewer #2. Why I agree that bottle net, is a very exciting and original tool, the results here are still too poor to be used alone or to state that silicon is the trigger of the bloom senescence and sinking.
I disagree with the authors when they state that they include a sufficient amount of data to strengthen this paper. To my point of view, the paper rely too much on two experiments that were not robust enough to conclude anything, especially for the one where we could find some opposite results in the literature (which is not discussed at all). At the minimum this paper should discuss the problem with the experimental set up.

Authors.- The major contribution of the manuscript is the innovative relationship between the health status of diatoms in the photic layer and the large stock of diatoms exported below this layer when resource-depletion leads to poor diatom health state in the photic layer, delivered from the use of the bottle net. In contrast, the experiments presented have simply a supporting role, but are not central to the argument.

The argument that the data set does not allow to conclude that silicon is the trigger of the bloom senescence and sinking was already addressed and was removed from the previous version, so we are surprise the reviewer continues to criticize such argument, which is no longer presented.

We agree that the experiments ran on board are limited in number but are, however, a relevant contribution examining both processes, sinking and cell death (under aphotic conditions), on

natural communities at conditions close to '*in situ*". The work on board a research vessel has limitations, particularly so in the Arctic where changing conditions impose changing cruise plans, but offers the opportunity to evaluate responses of natural communities at '*in situ'* conditions. Most literature data on the topic came from laboratory axenic cultures growing in optimized conditions, that do not exist at the sea.

We agree, indeed, that the experimental results should be compared to other evidence present in the literature, which we now do. However, the evidence present in the literature is consistent with our results, not opposite as the reviewer asserts:

Our results showing mortality rates in the dark of natural arctic diatoms populations are not in conflict with the literature. Vegetative cells have been shown to be unable to survive in darkness (e.g., Figure 1 below from Segovia et al. 2003) and only populations producing resting stages, e.g. spores, survive. We note, however, that not all diatoms are able to produce resting stages. We provide a more detailed assessment of the experimental evidence in response to the comment on this experiment below, and we have now added a paragraph in the discussion comparing our results with those of other experiments in the discussion section.

Reviewer #2. Diatom survival to darkness:
I am still not convince by this experiment that aim at measuring the survival of diatom in the dark…
First, if I understood correctly it has only be done once and only at St 3.
Authors.- We indicated in the methods that information: the experiment was made with the plankton community sampled at station #3.
Reviewer #2. What was the health status of the cells at the start of the experiment?
Authors.- It was a reasonably healthy community (the % of living cells, 63.64 %, was already indicated in Table 1).

Action.- We added more information to contextualize this experiment (p 7, starting line 16).

Reviewer #2. Second, I am not sure that the experimental protocol was sufficiently robust to conclude anything from it.

Authors.- The experiment aimed to test natural community cell death at darkness as found by phytoplankton when transiting to the aphotic layer. Our experiment is a valuable test because most data published on the topic (including those papers cited by the reviewer) are from "*in vitro*" assays ran in the laboratory, under conditions far from those found at sea: monospecific cultures growing in culture media with high nutrients, axenic, and other optimized conditions, and able to produce resting stages.
Action.- As stated above, try to add more context as to the initial condition of the cells in this experiment.

Reviewer #2. Third, the results are not at all compared to other study where the authors stated something totally different from a more robust experimental set up:
Why are these results so different from the one by Smayda and Mitchell-Innes 1974 showing survival of diatoms for 90 days in the dark?

And by the study by Lewis et al. 1999 where some diatoms even survive up to 112 month?

Authors.- We disagree with the reviewer assessment. Careful reading of the papers the reviewer cites show that our results showing arctic diatoms mortality rates in the dark are not in conflict with the literature.

First, some of the papers the reviewer cite do not focus on the survival/mortality of the phytopklankton cells in the community, but on the capacity of resting stages, often sampled from sediments, to germinate and give rise to a population when placed under light, which can hardly be compared to the experiments we report. In previous studies different authors identified that vegetative cells could not survive in darkness, and populations must produce resting stages:

Smayda and Mitchell-Innes (1974), and Lewis et al. (1999) experimentally assessed the capability of resting stages of phytoplankton to resume growth after placement in light, which they referred to as "survival". Note that we use "survival time" to refer to the time for the population of vegetative cells to be reduced to half under darkness, which is very different from the meaning of "survival" in those experiments (i.e. it suffices for one resting stage to become viable in their experiments for a population to be developed when transferred to the light), but this is no way in conflict with our results.

Moreover, Lewis et al. 1999, did not work with phytoplankton communities, they collected cysts from sediments and germinated in the lab. Cysts are morphological and physiologically different from vegetative phytoplankton cells, and the finding that some cysts from sediments can be germinated is not at all in conflict with results showing high mortality rates of vegetative cells under darkness.

However, Smayda and Mitchell-Innes (1974) also evaluated the mortality of vegetative cells of *Chaetocesos curvissetum* under darkness, which can indeed be compared to our experiment. We, therefore, recalculated the corresponding mortality rates from the data Smayda and Mitchell-Innes (1974) provided to yield a mortality rate in darkness of vegetative *Chaetocesos curvissetum* cells of 0.19 d^{-1}, very close to the rates we found for "natural diatom populations" in our paper. Hence, the comparable experiment presented by Smayda and Mitchell-Innes (1974) is consistent with our findings.

A more thorough assessment of the recent literature reveals that Segovia et al. 2003 and Segovia and Berges 2009 described the he processed involved in phytoplankton cell death in monospecific cultures under darkness, using molecular tools and/or differentiate living from dead vegetative cells, concluding that vegetative cells are unable to survive in the dark. The photograph below (Segovia et al. 2003, Fig. 1), is compeling as to the outcome of exposure of the culture to 8 days of darkness.

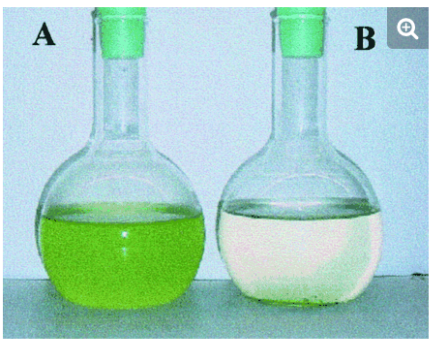


Fig. 1. Download figure | Open in new tab | Download powerpoint

Effects of light deprivation in *D. tertiolecta*. A, Actively growing culture. B, Culture after 8 d in darkness.

Agusti et al. (2015) is, to the best of our knowledge, the only paper reporting mortality rates in the dark of natural (subtropical) phytoplankton communities. The rates reported (from 0.06 to 0.27 d^{-1}) are also consistent with those we report for the Arctic community studied.

Provided these results, we find no basis on the reviewer argument that our results are inconsistent with the literature. They are indeed perfectly consistent.

Action: We added a discussion of previously reported experiments and cited the literature on the topic. In p 7, starting line 20, we added the following paragraph: *"Smayda and Mitchell-Innes (1974) also reported the decrease in viable cells after darkness: "After 6 days of dark incubation, the number of viable cells of Chaetoceros curvisetus recognizable decreased from 760 to 240 cells per ml", representing a decay rate of 0.19 d^{-1} (i.e. 50% loss of cells in 3.6 days) comparable to the rate reported here. Other studies also reported cell mortality in darkness (Segovia and Berges 2009; Agusti et al. 2015), also yielding mortality rates close to those reported here. Phytoplankton vegetative cells do not survive under darkness (Smayda and Mitchell-Innes 1974; Segovia and Berges 2009; Segovia et al. 2003) and only resting spores or resting cells are able to survive in the dark (Ignatiades and Smayda 1970; Smayda and Mitchell-Innes 1974; Peters and Thomas 1996)."*

We also added more information in the methods section and in the discussion to highlight the relevance of this experiment.

In p 7, lines 18-19: *"This result, although limited to a single experiment, was consistent among the major genera and functional groups analyzed and reflected survival at "in situ" conditions in the aphotic layer*."

Reviewer #2. How the same cells would survive in the same conditions but with light?

Authors.- As we indicated in the previous revision, our aim was not to test the survival of the phytoplankton on the photic layer, but to assess what is their likely fate once they fall below the photic layer. Whether they grow, remain stable or decay in the light is not relevant to understanding what is their fate in the aphotic layer.

Action: We already specified this aspect in the previous revised manuscript in response to the reviewer's comments, but have modified the statement of the aim of the experiments in the Methods section as (p4, lines 27-29): "*The mortality rates of living phytoplankton cells expected when transferred from the photic to the aphotic layer were examined at station #3 with vertical tows from the photic layer.* ".

Reviewer #2. What was the cell concentration in the experiment?

Action: In p 6, last paragraph, we added the following information: *"The incubation was performed close to the temperature below the photic layer that averaged 2.978 $^{o}C \pm 0.003$ at station #3, suggesting that trends were not driven by thermal effects. The cell concentration at the onset of the experiment was 298 cells ml^{-1}."*

Reviewer #2. You started with a very limiting silicate concentration, so how could you state that darkness is responsible for the death of the cells? Multiple nutrient stress in the batch may have be the trigger for the diatom death…

Authors.- We already modified the manuscript (in the previous revision round) to avoid such confusion. Darkness, low nutrients, the presence of other microorganisms including viruses and parasites, among other threats are the conditions at the aphotic layer of the ocean for phytoplankton. Our experiment, testing cell death of natural populations close to "*in situ*" conditions, represent an original contribution. We added a paragraph in the revised manuscript to improve the discussion and highlight the value of these results. Note, however, that the results obtained in this experiment are consistent with those in previous papers, so it is, therefore, logical to expect that darkness contributed to the mortality observed.

Action: In p 8, lines 27-31we added the following paragraph: "*Beyond the stressor of continuous darkness, the fast decay rates observed here under aphotic experimental conditions may have been influenced, in addition to darkness, by low nutrients availability, and/or the possible presence of pathogens or parasites, suggesting that survival of natural populations below the photic layer may be lower than expected in the dark from axenic, high- nutrients "in vitro" studies with cultures, aspect already pointed by Ignitiades and Smayda (1970).*

Reviewer #2. The authors already observed some living diatoms really deep in the water column during the Malaspina expedition, so why would it be different in arctic?

Authors.- We, again, emphasize that there is no conflict between these observations, as we also report in our paper that between 0.5 % and 50% of diatoms retrieved from the aphotic layer (50 to 400 m) in our Arctic study were living (Malaspina samples were from 2000-4000 m depth, Agusti et al. 2015). Hence, our observations and those from the Malaspina Expedition are consistent.

Action: Table 2 shows the percentage of aphotic living cells to vary between 0.5 and 50 %.

Reviewer #2. Diatom sinking triggered by the health status of the cells:
As there was more than one sampling, it could have been nice at least to have the composition of the sample at the different time. However, the authors need to be a lot more careful in their discussion as they only tested one community once. While the idea behind is interesting, it is not enough experiment to conclude.

Authors.- We agree that it would have been nice to conduct more experiments. However, since there was ample prior evidence that dead cells sink faster (elegantlty demonstrated by Smayda, 1971) we believe that a single experiment, showing that these findings also apply to the Arctic community tested, sufficed.

Action: The experiment was not designed to assess how the community may have changed inside the experimental chamber (although we reasonabluy expect no growth under the dark conditions in the chamber. p 7, second paragraph: *"The experiment conducted, although limited in number, was tested in a natural community and showed that dead cells sank much faster than living ones in a field assemblage."*

P5
Line 28: Fig. 2 should be Fig. 3
Fig. 2 was cited properly.

Line 36: Navicula does not dominate the Barent Sea the composition look much alike St3
Action: We revised this statement. The sentence was modified to avoid confusion, Navicula was found at the polar front, and did not dominate the community. The sentence now reads: "with a larger contribution of other taxa, including *Navicula pelagica* (station #9, Fig. 4)."
P6
Line 20: what was the main species after one hour?
Action: -P6 was revised. After one hour, Coscinodiscus was dominant at the bottom, as already indicated.

Line 30 : how to concile with result showing some Thalassiosira starting back in culture after 70 days in the dark?

- We added a new paragraph (in pg 7-lines 22-30) to discuss the literature on the topic. Careful reading of the paper the reviewer extracts this statement from shows that there is indeed not conflict between our results and those observations: *Thalassiosira* vegetative cells died, some vegetative cells, however, led to resting stages were that formed after several days in darkness and the resulting cysts were able to grow when reillumined after 70 days in darkness.

P7Line 29: "depleted silicic acid silicic acid limits diatom Si uptake to such a degree that growth must slow, Krause et al" please add a coma or a colon for clarity
Line 30: but you show that they stay alive only shortly after they leave the photic zone
Line 31-33: I don't understand your sentence here that state that diatom mortality is triggered by Si limitation, especially as what you explained in the next paragraph is more like Si limitation trigger the loss of buoyancy and the darkness trigger the death of the cell, no?
Authors: The reviewer is correct that the narrative may lead to confusion. The term mortality referring to the consequences of acute Si limitation was misleading, as we referred to high mortality rate, as reflected in 30-50% of the diatom cells in Si-depleted photic layers to be dead.

The rest are likely to be in poor condition. Available evidence suggests that this leads to loss of buoyancy capacity that characterized healthy cells, leading to rapid sinking of the bloom. A fraction of the cells falling below the photic layer is still alive, as shown in Table 1 (0.5 to 50%), but most of these will die under the aphotic conditions. Overall we feel that the discrepancies with the reviewer stem from confusion between mortality, interpreted by the reviewer to represent the death of 100%, which is consistent with the data we show that characterizes both populations in the photic and aphotic layer as consisting of living and dead cells in variable proportions (i.e. dead or alive is a binary state for individual cells, but a probability for the population). Hence we refer to mortality rates throughout the paper.

Action: p. 8, lines 22- 25: We revised and corrected the text to avoid confusion: " *A large fraction (30 – 50%) of the diatom cells in silicon-depleted photic layer were dead, pointing at acute silicic acid limitation as the likely factor triggering partial mortality, while the remaining cells are likely to be senescent. Unhealthy diatoms would then lose the capacity to actively regulate buoyancy that characterizes healthy diatom cells (Smayda, 1970), leading to rapid sinking of the bloom."*

and: "*The potential for rapid sedimentation is enhanced, due to the higher silica quotas for polar diatoms (Lomas et al. 2019) compared to lower-latitude diatoms (Brzezinski 1985)."*

P8

Line 10: aphotic instead of photic?

