

Reply to RC1

This study aims to demonstrate in the Kuroshio area near northern East China Sea, that turbulence-induced nitrate flux can stimulate phytoplankton production in this seemingly oligotrophic ocean, while microzooplankton respond quickly to graze down the phytoplankton. As a consequence, high phytoplankton biomass is not observable. The authors used turbulence and nitrate sensor to demonstrate the nitrate flux, use nutrient enrichment experiments to demonstrate effects of nitrate flux on phytoplankton growth, and dilution experiments to measure microzooplankton grazing. This work is really interesting and deserves publishing in Biogeosciences. I have following comments that aim to help improve this manuscript.

>We appreciate your kind comments to our findings. As shown in BGD, we indicated point-by-point response to the following comments. Some responses to RC1 at the last time (BGD) were little changed after receiving the RC2 and RC3, but the revised phrases are substantially same. Hopefully, these are enough responses to your comments and suggestions.

Main concerns:

1. Potential effects of microzooplankton:phytoplankton ratio on the enrichment experiments: Table 1 shows that the chl-a and microzooplankton standing stock at the beginning of the incubations varied. The relative abundance of microzooplankton to phytoplankton may change the strength of top-down control. I wonder if adding microzooplankton:chl-a ratio or standing stock of microzooplankton and chl-a density to the regression analysis (Figure 5) can further explain the variation of phytoplankton growth after enrichment.

>As we mentioned at BGD, we computed correlation between the slope of phytoplankton growth rates to the nutrients gradients and micro-sized heterotrophs biomass. Because no significant correlation was found for any size fractions to micro-sized heterotrophs, we have deleted these results from the manuscript. However, since some readers might have similar point of view, we added these results in Figure 5 and some descriptions in the revised manuscript as follows (L205-217).

“The slope of a linear regression between growth rates of the size-fractionated chlorophyll and the logarithms of the nitrate enrichments at each incubation provided a metric of the sensitivity of their growth rates to nutrient supply. As shown in Supplement Fig 1, the steeper slopes were found at some stations in the upstream Kuroshio in the Tokara Strait compared with those at the other stations, suggesting that apparent phytoplankton growths were variable with the nutrients concentrations or predatory impacts at the beginning of the incubations. To explain whether growth rates of the size-fractionated chlorophyll might be variable with initial nutrients concentrations (bottom-up control) or predator biomasses (top-down control) at the beginning of the experiments, the slopes were compared to the nitrate+nitrite (Fig 5a) and phosphate concentrations (Fig 5b) and micro-heterotrophs biomass (Fig 5c) in the ambient seawater without enrichment. No significant correlation was found for all size-fractionated chlorophyll to the micro-sized heterotrophs biomass. On the other hand, there was a negative correlation of the slopes for all size-fractions to the nitrate plus nitrite or phosphate concentrations, indicating that the stimulation of their growth rates by nutrients supply was greater for all size-fractionated chlorophyll under more oligotrophic conditions. Thus, the variations in phytoplankton growth rates are likely associated with nutrients concentrations at the beginning of the incubations.”

2. Enrichment experiments that did not exhibit clear effect of ambient nutrient on phytoplankton growth enhancement to enrichment (Lines 161-167 and Figure 5): Indeed there is a negative trend between phytoplankton growth-enrichment regression slope and $[\text{NO}_3^- + \text{NO}_2^-]$ or $[\text{PO}_4^{3-}]$ in control experiments. However, the plankton communities that have small regression slopes and low r^2 ($r^2 < 0.5$; F01 and K08 in Fig. 5 and Table 1, which I labeled in the figure below) experienced quite different in situ nutrient condition, and only K08 seems to drive the negative trend. I would like to know if the negative trend remains after removing these two sets of low- r^2 points. Furthermore, is there any possible explanation why the two incubations under low and high nutrient concentration reacted similarly to nutrient enrichment?

>As you suggested, correlation coefficients are much low (-0.014 to -0.778) and no significant if the slope of the phytoplankton growth rates at both stations are removed from Figure 5. According to the results in Table 2, phytoplankton growths at both stations tended to be higher than those at the other stations and positive even under no enrichment, particularly for micro-sized phytoplankton. We reported that larger phytoplankton predominated in coastal waters were often entrapped in frontal eddies and meanders of the Kuroshio around the study sites and advected into the Kuroshio (Kobari et al. 2019, Geophysical Monograph 243: 223-243). Probable explanations are that growths of phytoplankton communities at both stations are already stimulated with the advected coastal waters before our bottle experiments and nutrients are consumed for those phytoplankton communities particularly at K08. There is no evidence to support such hypothesis, however, we could not add further explanations in the revised manuscript.

3. “Intra-guild” predation within microzooplankton community (Line 158-160): The results indicate that enrichment slightly increased the growth rate of nauplii but not always increase ciliate growth, especially when enrichment is low. According to the biomass change of the three types of microzooplankton to enrichment, the increase of nauplii is not as significant as ciliates when enrichment is high (Figure 3). I think, maybe the intraguild predation of ciliates by nauplii inhibit the growth of ciliates when ciliate growth enhanced by low enrichment was not strong enough to compensate their mortality by nauplii feeding. As the enrichment increase further, fast growing ciliates can outgrow the consumption by large nauplii that grow and react more slowly to environmental change, and thus ciliate growth and biomass accumulation increase. If the body size ratio between nauplii and ciliates in the incubations fit the predator-prey mass ratio of nauplii (Hansen et al. 1994), this is possible to happen.

>This might be another possibility. Based on our data sets, the ratio of mean equivalent spherical diameter of body mass between copepod nauplii (88 μm) and naked ciliates (16 μm) was estimated to be 5:1 and much different from to the predator-prey mass ratio (i.e., 18:1) reported by Hansen et al. (1994). As described above, no significant correlation was found for the growth response of phytoplankton to nutrients gradients. We think that such intraguild predation of copepod nauplii on naked ciliates would not happen in the bottles. However, we added such explanations in the revised manuscript as follows (L273-L283).

“On the other hand, “intra-guild” predation within micro-heterotrophs community might be another explanation on the less clear pattern of their standing stocks and growth rates. Growth rates of copepod nauplii were always higher than those of naked ciliates, especially under no or less nitrate supply. The ratio of mean equivalent spherical diameter of body mass between copepod nauplii (88 μm) and naked

ciliates (16 μm) was estimated to be 5:1 and much different from to the predator-prey mass ratio (i.e., 18:1, Hansen et al., 1994). Thus, such intraguild predation of copepod nauplii on naked ciliates would not happen in the bottles. More importantly to no or less clear pattern of the growth of micro-heterotrophs, the results from the simultaneously conducted experiments imply that phytoplankton productivity is stimulated by the turbulent nitrate flux and rapidly grazed by microzooplankton but standing stocks and growths of micro-heterotrophs are not elevated during 3 days in the Kuroshio Current.”

