

Interactive comment on “A meta-analysis on environmental drivers of marine phytoplankton C : N : P” by Tatsuro Tanioka and Katsumi Matsumoto

Anonymous Referee #2

Received and published: 18 December 2019

General Comments

This study addresses the very important topic of stoichiometric variability in marine phytoplankton. Understanding the magnitude and drivers of this variability as well as its taxonomic variation are essential for developing new and more accurate global biogeochemical models. The authors take a novel approach to this problem by performing a meta-analysis through which they calculate a sensitivity factor for major stoichiometries (N:C, P:C, and N:P) in response to a suite of environmental drivers. The goal of such a quantitative approach - to estimate the group-specific response of these stoichiometries to expected changes in ocean conditions - is laudable. However, there

[Printer-friendly version](#)

[Discussion paper](#)



are several major flaws in how this approach is applied and how studies are selected and screened for this meta-analysis that would need to be addressed for this to be published in Biogeosciences. Additionally, these major flaws in approach receive little or no discussion throughout the manuscript. The authors present their approach to estimating a response to an environmental condition as more nuanced and informative than simply calculating a response between two end points or experimental treatments. While those simplistic, past approaches have numerous limitations, they were generally acceptable for meta-analyses due to two major challenges: 1) the high variability in experiment conditions of individual studies; and 2) the fact that some environmental drivers may produce linear or at least monotonic responses within a range of natural variability (e.g. the response to nutrient availability), while other drivers produce responses that are distinctly antitonic (e.g. temperature and irradiance). Essentially the authors have suggested a more complex metric for such meta-analyses without addressing these two major challenges. As a result, ambient nutrient concentrations are treated as a measure of a study species' nutrient status that is comparable across different experiment types (semi-continuous batch vs. chemostat), which is inappropriate for several reasons (addressed below in my specific comments). The flaws of this approach are not discussed in the manuscript and the approach is used to make the study's strongest conclusion, that diatom P:C and N:C are particularly sensitive to N and P availability. It should be added that this result is based on meta-analysis of only four studies, one of which was on a dinoflagellate and incorrectly categorized. This approach also results in deeming a given stoichiometry as sensitive to a driver like irradiance or temperature if that stoichiometry has a monotonic response to these drivers. Considering that the responses of phytoplankton to light and temperature are distinctly non-linear and antitonic (usually displaying a clear central optimum), this approach seems very flawed. Considering its novelty and potential value, the approach used by the authors should not be discarded, but refinement and far more discussion of its limitations would be necessary to present it in a manuscript. The computational needs of the sensitivity factor that the authors use (requiring experiments where the

[Printer-friendly version](#)[Discussion paper](#)

response to at least 3 levels of an environmental driver were measured) also seems to have resulted in a meta-analysis of a somewhat limited number of studies. While this criteria is strict, there is no study selection criteria mentioned that address the many other confounding factors that could differ among studies and little or no discussion of such factors. Along with this lack of evaluation of the original studies used in the meta-analysis, there is also little comparison of the results of this work to the findings of several other narrative reviews and quantitative meta-analyses of phytoplankton stoichiometry, most of which considered a larger number of original studies. These past studies are generally just mentioned for comparison of approaches, but not their results are not critically evaluated in light of the authors' contributions to this topic. As mentioned above, there also seems to be several studies that were incorrectly categorized, with non-diatom species appearing to be grouped with diatoms in the group-specific meta-analyses. In addition to an explanation of the issues noted above, there are several other issues noted in my specific comments below.

Specific Comments

Abstract

Line 18-20: It seems overly simplistic to imply that the temperature response of cyanobacteria is responsible for global P:C patterns without acknowledging the effect of macronutrient availability, which you have also shown to have a strong effect on P:C and N:C. The global patterns in C:N:P (lower P:C and N:C in subtropics, higher in sub polar and upwelling regions) has also been attributed to macronutrient availability and phytoplankton biogeography with the relative impact of all three drivers being a rich and contentious area of research. Linking your findings to this on-going area of study should either be excluded from the abstract or addressed in a more complete fashion by noting that the macronutrient sensitivity of diatom C:N:P and the temperature sensitivity of cyanobacteria C:N:P you observe are both helpful in explaining the persistent global patterns in C:N:P.