Action: We have changed this in the revised manuscript.

New references

Brzezinski, M. A.: The Si: C: N ratio of marine diatoms: Interspecific variability and the effect of some environmental variables 1. Journal of Phycol. 21, no. 3, 347-357, 1985.

Ignatiades, L., and Smayda, T. J.: Autecological studies on the marine diatom rhizosolenia fragilissima bergon. ii. enrichment and dark viability experiments 1. Journal of Phycology 6, 357-364, 1970.

Lomas, M.W., Baer, S.E., Acton, S., Krause, J.W.: Pumped up by the Cold: Elemental Quotas and Stoichiometry of Polar Diatoms. *Frontiers in Marine Science*, doi: 10.3389/fmars.2019.00197, 2019.

Peters, E., and Thomas, D.N.: Prolonged darkness and diatom mortality I: Marine Antarctic species. Journal of Experimental Marine Biology and Ecology, 207, 25-41, 1996.

Segovia M., Haramaty, L., Berges, J. A., and Falkowski, P.G.: Cell Death in the Unicellular Chlorophyte *Dunaliella tertiolecta*. A Hypothesis on the Evolution of Apoptosis in Higher Plants and Metazoans. Plant Physiology, 132, 9-105, 2003; **DOI:** 10.1104/pp.102.017129.

Segovia, M. and Berges, J. A.: Inhibition of caspase-like activities prevents the appearance of reactive oxygen species and dark-induced apoptosis in the unicellular chlorophyte *Dunaliella tertiolecta*1. Journal of Phycology, 45, 1116-1126, 2009. doi:10.1111/j.1529-8817.2009.00733.x

Smayda, T. J., and Mitchell-Innes, B.: Dark survival of autotrophic, planktonic marine diatoms. Marine Biology, 25, 195-202, 1974.

Arctic (Svalbard Islands) Active and Exported Diatom Stocks and Cell Health Status

Susana Agustí[1,*], Jeffrey W. Krause[2,3], Israel A. Marquez[2,3], Paul Wassmann[4], Svein Kristiansen[4], and Carlos M. Duarte[1,5]

[1] King Abdullah University of Science and Technology, Thuwal, 23955-6900, Kingdom of Saudi Arabia
[2] Dauphin Island Sea Lab, Dauphin Island, 36528-4603 United States
[3] Department of Marine Sciences, University of South Alabama, Mobile, 36688-0002 United States
[4] Department of Arctic and Marine Biology, UiT The Arctic University of Norway, 9037 Tromsø, Norway
[5] Arctic Research Centre, Department of Biosscience, Aarrhus University, C.F. Mollers Alle 8, DK-8000 Arhus C, Denmark

* *Correspondence to:* Susana Agustí (susana.agusti@kaust.edu.sa)

Abstract. Diatoms tend to dominate the Arctic spring bloom, a key event in the ecosystem including a rapid decline in surface-water pCO_2. While a mass sedimentation event of diatoms at the bloom terminus is commonly observed, there are few reports on the status of diatoms' health during Arctic blooms and its possible role on sedimentary fluxes. Thus, we examine the idea that the major diatom-sinking event which occurs at the end of the regional bloom is driven by physiologically deteriorated cells. Using the Bottle-Net system, we quantified diatom stocks, below and above the photic zone, concurrent with their cell health status. The communities were sampled around the Svalbard Islands and encompassed pre- to post-bloom conditions. A mean of 24.2 ± 6.7 % (SE) of the total water column (max. 415 m) diatom standing stock was found below the photic zone, indicating significant diatom sedimentation. The fraction of living diatom cells in the photic zone averaged 59.4 ± 6.3 % but showed the highest mean percentages (72.0 %) in stations supporting active blooms. In contrast, populations below the photic layer were dominated by dead cells (20.8 ± 4.9 % living cells). The percentage of diatoms' standing stock found below the photic layer was negatively related to the percentage of living diatoms in the surface, indicating that healthy populations remained in the surface layer. Ship-board manipulation experiments demonstrated that 1) dead diatom cells sank faster that living cells, and 2) diatom cell mortality increased in darkness, showing an average half-life among diatom groups of 1.025 ± 0.075 days. The results conform to a conceptual model where diatoms grow during the bloom until resources are depleted and supports a link between diatom cell health status (affected by multiple factors) and sedimentation fluxes in the Arctic. Healthy Arctic phytoplankton communities remained at the photic layer, whereas the physiologically compromised (e.g. dying) communities exported a large fraction of the biomass to the aphotic zone, fuelling carbon sequestration to the mesopelagic and material to benthic ecosystems.

Formatted: English (UK)
Formatted: English (UK)
Formatted: Font: Italic, English (UK)
Formatted: English (UK)
Formatted: English (UK), Subscript
Formatted: English (UK)
Deleted: Large sinking of diatoms is expected at the end of the bloom driven by deteriorated cell status associated to nutrients (silicon) depletion. However, t
Formatted: English (UK)
Deleted: Here
Deleted: y, using the Bottle-Net, Arctic
Formatted: English (UK)
Deleted: and assess their
Deleted: a broad diversity of conditions and
Deleted:
Deleted: stages
Formatted: English (UK)
Deleted: bout 1/4 (mean±SE
Formatted: English (UK)
Deleted: sinking of diatoms in the area
Deleted: ’
Deleted: A
Deleted: n experiment on board in a tall (1.35 m) sedimentation column confirmed that
Deleted: from the Arctic community
Deleted: ones.
Deleted: Also, diatoms
Deleted: d
Deleted: half life
Deleted: of
Deleted: -1
Formatted: English (UK)
Deleted: depleted, and
Formatted: English (UK)
Formatted: English (UK)
Deleted: arctic
Deleted: dying
Deleted: fuelling
Formatted: English (UK)
Deleted: and

1. Introduction

Diatoms can support most of the Arctic primary production during the spring phytoplankton bloom (Krause et al. 2018), the key event setting the ecosystem and driving the intense carbon uptake characteristic of the Arctic (Vaquer-Sunyer et al. 2013). However, silicic acid concentrations [$Si(OH)_4$] are characteristically low in the European Sector of the Arctic, due to the inflow of Si-depleted Atlantic water (Rey 2012). In the Svalbard Island region, Krause et al. (2018) showed diatoms to be limited by [$Si(OH)_4$] at the spring bloom and suggested that silicon limitation could collapse a diatom bloom before nitrogen when spring conditions favor diatoms, instead of the haptophyte *Phaeocystis*. A similar observation was made during the spring bloom in Southern Greenland, whereby diatom depletion of [$Si(OH)_4$] collapsed the bloom with ~4 µmol L^{-1} remaining nitrate (Krause et al. in review).

The termination of the Arctic spring bloom is characterized by rapid sinking of diatom cells, leading to high sedimentary fluxes in the spring (Oli et al., 2002; Wassmann et al., 2006; Bauerfeind et al., 2009), precluding this production from being recycled in the upper ocean. The apparent rapid sinking of the senescent diatom bloom appears to sustain the depletion of CO_2 in surface waters initiated by the bloom and drive strong atmospheric CO_2 uptake (Bates et al., 2009), as average pCO_2 values post bloom being typically below 300 ppm —with some values as low as 100 ppm (Takahashi et al., 2002; Holding et al., 2015).

Factors regulating diatom sedimentation have been explored for decades; however, there are few published reports on the status of diatoms' health in the Arctic during blooms and the possible role deteriorated cell health status may play in driving sedimentary fluxes. Alou-Font et al. (2016) observed large variability in the health status of phytoplankton in the Canadian Arctic, influenced by the light and temperature conditions, but not by nitrate concentration —typically thought to be the main yield-limiting nutrient. More recent studies which have observed diatom silicic acid depletion prior to nitrate limitation suggest that nutrient limitation may be a physiological stressor which could initiate diatom sedimentation (Krause et al. 2018, 2019). Silicon limitation has also been shown to affect both autolysis (i.e. cell death) and the potential to form aggregates (which facilitate sinking) in *Coscinodiscus wailesii* cultures, whereas the latter was less pronounced under nitrogen limitation (Armbrecht et al. 2014). Given the field observations, and seminal culture-based experimental work, we suggest that rapid sinking of diatoms at the end of the Arctic spring bloom is driven by a deterioration of cell status, leading to cell mortality.

Because of diatoms' obligate silicon requirement, its depletion in the water column would exclusively affect their physiology, and potentially, their biogeochemical fate. Lomas et al. (2019) recently demonstrated that polar diatoms have high elemental density (i.e. element content per unit biovolume) relative to low-latitude diatoms, and this is especially true for silicon content (consistent with results from Baines et al. 2010). Therefore, short-term changes in diatom physiology, e.g. responses to nutrient stress, may favour rapid sinking of polar diatoms much more than in temperate diatom species. While one could examine diatom cells from sediment traps, which is a

Deleted: Recently

Commented [JK1]: Probably good to add this now, to help solidify the idea of Si depletion and diatom mortality.

Krause, J.W., Schulz, I.K, Rowe, K.A., Dobbins, W., Winding, M., Sejr, M., Duarte, C.M., Agustí, S. in review. Silicon limitation drives bloom termination and carbon sequestration in an Arctic bloom. Scientific Reports.

Formatted: English (UK)

Formatted: English (UK)

Deleted: e

Deleted: is, thus, responsible for

Deleted: CO_2

Deleted: ,

Deleted: with

Deleted: and

Deleted: reported in the European sector of the Arctic

Deleted: suggests driving strong atmospheric CO_2 uptake (Bates et al., 2009)

Deleted: Current understanding of spring diatom-bloom dynamics in the Arctic suggest that rapid sinking of diatoms at the end of the Arctic spring bloom is driven by a deterioration of cell status, leading to cell mortality. H

Deleted: a

Deleted: Arctic

Deleted: with silicon depletion

Deleted: found

Formatted: Font: Italic, English (UK)

Formatted: English (UK)

Deleted:

Commented [JK2]: Lomas, M.W., Baer, S.E., Acton, S., Krause, J.W. 2019. Pumped up by the Cold: Elemental Quotas and Stoichiometry of Polar Diatoms [1]

Formatted: English (UK)

Formatted: English (UK)

Commented [JK3]: Baines, Stephen B., Benjamin S [2]

Formatted: English (UK)

Formatted: English (UK)

Formatted: English (UK)

Formatted: English (UK)

Formatted: English (UK)

Formatted: English (UK)

Deleted: Use of

Deleted: are

Formatted: English (UK)

standard approach used to explore diatoms' sinking fluxes, this methodology precludes accurate analysis of physiological health due to both the time required to collect cells (i.e. cell status can change) and trap fixatives (necessary to avoid "swimmers" from consuming sedimented material) lead to mortality of all cells. A new instrument, the Bottle-Net, has been applied to address this methodological gap. The Bottle-Net is a plankton net fitted inside a Rosette sampling system that can be used to collect plankton samples at depth without a prolonged deployment. This system was recently used to assess the stock and health status of microplankton in deep waters across the subtropical and tropical ocean (Agusti et al., 2015). Using the Bottle-Net at stations around the Svalbard Islands, we examined diatom stocks within and below the photic layer and assessed their health status along contrasting stages of bloom development. We also conducted two exploratory experiments to test the hypotheses that dead diatom cells in the field sink faster than living ones, based on previous culture experiment results (Smayda, 1971), and that field diatoms can die rapidly upon falling below the photic layer.

2. Methods

2.1 Sampling and study area.

The study was conducted between May 17 and 29 of 2016 on board the R/V *Helmer Hanssen*. The cruise started in the southwestern fjords of Svalbard Islands transited northward toward Erik Eriksen Strait and then south towards stations near the Polar Front and the Barents Sea (Fig. 1).

Vertical profiles with a Seabird Electronics 911 plus CTD, provided with an oxygen sensor, fluorometer, turbidity meter and PAR sensor (Biospherical/LI-CORR, SN 1060) were conducted at all stations sampled. Water samples were collected using a 12 five-liter Niskin bottles installed on a rosette sampler. Water samples were taken between the surface and the bottom (max. 500 meters) for analysis of nutrients, diatom silica, productivity, and other properties (Krause et al. 2018). We calculated the upper mixed layer (UPM) an index of the stability of surface water column, as the shallowest depth at which water density (σ_θ) differs from surface values by more than 0.05 kg m^{-3} (Mura et al. 1995).