4. Stoichiometry of nutrient supply in Kuroshio (Lines 82-83): The enrichment and dilution experiments supplied phytoplankton with nitrate and phosphate molar concentration in 15:1 ratio (slightly N-limited, relative to the Redfield ratio 16:1). Did this ratio mimic the inorganic N:P concentration ratio or N:P flux by turbulent mixing in Kuroshio? Since this study focus on the nitrate supply from turbulent mixing, I expect that N should be limited. Nevertheless, I would like to know more about the stoichiometric condition of this study area and its potential effect on phytoplankton growth.

>You are right. As you can find in Table 1, the ratios of nitrate/nitrite and phosphate molar concentrations showed N-limited conditions at ambient waters excepted for one station. As reported by Hasegawa et al. (2019, Geophysical Monograph 243: 191-205), 15:1 was measured in the ECS-Kuroshio and defined for the stoichiometric ratio of nutrients enrichment in our bottle experiments. On the other hand, in my knowledge, no information on the stoichiometric effects on phytoplankton growth is available in the ECS-Kuroshio.

5. I will appreciate data to demonstrate the accuracy of in situ nitrate sensor (e.g. comparing with measurements using water collected by sampling bottles). This issue is particularly important when nitrate concentration is low in the water.

>The measurement methodology for in situ nitrate sensor is just published in Japanese journal (Hasegawa et al. 2019, Bull Coast Oceanogr 27: 59-64). We added detail explanation as follows referring the previous report (L86-110). We also demonstrated the supplement figure 1.

“The nitrate sensor was calibrated with the observed nitrate concentrations (Supplement Fig. 1). Since the precision of the nitrate sensor used in this study is low as 0.37 mmol m^{-3} (estimated by Hasegawa et. al., 2019), and the sampling rate ($\sim 2 \text{ samples m}^{-1}$ for the sensor deployment speed of 0.5 m s^{-1}) was coarse; if we calculate the vertical gradient from the raw data, the noise level would be too high for resolving the normal background nitrate stratification of $O(10^{-1} \text{ mmol m}^{-4})$. Therefore, need to set the vertical smoothing (averaging). Using the sensor value C_s , real value C_r , sensor precision P (0.37 mmol m^{-3}), vertical deployment speed of sensor w , sampling frequency f and averaging bin size Δz , the bin averaged vertical gradient of sensor value can be written as

$$\frac{\partial \bar{C}_s}{\partial z} \sim \frac{\bar{C}_r - \bar{C}_{r_{i-1}}}{\Delta z} \pm P \sqrt{\frac{2w}{\Delta z^3 f}} \quad (1)$$

where, $f = 1 \text{ Hz}$, $\bar{w} = 0.5 \text{ m s}^{-1}$ in this study. The second term of the right side of Eq. (1) indicates the expected precision of the bin averaged vertical gradient of nitrate (see the detailed discussions in Hasegawa et. al., 2019). In this study, we took $\Delta z = 10 \text{ m}$ to resolve the realistic vertical gradient with

the expected error size in O (10^{-2} mmol m^{-4}). Total of sixteen nitrate and the turbulence diffusivity profiles obtained among the stations at KG1515 cruise by T/S Kagoshima-maru across the Kuroshio path were averaged, then the profiles of the gradient of the averaged nitrate, and the averaged turbulence diffusivity were multiplied for each depth to get the averaged turbulent nitrate fluxes. Both parameters were binned and averaged within 10-meter intervals. The vertical gradient of the averaged nitrate profile (C_{NO_3}) and the averaged vertical diffusivity profile (K_z) were then multiplied at each depth (z) to estimate the area-averaged vertical turbulent nitrate flux (F_{NO_3}) with the following equation:

$$F_{NO_3} = -K_z \times \partial C_{NO_3} / \partial z \quad (2)$$

In recent years, there is an active discussion about the importance of diapycnal advective flux associated with the diffusive flux (e.g., Du et al., 2017); however, in the present study, we assumed that the important nutrient flux was the one across the euphotic depth, not through the density layer, which was transformed by the turbulent mixing. In addition, as our studied regions were frontal regions unlike the South China Sea, where the Kuroshio flows over the seamounts, density fluctuations should be caused not only by turbulent mixing but also by advection and the movement of the fronts. Accordingly, we focus our discussions on the vertical turbulent nutrient flux using cartesian coordinate, rather than diapycnal flux using isopycnal coordinate.”

6. English needs substantial polishing to ensure correct grammar and wording. Some sentences are difficult to understand.

>We checked all phrases in the manuscript again and revised the incorrect grammars and words.

Editorial comments:

Abstract:

I have concerns on "rapid trophic transfer" in the title. The authors show evidence of rapid microzooplankton consumption of phytoplankton, but did not show evidence of trophic transfer. Suggested title: "Phytoplankton growth and consumption by microzooplankton stimulated by turbulent nitrate flux suggest rapid trophic transfer in the oligotrophic Kuroshio

>The title was revised as you suggested.

The writing of Abstract is confusing. Readers cannot tell what are the results obtained from the experiments, what are the results from other studies, and what are the inferences from those results. I think these issues need to be clearly clarified in Abstract.

Line 29: I cannot understand this sentence, and what the authors intend to say.

>This sentence is revised as follows (L28-30).

“Even though vulnerable life stages of major foraging fishes have a risk to be entrapped by frontal eddies and meanders and encountered under the low food availability, they have life cycle strategies to grow and recruit around the Kuroshio Current.”

Line 31: This conclusion sentence is inference based on the results and should be written as so.

>We revised the phrase like this (L30-31).

“Here we report that phytoplankton growth and consumption by microzooplankton is stimulated by turbulent nitrate flux amplified with the Kuroshio Current.”

Line 35: “were simulated”

>We revised (L34).

Line 35: “Results of dilution ...

>We added (L34).

Line 37: Please explain what you mean by "invisible".

>We wanted to mean “phytoplankton and microzooplankton productivity have long been undetectable by satellite images and oceanographic observations”. Since the readers might be confused, however, we deleted this word, “invisible” (L36).

Introduction

Line 40: I cannot understand what is "originates to".

>We revised this phrase like this (L39-40).

“The Kuroshio enters the East China Sea from the east of Taiwan and flows along the continental slope until it passes through the Tokara Strait into the western North Pacific (Fig 1a).”

Line 43: In spite of such “seemingly” unproductive

>Yes, we added (L42).

Line 46: I cannot understand this sentence.

>We revised as follows (L46-49).

“Highly vulnerable early life stages of many foraging species have a risk to grow and recruit under the oligotrophic and unproductive waters in the ECS-Kuroshio (hereafter called the “Kuroshio Paradox”: Saito, 2019), even if the warm temperatures of the Kuroshio Current could enhance cellular metabolic processes and then growth.”

Methods:

Line 78: Please explain the motivation of using nutrient gradient in experiment in this paragraph, so that the readers can follow the logic flow better.

>We mentioned the motivation just before this sentence. However, as you suggested, we explained the motivations for EXPa and EXPb just before the section of “Experimental setup” as follows (L111-115).