[Printer-friendly version](#)[Discussion paper](#)

Introduction

Line 43-45: This sentence should be supported by citations. It is not clear which of the citations in the previous sentence (if any) are the sources for this information.

Line 53-55: This statement is vague and detailed specific support for this should be given. It's worth clarifying why previous studies have not yielded a broader understanding of how phytoplankton C:N:P varies across taxa and environmental conditions (and thus justifying your meta-analysis). Also, the inherent genetic differences among taxa don't simply correspond to differences in environmental responses, they correspond to inherent differences in steady-state C:N:P under ideal conditions among major phytoplankton groups (Quigg et al. 2003; Garcia et al. 2018) that likely reflect basic differences in cellular structure and size (Finkel et al. 2016a; Finkel et al. 2016b). See references below.

ââ Quigg, A., Finkel, Z. V., Irwin, A. J., Rosenthal, Y., Ho, T. Y., Reinfelder, J. R., ... & Falkowski, P. G. (2003). The evolutionary inheritance of elemental stoichiometry in marine phytoplankton. *Nature*, 425(6955), 291. ââ Garcia, N. S., Sexton, J., Riggins, T., Brown, J., Lomas, M. W., & Martiny, A. C. (2018). High variability in cellular stoichiometry of carbon, nitrogen, and phosphorus within classes of marine eukaryotic phytoplankton under sufficient nutrient conditions. *Frontiers in microbiology*, 9, 543. ââ Finkel, Z. V., Follows, M. J., Liefer, J. D., Brown, C. M., Benner, I., & Irwin, A. J. (2016a). Phylogenetic diversity in the macromolecular composition of microalgae. *PLoS One*, 11(5), e0155977. ââ Finkel, Z. V., Follows, M. J., & Irwin, A. J. (2016b). Size-scaling of macromolecules and chemical energy content in the eukaryotic microalgae. *Journal of Plankton Research*, 38(5), 1151-1162.

Line 55-58: In addition to the point made in the previous comment, there are many reasons why it is hard to draw consensus from the various studies of phytoplankton C:N:P, but an inconsistency of statistical analyses seems like one of the least compelling of these reasons. What about the differences in how experimental treatments are ap-

BGD

Interactive
comment

Printer-friendly version

Discussion paper



plied, particularly for macronutrient limitation (e.g. steady-state vs batch cultures and differences in the duration of nutrient stress)? What about confounding experimental conditions (e.g. bacterial contamination, low CO₂ availability/high pH in dense batch cultures)? Or more simply, the fact that many studies only measure one or two of the three major elements and few measure the biochemical components that determine elemental quotas. These are all factors that make understanding how phytoplankton C:N:P varies across taxa and conditions difficult when using existing literature and seem much more important than the selection of statistical analyses. Not mentioning these factors in the introduction and, more importantly, in the methods section when considering selection criteria is a major omission in this paper.

Line 59-67: This paragraph seems mostly unnecessary. The value of a quantitative meta-analysis is self-evident for the audience and can be stated by a simple statement of the goal of this work later in the introduction. Shortening this also leaves more room for more helpful introductory information regarding the causes of phytoplankton C:N:P variability or the factors that make this meta-analysis challenging (see previous two comments).

Line 69-72: While previous meta-analyses that focus on only one environmental driver are indeed limited, these studies must still have some value or informative conclusions. This introduction contains no mention of the actual findings or major conclusions of these previous studies. Addressing the findings and relative value of previous, similar work should be a fundamental part of any introduction. Again, addressing this omission seems more helpful than the paragraph explaining why meta-analyses are valuable.

Line 76: The sentence contained here is incomplete and seems like a typo.

Methods

Line 93: For readers who might not be familiar with search operators, you should define “TS” as in its first usage as a field tag for “topic” (or some other appropriate definition).

[Printer-friendly version](#)

[Discussion paper](#)



Line 94-100: As with the previous comment, it would be good to explain the meaning of “*” as a wildcard search operator.

Line 94-100: These descriptions of search terms are not accessible when listed in a paragraph. This information should be placed in a table.