At eight of the stations (Fig. 1) microphytoplankton samples were collected by using two Bottle-Net devices installed on the rosette sampler. The Bottle-Net is a new oceanographic device developed for the Malaspina 2010 Circumnavigation Expedition, which consists of a 20-µm conical plankton net housed in a cylindrical PVC pipe and is designed to be mounted in the place of a Niskin bottle on the rosette sampler. The Bottle-Net cover (on top) hermetically opens/closes bottle remotely, using the rosette's carousel bottle-firing mechanism, thereby initiating/terminating sample collection, the casing is open at the bottom to allow the water filtered through the internal plankton net to flow out (Agusti et al 2015). The Bottle-Net is lowered with the top cover closed, opened at the desired bottom depth (D_b, m) during the ascension of the rosette, and then the top cover closed again at the upper depth (D_u, m) of the water column to be sampled. This results in one integrated sample, from D_b to D_u, per deployment. Two Bottle-Nets were used mounted in the rosette sampling system, one to collect phytoplankton at the aphotic zone and the second to collect the community in the upper water column. The two layers were selected by combining the information on light penetration (PAR sensor) and chlorophyll *a* fluorescence obtained during the

Deleted: the tools …sed to explore diatom'…' sinking fluxes thus far… this methodology precludes accurate these analysis of physiological health due to both analyses as … [3]

Formatted: English (UK)

Deleted: Recently, however… a…new instrument, the Bottle-Net, has been applied to address this methodological gap. The Bottle-Net is a plankton net fitted inside a Rosette sampling system that can be used to collect plankton samples at depth without a prolonged deployment. This system,…was recently used to assess the stock and health status of microplankton in deep waters across the subtropical and tropical ocean (Agusti et al., 2015). Here we quantified, u… Using the Bottle-Net at stations around the Svalbard Islands, we examined Arctic …iatom stocks within and below and above …he photic layer and assessed their health status in communities sampled …long contrasting stages of bloom development around the Svalbard Islands… … [4]

Formatted … [5]

Deleted: cells

Formatted: English (UK)

Deleted: i

Formatted: English (UK)

Deleted: sigma

Formatted … [6]

Deleted: *t*

Formatted: English (UK)

Deleted: i… the rosette sampler. The Bottle-Net is a new oceanographic device developed for the Malaspina 2010 Circumnavigation Expedition, which consists of a 20-µm… [7]

Formatted … [8]

Deleted: case is opened at the bottom to allow the water … [9]

Formatted: English (UK)

Deleted: the t

Formatted … [10]

Deleted: …loses or opens the …ottle, … [11]

Formatted: English (UK)

Deleted: through

Formatted … [12]

Deleted: to expose the upper opening of the net

Formatted: English (UK)

Deleted: …ottle-Net is lowered mounted on the rosette … [13]

Formatted: English (UK)

Deleted: of the tow, which is conducted

Formatted: English (UK)

Deleted: with t

Formatted … [14]

downward CTD cast. The upper layer included the thickness of the photic layer to the depth when chlorophyll fluorescence faded away, which typically corresponded with very low levels of PAR (e.g. ~0.1–1% of surface irradiance). For the aphotic zone, one Bottle-Net was remotely opened and started filtering water when the rosette reached the maximum depth at each station, and it collected until reaching the depth ten meters below the maximum depth of photic zone. The second Bottle-Net was opened at the bottom of the photic layer and maintained open until reaching the water surface. Once on deck, the Bottle-Nets were gently rinsed with filtered seawater to retrieve the sample from the collector. Sampled volume was estimated as the product between the cross-sectional area of the mouth of the Bottle-Net and the vertical distance covered by the device from the start of the ascension to the closure of the top cover (D_b to D_u). The Bottle-Net presents an aspect ratio of 4, to avoid resuspension of materials filtered, displaying an efficiency of filtration of 96% for deep tows (2000–4000 m) at towing velocities around to 30 m min^{-1} i.e. standard rosette retrieval velocities (Agusti et al., 2015).

2.2 Microplankton abundance and viability

Bottle-Net sub samples were processed to identify living and dead phytoplankton cells. The freshly-collected samples were filtered onto 0.8-µm pore size black Nucleopore filters, stained with the Bac-light viability Kit, placed on slides and frozen at -80ºC until examination under epifluorescence microscopy. Another fraction of the sample collected by the Bottle-Net was fixed with formalin for further examination at the laboratory. The observed diatoms were classified to genera. The percentage of living or dead cells relative to the total (i.e. dead plus living) was calculated for the total community and by genera.

The Bac-light viability Kit (Molecular Probes ™ Invitrogen) is a double staining technique to test cell membrane permeability and is proven to be an effective method for determining phytoplankton viability (Llabrès and Agusti 2008, Agustí et al. 2015). When excited with blue light under the epifluorescence microscope, living phytoplankton cells with intact membranes fluoresce green (Syto 9, nucleic acid stain) and dead phytoplankton cells with compromised membranes fluoresce red (Propidium Iodine, nucleic acid stain). The samples were examined under blue light, most on board the research vessel, using a Partec CyScope® high power blue (470 nm) and green (528 nm) LED-illuminated epifluorescence microscope. In the laboratory at KAUST, all samples were examined using a Zeiss AxioObserver Z1 epifluorescence LED-illuminated microscope (Colibri 7 LED system). The fluorescence of the stained cells is well preserved at -80ºC for several months, and samples were transported frozen between the port of arrival (Tromsø, Norway) and KAUST.

2.3 Decay and sinking rates of living microphytoplankton cells

The mortality rates of living phytoplankton cells expected when transferred from the photic to the aphotic layer were examined at station #3 with vertical tows from the photic layer. An aliquot of the photic-layer microphytoplankton sample was resuspended in 2 L of 0.7 µm filtered surface water and incubated in the dark at 4ºC for 7 days; this simulated sedimentation from the photic layer into the aphotic layer. The community was sampled at the onset of the experiment and during set time intervals (i.e. 1, 3, 5, and 7 days). Immediately after sampling, cells were stained with the vital stain Bac-light Kit, then prepared and examined under epifluorescence

Deleted: below the1
Formatted ... [15]
Formatted ... [16]
Deleted: The aphotic zone was selected as the layer starting ... [17]
Deleted: When the rosette reached the maximum depth at ... [19]
Formatted ... [18]
Formatted ... [20]
Deleted: until rising to the upper target depth for the ... [21]
Formatted ... [22]
Deleted: in order
Formatted ... [23]
Deleted: -…000 m) at towing velocities around to 30 m ... [24]
Formatted ... [25]
Formatted ... [27]
Deleted: sample
Formatted ... [28]
Deleted: as…stained with the vital stain Back-light Kit ... [29]
Formatted ... [30]
Moved (insertion) [1]
Deleted: A freshly-sampled fraction of each
Formatted ... [31]
Formatted ... [26]
Deleted: black ….8-µm pore size black Nucleopore of ... [32]
Moved (insertion) [2]
Moved up [1]: The freshly-collected samples were filtered
Formatted ... [33]
Deleted: under
Formatted ... [34]
Deleted: Blue
Formatted ... [35]
Deleted: G…een (528 nm) led ... [36]
Formatted ... [37]
Deleted: , and a…l samples were examined at the KAUST ... [38]
Formatted ... [39]
Deleted: led
Formatted ... [40]
Deleted: led
Formatted ... [41]
Deleted: to
Formatted ... [42]
Deleted: the laboratory were maintained frozen during the ... [43]
Formatted ... [44]
Deleted:
Moved up [2]: Another fraction of the sample collected by
Formatted ... [45]
Deleted: The darkness-induced cellular mortality rates of ... [46]
Formatted ... [47]
Deleted: at increasing
Formatted ... [48]
Deleted: , …tained fresh ... [49]

microscope (as described above) to quantify the proportion of living cells in the community. The half-life (i.e. the time required for the number of living cells to decline by 50%) and the decay rate for each living-cell population were then calculated from the decline in living cells over time.

An experiment to test whether dead diatom cells sink faster than living cells was conducted shipboard using a sinking column (30 cm diameter, 1.35 m height, internal volume of 95 litres). The chamber was placed on deck, filled with 20 µm-filtered surface seawater, and left undisturbed for ~1 hour before starting the experiment. Microplankton collected in a vertical net tow (20-µm mesh) from the photic layer of Erik Eriksen Strait (close to the position of station #7) was resuspended in 1-litre of 0.7-µm filtered seawater and gently added at the surface of the sinking column. A fresh subsample of the initial community, which was added to the surface of the chamber, was stained with the Bac-light Kit and the diatoms were examined for identification and the percentage of living/dead cells as described above. The samples at the bottom of the sinking column (sampling port located 1.35 m below the surface) were collected at intervals of time of 0 (time when the sample was added at the surface), 1, 4 and 12 hours after the initial time, and processed similar to the initial community material.

3. Results

The stations sampled encompassed a spectrum of bloom conditions. Station 4 (off the Western Svalbard shelf) waters were pre-bloom, as indicated by low diatom stocks, high dissolved inorganic nutrient concentrations (photic layer concentrations $Si(OH)_4$ = 4.15 ± 0.04 µmol Si L^{-1}, NO_3 + NO_2 = 9.43 ± 0.09 µmol N L^{-1}, Table 1) and relatively low stratification (Table 1). All other stations sampled were characterized by comparatively depleted nutrient concentrations (photic layer concentrations $Si(OH)_4$= 0.99± 0.30 µmol Si L^{-1}, NO_3 + NO_2= 1.93 ± 0.76 µmol N L^{-1}, Table 1), thereby representing communities that were either in advanced blooming stages or were senescent after blooming. Stations 6 (SW Svalbard shelf) and 8 (E Svalbard shelf) supported actively blooming diatom populations, with the highest chlorophyll *a* concentration (10.5 µg Chl *a* L^{-1} for station 8, as described in Krause et al. 2018), and a large fraction of living diatom cells (about 70%, Table 1). Both locations had the highest stratification among the stations, as indicated by the low UPM values (Table 1). In contrast, Station 9 (Polar Front) supported a senescent diatom population in post-bloom phase, as indicated by depleted nutrient pools and a low percentage of living diatom cells (46.0 %, Table 1). The highest mixing was observed at station sampled at the Barents Sea (Table 1). The percentage of living cells was not significantly correlated with the concentration of NO_3 + NO_2 (2-tail test, r = -0.54, P = 0.17) or $Si(OH)_4$ (2-tail test, r =-0.69, P = 0.06).

Taxonomic classification under epifluorescence microscopy is not particularly accurate, but we were able to unambiguously identify different diatom genera, and some species. The more abundant genera found in the samples were *Thalassiosira* spp., differentiated between large (L *Thalassiosira*) and small (*Thalassiosira*) morphotypes; *Chaetoceros* spp., with a large representation of *Chaetoceros socialis;* pennate diatoms including

Formatted ... [50]
Deleted: to
Deleted: for any one diatom taxa
Deleted: run on
Deleted: board
Formatted ... [51]
Formatted ... [52]
Formatted ... [53]
Formatted ... [54]
Deleted: of
Formatted ... [55]
Deleted: and…1.35 m height, representing an ... [56]
Formatted ... [57]
Deleted: sinking …xperiment. Microplankton collected from ... [58]
Deleted: ,
Deleted: …itrer…of 0.7-µm filtered seawater and carefully ... [59]
Formatted ... [60]
Deleted: broad diversity…pectrum of of ... [61]
Formatted ... [62]
Deleted: ,
Formatted ... [63]
Deleted: including a station where the spring bloom had ... [64]
Formatted ... [65]
Deleted: and
Formatted ... [66]
Deleted: with
Formatted ... [67]
Deleted:
Formatted ... [68]
Deleted: er
Formatted ... [69]
Deleted:
Formatted ... [70]
Deleted: station …ocations hadshowed ... [71]
Formatted ... [72]
Deleted: sampled… as indicated by the lower…UPM ... [73]
Formatted ... [74]
Deleted: .
Formatted ... [75]
Formatted ... [76]
Formatted ... [77]
Deleted: , that dominated the microphytoplankton ... [78]

colonies of *Fragilariopsis* spp., *Navicula pelagica, Pseudo-nitzschia* sp., less abundant but identifiable cells of *Amphiprora hyperborean*, and *Coscinodiscus* sp. among others.

The living (green fluorescence) and dead (red fluorescence) cells were clearly identified under the LED-illumination of the epifluorescence microscopes used (Fig. 2). The fraction of living diatom cells in the photic layer averaged 59.4 ± 6.3 %, but ranged broadly, from 20.9 % in station 4, in pre-bloom state, to 72.0 % in station 5, which supported an active bloom. In contrast, the population sinking below the photic layer was comprised mostly of dead cells (20.8 ± 4.9 % living cells, Fig. 2). Indeed, the fraction of living diatoms was consistently greater in the photic layer than in the diatom stock sinking below the photic layer (Wilcoxon ranked sign test, P = 0.0078, Fig. 3), a pattern consistent across taxa found in at least four of the stations (large celled *Thalassiosira* sp., P = 0.02, N = 4, *Fragilariosis* sp., P = 0.005, N = 6; *Chaetoceros* sp., P = 0.0054, n = 6; Fig. 3), but the percent living cells in the photic layer and below this layer was not significantly different for the small-celled *Thalassiosira* (P = 0.09, N = 6).

Among stations, there was significant variability in the diatom assemblage structure. Earlier cruise stations were dominated by *Fragilariopsis* spp. and *Chaetoceros* spp. This changed at stations 6 through 8, where communities were dominated by *Fragilariopsis* spp. and *Thalassiosira* spp., these wre also the areas with the highest diatom biomass observed (station #8, Fig. 4, Krause et al. 2018). Community composition changed at the Polar Front and Barents Sea stations (Fig. 4) with a larger contribution of other taxa, including *Navicula pelagica* (station #9, Fig. 4). The diversity of the diatoms found at the aphotic zone differed in several stations from that found at the photic layer (Fig. 4). The large celled *Thalassiosira* sp. colonies dominated the aphotic community in several stations although they were not dominant at the photic community (Fig. 4). At station #4, the community sampled was more diverse at the aphotic than at the photic layer (Fig. 4). The stock of diatoms that had sunk below the photic layer comprised, on average, 24.2 ± 6.7 % of the total water column stock, with the proportional contribution ranging considerably among groups (Fig. 5). The proportion of biomass of the large-celled *Thalassiosira* colonies in the aphotic layer was the largest and *Chaetoceros* spp. the smallest (Fig. 5). Station #4 (pre-bloom status) had a larger proportion of diatom biomass in the aphotic layer and station #8, diatom bloom station, the lowest. At station #8, however, the photic-zone population of the dominant *Thalassiosira* species contained 54.8 % of living cells and was paralleled with a significant contribution of dead cells at the aphotic layer (Fig. 4), suggesting the collapse of the bloom had already been initiated despite the considerable photic-layer biomass. Similarly, *Fragilariopsis* senescence at the photic layer of station #3 (only 35.1 % of cells were alive at the photic layer) helps explain its larger contribution than in the aphotic layer (Fig. 4). There was a significant negative relationship between the percent of the diatom stock population that had sunk below the photic layer and the percent of living cells in the photic layer (R^2 = 0.39, P <0.001, Fig. 5b), indicating that healthy, actively growing populations largely remain in the surface, whereas senescent ones sink out of the photic layer.