“Two different types of bottle incubations were performed in the present study. For phytoplankton and micro-heterotrophs growth rates in response to in situ nitrate influx by turbulent mixing, bottle incubations with nutrient gradients (EXPa) were conducted at 8 stations in November 2016 and 2017. For microzooplankton grazing on phytoplankton, the dilution experiments (EXPb) followed by

Landry and Hasset (1982) were done at 8 stations in November 2017 (Fig 1b, Table 1).”

Typically in dilution exp, nutrients were amended in all bottles of the 4 dilution factors. Then, to evaluate whether nutrient limitation exists, additional no nutrient amended exp is conducted for non-diluted bottles (100%). Is this the protocol in the EXPb? Please clarify. If the authors did not follow this protocol, please explain why.

>Non-diluted bottles without nutrients were made for EXPb due to comparisons of phytoplankton growths between enriched and non-enriched series. Thus, we revised the explanation on dilution experiments like this (L131-132).

“For evaluating nutrient limitation on phytoplankton growth, no enrichment was conducted for triplicate non-diluted bottles (100%) for EXPb.”

Line 100: Please explain how the chl_a data from different size fraction was obtained in this section.

>We described the size fractions as follows (L145-146).

“Size fractions were defined as Pico for chlorophyll smaller than 2 μm, Nano for chlorophyll between 2 and 11 μm and Micro for chlorophyll larger than 11 μm”

Line 120: Please clarify the difference between the C_t in equation (2) and (3). The explanation is confusing.

>We used C_t and C_o for EXPb (L161).

Results

Line 131: confidence interval of “what”?

>We revised this phrase like this (L172-173).

“We obtained 16 pairs of vertical profiles for turbulent diffusivity and nitrate concentrations and estimated the averages and 95 percent confidence intervals of the vertical profiles.”

Line 136: what is "O"? I cannot understand this sentence.

>"O" means “order level”. Such descriptions are likely common for physical oceanography (L176).

Line 164: Is the “N concentration” the nitrate concentration in the control groups at the start of incubation, i.e. the nitrate concentration in the ambient seawater without enrichment?

>Yes, we changed “at the start of the incubations” into “in the ambient seawater without enrichment” (L212).

Line 179: do you mean "gen'=gmax-m"?

>Yes, we do. We did not change the phrase.

Line 184: Do you mean gen here when referring to net growth rate?

>Yes, we do.

Discussion

Line 191: should be "'previous", not previously

>We revised it (L241).

Line 225: This sentence is confusing. Previous sentence said that "microzooplankton standing stocks and growths are not elevated".

>We revised the phrase like this (L281-283).

“Increase of micro-heterotrophs standing stocks and their trophic transfer to mesozooplankton might be found in the further downstream of the Kuroshio Current.”

Line 235: Because microzooplankton growth rate and standing stocks are NOT significantly elevated, I am NOT sure that the authors can conclude the "rapidly transferred to microzooplankton via their grazing".

>We revised the phrase as follows (L290-292).

“Our study has provided the first experimental evidence that phytoplankton standing stocks and growths are stimulated by turbulent nutrient fluxes and rapidly grazed by microzooplankton.”

Figures:

Figure 2a: The unit of the orange curve seems to be the vertical gradient of nitrate, not the concentration. Please confirm whether this is the concentration or gradient curve.

>We revised “nitrate gradient curve” in the caption (L466).

Figure 3b and 4b: Please use a different set of colors or shading to present the microzooplankton data. It is a little bit difficult to recognize the difference between subplots a and b in these two figures.

>We changed the colors (see revised Figures 3b and 4b).

Figure 5: The color used to present the r values should be consistent to the color used in Figure 3, 4, and 6 (micro = red, nano = green, and pico = yellow). I found that the colors of the points used in this figure correspond to the right size classes but colors of the captions on this figure seem not (micro = green, nano = red, pico = black).

>We used same colors among the figures (see revised Figure 5).

Reply to RC2

This is an interesting study seeking to solve the so-called Kuroshio Paradox. As a physical oceanographer with expertise in small-scale ocean physics I am not in a position to comment on the biological part of this paper, but I do have fundamental concerns on the physics the authors employed in this study.

>We appreciate your kind comments to our findings. As shown in BGD, we indicated point-by-point response to the following comments. Some responses to RC2 at the last time (BGD) might be little changed after receiving the RC3 and editor comments, but the revised phrases are substantially same. Hopefully, these are enough responses to your comments and suggestions.

First of all, turbulent diffusivity was not "measured", but rather estimated involving important physical assumptions, such as isotropy of small-scale (3D) turbulence for the estimation of the turbulent kinetic energy (TKE) dissipation rate from microscale velocity shear measurements, and the Osborn formula (i.e., a local energy balance assuming constant mixing efficiency) for the estimation of diffusivity from the TKE dissipation rate. These and the procedures of data processing should be explained at least briefly in the manuscript. This is in particular necessary given the interdisciplinary nature of the work; the readers with different backgrounds should be able to well appreciate the foundations of the numbers that the authors use to support their points.

>As RC2 suggested, detail descriptions were added at the Materials and Methods section in the revised manuscript as follows (L86-103).

“The nitrate sensor was calibrated with the observed nitrate concentrations (Supplement Fig. 1). Since the precision of the nitrate sensor used in this study is low as 0.37 mmol m^{-3} (estimated by Hasegawa et. al., 2019), and the sampling rate ($\sim 2 \text{ samples m}^{-1}$ for the sensor deployment speed of 0.5 m s^{-1}) was coarse; if we calculate the vertical gradient from the raw data, the noise level would be too high for resolving the normal background nitrate stratification of $O(10^{-1} \text{ mmol m}^{-4})$. Therefore, need to set the vertical smoothing (averaging). Using the sensor value C_s , real value C_r , sensor precision P (0.37 mmol m^{-3}), vertical deployment speed of sensor w , sampling frequency f and averaging bin size Δz , the bin averaged vertical gradient of sensor value can be written as

$$\frac{\partial \overline{C_s}}{\partial z} \sim \frac{\overline{C_r}_i - \overline{C_r}_{i-1}}{\Delta z} \pm P \sqrt{\frac{2\overline{w}}{\Delta z^3 f}} \quad (1)$$

where, $f = 1 \text{ Hz}$, $\overline{w} = 0.5 \text{ m s}^{-1}$ in this study. The second term of the right side of Eq. (1) indicates the expected precision of the bin averaged vertical gradient of nitrate (see the detailed discussions in Hasegawa et. al., 2019). In this study, we took $\Delta z = 10 \text{ m}$ to resolve the realistic vertical gradient with the expected error size in $O(10^{-2} \text{ mmol m}^{-4})$. Total of sixteen nitrate and the turbulence diffusivity profiles obtained among the stations at KG1515 cruise by T/S Kagoshima-maru across the Kuroshio path were averaged, then the profiles of the gradient of the averaged nitrate, and the averaged turbulence diffusivity were multiplied for each depth to get the averaged turbulent nitrate fluxes. Both parameters were binned and averaged within 10-meter intervals. The vertical gradient of the averaged nitrate profile (C_{NO_3}) and the averaged vertical diffusivity profile (K_z) were then multiplied at each depth (z) to estimate the area-averaged vertical turbulent nitrate flux (F_{NO_3}) with the following equation:

$$F_{NO_3} = -K_z \times \partial C_{NO_3} / \partial z \quad (2)$$

Moreover, and more crucially, although it has been customary (in the biogeochemical literature particularly) to estimate diapycnal turbulent fluxes considering only the diffusive flux (i.e., equation (1) in the manuscript), it is now well recognized that this is fundamentally improper, because there is always a diapycnal advective flux associated with the diffusive flux. The physical reason is in fact quite straightforward, that is, diapycnal mixing induces fluxes not only of passive properties such as nutrients, but also of the buoyancy, so that the density of the water parcel is changed due to mixing, and thus a diapycnal advective velocity is induced. These ideas have in fact been rigorously elaborated by Trevor McDougall in 1980s (albeit apparently with insufficient attentions), and the biogeochemical implications have recently been explained by Du et al. (2017). It would be very interesting to see how the refined estimate would affect the authors' results.

>Brief explanations were added at the Materials and Methods section in the revised manuscript as follows (L104-110).

“In recent years, there is an active discussion about the importance of diapycnal advective flux associated with the diffusive flux (e.g., Du et al., 2017); however, in the present study, we assumed that the important nutrient flux was the one across the euphotic depth, not through the density layer, which was transformed by the turbulent mixing. In addition, as our studied regions were frontal regions unlike the South China Sea, where the Kuroshio flows over the seamounts, density fluctuations should be caused not only by turbulent mixing but also by advection and the movement of the fronts. Accordingly, we focus our discussions on the vertical turbulent nutrient flux using cartesian coordinate, rather than diapycnal flux using isopycnal coordinate.”

Reply to RC3

This manuscript suggests the potential mechanism to explain the biological richness (higher trophic level food web) of Kuroshio based on the indirect experimental results of cultured growth rate estimated by size fractionated Chl.a and mortality estimated by grazing pressure of microzooplankton. These indirect approaches are interesting and might be valuable, however I think further explanation or evidences are necessary to make readers agree to the authors conclusion. I also agree with this manuscript for the possible publication in Biogeosciences after moderate revision. The substantial comments are as follows:

>As shown in BGD, we indicated point-by-point response to the following comments. Some responses to RC3 at the last time (BGD) might be little changed after receiving the editor comments, but the revised phrases are substantially same. Hopefully, these are enough responses to your comments and suggestions.

Introduction 1: The current version looks too simply. Why don't authors add the research background of this study citing references? For example, the importance of fish resources from Kuroshio is not described in this version and the significance of fish catch in the Kuroshio to the entire the North Pacific or global. In addition, what kind of lower trophic level organisms compose of assemblages of phytoplankton and zooplankton in the study area? What nutrient regulates the primary production in this study area N? or P? Etc....

>Thanks for suggestions. Just after this manuscript was submitted to Biogeosciences, the review papers have been published. Based on these results, we added more description on the research background. These revisions were highlighted in yellow (L42-62).

“In spite of such seemingly unproductive conditions, the Kuroshio in the East China Sea (ECS-Kuroshio) is neighboring major spawning and nursery grounds for foraging species such as sardine (Watanabe et al., 1996), jack mackerel (Sassa et al., 2008), and chub mackerel (Sassa and Tsukamoto, 2010), and common squid (Bower et al., 1999). Indeed, good fishing grounds have been formed for various fishes and squid using the Kuroshio and their catches composed more than half of total catch in Japan (Saito, 2019). Highly vulnerable early life stages of many foraging species have a risk to grow and recruit under the oligotrophic and unproductive waters in the ECS-Kuroshio (hereafter called the “Kuroshio Paradox”: Saito, 2019), even if the warm temperatures of the Kuroshio Current could enhance cellular metabolic processes and then growth. It has been believed that survival of these early stages is supported by high plankton productivity on the continental shelf and in the Kuroshio front (Nakata et al., 1995). However, such good food availability is spatially limited and greatly variable because the Kuroshio Current often meanders (Nakata and Hidaka, 2003). Otherwise, the coastal water mass is sometimes entrapped and transported into the Kuroshio and more pelagic sites (Nakamura et al., 2006; Kobari et al., 2019). Use of waters in the vicinity of the oligotrophic Kuroshio as a nursery and feeding ground would therefore appear to be a risky strategy unless there is a mechanism that enhance biological production in the Kuroshio.

There is increasing information on community structure of phyto- and zooplankton in the Kuroshio. Pico- to nano-autotrophs contributed to phytoplankton standing stocks in the Kuroshio and predominant components were cellular cyanobacteria like *Prochlorococcus* and *Synechococcus*, haptophytes and diatoms (Hasegawa et al., 2019; Endo and Suzuki, 2019). Heterotrophic bacteria and calanoid copepods

contributed to heterotrophs biomass in the Kuroshio, while microzooplankton biomass were minor (Kobari et al., 2019). Based on the mass balance model, mesozooplankton standing stocks were supported by micro- and nano-autotrophs and microzooplankton (Kobari et al., 2019). However, we have little knowledge how biogeochemical processes and trophodynamics support plankton community in the Kuroshio.”

Introduction 2: Nutrient supply mechanism by turbulent mixing or other physical processes should be more explained citing references because there is a large gap between the paragraph 1 and 2 in the current introduction.

Introduction 3: Why is Tokara Strait important in the Kuroshio track area? Is there any geographical characteristics or bottom topographic characteristics? Is the area of Tokara Strait hot spot of turbulent mixing? Is there any other hot spot of turbulent mixing in the Kuroshio track area? Please explain the above questions in the revised manuscript because the readers who are not familiar with Kuroshio and the North Pacific would not understand the significance of research of Tokara Strait.

>The two issues are associated each other. We added more description on the nutrients supply mechanisms and importance of the Tokara Strait before the last paragraph in Introduction section. The information was also based on the recent review papers as mentioned above (L63-71).

“In recent years, some mechanisms have been found for nutrients supply to the oligotrophic Kuroshio waters. The Kuroshio nutrient stream contributed significantly to productivity in the euphotic layer, similarly to the “nutrient stream” along the Gulf Stream (Komatsu and Hiroe, 2019). Turbulence around the Kuroshio appeared to be important for upward nutrients supply in the Kuroshio (Nagai et al., 2019). Frontal disturbances also contributed to nutrients supply to the surface layer in the Kuroshio (Kuroda, 2019). Moreover, the Island Mass Effect was produced by the Kuroshio Current around the archipelagic topography and induced upward nutrients supply (Hasegawa, 2019). These nutrients supplies have been suggested to stimulate biological productivity in the Kuroshio. In the wide Kuroshio track area, these nutrients supplies can happen particularly around the Tokara Straits due to the extensive frontal disturbances (Nakamura et al., 2006) and strong turbulence (Tsutsumi et al., 2017; Nagai et al., 2017, 2019).”