Study Selection Criteria: The way in which studies were selected for the meta-analysis and the lack of analysis or discussion of the confounding factors that various studies present are where some of my strongest critiques lie. I’ve presented these critiques as a list below:

- Limitation of 3 experimental levels: The value of setting the study selection criteria to 3 experimental levels for each environmental factor of interest seems overstated. The terms X and Y (the fractional response and fractional change in conditions) could be calculated with just two experimental levels for each experimental unit. Granted this does not allow the error associated with a linear regression of 3 X and Y values to be used or for a non-linear response to be detected, but I would question the value of such an error term or description of a non-linear response that was based on a linear regression of only three points. Give the limits of this additional explanatory power, this criterion seems unnecessarily limiting (see next points).
- Excluding valuable studies: Having only two levels of an experimental factor is not the major failing of most studies of phytoplankton elemental composition. There are many studies that I would deem of high quality that would have made excellent additions to this meta-analysis that only use two experimental levels for a given type of nutrient stress (e.g. Bertilsson et al. 2003; Fu et al. 2007 J. Phycol.). Considering this, the criteria of 3 levels unnecessarily diminishes the data density of the meta-analysis. Again, perhaps a better explanation of the selection criteria and meta-analysis calculations is needed if I am mistaken. It seems like a meta-analysis that utilizes a greater number of individual experimental units by including experiment with only two levels would have much greater breadth and power.
- Not addressing major confounding factors: The more important failing in studies of phytoplankton C:N:P is the lack of consistent experimental conditions or poorly described conditions. Many studies do not offer verification that

some desired growth state was successfully applied, particularly in the case of N or P stress. For example, many studies do not describe the growth rate at a given experimental level of a limiting nutrient. How an author defines N or P starvation or to what extent these conditions were applied (e.g. were they applied until growth ceased, or just until growth slowed) can greatly affect the observed response. Additionally, many nutrient starvation experiments are done in dense batch cultures where the additional stressors of light limitation, high pH, and low carbon availability arise as cultures increase in density and coincide with the onset of nutrient starvation. I mention this not to say that the authors should have determined such confounding factors in every study (in many cases, experimental conditions are not described well enough to do this), but rather to point out that such factors are not addressed at all in the selection criteria. In other words, a poorly executed study that did not fully apply nutrient starvation (even across 3 levels) would be included, but a well-described and well-executed experiment across only 2 levels (e.g. nutrient replete vs. nutrient starved) would be excluded. Again, this gets to the point that basing s-factors on a linear regression of 3 or more experimental levels has applied a major constraint on the meta-analysis and the value of this constraint is unclear, yet other major confounding factors are not addressed in the selection criteria.

S-factor Calculation for Meta-Analysis: My other major critiques pertain to how s-factor was calculated, particularly for macronutrient stress experiments. Again, I've presented these critiques as a list below:

- How was standard error propagated when calculating s-factors? Does the error reflect both the error associated with each P:C or N:C measurement and the error associated with the regression of X and Y for each experimental unit? How the error associated with the original measurements was accounted for and propagated must be described (if this was done).
- With respect to the error associated with the weighted mean s-factors, I realize that the metafor R package is used for this calculation, but some general description of how this package calculates error should be provided. In other words, you should be explicit about what the error bars shown in the figures actually mean.
- It is not at all clear how

[Printer-friendly version](#)[Discussion paper](#)

the fractional change in nitrate or phosphate stress was calculated. Was this simply based on the ambient nitrate or phosphate concentration reported for each experimental level? If so, how can the level of N stress be determined if ammonium or nitrate are not accounted for? – Batch, semi-continuous batch, and continuous chemostat experiments were used in the meta-analysis of macronutrient response. I do not understand how a simple measurement of ambient inorganic nutrient concentration can be used to determine experimental levels of N or P stress across these different experiment types. Even between a semi-continuous batch experiment where authors claim cultures are in balanced growth and a chemostat experiment, the measured nutrient concentrations or nutrient concentrations in fresh or inflow media mean different things with respect to extent of nutrient stress. In other words, moving from a nitrate concentration of 1.0 to 0.2 would mean very different things depending on whether they are in semi-continuous or continuous mode, the concentration of other forms of dissolved inorganic nitrogen (ammonium, nitrite) or what the concentration of other potentially limiting nutrients are. The extent of nutrient stress cannot be compared between these different growth modes based on dissolved nutrient concentrations alone. Some would argue the extent of nutrient stress cannot be compared across these growth modes at all, and thus they can't be pooled into one type of meta-analysis. Again, a strict criterion of 3 experimental levels has been applied in this meta-analysis to serve a computational need, but other major confounding factors have been ignored. Additionally, these 3 experimental levels have been used to calculate a fractional change in conditions that does not have a consistent meaning across experiment types. The only way to deal with these problems while still using the current meta-analysis approach (s-factors, based on 3 experimental levels) would be to separate experimental units based on their growth mode and apply a more rigorous means of determining experimental levels of nutrient stress (i.e. growth rate) in the semi-continuous and continuous growth experiments. – Similar problems with the s-factor calculation of using a linear fractional change in growth conditions also apply to temperature and irradiance. Such a formulation ignores the growth optimum of a particular species or strain and thus treats