The suggestion that dead diatom cells sink faster than living cells was tested experimentally. Initially, only 6.7 % of the cells of the *Flagilariosis* sp. and *Thalassosira* sp. colonies dominating the community tested were dead. However, all cells settling to the bottom of the sedimentation chamber within 1 h of the experiment start were dead, including large *Coscinodiscus* sp. cells (Fig. 6). The population of cells settling to the chamber bottom 4 h and 12 h following addition of the fresh, healthy community, was also largely dominated by dead *Flagilariosis* sp. and *Thalassosira* sp. colonies, 82.2 and 71.7%, respectively. And the fraction of living cells which had settled the height

Formatted ... [79]
Deleted: P
Formatted ... [80]
Deleted: The diatom community at the beginning of the ... [81]
Deleted: -7-
Deleted: to
Formatted ... [82]
Deleted: , and c
Formatted ... [83]
Formatted ... [84]
Formatted ... [85]
Deleted: that dominated the
Formatted ... [86]
Deleted: where the largest diatom bloom was found
Formatted ... [87]
Deleted: larger
Deleted:
Deleted: included in "Other", …ig. 4). The diversity of the ... [88]
Deleted: is
Formatted ... [89]
Deleted: fraction …anging considerably amongbetween ... [90]
Formatted ... [91]
Deleted: large
Formatted ... [92]
Deleted: that had sunk below the…n the a ... [93]
Formatted ... [94]
Deleted: ,…and that of …*haetoceros* spp. the smallest (Fig. ... [95]
Formatted ... [96]
Deleted: showed the
Formatted ... [97]
Deleted: the
Formatted ... [98]
Deleted: below the a
Formatted ... [99]
Deleted:
Formatted ... [100]
Deleted: supporting the largest
Formatted ... [101]
Deleted: initiation of the
Formatted ... [102]
Deleted: standing in the photic layer… Similarly, ... [103]
Formatted ... [104]
Moved (insertion) [3]
Deleted: cells (
Formatted ... [105]
Deleted: , dominated by *Flagilariosis* sp. and *Thalassosira* ... [106]
Moved up [3]: *Flagilariosis* sp. and *Thalassosira* sp.
Deleted: whereas
Formatted ... [107]

of the chamber proportionally increased with time (Fig. 6). These experimental results indicated that dead diatom cells among the groups examined sink faster than living cells.

The experiment testing diatom survival in aphotic zone indicated that once diatom cells sink below the photic layer, they would die rapidly. The incubation was performed close to the temperature below the photic layer that averaged 2.978 ºC ± 0.003 at station #3, suggesting that trends were not driven by thermal effects. The cell concentration at the onset of the experiment was 298 cells ml^{-1}. The half-life (i.e. percent of living cells reduced to half) survival times were remarkably uniform across diatom taxa, ranging from 0.9 days, for *Thalassiosira* sp. to 1.3 days for *Coscinodiscus* sp., depending on species (Fig. 7). Once dead, the cells lysed; half-life periods for cell death and lysis after transfer into aphotic darkness increasing from 1.6 days, for the smaller *Flagilariosis* sp. cells, to 5.3 days for the largest *Thalasiosira* sp. cells (Fig. 7).

4. Discussion

The results presented confirm that active and healthy diatom populations, as those actively growing during the spring bloom, are associated with relatively small stocks of fast-sinking diatoms. In contrast, unhealthy diatom populations, such as those present before blooming has initiated or in the senescent phase of the bloom, characterized by a large fraction of dead cells, support comparatively larger pools of sinking diatoms.

These observations are consistent with early reports, based mostly on laboratory cultures, indicating that dead diatom cells sink faster than living ones (Smayda, 1971). The experiment conducted, albeit at only one station, showed that dead cells sank much faster than living ones in a field assemblage with considerable diversity in species and physiological condition. Indeed, whereas the dominant populations tested were dominated by living, healthy cells, only dead cells were collected at the bottom of the sedimentation chamber over the first few hours of the experiment, and the proportion of living cells collected increased over time. Moreover, our experimental assessment of diatom survival incubated at aphotic conditions suggested that once sinking below the photic layer, diatoms cells could die at half-lives of 21.8 to 30.2 hours across species. This result, although limited to one experiment, was consistent among the major genera and functional groups analysed and reflected survival at "in situ" conditions. Smayda and Mitchell-Innes (1974) also reported the decrease in viable cells after darkness: "*After 6 days of dark incubation, the number of viable cells of Chaetoceros curvisetus recognizable decreased from 760 to 240 cells per ml*", representing a decay rate of 0.19 d^{-1} (i.e. 50% loss of cells in 3.6 days) *comparable to the rate reported here*. Other studies also reported rates of cell mortality in darkness close to those found here (Segovia and Berges 2009; Agusti et al. 2015). Phytoplankton vegetative cells do not survive under darkness (Smayda and Mitchell-Innes 1974; Segovia and Berges 2009; Segovia et al. 2003) and only resting spores and resting cells are able to survive in the dark (Ignitiades and Smayda 1970; Smayda and Mitchell-Innes 1974; Peters and Thomas 1996).

Beyond the stressor of continuous darkness, the fast decay rates observed here under aphotic experimental conditions could also have been influenced, in addition to darkness, by the low nutrient availability and/or the possible presence of pathogens or parasites, suggesting that survival of natural populations below the photic layer may be lower than expected in the dark from axenic, high- nutrients "in vitro" studies with cultures, as already pointed by Ignitiades and Smayda (1970). The $[Si(OH)_4]$ at station #3 was 0.75 µmol Si L^{-1} and $[NO_3 + NO_2]$ was 1.79 µmol N L^{-1}; Lomas et al. (2019) demonstrated that polar diatoms have higher Si:N requirements (>1.5

Deleted: retrieved in the lower sampling port after ... [108]
Formatted ... [109]
Deleted: light conditions conducted
Formatted ... [110]
Deleted: median…alf-life (i.e. percent of living cells ... [111]
Deleted: although limited in number
Formatted ... [112]
Deleted:
Deleted: sunk
Formatted ... [113]
Deleted:
Deleted: analyzed
Formatted ... [114]
Deleted: tes…eflectedted ... [115]
Formatted ... [116]
Commented [SRA4]: Smayda, T. J., Mitchell-Innes, B. ... [117]
Formatted ... [118]
Deleted: .
Formatted ... [119]
Deleted:
Formatted ... [120]
Commented [SRA5]: Segovia, M. and Berges, J. A. ... [121]
Formatted ... [122]
Deleted: hardly
Formatted ... [123]
Commented [SRA6]: Cell Death in the Unicellular ... [124]
Formatted ... [125]
Deleted: the mechanisms of survival imply the formation of
Formatted ... [126]
Commented [SRA7]: Ignatiades, L., & Smayda, T. J. ... [127]
Formatted ... [128]
Formatted ... [129]
Deleted: T
Formatted ... [130]
Deleted: were influenced, moreover by darkness,
Deleted:
Formatted ... [131]
Formatted ... [132]
Commented [JK8]: Susana, ... [133]
Formatted ... [134]
Formatted ... [135]

mol:mol) than lower latitude diatoms, thus, nutrients data would predict Si would be the yield limiting relative to N. The progressive [$Si(OH)_4$] limitation likely slowed diatom growth in the field condition prior to the experiment but living diatoms at the initial condition were still a majority (i.e. 63.5%) of the quantifiable cells.

Cell abundances observed were consistent with previous studies. In the Canadian Arctic, living cells in open-water and ice-covered stations represented the 57.3 ± 5.8% and 48.0 ± 3.9% (± SE), respectively (Alou–Font et al., 2006), similar to proportions in our study. The percentage of living cells was higher during the bloom periods than periods before and after (Alou-Font et al. 2006). This trend appeared to be driven by light and low nutrient concentrations (Alou–Font et al., 2006). The quantification of the percent of living cells in our study helped to identify the different stages of the Arctic spring bloom among the stations sampled. A pre-bloom situation with low cell abundance and a small percentage of living cells was found at station #4 located further west of Svalbard Islands, where silicic acid and nitrogen concentrations were higher and mixing was more significant than in other arctic stations. The healthiest diatom community was observed at station #5, where the high stratification and $Si(OH)_4$ concentration above the half saturation constant (Ks) of 2 µM (from kinetic experiments in the same region by Krause et al. 2018) helped the diatoms to grow actively. The highest cell abundance was observed at station #8, but this persisted with a lower percentage of living diatoms and the $Si(OH)_4$ concentration well below the Ks value indicated that the bloom was reaching the maximum capacity, although diatom sinking was still low. A post-bloom situation was identified at the polar front community, with similar percentages of living cells at the photic and aphotic zones as a result of high sinking induced by Si and nitrogen limitation, as suggested by the lower $Si(OH)_4$ K_S of 0.8 µM (Krause et al. 2018). The diatom community captured by the Bottle Net below the photic layer was consistent with the limited, but comparable, data obtained by sediment traps deployed in the area which also indicated *Fragilariopsis and Thalassiosira* species to be the dominant contributors to Si and biomass export (Krause et al. 2018).

Given the range of bloom development represented among out stations, the results presented conform to a conceptual model where nutrients, including Si (Rey 2012; Krause et al., 2018), and mixed layer drives the growth of diatoms during the Arctic spring bloom (Wassmann et al., 1997; Reigstad et al 2002). For diatoms, Si depletion results in two potential physiological issues: yield limitation (i.e. diatom standing stock is too high to be supported by the available silicic acid) and intense kinetic/growth limitation (i.e. depleted silicic acid limits diatom Si uptake to such a degree that growth must slow, Krause et al., 2018). Thus, such a situation would stimulate mass sedimentation, suggested to be an evolutionary adaptation to help diatoms communities persist when nutrients are limiting (Raven and Waite, 2004). A large fraction (30 – 50%) of the diatom cells in silicon-depleted photic layer were dead, pointing at acute silicic acid limitation as the likely factor triggering partial mortality, while the remaining cells are likely to be senescent. Unhealthy diatoms would then lose the capacity to actively regulate buoyancy that characterizes healthy diatom cells (Smayda, 1970), leading to rapid sinking of the bloom. Acute silicic acid limitation, is identified, therefore, as the event leading to loss of the capacity to actively regulate buoyancy that characterizes diatom cells (Smayda, 1970), and rapid sinking of the bloom. The potential for rapid sedimentation is enhanced, due to the higher silica quotas for polar diatoms (Lomas et al. 2019) compared to lower-latitude diatoms (Brzezinski 1985).

Deleted: Assuming that the diatoms silica per cell ranged ... [136]
Formatted ... [137]
Deleted: as already pointed by Ignitiades and Smayda ... [138]
Formatted ... [139]
Deleted: The averaged living cells found in the photic layer ... [140]
Formatted ... [141]
Deleted: e
Formatted ... [142]
Deleted: where the
Formatted ... [143]
Deleted: the …pen- ... [144]
Formatted ... [145]
Deleted: s stations…and ice- ... [146]
Formatted ... [147]
Commented [JK9]: 2016 reference, please correct
Formatted ... [148]
Moved (insertion) [4]
Deleted: ,…and decreased…han periods before and after ... [149]
Commented [JK10]: 2016 reference, please correct
Deleted: High and low light within the water column and ... [150]
Formatted ... [151]
Deleted: s
Commented [JK11]: 2016 reference, please correct
Formatted ... [152]
Formatted ... [153]
Deleted:
Moved up [4]: The percentage of living cells was higher
Formatted ... [154]
Deleted: Q
Deleted: %
Formatted ... [155]
Deleted: arctic
Formatted ... [156]
Deleted: at
Formatted ... [157]
Deleted: e
Formatted ... [158]
Deleted: %
Formatted ... [159]
Deleted: …oncentration well below the Ks value indicated ... [160]
Formatted ... [161]
Deleted: When compared across the contrasting ... [162]
Formatted ... [163]
Deleted: silicic acid
Formatted ... [164]
Formatted ... [165]
Deleted: Diatom mortality, likely triggered by
Formatted ... [166]
Deleted: a
Formatted ... [167]
Commented [JK13]: Brzezinski, Mark A. "The Si C ... [168]
Deleted: .
Formatted ... [169]

Diatoms have been shown to have a remarkable metabolic capacity to regulate buoyancy (Gemmel et al., 2016), both maintaining zero (Gemmel et al., 2016) and positive buoyancy (e.g. Villareal et al., 2014) involving regulation through the production of osmolytes (Gradmann and Boyd, 2002), which plays an important role in exploiting nutrient patchiness within the photic layer (Villareal et al. 2014). Diatom sinking rates are inversely related to growth rate (Gemmel et al., 2016), so that silicon depletion is expected to result in increased sinking rates, despite field diatoms reducing their silica per cell when kinetically limited by silicic acid (McNair et al. 2018). There is experimental demonstration that silicon depletion plays the most important role, compared to nitrogen or phosphorus, in triggering rapid sinking of diatom cells, indicating that biochemical aspects of silicon metabolism are particularly important to diatom buoyancy regulation (Bienfang et al. 1982). N:P ratios in this region do not suggest that phosphorus plays a limiting role in primary production, and when silicic acid is depleted, enough nitrate remains to fuel growth of other phytoplankton groups (e.g. *Phaeocystis*, Krause et al., 2018). Once diatoms lose their capacity to regulate buoyancy and sink below the photic layer, they die rapidly and are unable of ascending back to the photic layer, resulting in the rapid sinking fluxes that drives high sedimentation rates characteristic of the termination of the Arctic spring bloom (Oli et al., 2002; Wassmann et al., 2006, Bauerfeind et al., 2009). Rapid sinking of the Arctic spring bloom, in turn, precludes carbon recycling in the photic layer, thereby leading to undersaturated pCO_2 driving the large atmospheric CO_2 uptake characteristic of the European sector of the Arctic during this season which do not equilibrate with the atmosphere until months later (Bates et al. 2009, Takahashi et al., 2002; Holding et al., 2015).