Results 1: The manuscript described that nitrate flux induced by turbulent mixing at the subsurface Chl maximum was observed as 0.788 mmol m⁻² d⁻¹ in the Tokara Strait (150 km wide) and authors assumed that the same concentration was kept during 5 days. What potential physical mechanism does keep almost same nitrate concentration at the Chl maximum layer during week?

>Our assumptions are based on the direct observations of turbulence (see Tsutsumi et al., 2017; Nagai et al., 2017). The strong turbulence was likely kept when the Kuroshio Current passed over the Tokara Strait due to the narrow and shallow topography with many islands and seamount. Also, our assumption of the nitrate supply might be conservative in the ambient waters because the upward nutrients supplied with the Island Mass Effect was not considered here.

Results 2: In terms of gradient enrichment experiment and dilution experiment, the please add further

descriptions of the details e.g., methods themselves and what purpose are achieved by these methods etc.

>In the revised manuscript, we mentioned them briefly at each paragraph but added clearer descriptions of the purpose and results achieved at the beginning and end of the phrases as follows. Since the revised phrases are found everywhere, they are highlighted in yellow as follows.

Gradient enrichment experiments

L181-182

To evaluate how these turbulent nitrate fluxes measured in the Tokara Strait increase the standing stocks of phytoplankton and micro-heterotrophs in the Kuroshio, we conducted bottle incubations of the phytoplankton and micro-heterotrophs communities enriched with the different nutrient concentrations (EXPa).

L202-204

Thus, the standing stocks of phytoplankton and micro-heterotrophs were likely increased within the range of the turbulent nitrate fluxes measured in the Tokara Strait.

L209-211

To explain whether growth rates of the size-fractionated chlorophyll might be variable with initial nutrients concentrations (bottom-up control) and predator biomasses (top-down control) at the beginning of the experiments, the slopes were compared to the nitrate+nitrite (Fig 5a) and phosphate concentrations (Fig 5b) and micro-heterotrophs biomass (Fig 5c) in the ambient seawater without enrichment.

L216-217

Thus, the variations in phytoplankton growth rates are likely associated with nutrients concentrations at the beginning of the incubations.

Dilution experiments

L220-221

To evaluate how much and which size-fractionated phytoplankton was removed by microzooplankton grazing, the dilution experiments were conducted simultaneously to the gradient enrichment experiments.....These results imply that gen of all size-fractionated chlorophyll balances the microzooplankton grazing mortality with the maximum growth. Particularly for the nano-fractionated chlorophyll, the net growth rates were slightly low due to the mortality rates by microzooplankton grazing exceeded the maximum growth rates.

Results 3. Lines 161-167: I could not understand what authors would like to describe in this paragraph. Especially, the sentence of the line 163 (To explain ...) seems quite to be abrupt. The more explanation needs for Fig. 5. Does the fig 5 show the data comparing among all stations? Why can the Fig. 5 be used to explain the difference in growth rate of size fractionated Chl. a among stations? Please explain more details of the similarity or difference of characteristics among stations. In addition, no Supplement Fig.1 is attached in the manuscript.

>We added more descriptions on the reason why we compared the slope of a linear regression of phytoplankton growths to nutrients supply using supplement Fig. 1 as follows. At the platform of

Biogeosciences, supplement materials seem to be provided with different files from the manuscript. You can find the Supplement (205KB) below the manuscript PDF or XML files at the website (L205-212).

The slope of a linear regression between growth rates of the size-fractionated chlorophyll and the logarithms of the nitrate enrichments at each incubation provided a metric of the sensitivity of their growth rates to nutrient supply. As shown in Supplement Fig 1, the steeper slopes were found at some stations in the upstream Kuroshio in the Tokara Strait compared with those at the other stations, suggesting that apparent phytoplankton growths were variable with the nutrients concentrations or predatory impacts at the beginning of the incubations. To explain whether growth rates of the size-fractionated chlorophyll might be variable with initial nutrients concentrations (bottom-up control) or predator biomasses (top-down control) at the beginning of the experiments, the slopes were compared to the nitrate+nitrite (Fig 5a) and phosphate concentrations (Fig 5b) and micro-heterotrophs biomass (Fig 5c) in the ambient seawater without enrichment.

Discussion 1: Line205: Why is microzooplankton standing stock in the Tokara Strait of the Kuroshio track low, although the grazing pressure of phytoplankton by microzooplankton are relatively large? Is there any evidence or previous studies to indicate the rapid energy transfer of the microzooplankton to larger size organisms? Please give the potential mechanism in the revised version.

>Unfortunately, there is no direct evidence why microzooplankton biomass was low in the Kuroshio, excepted for the indirect evidence that microzooplankton might be removed by mesozooplankton predation based on the carbon flow among various components (Kobari et al., 2019). Thus, we added this brief information there (L255-257).

Microzooplankton standing stocks in the Kuroshio Current at the Tokara Strait were lower than those on the continental shelf of the ECS (Chen et al., 2003) and might be removed by mesozooplankton predation (Kobari et al., 2019). These results expected low microzooplankton grazing on phytoplankton. On the other hand, we have conducted the other bottle experiments to evaluate how much microzooplankton was removed by mesozooplankton predations. As you expected, the results from the bottle experiments demonstrated that naked ciliates predominated in microzooplankton biomass were removed by mesozooplankton predation. These results are recently submitted but could not be mentioned more here.

Discussion 2: Line219-220: The sentence of this line is abrupt because there is no evidence or discussion in terms of the large variation in microzooplankton standing stocks among stations (L262).

>Large variations in microzooplankton standing stocks among the stations were already shown in Table 1, and thus we added “Table 1” in this sentence.

1 **Phytoplankton growth and consumption by microzooplankton**
2 **stimulated by turbulent nitrate flux suggest rapid trophic transfer**
3 **in the oligotrophic Kuroshio**

4
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27 **Abstracts.** The Kuroshio Current has been thought to be biologically unproductive due to oligotrophic conditions and
28 low plankton standing stocks. Even though vulnerable life stages of major foraging fishes have a risk to be entrapped by
29 frontal eddies and meanders and encountered under the low food availability, they have life cycle strategies to grow and
30 recruit around the Kuroshio Current. Here we report that phytoplankton growth and consumption by microzooplankton
31 is stimulated by turbulent nitrate flux amplified with the Kuroshio Current. Oceanographic observations demonstrate that
32 the Kuroshio Current topographically enhances significant turbulent mixing and nitrate influx to the euphotic zone.
33 Gradual nutrient enrichment experiments show growth rates of phytoplankton and micro-heterotrophs communities were
34 stimulated within a range of the turbulent nitrate flux. Results of dilution experiments imply a significant
35 microzooplankton grazing on phytoplankton. We propose that these rapid and systematic trophodynamics enhance
36 biological productivity in the Kuroshio.