[Printer-friendly version](#)[Discussion paper](#)

an extremely non-linear response as something that can be compared across studies and taxa with a simple linear relationship. Consider a scenario where an experiment measured N:C at four temperatures in a species with growth optimum of 22C and had the following result: 15C = 0.14, 20C=0.154, 25C=0.156, and 30C=0.14. An s-factor calculated as a linear regression of X and Y from this experiment would be very small in magnitude and imply that this species is insensitive to temperature changes, when in fact these are actually large changes in N:C with respect to global conditions and what is generally observed in temperature responses. This experiment also shows that N:C declines at supraoptimal temperatures, the most relevant result with respect to climate change scenarios, but something that would be missed by the s-factor. In other words, the s-factor is a poor metric for a biological variable that does not have a monotonic response to some condition as is the case with light and temperature responses. Also, depending on the light or temperature levels selected in a given experiment with respect to the study species growth optimum, a fractional change in these conditions means very different things and are not directly comparable.

Line 147-149: the symbol used to denote dissolved iron should be a mathematical prime symbol (Fe'), not an apostrophe or single quotation mark.

Line 150-151: “only selected experiments where NO₃ concentrations were kept constant.” This is either a writing error or a misunderstanding of the experiments selected. The non-limiting macronutrient was not kept constant in many of the experiments selected and this is rarely achieved even in chemostat experiments (see the nutrient concentrations described in Leonardos and Geider 2004 for example). Again, the selection criteria and calculation of fractional change for macronutrient stress experiments is either poorly described, problematic, or both.

Results

Figures 2 – 5: The structure of the figures seems likely to confuse readers. Tables are often arranged such that inclusive categories are listed above subcategories. When

[Printer-friendly version](#)[Discussion paper](#)

first looking at figure 1, I see “Diatoms” in bold and then genus names for various eukaryotes below it and was disoriented for a moment. The figures may be more intuitive if you listed an inclusive group (e.g. “Diatoms”) and then listed taxa within that group immediately below it with an indentation. Also, why are the figures arranged as nutrient limitation (Fig. 2), Light (Fig. 3), Temperature (Fig. 4), nutrient-limitation (Fig. 5). I understand if this was done because there is very limited data for Iron limitation, but a more logical arrange of the figures would be better for comparison. There also appears to be a few taxonomic assignment errors in the meta-analysis based on the figures. *Alexandrium minutum* (a dinoflagellate) is listed among the diatoms in the Figure 2, *Chlorella* sp. (a chlorophyte) is listed among the diatoms in Figure 3, and *Phaeocystis* (a haptophyte) is listed among the diatoms in Figure 4. Does this error extend to the meta-analysis or was this an error in figure preparation? There also seems to be errors or inconsistencies in how studies were characterized with respect to N or P limitation. For example, why is Leonardos and Geider 2004 only listed among “Phosphate” experiments. This is a chemostat study that spanned both N-limited, balanced growth and P-limited balanced growth and thus could also be included with the “Nitrate” and “Nitrate/Phosphate” meta-analyses. The fact that these chemostats were controlled by manipulating inflow phosphate is irrelevant and does not make them simply “phosphate” experiments. Neither nitrate or phosphate values were constant across experimental levels in this experiment, what matters is that these were chemostats where inflow N:P was manipulated. I did not closely examine every study in the meta-analysis, but I am concerned that other such inconsistencies are present.

Discussion Line 230: the word “the” before “chemical” should be removed

Line 241: “making of . . . reductase”. Do you mean “reductant” (i.e. NADPH) rather than reductase (an enzyme)?

Line 243-246: These are specific statements that should be supported with references.