A large fraction of the total water column phytoplankton biomass was observed below the photic layer, representing on average 24% ± 6.7 (±SE) of the surface diatom populations in the study area. This considerable proportion can be explained by high diatom export from the photic zone, opposed to lateral advection. This is consistent with the high rates of biogenic silica (proxy for diatom biomass) export at stations 4, 7-8 and 10, rates were a factor of four higher than integrated diatom silica production in the upper water column and represented up to 40% of the integrated diatom silica standing stock (Krause et al., 2018). These cruise trends are in agreement with the observation of large sinking events in the Arctic as reported for ice diatoms (Boetius et al., 2014; Aumack et al., 2014) associated to ice melting in the Arctic, which represent a large carbon supply to benthic communities in the Arctic shelves (Moran et al., 2005; Tamelander et al., 2006). While we do not report data for an ice-diatom assemblage, data for the same cruise showed that ice diatoms silicon uptake rates near stations #7, #8 were strongly limited by $Si(OH)_4$ concentration in the surface waters, likely to a degree limiting their growth (Krause et al., 2018), and previous studies have noted their susceptibility to silicon limitation (Cota et al. 1990; Smith et al. 1990). Our results show that healthy phytoplankton communities remained at the photic layer, although dying communities exported a large fraction of the biomass (up to 65%) to the aphotic zone.

In summary, the results presented here support a link between diatom cell health status, likely driven by progressive nutrient limitation, and sedimentation fluxes in the Arctic. Whereas the link between diatom health status and sinking rates has long been established (Smayda, 1971), the evidence corresponded to algal cultures in the laboratory. This is the first demonstration of these ideas using natural diatom communities in this region —partially due to the logistical challenges of assessing both viability and settling in the field. Our conceptual model suggests that deterioration of diatom health, such as occurring when reaching acute silicon or other resources limitation along

Formatted: English (UK)

Formatted: English (UK)

Deleted: of the phytoplankton biomass below the photic layer …

Deleted: should

Deleted: of diatoms sinking

Deleted: to the sea bottom.

Formatted: English (UK)

Deleted: a large

Formatted: English (UK)

Formatted: English (UK)

Formatted: English (UK)

Deleted: and that must

Formatted: English (UK)

Commented [JK14]: Smith, R. E., Harrison, W. G., Harris, L. R. & Herman, A. W. Vertical fine structure of particulate matter and nutrients in sea ice of the high Arctic. *Canadian Journal of Fisheries and Aquatic Sciences* **47**, 1348-1355 (1990).

Cota, G. F., Anning, J. L., Harris, L. R., Harrison, W. G. & Smith, R. E. Impact of ice algae on inorganic nutrients in seawater and sea ice in Barrow Strait, NWT, Canada, during spring. *Canadian Journal of Fisheries and Aquatic Sciences* **47**, 1402-1415 (1990).

Formatted: English (UK)

Commented [JK15]: Based on table 1, I only calculate a maximum of 54% at station 4.

Formatted: English (UK)

Formatted: English (UK)

Deleted: , and was lacking for

Formatted: English (UK)

Deleted: D

Formatted: English (UK)

the spring bloom, leads to loss of the capacity to regulate buoyancy and leads to rapid sinking, with cells exported below the photic layer dying quickly. Understanding the role of cell health status, and the role of silicic acid depletion, in the regulation of diatom sinking rates is fundamental to mechanistically understand the biological pump in the Arctic and its response to future changes.

Acknowledgements

This research was supported by King Abdullah University of Science and Technology through baseline funding BAS/1/1072-01-01 and BAS/1/1071-01-01 to SA and CMD, respectively; the ARCEx project funded by industry partners and the Research Council of Norway (project #228107) to PW; and funding provided by Dauphin Island Sea Lab to J. Krause. We thank the science team and crew of the R/V *Helmer Hanssen*, as well as S. Øygarden, E. Kube, A. Renner, D. Vogedes, H. Foshaug, S. Acton, D. Wiik, B. Vaaja and W. Dobbins for logistic support.

References

Agusti, S., González-Gordillo, J. I., Vaqué, D., Estrada, M., Cerezo, M.I., Salazar, G., Gasol, J.M. and Duarte, C. M. : Ubiquitous healthy diatoms in the deep sea confirms deep carbon injection by the biological pump, Nature Communications 6:7608. DOI: 10.1038/ncomms8608, 2015.

Alou-Font, E., Roy, S., Agustí, S. and Gosselin, M.: Cell viability, pigments and photosynthetic performance of Arctic phytoplankton in contrasting ice-covered and open-water conditions during the spring-summer transition, Marine Ecology Progress Series, 3, 543:89-106, 2016.

Aumack, C. F., A. R. Juhl, and C. Krembs. Diatom vertical migration within land-fast Arctic sea ice. Journal of Marine Systems 139 (2014): 496-504.

Bates, N. R., and Mathis, J. T. The Arctic Ocean marine carbon cycle: Evaluation of air–sea CO2 exchanges, ocean acidification impacts and potential feedbacks. Biogeosciences 6, 2433–2459, 2009.

Bauerfeind, E., Nöthig, E.-M., Beszczynska, A., Fahl, K., Kaleschke, L., Kreker, K., Klages, M., Soltwedel, T., Lorenzen, C. and Wegner, J. :Variations in vertical particle flux in the Eastern Fram Strait (79°N/4°E) during 2000-2005. Results from the Deep-Sea Long-Term observatory HAUSGARTEN, *Deep-Sea Research I* **56,** 1471-1487, 2009.

Bienfang, P. K., Harrison, P. J., and Quarmby, L. M.: Sinking rate response to depletion of nitrate, phosphate and silicate in four marine diatoms, Marine Biology, 67(3), 295-302, 1982.

Boetius, A., Albrecht, S., Bakker, K., Bienhold, C., Felden, J., Fernández-Méndez, M., ... and Nicolaus, M.: Export of algal biomass from the melting Arctic sea ice. Science, 339(6126), 1430-1432, 2013.

Brzezinski, M. A.: The Si: C: N ratio of marine diatoms: Interspecific variability and the effect of some environmental variables 1. Journal of Phycol, 21, no. 3, 347-357, 1985.

Gradmann D., and Boyd C.M.: Impact of osmolytes on buoyancy of marine phytoplankton, Mar Biol. ,141: 605–618, 2002.

Holding, J., Duarte, C. M., Sanz-Martín, M., Mesa, E., Arrieta, JM., Chierici, M., Hendriks, I., García-Corral, L.S., Regaudie-de-Gioux, A., Delgado, A., Reigstad, M., Wassmann, P., and Agustí, S.: Temperature dependence of CO2-enhanced primary production in the European Arctic Ocean. Nature Climate Change, 5 (12), 1079-1082, 2015.

Ignatiades, L., and Smayda, T. J.: Autecological studies on the marine diatom rhizosolenia fragilissima bergon. ii. enrichment and dark viability experiments 1. Journal of Phycology 6, 357-364, 1970.

Krause, J., Duarte, C.M., Marquez, I.A., Assmy, P., Fernandez-Mendez, M., Wiedmann, I., Wassmann, P., Kristiansen, S., and Agusti, S.: Biogenic silica production and diatom dynamics in the Svalbard region during spring. Biogeosciences, 15, 6503–6517, 2018. https://doi.org/10.5194/bg-15-6503-2018.

Krause, J.W., Schulz, I.K, Rowe, K.A., Dobbins, W., Winding, M., Sejr, M., Duarte, C.M., Agustí, S.: Silicon limitation drives bloom termination and carbon sequestration in an Arctic bloom. Scientific Reports. In review.

Llabrés, M., and Agustí, S.: Extending the cell digestion assay to quantify dead phytoplankton cells in cold and polar Waters, Limnol. Oceanogr. Meth. 6, 659–666, 2008.

Formatted: English (UK)

Formatted: Font: Not Italic, Complex Script Font: Not Italic, English (US)

Formatted: Font: Not Italic, Complex Script Font: Not Italic

Formatted: English (US)

Formatted: English (US)

Formatted: English (UK)

Deleted: ;

Deleted: ;

Formatted: Font: Not Italic, Complex Script Font: Not Italic, English (UK)

Formatted: English (UK)

Formatted: Font: Not Italic, Complex Script Font: Not Italic, English (UK)

Formatted: English (UK)

Field Code Changed

Formatted: Hyperlink, Font: 12 pt, Complex Script Font: 12 pt, English (UK)

Deleted: Biogeosciences Discussions, doi: 10.5194/bg-2018-226, Biogeosciences In press, 2018

Formatted: English (UK)

Formatted: English (US)

Formatted: English (UK)

Formatted: Font: Not Italic, Complex Script Font: Italic, English (UK)

Formatted: Complex Script Font: Italic, English (UK)

Formatted: Font: Not Bold, Complex Script Font: Not Bold, Italic, English (UK)

Formatted: Complex Script Font: Italic, English (UK)

Lomas, M.W., Baer, S.E., Acton, S., Krause, J.W.: Pumped up by the Cold: Elemental Quotas and Stoichiometry of Polar Diatoms. *Frontiers in Marine Science*, doi: 10.3389/fmars.2019.00197, 2019.

McNair, H. M., Brzezinski, M. A., Krause, J. W.:Diatom populations in an upwelling environment decrease silica content to avoid growth limitation. Environmental Microbiology, in press, 2018.

Moran, S. B., Kelly, R. P., Hagström, K., Smith, J. N., Grebmeier, J. M., Cooper, L. W., ... & Maslowski, W.: Seasonal changes in POC export flux in the Chukchi Sea and implications for water column-benthic coupling in Arctic shelves, Deep Sea Research Part II: Topical Studies in Oceanography, 52(24-26), 3427-3451, 2005.

Mura, M.P., M. P. Satta and Agustí, S.: Water-mass influences on summer Antarctic phytoplankton biomass and community structure, Polar Biol, 15 (15-20), 1995.

Olli, K., P. Wassmann, P. Ratkova, T. Arashkevich, E. and Pasternak, A.: Seasonal variation in vertical flux of biogenic matter in the marginal ice zone and the central Barents Sea, Journal of Marine Systems, 38: 189-204, 2002.

Peters, E., and Thomas, D.N.: Prolonged darkness and diatom mortality I: Marine Antarctic species. Journal of Experimental Marine Biology and Ecology, 207, 25-41, 1996.

Raven, J. A., Waite, A. M.: The evolution of silicification in diatoms: inescapable sinking and sinking as escape? New phytologist, 162(1), 45-61, 2004.

Reigstad, M., Wassmann, P., Riser, C. W., Øygarden, S., and Rey, F.: Variations in hydrography, nutrients and chlorophyll a in the marginal ice-zone and the central Barents Sea, J. Marine Syst., 38, 9–29, 2002.

Rey, F.: Declining silicate concentrations in the Norwegian and Barents Seas, ICES Journal of Marine Science, 69, 208-212, 2012.

Segovia M., Haramaty, L., Berges, J. A., and Falkowski, P.G.: Cell Death in the Unicellular Chlorophyte *Dunaliella tertiolecta*. A Hypothesis on the Evolution of Apoptosis in Higher Plants and Metazoans, Plant Physiology, 132, 9-105, 2003; **DOI:** 10.1104/pp.102.017129.

Segovia, M. and Berges, J. A.: Inhibition of caspase-like activities prevents the appearance of reactive oxygen species and dark-induced apoptosis in the unicellular chlorophyte *Dunaliella tertiolecta*1, Journal of Phycology, 45, 1116-1126, 2009. doi:10.1111/j.1529-8817.2009.00733.x

Smayda, T. J.: The suspension and sinking of phytoplankton in the sea, Oceanogr. Mar. Biol. 8, 353–414, 1970.

Smayda, T. J.: Normal and accelerated sinking of phytoplankton in the sea, Mar. Geol 11, 105–122, 1971.

Smayda, T. J., and Mitchell-Innes, B.: Dark survival of autotrophic, planktonic marine diatoms, Mar. Biol., 25, 195-202, 1974.

Smith, R. E., Harrison, W. G., Harris, L. R. & Herman, A. W.: Vertical fine structure of particulate matter and nutrients in sea ice of the high Arctic. *Canadian Journal of Fisheries and Aquatic Sciences* **47**, 1348-1355, 1990.

Takahashi, T. et al.: Global sea–air CO2 flux based on climatological surface ocean pCO2 , and seasonal biological and temperature effects. Deep Sea Res. II 49, 1601–1622, 2002.

Tamelander, T., Renaud, P. E., Hop, H., Carroll, M. L., Ambrose Jr, W. G., & Hobson, K. A.: Trophic relationships and pelagic–benthic coupling during summer in the Barents Sea Marginal Ice Zone, revealed by stable carbon and nitrogen isotope measurements, Marine Ecology Progress Series, 310, 33-46, 2006.

Deleted: ¶
Formatted: English (UK)
Deleted: logy
Formatted: Font: Not Italic, Complex Script Font: Not Italic, English (UK)
Formatted: English (UK)
Formatted: English (UK)
Formatted: English (UK)
Deleted: .
Deleted: (
Deleted:)
Formatted: Font: Not Italic, Complex Script Font: Not Italic, English (UK)
Formatted: English (UK)
Formatted: Font: Not Italic, Complex Script Font: Not Italic, English (UK)
Formatted: English (UK)
Formatted: English (UK)

Vaquer-Sunyer, R. et al. Seasonal patterns in Arctic planktonic metabolism (Fram Strait—Svalbard region). Biogeosciences 10, 1451–1469, 2013.

Villareal, T. A., Pilskaln, C. H., Montoya, J. P., and Dennett, M.: Upward nitrate transport by phytoplankton in oceanic waters: balancing nutrient budgets in oligotrophic seas, PeerJ, 2, e302, 2014.

Wassmann, P., Egge, J. K., Reigstad, M., and Aksnes, D. L.: Influence of dissolved silicate on vertical flux of particulate biogenic matter, Mar. Pollut. Bull. 33: 10-21,1997.

Wassmann, P., Slagstad, D., Rise CWr, and Reigstad, M.: Modelling the ecosystem dynamics of the Barents Sea including the marginal ice zone II. Carbon flux and interannual variability, Journal of Marine Systems, 59, 1-24, 2006.

Formatted: English (UK)

Deleted: .

Deleted: .

Deleted: .

Figure headings

Figure 1: ARCEx cruise study area with the insert showing the station number and location (green dot) around the Svalbard Islands.