37 **1 Introduction**

38 The Kuroshio Current is the western boundary current of the North Pacific Subtropical Gyre (Qiu, 2001; Hu et
39 al., 2015). The Kuroshio enters the East China Sea **from the east of Taiwan** and flows along the continental slope until it
40 passes through the Tokara Strait into the western North Pacific (Fig 1a). The Kuroshio has been thought to be biologically
41 unproductive because ambient nutrient concentrations and plankton standing stocks in its waters are low (Guo, 1991;
42 Hirota, 1995). In spite of such **seemingly** unproductive conditions, the Kuroshio in the East China Sea (ECS-Kuroshio)
43 is neighboring major spawning and nursery grounds for foraging species such as sardine (Watanabe et al., 1996), jack
44 mackerel (Sassa et al., 2008), and chub mackerel (Sassa and Tsukamoto, 2010), and common squid (Bower et al., 1999).
45 **Indeed, good fishing grounds have been formed for various fishes and squid using the Kuroshio and their catches**
46 **composed more than half of total catch in Japan (Saito, 2019). Highly** vulnerable early life stages of many foraging species
47 **have a risk to** grow and recruit **under** the oligotrophic and unproductive waters **in** the ECS-Kuroshio (hereafter called the
48 “Kuroshio Paradox”: Saito, 2019), even if the warm temperatures of the Kuroshio Current could enhance cellular
49 metabolic processes and then growth. It has been believed that survival of these early stages is supported by high plankton
50 productivity on the continental shelf and in the Kuroshio front (Nakata et al., 1995). However, such good food availability
51 is spatially limited and greatly variable because the Kuroshio Current often meanders (Nakata and Hidaka, 2003).
52 Otherwise, the coastal water mass is sometimes entrapped and transported into the Kuroshio and more pelagic sites
53 (Nakamura et al., 2006; Kobari et al., 2019). Use of waters in the vicinity of the oligotrophic Kuroshio as a nursery and
54 feeding ground would therefore appear to be a risky strategy unless there is a mechanism that enhance biological
55 production in the Kuroshio.

56 **There is increasing information on community structure of phyto- and zooplankton in the Kuroshio. Pico- to**

57 nano-autotrophs contributed to phytoplankton standing stocks in the Kuroshio and predominant components were cellular
58 cyanobacteria like *Prochlorococcus* and *Synechococcus*, haptophytes and diatoms (Hasegawa et al., 2019; Endo and
59 Suzuki, 2019). Heterotrophic bacteria and calanoid copepods contributed to heterotrophs biomass in the Kuroshio, while
60 microzooplankton biomass were minor (Kobari et al., 2019). Based on the mass balance model, mesozooplankton
61 standing stocks were supported by micro- and nano-autotrophs and microzooplankton (Kobari et al., 2019). However, we
62 have little knowledge how biogeochemical processes and trophodynamics support plankton community in the Kuroshio.

63 In recent years, some mechanisms have been found for nutrients supply to the oligotrophic Kuroshio waters. The
64 Kuroshio nutrient stream contributed significantly to productivity in the euphotic layer, similarly to the “nutrient stream”
65 along the Gulf Stream (Komatsu and Hiroe, 2019). Turbulence around the Kuroshio appeared to be important for upward
66 nutrients supply in the Kuroshio (Nagai et al., 2019). Frontal disturbances also contributed to nutrients supply to the
67 surface layer in the Kuroshio (Kuroda, 2019). Moreover, the Island Mass Effect was produced by the Kuroshio Current
68 around the archipelagic topography and induced upward nutrients supply (Hasegawa, 2019). These nutrients supplies
69 have been suggested to stimulate biological productivity in the Kuroshio. In the wide Kuroshio track area, these nutrients
70 supplies can happen particularly around the Tokara Straits due to the extensive frontal disturbances (Nakamura et al.,
71 2006) and strong turbulence (Tsutsumi et al., 2017; Nagai et al., 2017, 2019).

72 Here we report phytoplankton productivity and subsequent microzooplankton grazing stimulated by turbulent
73 nitrate flux that can happen in the Kuroshio Current. Oceanographic observations demonstrate a significant nitrate flux
74 caused by turbulent mixing in the Tokara Strait of the ECS-Kuroshio. Nutrient-amended bottle incubation experiments
75 show phytoplankton and micro-heterotrophs growths elevated within a range of this turbulent nitrate flux and significant
76 grazing of microzooplankton on phytoplankton.

77

78 2 Materials and methods

79 2.1 Onboard observations and experiments

80 All oceanographic observations and bottle incubations were done in the Kuroshio Current where it passes through
81 the Tokara Strait. Samplings for nitrate concentrations and measurements of turbulent diffusivity were conducted at 14
82 stations along the 2 lines across the Kuroshio Current (Fig 1a) during cruises of the T/S *Kagoshima-maru* in November
83 2015.

84 The nitrate profiles were measured by a nitrate sensor (Deep SUNA V2) attached on a SBE-9plus CTD system.
85 The turbulence diffusivity was estimated from microstructure measurements by TurboMAP-L (JFE Advantech Co. Ltd.)
86 based on Osborn (1980)'s formula, which were deployed instantly after each CTD cast for the same stations. The nitrate
87 sensor was calibrated with the observed nitrate concentrations (Supplement Fig. 1). Since the precision of the nitrate
88 sensor: P in this study is 0.37 mmol m^{-3} (estimated by Hasegawa et. al., 2019), if we calculate the vertical gradient from
89 the raw data, the noise level would be too high for resolving the normal background nitrate stratification of $O(10^{-1} \text{ mmol}$
90 $\text{m}^{-4})$. Therefore, we need to apply the vertical averaging on the sensor data for reducing the sensing error. Using the sensor
91 value: C_s , real concentration: C_r , vertical deployment speed of sensor: w , sampling frequency: f , and averaging bin size:
92 Δz , the bin averaged vertical gradient of sensor value can be written as

$$93 \frac{\partial \overline{C_s}}{\partial z} \sim \frac{\overline{C_r} - \overline{C_r}_{i-1}}{\Delta z} \pm P \sqrt{\frac{2\overline{w}}{\Delta z^3 f}} \quad (1)$$

94 where, $f = 1 \text{ Hz}$, $\overline{w} = 0.5 \text{ m s}^{-1}$ in this study. The second term of the right side of Eq. (1) indicates the expected precision
95 of the bin averaged vertical gradient of nitrate (see the detailed discussions in Hasegawa et. al., 2019). In this study, we
96 took $\Delta z = 10 \text{ m}$ to resolve the realistic vertical gradient with the expected error size in $O(10^{-2} \text{ mmol m}^{-4})$. Total of
97 sixteen nitrate and the turbulence diffusivity profiles obtained among the stations at KG1515 cruise by T/S *Kagoshima-*