[Printer-friendly version](#)[Discussion paper](#)

Line 237-238 and other parts of paragraph: There seems to be a misunderstanding of the term “balanced growth”. A natural population or culture can be both nutrient-limited and in steady-state, balanced growth if the limiting nutrient is supplied at a consistent rate. Despite the various factors that limit phytoplankton growth and the natural conditions that represent clearly unbalanced growth (spring blooms), a balanced growth model of natural populations (the “steady-state ocean”) is still very relevant for the vast subtropical oceans where consistent and actively growing populations occur amidst apparent chronic nutrient limitation.

Line 282: This should be corrected to “we observe a consistent trend” or “we observe consistent trends”

Line 296: I think “. . .the level. . .” should be changed to “. . .the same level. . .”. If this is not just a typo, than this sentence should rewritten and clarified

Line 298: the phrase “number of. . .” or “abundance of. . .” should be placed before “. . .ribosomes”

Line 300: revise to “. . .in a cell, resulting in. . .” or “. . .in a cell and result in. . .”

Line 309: The Garcia reference is not appropriate here. References that actually describe this mechanism should be cited: • Dortch, Q., Clayton, J. R., Thoresen, S. S., & Ahmed, S. I. (1984). Species differences in accumulation of nitrogen pools in phytoplankton. *Marine Biology*, 81(3), 237-250. • Lourenço, S. O., Barbarino, E., Lavín, P. L., Lanfer Marquez, U. M., & Aidar, E. (2004). Distribution of intracellular nitrogen in marine microalgae: calculation of new nitrogen-to-protein conversion factors. *European Journal of Phycology*, 39(1), 17-32. • Grover, J. P. (1991). Resource competition in a variable environment: phytoplankton growing according to the variable-internal-stores model. *The American Naturalist*, 138(4), 811-835. • Tozzi, S., Schofield, O., & Falkowski, P. (2004). Historical climate change and ocean turbulence as selective agents for two key phytoplankton functional groups. *Marine Ecology Progress Series*, 274, 123-132. • Talmy, D., Blackford, J., Hardman-Mountford, N.

[Printer-friendly version](#)[Discussion paper](#)

J., Polimene, L., Follows, M. J., & Geider, R. J. (2014). Flexible C: N ratio enhances metabolism of large phytoplankton when resource supply is intermittent.

Line 320: The word “and” should be inserted after “significantly”

Line 328: “Large stoichiometry sensitivity. . .” should be changed to “The larger stoichiometric sensitivity. . .” or “The larger sensitivity of P:C. . .”

Line 339-340: “Excess carbon. . .” – this sentence is a non-sequitur and should be modified to connect with the topic of irradiance effects.

Line 349-350: This statement may not be true and should be supported by some reference. The light harvesting apparatus will still be expected to be down-regulated under N-replete conditions in order to avoid oxidative stress and photodamage and also to maximize growth rate and N allocation.

Line 351-355: Amidst all these explanations of why irradiance has little effect on C:N:P, there is a fundamental explanation that has not been addressed. Although N-content may be expected to decline as irradiance increases due to a down regulation of the light harvesting apparatus, one could also expect an increase in N allocation to other cellular functions including nutrient uptake, biosynthesis, and repair of the light harvesting apparatus in order to match an increase in C-fixation. This shift in N allocation from light harvesting content to nutrient acquisition and biosynthesis is essential to an increase in growth rate with irradiance and could be expected at light levels that are below some photoinhibitory level. I don't know if this reallocation of N is sufficient to offset the expected decline in N content due down regulation of the light harvesting apparatus, but at least this is based on fundamental biological processes rather than critiques of experimental conditions that are not followed by any details or substantiation.

Line 351-364: It seems odd that the variation in experimental conditions is invoked here to explain the limited the effect of irradiance on C:N:P, but this was not addressed with respect to macronutrient limitations. It seems logically inconsistent to note these

[Printer-friendly version](#)[Discussion paper](#)

methodological issues only when a clear effect is not found.

Line 359-360 and 372-373: “We speculate. . .” – Aren’t these concepts easy to verify or discuss further considering the small number of studies used in the meta-analyses rather than just speculate? Were the experiments used for the irradiance meta-analysis diel or continuous light. What proportion were continuous light? Were these experiments mostly done at optimal temperature? Also, I thought your selection criteria examined studies where irradiance was manipulated, but nutrient status was not. How can nutrient status then be invoked as a possible confounding factor? It seems more reasonable and conservative to assume that irradiance simply does not have strong effect on P:C?