Figure 2: Photographs of the natural Arctic diatoms sampled with the Bottle-Net observed under epifluorescence microscopy and stained with the Bac-light Kit. (a) Colonies of *Thalassosira* sp. showing green fluorescence corresponding to living cells. (b) Colonies of *Fragilariopsis* sp. showing dead cells (red fluorescence, vertical-left colonies) and living cells (green fluorescence, transversal-right colony). (c) Surface layer community composed of multiple diatom genera (*Chaetoceros* sp., *Fragilariopsis* sp., *Thalassiosira* sp., pennates), showing blend of living cells (green fluorescence) and dead cells (red fluorescence). (d) Aphotic zone sample showing dead colonies (red fluorescence) of *Fragilariopsis* sp. and *Thalassiosira* sp. (two-cell colony in the bottom-right of the photo).

Figure 3: Box plots showing the distribution of the percentage of living diatoms in the photo (blue) and aphotic (brown) layers. Percentage of living cells among (a) the total diatom community and (b) for the populations of the most abundant diatom taxa observed during the cruise. The asterisks indicate significant differences between photic and aphotic zones ($p < 0.05$). Boxes encompass the central 50% of the data, the horizontal line inside the box represents the median, and vertical bars encompass 90% of the data.

Figure 4: Pie charts showing the diatom community within the photic and aphotic zones among stations. Colours correspond to different taxa and the numbers indicate the percentage of cells relative to the total community.

Figure 5: (a) The proportion (mean ± SE) of the water-column stock observed in the aphotic zone (relative to the sum of the aphotic and photic zones) among the different diatom taxa. (b) The relationship between the percentage of living diatoms cells among the different populations in the photic layer and the proportion of the water-column population stock observed in the aphotic zone. The line represents the fitted linear regression ($R^2 = 0.39$, $P < 0.001$).

Figure 6: Diatom cell viability among the sinking cells. The initial percentage of dead cells corresponds to the fresh photic-zone Arctic microplankton sample (see Methods) and added to the surface of the sinking column (1.35 m height) at time 0. The percentages of dead cells at the bottom of the sinking column were collected at intervals of time of 0, 1, 4 and 12 hours after sample inoculum addition.

Figure 7: Decay in the cell abundance of living (blue diamonds) and total (orange squares) diatoms when exposed to aphotic zone light conditions. (a) large celled *Thalassiosira* sp. sp. (b) *Fragilariopsis* spp. (c) *Thalassiosira* sp. (d) Pennate diatom. The solid black lines and equations show the fitted linear regressions for the percent of living cells (blue box, all fitted lines significant $p < 0.05$) and total population cells (orange box, none of the fitted lines were significant $p > 0.05$).

Deleted: S
Formatted ... [170]
Deleted: ,…with the insert showing the station number and ... [171]
Formatted ... [172]
Deleted: locations as
Formatted ... [173]
Deleted: s… , and labeled with the station number. ... [174]
Formatted ... [175]
Deleted:
Formatted ... [176]
Deleted: by
Formatted ... [177]
Deleted:
Formatted ... [178]
Deleted: s from different…genera (*Chaetoceros* sp., ... [179]
Formatted ... [180]
Deleted: (
Formatted ... [181]
Deleted: living
Formatted ... [182]
Deleted:) and
Formatted ... [183]
Deleted: (dead cells)
Formatted ... [184]
Deleted: s
Deleted: encountered …n the photoupper layer ... [185]
Formatted ... [186]
Deleted: zone
Formatted ... [187]
Deleted: for
Formatted ... [188]
Deleted: at the different sampling stations encountered at
Formatted ... [189]
Deleted: The …colors ... [190]
Formatted ... [191]
Deleted: d…the percentage of cells with respect to…d ... [192]
Formatted ... [193]
Deleted: in the
Deleted: population …tock observed found ... [194]
Formatted ... [195]
Deleted: for
Formatted ... [196]
Deleted: for
Formatted ... [197]
Deleted: found
Formatted ... [198]
Deleted: C
Formatted ... [199]
Deleted: of the diatoms quantified…mong during ... [200]
Formatted ... [201]
Deleted: experiment
Formatted ... [202]
Deleted: corresponded
Formatted ... [203]
Deleted: a
Formatted ... [204]
Deleted: (20 µm) …ample (collected ... [205]
Formatted ... [206]

Table 1. Stations number and location, averaged (±SE) photic layer temperature, salinity, upper mixed layer (UPM) depth, nutrients, and measurements made with the Bottle-Net (BN) in the photic and aphotic zones, indicating the depth of the tows, and the abundance and percentage of living diatoms found at the two layers.

Station	Latitude N	Longitude E	Temperature (°C)	Salinity (psu)	UPM (m)	NO_3+NO_2 (µM)	PO_4 (µM)	$Si(OH)_4$ (µM)	BN Photic (range, m)	BN Aphotic (range, m)	Photic diatoms (cells m^{-2})	Aphotic diatoms (cells m^{-2})	Photic living diatoms (%)	Aphotic living diatoms (%)
# 3, Bellsund Hula	77 28.09	13 27.483	0.81 ± 0.33	34.48 ± 0.083	14.5	1.79 ± 1.52	0.27 ± 0.11	0.75 ± 0.45	45-0	197-55	3.160E+07	6.843E+06	63.54	21.70
# 4, Bredjupet	77 03.356	13 23.369	4.64 ± 0.025	35.0 ± 0.001	65.5	9.44 ± 0.097	0.63 ± 0.019	4.16 ± 0.046	60-0	415-100	3.04E+05	1.63E+05	20.93	9.47
# 5, Inngang Hornsund	76 58.73	15 44.113	-0.54 ± 0.035	34.27 ± 0.037	24.2	5.66 ± 0.019	0.34 ± 0.078	2.45 ± 0.40	50-0	220-80	3.20E+07	3.00E+05	72.03	0.50
# 6, Hornsund Dypet	76 51.244	15 13.143	-0.034 ± 0.1	28.98 ± 4.5	9.8	0.49 ± 0.37	0.17 ± 0.03	0.36 ± 0.118	50-0	220-60	2.01E+09	4.69E+08	70.03	8.31
# 7, Erik Eriksen Strait	79 09.986	26 02.20	-1.44 ± 0.093	34.29 ± 0.04	35.9	0.03 ± 0.026	0.16 ± 0.01	0.07 ± 0.012	50-0	260-70	1.25E+07	1.13E+06	61.12	26.99
# 8, Erik Eriksen Strait	79 10.479	26 27.518	-1.31 ± 0.088	34.22 ± 0.4	3.0	2.23 ± 1.64	0.15 ± 0.077	0.57 ± 0.40	50-0	245-70	2.47E+10	5.56E+06	69.79	31.27
# 9, Polar Front	77 15.308	29 29.243	2.04 ± 0.099	34.7 ± 0.027	34.0	0.14 ± 0.034	0.204 ± 0.022	1.29 ± 0.17	50-0	180-60	2.76E+07	2.27E+06	45.97	50.00
# 10, Barents Sea	76 13.513	29 43.710	4.06 ± 0.044	34.9 ± 0.001	75.0	3.21 ± 0.20	0.345 ± 0.03	1.48 ± 0.156	50-0	180-60	1.45E+08	2.35E+07	71.77	13.14

Formatted: English (UK)

Formatted: English (UK)

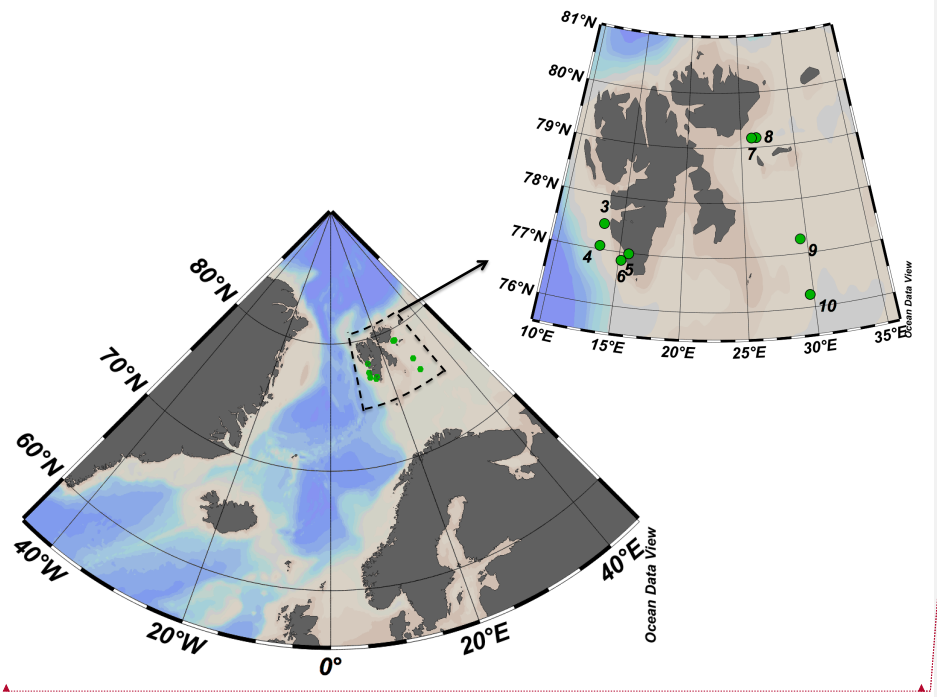


Figure 1

Formatted: English (UK)

Formatted: English (UK)

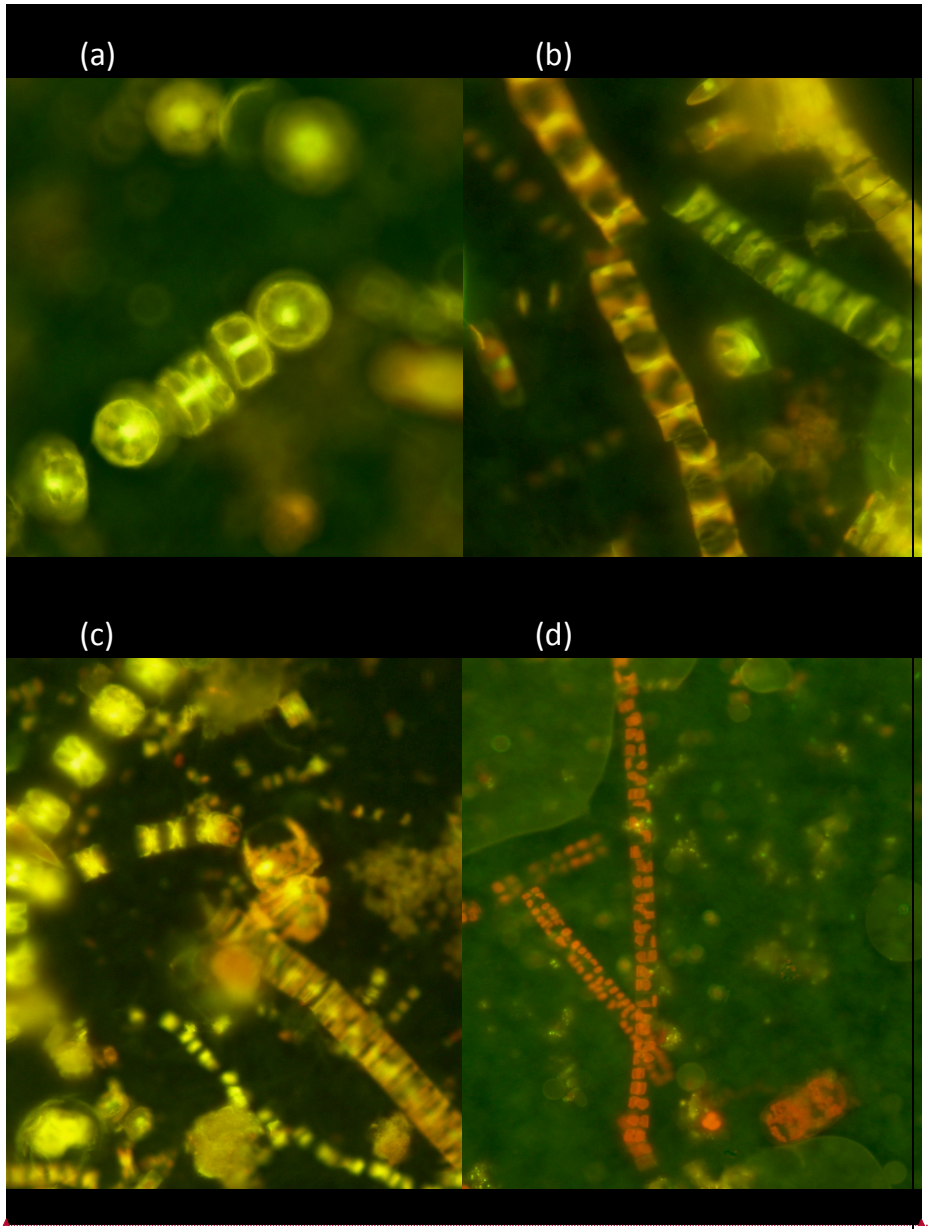


Figure 2

Formatted: English (UK)

Formatted: English (UK)

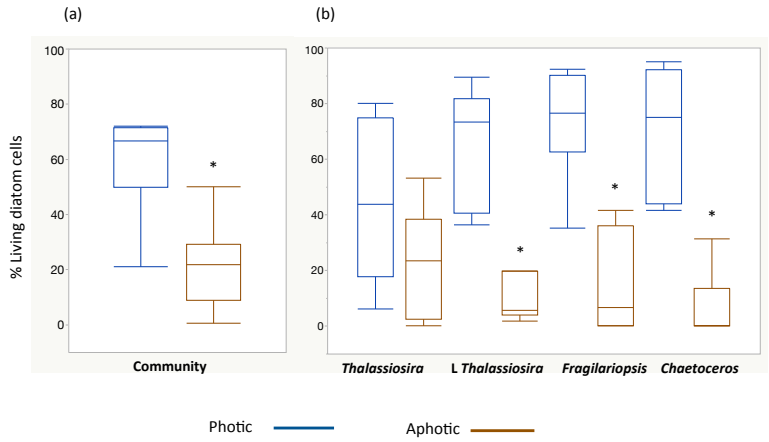


Figure 3

Formatted: English (UK)

Formatted: English (UK)