98 maru across the Kuroshio path were averaged, then the profiles of the gradient of the averaged nitrate, and the averaged
99 turbulence diffusivity were multiplied for each depth to get the averaged turbulent nitrate fluxes. Both parameters were
100 binned and averaged within 10-meter intervals. The vertical gradient of the averaged nitrate profile (C_{NO_3}) and the
101 averaged vertical diffusivity profile (K_z) were then multiplied at each depth (z) to estimate the area-averaged vertical
102 turbulent nitrate flux (F_{NO_3}) with the following equation:

$$103 \quad F_{NO_3} = -K_z \times \partial C_{NO_3} / \partial z \quad (2)$$

104 In recent years, there is an active discussion about the importance of diapycnal advective flux associated with the diffusive
105 flux (e.g., Du et al., 2017); however, in the present study, we assumed that the important nutrient flux was the one across
106 the euphotic depth, not through the density layer, which was transformed by the turbulent mixing. In addition, as our
107 studied regions were frontal regions unlike the South China Sea, where the Kuroshio flows over the seamounts, density
108 fluctuations should be caused not only by turbulent mixing but also by advection and the movement of the fronts.
109 Accordingly, we focus our discussions on the vertical turbulent nutrient flux using cartesian coordinate, rather than
110 diapycnal flux using isopycnal coordinate.

111 Two different types of bottle incubations were performed in the present study. For phytoplankton and micro-
112 heterotrophs growth rates in response to *in situ* nitrate influx by turbulent mixing, bottle incubations with nutrient
113 gradients (EXP_a) were conducted at 8 stations in November 2016 and 2017. For microzooplankton grazing on
114 phytoplankton, the dilution experiments (EXP_b) followed by Landry and Hassett (1982) were done at 8 stations in
115 November 2017 (Fig 1b, Table 1).

116

117 2.2 Experimental setup

118 Seawater samples for all experiments were obtained using 2.5-L Niskin-X bottles attached to a conductivity-
119 temperature-depth profiler and carousel multisampling system (CTD-CMS: Sea-Bird SBE-9plus). The samples were
120 transferred by gravity filtration using a silicon tube with a nylon filter (0.1-mm mesh opening) into the incubation bottles
121 for EXP_a and EXP_b.

122 EXP_a was performed using duplicate 2.3-L polycarbonate bottles without added nutrients and with a mixture of
123 nitrate (NaNO₃) and phosphate (KH₂PO₄) in an atomic N:P ratio of 15:1. The nitrate concentrations were either 0 (control),
124 0.05, 0.15, 0.5, 0.75, 1.5, or 5 μmol L⁻¹. Assuming that the turbulent nitrate supplies at the subsurface chlorophyll
125 maximum observed in the Tokara Strait (*O*: 0.788 mmol m⁻² d⁻¹, see Results) were continued during 5.3 days when the
126 Kuroshio Current (0.33 m s⁻¹, Zhu et al., 2017) passed over the Tokara Strait (150 km) and consumed by phytoplankton
127 in a 10-m thick layer, they were equivalent to the nitrate enrichment of 0.41 μmol L⁻¹.

128 EXP_b was conducted using triplicate 1.2-L polycarbonate bottles with microzooplankton as grazers and involved
129 four dilution factors (10, 30, 60, and 100%) of the microzooplankton standing stocks in the original water samples. These
130 treatment bottles were enriched with 3 μmol L⁻¹ nitrate (NaNO₃) and 0.2 μmol L⁻¹ phosphate (KH₂PO₄) to promote
131 phytoplankton growth. For evaluating nutrient limitation on phytoplankton growth, no enrichment was conducted for
132 triplicate non-diluted bottles (100%) for EXP_b.

133 All incubation tools were soaked in 10% HCl and rinsed with surface seawater at each station before use (Landry
134 et al., 1995). All experimental bottles were incubated for 72 h for EXP_a and 24 h for EXP_b in a water bath with running
135 surface seawater for temperature control and covered by a nylon mesh screening (5-mm mesh opening screening to reduce
136 irradiance to 75% of the surface irradiance. Note that the phytoplankton growth in the incubation bottles might be

137 overestimated due to the weaker irradiance at subsurface than those under the incubation conditions.

138

139 **2.3 Sample analysis**

140 Chlorophyll *a* concentrations were determined at the beginning and end of the incubations for EXP_a and EXP_b.
141 Subsamples of 500 to 1000 mL were filtered through a nylon mesh (11- μm mesh opening: Millipore NY1104700) and a
142 glass-fiber filter (2- μm : Whatman GM/F, 0.7- μm : Whatman GF/F) for EXP_a and through a glass-fiber filter (GF/F) for
143 EXP_b at a pressure less than 20 kPa. Photosynthetic pigments were extracted overnight in *N,N*-dimethylformamide at –
144 20°C in the dark, and the chlorophyll *a* concentrations were determined with a fluorometer (Turner Designs 10AU or
145 TD700). Size fractions were defined as Pico for chlorophyll smaller than 2 μm , Nano for chlorophyll between 2 and 11
146 μm and Micro for chlorophyll larger than 11 μm .

147 Micro-sized heterotrophs in the incubation bottles at the beginning of EXP_a and EXP_b were examined.
148 Subsamples of 500 mL were collected and fixed with 3% acid Lugol's solution. We identified and counted three taxonomic
149 groups of the micro-heterotrophs community with an inverted microscope (Leica Leitz DMRD). Some marine planktonic
150 ciliates and flagellates are known to be mixotrophs (Gaines and Elbrächter, 1987), but we assumed naked ciliates and
151 tintinnids to be heterotrophic in the present study. The sizes of cells or individuals were measured, biovolume was
152 computed based on geometric shape, and the carbon content was estimated using conversion equations (Put and Stoecker,
153 1989; Verity and Langdon, 1984; Parsons et al., 1984).

154

155 **2.4 Rate calculation**

156 Growth rates ($g: d^{-1}$) in the incubation bottles of EXP_a and EXP_b were calculated from size-fractionated
157 chlorophyll *a* concentrations ($\mu\text{g L}^{-1}$) or standing stocks ($\mu\text{gC L}^{-1}$) of micro-heterotrophs groups identified at the

158 beginning (C_o) and end (C_t) of the incubations period (t : days):

$$159 \quad G = [\ln(C_t) - \ln(C_o)] / t \quad (3)$$

160 Apparent growth rates in the incubation bottles of EXP_b were calculated using the following model (Landry et al., 1995):

$$161 \quad C'_t = C'_o \times \exp[(g_{max} - m) \times t] \quad (4)$$

162 where g_{max} and m are the maximum growth rate of size-fractionated phytoplankton (d^{-1}) and their mortality rate by

163 microzooplankton grazing (d^{-1}), respectively. The maximum growth rate and mortality rate were determined with a linear