Line 420-422: The time range of selected studies seems like a very weak argument. Wouldn’t the selection criteria for the studies used in each meta-analysis also have a strong effect on the result. Also couldn’t you simply split your analysis between these time ranges to see how it compares to the Yvon-Durocher study? This seems like another speculation that could be very easily examined.

Line 432-434: The sentence here is incomplete or a fragment and should be revised.

Line 436: This seems like an erroneous assumption. Couldn’t a non-significant effect of iron on stoichiometry also be due to variable and contrasting effects of iron on cellular C and N or reflect the small number of studies examined!?

Line 467-470: Cause and effect seem to be mixed up here. Sea surface warming is driven by air temperature, which in the long-term is driven by radiative forcing (greenhouse effect) rather than visible light. Also changes in incident irradiance at the sea surface are expected to be far smaller than changes in sea surface temperature due to climate change. Surface warming drives stratification, which then results in greater overall light intensity and lower nutrient availability for phytoplankton trapped in a more shallow surface mixed layer. Also some references should be provided in this section.

BGD

Interactive
comment

Printer-friendly version

Discussion paper



Line 474: The word “out” should be placed after “carried”

Line 482-493: This discussion of organic matter decoupling is a bit muddled and unclear. I point out specific problems below. Generally, the value of this paragraph and its connections to the main point of this work are not clear. Is point here simply that P:N:C of cultured phytoplankton analysed here do not directly correspond to ocean particulate matter P:N:C due to the presence of detritus and decomposition?

Line 484: “organic matter accumulation and remineralization”. Are implying that detritus plays a role in bulk organic matter P:N:C? If so, this should be stated directly. Amongst the possible causes of decoupling between expected phytoplankton stoichiometry and measured bulk organic matter stoichiometry, detrital material is likely very important and not addressed. Some helpful references:

Å Karl D.M., Dobbs F.C. (1998) Molecular Approaches to Microbial Biomass Estimation in the Sea. In: Cooksey K.E. (eds) Molecular Approaches to the Study of the Ocean. Springer, Dordrecht Å Verity, P. G., Williams, S. C., & Hong, Y. (2000). Formation, degradation, and mass: volume ratios of detritus derived from decaying phytoplankton. Marine Ecology Progress Series, 207, 53-68.

Line 485-488: This sentence is unclear. One point of Martiny et al 2013a is the increase in C:N (or rather a decrease in N:C) of sinking organic matter (see Figure 4 therein). Aside from that point, it is not clear how sinking organic matter being close to Redfield composition predicts low N:C in phytoplankton.

Line 494-505: The study by Moreno et al. 2018 would be good to include here. It not only supports your point about the value of flexible stoichiometry in global biogeochemical models, it particularly highlights the more flexible P:C of diatoms as an important driver of global patterns

Å Moreno, A. R., Hagstrom, G. I., Primeau, F. W., Levin, S. A., & Martiny, A. C. (2018). Marine phytoplankton stoichiometry mediates nonlinear interactions between

[Printer-friendly version](#)[Discussion paper](#)

nutrient supply, temperature, and atmospheric CO₂. Biogeosciences (Online), 15(9).

Line 508: This point seems overstated and not in accordance with your results. Didn't you show that irradiance has no clear effect on P:C and only a weak effect on N:C?

510-516: This is an interesting suggestion. You have made other predictions based on your meta-analysis, so you should actually present a prediction using this power-law function if you are going to suggest it. Or at least use this function to highlight what terms need to be better constrained and/or what terms should be added (e.g. detrital contribution, decomposition) in order to properly apply a power-law formulation to ocean stoichiometry.

Line 520: remove the word "on"

Line 521: "...evolve under the climate change." is grammatically incorrect or a typo. "under the" could just be changed to "with" or one of many other revisions could be applied

Line 525: Remove the word "the".

References

Be sure to double-check reference formatting. Reference titles should not be in all caps (a frustrating result of citing articles from Journal of Phycology).

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2019-440>, 2019.

BGD

Interactive
comment

Printer-friendly version

Discussion paper