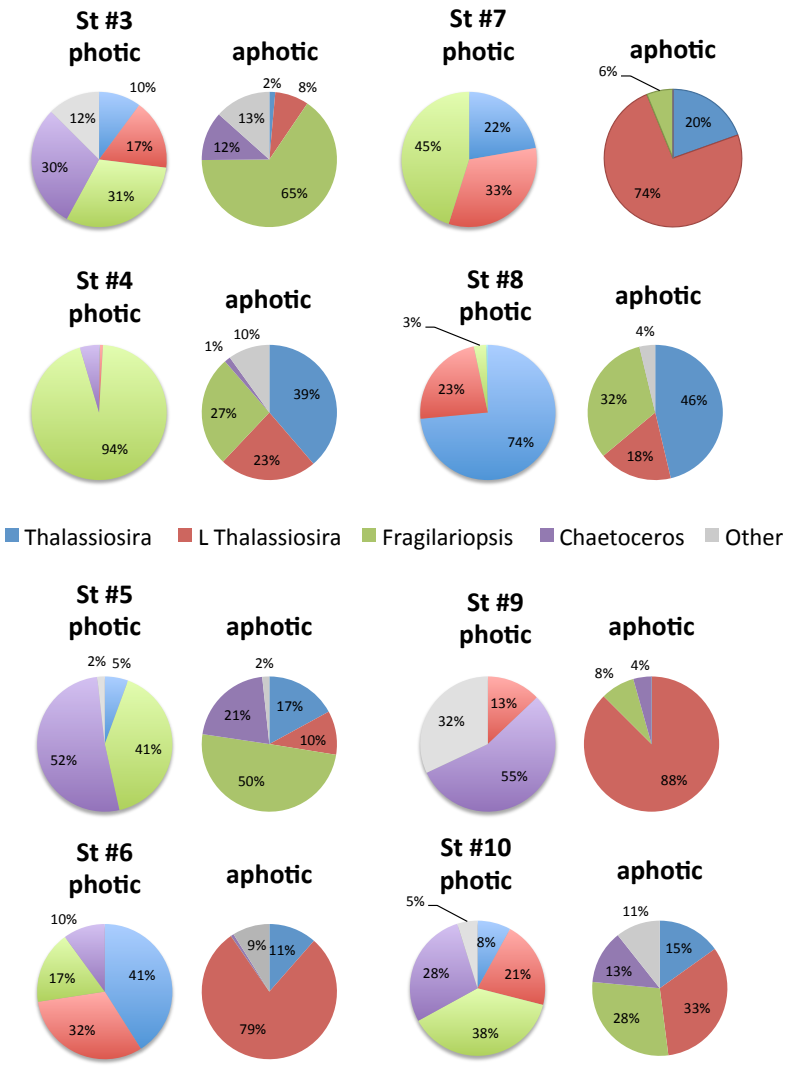


Figure 4

5

Formatted: English (UK)

Formatted: English (UK)

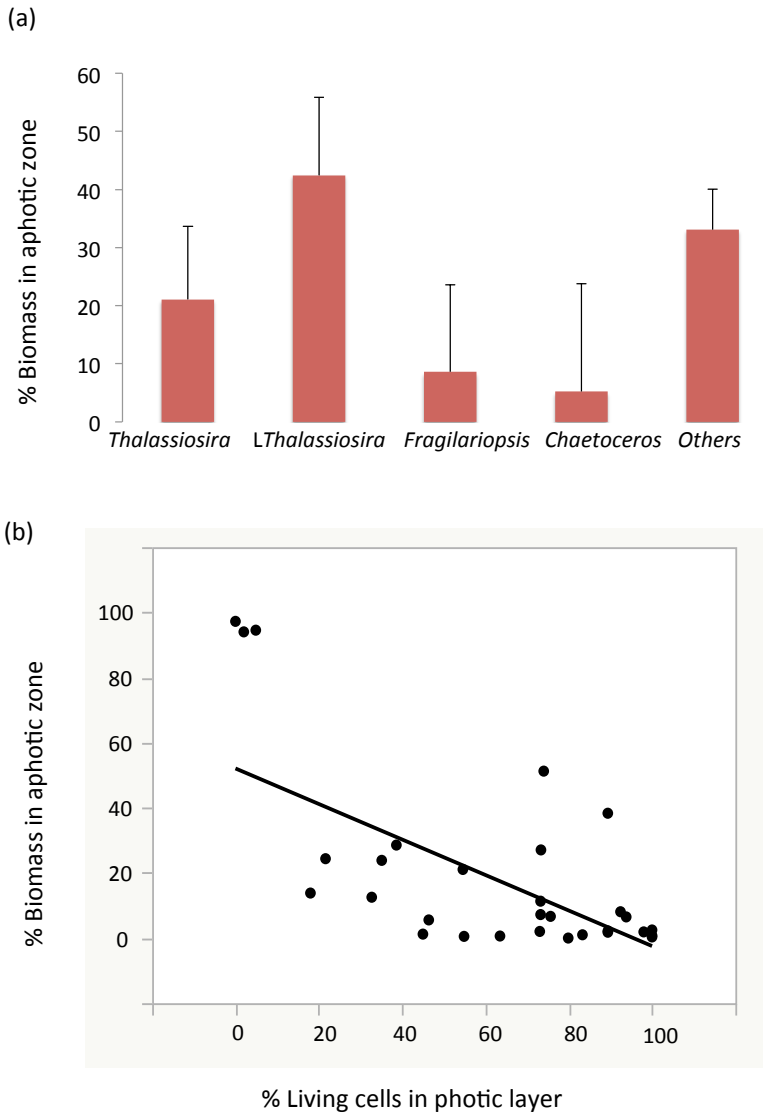


Figure 5

Formatted: English (UK)

Formatted: English (UK)

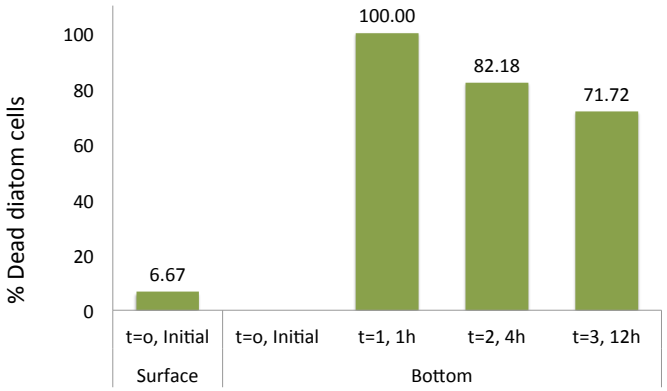


Figure 6

Formatted: English (UK)

Formatted: English (UK)

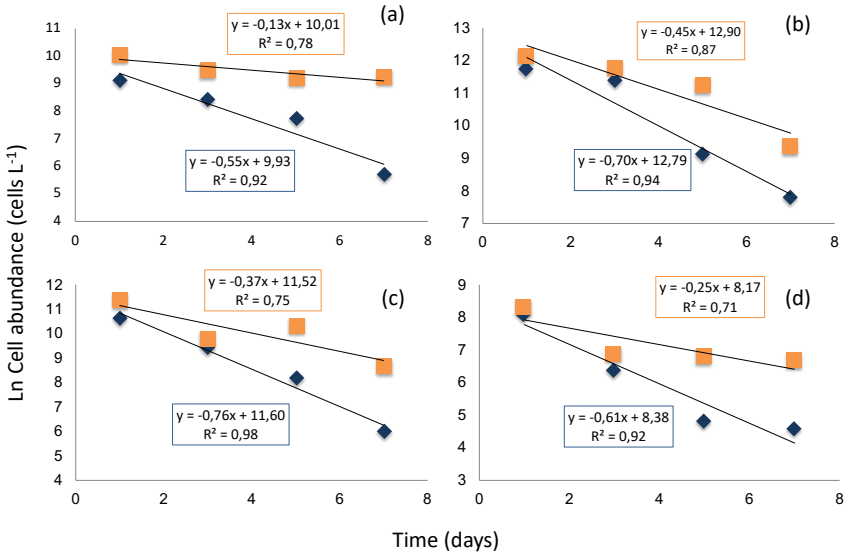
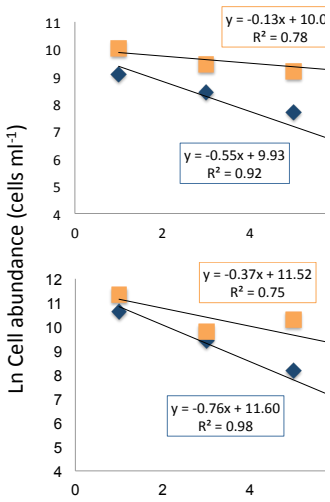


Figure 7



Deleted:

Formatted: English (UK)

Formatted: English (UK)

Page 10: [1] Commented [JK2] Jeffrey Krause 5/6/19 10:43:00 AM

Lomas, M.W., Baer, S.E., Acton, S., Krause, J.W. 2019. Pumped up by the Cold: Elemental Quotas and Stoichiometry of Polar Diatoms. *Frontiers in Marine Science*. doi: 10.3389/fmars.2019.00197

Page 10: [2] Commented [JK3] Jeffrey Krause 5/6/19 10:43:00 AM

Baines, Stephen B., Benjamin S. Twining, Mark A. Brzezinski, David M. Nelson, and Nicholas S. Fisher. "Causes and biogeochemical implications of regional differences in silicification of marine diatoms." *Global Biogeochemical Cycles*24, no. 4 (2010).

Page 11: [3] Deleted Jeffrey Krause 5/6/19 10:37:00 AM

Page 11: [3] Deleted Jeffrey Krause 5/6/19 10:37:00 AM

Page 11: [3] Deleted Jeffrey Krause 5/6/19 10:37:00 AM

Page 11: [3] Deleted Jeffrey Krause 5/6/19 10:37:00 AM

Page 11: [3] Deleted Jeffrey Krause 5/6/19 10:37:00 AM

Page 11: [4] Deleted Jeffrey Krause 5/6/19 10:38:00 AM

Page 11: [4] Deleted Jeffrey Krause 5/6/19 10:38:00 AM

Page 11: [4] Deleted Jeffrey Krause 5/6/19 10:38:00 AM

Page 11: [4] Deleted Jeffrey Krause 5/6/19 10:38:00 AM

Page 11: [4] Deleted Jeffrey Krause 5/6/19 10:38:00 AM

Page 11: [4] Deleted Jeffrey Krause 5/6/19 10:38:00 AM

Page 11: [4] Deleted Jeffrey Krause 5/6/19 10:38:00 AM

Page 11: [4] Deleted Jeffrey Krause 5/6/19 10:38:00 AM

Page 11: [4] Deleted Jeffrey Krause 5/6/19 10:38:00 AM

Page 11: [5] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 11: [5] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 11: [6] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

Subscript

Page 11: [6] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

Subscript

Page 11: [7] Deleted Jeffrey Krause 5/6/19 10:47:00 AM

Page 11: [7] Deleted Jeffrey Krause 5/6/19 10:47:00 AM

Page 11: [8] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 11: [8] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 11: [9] Deleted Jeffrey Krause 5/6/19 10:48:00 AM

Page 11: [10] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 11: [10] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 11: [10] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 11: [11] Deleted Jeffrey Krause 5/6/19 10:50:00 AM

Page 11: [11] Deleted Jeffrey Krause 5/6/19 10:50:00 AM

Page 11: [11] Deleted Jeffrey Krause 5/6/19 10:50:00 AM

Page 11: [12] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 11: [12] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 11: [12] Formatted	Jeffrey Krause	5/6/19 12:53:00 PM

English (UK)

Page 11: [13] Deleted	Jeffrey Krause	5/6/19 10:51:00 AM
Page 11: [13] Deleted	Jeffrey Krause	5/6/19 10:51:00 AM
Page 11: [13] Deleted	Jeffrey Krause	5/6/19 10:51:00 AM
Page 11: [14] Formatted	Jeffrey Krause	5/6/19 12:53:00 PM

English (UK)

Page 11: [14] Formatted	Jeffrey Krause	5/6/19 12:53:00 PM

English (UK)

Page 12: [15] Formatted	Jeffrey Krause	5/6/19 12:53:00 PM

English (UK)

Page 12: [16] Formatted	Jeffrey Krause	5/6/19 12:53:00 PM

English (UK)

Page 12: [17] Deleted	Jeffrey Krause	5/6/19 10:53:00 AM
Page 12: [18] Formatted	Jeffrey Krause	5/6/19 12:53:00 PM

English (UK)

Page 12: [19] Deleted	Jeffrey Krause	5/6/19 10:54:00 AM
Page 12: [20] Formatted	Jeffrey Krause	5/6/19 12:53:00 PM

English (UK)

Page 12: [21] Deleted	Jeffrey Krause	5/6/19 10:56:00 AM
Page 12: [21] Deleted	Jeffrey Krause	5/6/19 10:56:00 AM
Page 12: [22] Formatted	Jeffrey Krause	5/6/19 12:53:00 PM

English (UK)

Page 12: [23] Formatted	Jeffrey Krause	5/6/19 12:53:00 PM

English (UK)

Page 12: [24] Deleted	Jeffrey Krause	5/6/19 10:56:00 AM

Page 12: [24] Deleted Jeffrey Krause 5/6/19 10:56:00 AM

Page 12: [25] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 12: [25] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 12: [26] Formatted Jeffrey Krause 5/6/19 12:49:00 PM

Indent: First line: 1.27 cm

Page 12: [27] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 12: [28] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 12: [29] Deleted Jeffrey Krause 5/6/19 12:40:00 PM

Page 12: [29] Deleted Jeffrey Krause 5/6/19 12:40:00 PM

Page 12: [30] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 12: [31] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 12: [32] Deleted Jeffrey Krause 5/6/19 12:45:00 PM

Page 12: [32] Deleted Jeffrey Krause 5/6/19 12:45:00 PM

Page 12: [32] Deleted Jeffrey Krause 5/6/19 12:45:00 PM

Page 12: [32] Deleted Jeffrey Krause 5/6/19 12:45:00 PM

Page 12: [32] Deleted Jeffrey Krause 5/6/19 12:45:00 PM

Page 12: [33] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 12: [34] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 12: [35] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 12: [35] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 12: [36] Deleted Jeffrey Krause 5/6/19 12:45:00 PM

Page 12: [36] Deleted Jeffrey Krause 5/6/19 12:45:00 PM

Page 12: [37] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 12: [37] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 12: [38] Deleted Jeffrey Krause 5/6/19 12:46:00 PM

Page 12: [38] Deleted Jeffrey Krause 5/6/19 12:46:00 PM

Page 12: [39] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 12: [39] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 12: [40] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 12: [41] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 12: [41] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 12: [42] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 12: [43] Deleted Jeffrey Krause 5/6/19 12:48:00 PM

Page 12: [44] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 12: [45] Formatted Microsoft Office User 5/12/19 10:37:00 AM

Font: Not Italic, Complex Script Font: Not Italic

Page 12: [46] Deleted Microsoft Office User 5/12/19 10:37:00 AM

Page 12: [47] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 12: [47] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 12: [47] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 12: [48] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 12: [49] Deleted **Jeffrey Krause** **5/6/19 12:51:00 PM**

Page 12: [49] Deleted **Jeffrey Krause** **5/6/19 12:51:00 PM**

Page 13: [50] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 13: [50] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 13: [50] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 13: [50] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 13: [50] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 13: [51] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 13: [51] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 13: [52] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 13: [53] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 13: [54] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 13: [55] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 13: [56] Deleted **Jeffrey Krause** **5/6/19 12:53:00 PM**

Page 13: [56] Deleted Jeffrey Krause 5/6/19 12:53:00 PM

Page 13: [57] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 13: [58] Deleted Jeffrey Krause 5/6/19 12:54:00 PM

Page 13: [58] Deleted Jeffrey Krause 5/6/19 12:54:00 PM

Page 13: [59] Deleted Jeffrey Krause 5/6/19 12:54:00 PM

Page 13: [59] Deleted Jeffrey Krause 5/6/19 12:54:00 PM

Page 13: [59] Deleted Jeffrey Krause 5/6/19 12:54:00 PM

Page 13: [59] Deleted Jeffrey Krause 5/6/19 12:54:00 PM

Page 13: [59] Deleted Jeffrey Krause 5/6/19 12:54:00 PM

Page 13: [59] Deleted Jeffrey Krause 5/6/19 12:54:00 PM

Page 13: [59] Deleted Jeffrey Krause 5/6/19 12:54:00 PM

Page 13: [60] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 13: [61] Deleted Jeffrey Krause 5/6/19 12:57:00 PM

Page 13: [61] Deleted Jeffrey Krause 5/6/19 12:57:00 PM

Page 13: [62] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 13: [62] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 13: [63] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 13: [64] Deleted Jeffrey Krause 5/6/19 12:57:00 PM

Page 13: [65] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 13: [66] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 13: [67] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 13: [68] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 13: [69] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 13: [70] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 13: [71] Deleted Jeffrey Krause 5/6/19 12:59:00 PM

Page 13: [71] Deleted Jeffrey Krause 5/6/19 12:59:00 PM

Page 13: [72] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 13: [72] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 13: [73] Deleted Jeffrey Krause 5/6/19 12:59:00 PM

Page 13: [73] Deleted Jeffrey Krause 5/6/19 12:59:00 PM

Page 13: [73] Deleted Jeffrey Krause 5/6/19 12:59:00 PM

Page 13: [74] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 13: [75] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 13: [76] Formatted Microsoft Office User 5/10/19 10:52:00 AM

Font: 12 pt, Complex Script Font: 12 pt, English (US)

Page 13: [77] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 13: [78] Deleted Jeffrey Krause 5/6/19 1:08:00 PM

Page 14: [79] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [79] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [80] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [81] Deleted Jeffrey Krause 5/6/19 1:11:00 PM

Page 14: [82] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [83] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [83] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [84] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [85] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [85] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [86] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [86] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [87] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [87] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [88] Deleted Susana R. Agusti 4/19/19 9:31:00 AM

Page 14: [88] Deleted Susana R. Agusti 4/19/19 9:31:00 AM

Page 14: [89] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [90] Deleted Jeffrey Krause 5/6/19 1:14:00 PM

Page 14: [90] Deleted Jeffrey Krause 5/6/19 1:14:00 PM

Page 14: [91] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [92] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [93] Deleted Jeffrey Krause 5/6/19 1:15:00 PM

Page 14: [93] Deleted Jeffrey Krause 5/6/19 1:15:00 PM

Page 14: [94] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [95] Deleted Jeffrey Krause 5/6/19 1:15:00 PM

Page 14: [95] Deleted Jeffrey Krause 5/6/19 1:15:00 PM

Page 14: [95] Deleted Jeffrey Krause 5/6/19 1:15:00 PM

Page 14: [96] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [97] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [97] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [98] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [99] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [99] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [100] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [101] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [101] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [102] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [102] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [103] Deleted Jeffrey Krause 5/6/19 1:19:00 PM

Page 14: [103] Deleted Jeffrey Krause 5/6/19 1:19:00 PM

Page 14: [104] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [105] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 14: [106] Deleted Jeffrey Krause 5/6/19 1:21:00 PM

Page 14: [107] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 15: [108] Deleted Jeffrey Krause 5/6/19 1:21:00 PM

Page 15: [109] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 15: [109] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 15: [110] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK), Superscript

Page 15: [110] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK), Superscript

Page 15: [110] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK), Superscript

Page 15: [110] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK), Superscript

Page 15: [110] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK), Superscript

Page 15: [111] Deleted Susana R. Agusti 4/12/19 9:48:00 AM

Page 15: [111] Deleted Susana R. Agusti 4/12/19 9:48:00 AM

Page 15: [111] Deleted Susana R. Agusti 4/12/19 9:48:00 AM

Page 15: [112] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 15: [113] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 15: [114] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 15: [115] Deleted Microsoft Office User 5/12/19 10:34:00 AM

Page 15: [115] Deleted Microsoft Office User 5/12/19 10:34:00 AM

Page 15: [116] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 15: [117] Commented [SRA4] Susana R. Agusti 4/12/19 9:57:00 AM

Smayda, T. J., Mitchell-Innes, B.: Dark survival of autotrophic, planktonic marine diatoms. Marine Biology, 25, 195-202, 1974.

Page 15: [118] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 15: [118] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 15: [118] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 15: [118] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 15: [119] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 15: [120] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 15: [121] Commented [SRA5] **Susana R. Agusti** **4/20/19 11:51:00 AM**

Segovia, M. and Berges, J. A. (2009), INHIBITION OF CASPASE-LIKE ACTIVITIES PREVENTS THE APPEARANCE OF REACTIVE OXYGEN SPECIES AND DARK-INDUCED APOPTOSIS IN THE UNICELLULAR CHLOROPHYTE *DUNALIELLA TERTIOLECTA*1. Journal of Phycology, 45: 1116-1126. doi:10.1111/j.1529-8817.2009.00733.x

Page 15: [122] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 15: [122] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 15: [123] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 15: [123] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 15: [124] Commented [SRA6] **Susana R. Agusti** **4/20/19 12:08:00 PM**

Cell Death in the Unicellular Chlorophyte *Dunaliella tertiolecta*. A Hypothesis on the Evolution of Apoptosis in Higher Plants and Metazoans
María Segovia, Liti Haramaty, John A. Berges, Paul G. Falkowski
Plant Physiology May 2003, 132 (1) 99-105; **DOI:** 10.1104/pp.102.017129

Page 15: [125] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 15: [125] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 15: [126] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 15: [126] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 15: [127] Commented [SRA7] **Susana R. Agusti** **4/27/19 10:39:00 AM**

Ignatiades, L., & Smayda, T. J. (1970). AUTECOLOGICAL STUDIES ON THE MARINE DIATOM RHIZOSOLENIA FRAGILISSIMA BERGON. II. ENRICHMENT AND DARK VIABILITY EXPERIMENTS 1. *Journal of Phycology*, *6*(4), 357-364.

Page 15: [128] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 15: [128] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 15: [129] Formatted **Jeffrey Krause** **5/6/19 2:20:00 PM**

Indent: First line: 1.27 cm

Page 15: [130] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 15: [131] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 15: [131] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 15: [132] Formatted Microsoft Office User 5/12/19 1:54:00 PM

Font: Not Italic, Complex Script Font: Italic

Page 15: [132] Formatted Microsoft Office User 5/12/19 1:54:00 PM

Font: Not Italic, Complex Script Font: Italic

Page 15: [133] Commented [JK8] Jeffrey Krause 5/6/19 1:39:00 PM

Susana,
See if you agree with this assessment (next sentences).

Page 15: [134] Formatted Microsoft Office User 5/12/19 1:54:00 PM

Complex Script Font: Italic

Page 15: [135] Formatted Jeffrey Krause 5/6/19 2:01:00 PM

Subscript

Page 16: [136] Deleted Microsoft Office User 5/12/19 1:53:00 PM

Page 16: [136] Deleted Microsoft Office User 5/12/19 1:53:00 PM

Page 16: [137] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [137] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [138] Deleted Jeffrey Krause 5/6/19 2:19:00 PM

Page 16: [139] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [140] Deleted Jeffrey Krause 5/6/19 2:31:00 PM

Page 16: [141] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [142] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [142] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [143] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [143] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [144] Deleted Jeffrey Krause 5/6/19 2:32:00 PM

Page 16: [144] Deleted Jeffrey Krause 5/6/19 2:32:00 PM

Page 16: [145] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [146] Deleted Jeffrey Krause 5/6/19 2:32:00 PM

Page 16: [146] Deleted Jeffrey Krause 5/6/19 2:32:00 PM

Page 16: [147] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [147] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [148] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [148] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [149] Deleted Jeffrey Krause 5/6/19 2:33:00 PM

Page 16: [149] Deleted Jeffrey Krause 5/6/19 2:33:00 PM

Page 16: [149] Deleted Jeffrey Krause 5/6/19 2:33:00 PM

Page 16: [150] Deleted Jeffrey Krause 5/6/19 2:33:00 PM

Page 16: [151] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [152] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [153] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [154] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [155] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [156] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [157] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [158] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [159] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [160] Deleted Jeffrey Krause 5/6/19 2:35:00 PM

Page 16: [160] Deleted Jeffrey Krause 5/6/19 2:35:00 PM

Page 16: [160] Deleted Jeffrey Krause 5/6/19 2:35:00 PM

Page 16: [161] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [162] Deleted Jeffrey Krause 5/6/19 2:37:00 PM

Page 16: [162] Deleted Jeffrey Krause 5/6/19 2:37:00 PM

Page 16: [162] Deleted Jeffrey Krause 5/6/19 2:37:00 PM

Page 16: [163] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [164] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [165] Formatted Microsoft Office User 5/12/19 2:18:00 PM

Font: Not Italic, Complex Script Font: Not Italic

Page 16: [166] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 16: [167] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 16: [168] Commented [JK13] **Jeffrey Krause** **5/6/19 2:39:00 PM**

Brzezinski, Mark A. "The Si: C: N ratio of marine diatoms: Interspecific variability and the effect of some environmental variables1." *Journal of Phycology* 21, no. 3 (1985): 347-357.

Page 16: [169] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 22: [170] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 22: [171] Deleted **Jeffrey Krause** **5/6/19 3:03:00 PM**

Page 22: [171] Deleted **Jeffrey Krause** **5/6/19 3:03:00 PM**

Page 22: [172] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 22: [173] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 22: [173] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 22: [174] Deleted **Jeffrey Krause** **5/6/19 3:04:00 PM**

Page 22: [174] Deleted **Jeffrey Krause** **5/6/19 3:04:00 PM**

Page 22: [175] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 22: [176] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 22: [177] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 22: [178] Formatted **Jeffrey Krause** **5/6/19 12:53:00 PM**

English (UK)

Page 22: [179] Deleted **Jeffrey Krause** **5/6/19 3:18:00 PM**

Page 22: [179] Deleted **Jeffrey Krause** **5/6/19 3:18:00 PM**

Page 22: [180] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [180] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [181] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [182] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [183] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [183] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [184] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [185] Deleted Jeffrey Krause 5/6/19 3:20:00 PM

Page 22: [185] Deleted Jeffrey Krause 5/6/19 3:20:00 PM

Page 22: [186] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [187] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [187] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [188] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [188] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [189] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [189] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [190] Deleted Jeffrey Krause 5/6/19 3:07:00 PM

Page 22: [190] Deleted Jeffrey Krause 5/6/19 3:07:00 PM

Page 22: [191] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [191] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [192] Deleted Jeffrey Krause 5/6/19 3:07:00 PM

Page 22: [192] Deleted Jeffrey Krause 5/6/19 3:07:00 PM

Page 22: [192] Deleted Jeffrey Krause 5/6/19 3:07:00 PM

Page 22: [193] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [194] Deleted Jeffrey Krause 5/6/19 3:22:00 PM

Page 22: [194] Deleted Jeffrey Krause 5/6/19 3:22:00 PM

Page 22: [195] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [196] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [196] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [197] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [198] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [199] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [200] Deleted Jeffrey Krause 5/6/19 3:10:00 PM

Page 22: [200] Deleted Jeffrey Krause 5/6/19 3:10:00 PM

Page 22: [201] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [202] Formatted Jeffrey Krause 5/6/19 12:53:00 PM

English (UK)

Page 22: [203] Formatted	**Jeffrey Krause**	**5/6/19 12:53:00 PM**

English (UK)

Page 22: [204] Formatted	**Jeffrey Krause**	**5/6/19 12:53:00 PM**

English (UK)

Page 22: [205] Deleted	**Jeffrey Krause**	**5/6/19 3:11:00 PM**

Page 22: [205] Deleted	**Jeffrey Krause**	**5/6/19 3:11:00 PM**

Page 22: [206] Formatted	**Jeffrey Krause**	**5/6/19 12:53:00 PM**

English (UK)

Page 22: [207] Formatted	**Jeffrey Krause**	**5/6/19 12:53:00 PM**

English (UK)

Page 22: [207] Formatted	**Jeffrey Krause**	**5/6/19 12:53:00 PM**

English (UK)

Page 22: [208] Deleted	**Jeffrey Krause**	**5/6/19 3:25:00 PM**

Page 22: [208] Deleted	**Jeffrey Krause**	**5/6/19 3:25:00 PM**