164 regression of the apparent growth rate against dilution factors (X):

$$165 \quad g = g_{max} - mX \quad (5)$$

166 All parameters derived from EXP_a and EXP_b are listed in Table 2 and Table 3.

167

168 **3 Results**

169 **3.1 Oceanographic observations**

170 First, turbulent diffusivity and nitrate concentrations were measured in order to estimate the vertical turbulent

171 nitrate flux along the transects across the Kuroshio Current in the Tokara Strait, where a shallow ridge lies in the

172 Kuroshio's path. We obtained 16 pairs of vertical profiles for turbulent diffusivity and nitrate concentrations and estimated

173 the averages and 95 percent confidence intervals of the vertical profiles. The averaged chlorophyll-*a* profile (Fig 2a)

174 recorded with a light-emitting diode fluorometer on a TurboMAP-L profiler revealed a subsurface chlorophyll maximum

175 (SCM) at 60 m, which was almost coincident with a sharp increase in the nitrate concentration (i.e., the top of the

176 nitracline). Vertical diffusivity of O ($10^{-4} \text{ m}^2 \text{ s}^{-1}$, Fig 2b) was higher at 70 m compared with those in the layers between

177 80 and 130 m. Just below the SCM peak, relatively high nitrate concentrations and vertical diffusivity induced vertical

178 turbulent nitrate fluxes of O ($1 \text{ mmol m}^{-2} \text{ d}^{-1}$, Fig 2c).

179

180 3.2 Gradient enrichment experiments (EXP_a)

181 To evaluate how these turbulent nitrate fluxes measured in the Tokara Strait increase the standing stocks of
182 phytoplankton and micro-heterotrophs in the Kuroshio, we conducted bottle incubations of the phytoplankton and micro-
183 heterotrophs communities enriched with the different nutrient concentrations (EXP_a). The total chlorophyll *a*
184 concentrations at the beginning of the EXP_a averaged among the duplicate samples ranged from 0.15 to 0.52 $\mu\text{g L}^{-1}$ (Table
185 1). The pico-fractions defined as smaller than 2 μm and nano-fractions between 2 to 11 μm accounted for more than 80%
186 of the total chlorophyll *a* (Fig 3a). All size-fractionated chlorophyll *a* declined or changed little toward the end of the
187 incubations at the nitrate enrichments below 0.15 $\mu\text{mol L}^{-1}$, but they increased at the enrichments above 0.5 $\mu\text{mol L}^{-1}$. At
188 the beginning of the incubations, micro-heterotrophs standing stocks averaged among the duplicate samples ranged from
189 0.12 to 0.79 $\mu\text{g C L}^{-1}$ (Table 1). Naked ciliates accounted for 51 to 96% of the micro-sized heterotrophs biomass in terms
190 of carbon at the beginning of the incubations. Copepod nauplii were the second contributor to the micro-heterotrophs
191 biomass due to the low abundance and large individual body mass, and tintinnid ciliates were a minor component. The
192 standing stocks of all taxonomic groups in the micro-sized heterotrophs increased with the higher nitrate enrichments (Fig
193 3b), but the increasing patterns to nutrient gradient were less clear than those of the size-fractionated chlorophyll *a*
194 concentrations.

195 Based on these differences of the standing stocks between the beginning and end of the incubations, we
196 investigated the growth rates of chlorophyll and micro-heterotrophs. The growth rates of all size-fractionated chlorophyll
197 increased at the larger nitrate additions (Fig 4a). Growth rates were negative or close to zero for all size-fractions at the
198 enrichment below 0.15 $\mu\text{mol L}^{-1}$. However, the pico- and micro-sized chlorophyll revealed positive growth rates at the

199 nitrate concentrations above $0.5 \mu\text{mol L}^{-1}$, which were nearly equivalent to the turbulent nitrate fluxes observed in the
200 Tokara Strait (see Experimental setup). Because **micro-heterotrophs** growth rates varied among stations, the response of
201 **micro-heterotrophs** growth to nutrient gradient was ambiguous (Fig 4b). Growth rates were positive for copepod nauplii
202 at all nitrate enrichments and were higher for both naked and tintinnid ciliates at the larger nitrate enrichments. **Thus, the**
203 **standing stocks of phytoplankton and micro-heterotrophs were likely increased within the range of the turbulent nitrate**
204 **fluxes measured in the Tokara Strait.**

205 The slope of a linear regression between growth rates of the size-fractionated chlorophyll and the logarithms of
206 the nitrate enrichments at each incubation provided a metric of the sensitivity of their growth rates to nutrient supply. **As**
207 **shown in Supplement Fig 1, the steeper slopes were found at some stations in the upstream Kuroshio in the Tokara Strait**
208 **compared with those at the other stations, suggesting that apparent phytoplankton growths were variable with the nutrients**
209 **concentrations or predatory impacts at the beginning of the incubations.** To explain **whether** growth rates of the size-
210 fractionated chlorophyll **might be variable with initial nutrients concentrations (bottom-up control) or predator biomasses**
211 **(top-down control) at the beginning of the experiments,** the slopes were compared to the nitrate+nitrite (Fig 5a) and
212 phosphate concentrations (Fig 5b) **and micro-heterotrophs biomass (Fig 5c) in the ambient seawater without enrichment.**
213 **No significant correlation was found for all size-fractionated chlorophyll to the micro-sized heterotrophs biomass. On the**
214 **other hand, there was a negative correlation of the slopes for all size-fractions to the nitrate plus nitrite or phosphate**
215 **concentrations, indicating that the stimulation of their growth rates by nutrients supply was greater for all size-fractionated**
216 **chlorophyll under more oligotrophic conditions. Thus, the variations in phytoplankton growth rates are likely associated**
217 **with nutrients concentrations at the beginning of the incubations.**

218

219 3.3 Dilution experiments (EXP_b)

220 To evaluate how much and which size-fractionated phytoplankton was removed by microzooplankton grazing,
221 the dilution experiments were conducted simultaneously to the gradient enrichment experiments. The maximum growth
222 rates represented by the intercepts in the dilution experiments were relatively high for the nano-sized chlorophyll (Fig 6a),
223 while the difference was insignificant among the three size-fractions (ANOVA, $p>0.05$). These findings indicated that
224 growth potential under no microzooplankton grazing was slightly high for the nano-sized chlorophyll compared with
225 those for the pico- and micro-fractions. On the other hand, the slopes were representative of the mortality rates by
226 microzooplankton grazing and significantly higher for the nano-sized chlorophyll than those for the pico- and micro-sized
227 chlorophyll (ANOVA+Tukey, $p<0.05$), indicating the preference of microzooplankton grazing on the nano-sized
228 chlorophyll. To evaluate the impact of microzooplankton grazing on phytoplankton growth, we compared the three
229 different net growth rates, which were the observed net growth rates without enrichment (g_o) and with enrichment (g_{en})
230 in the non-diluted bottles, and the estimated net growth rates (g_{en}') subtracted the mortality rates (m) from the maximum
231 growth rates (g_{max}). All size-fractionated chlorophyll demonstrated g_o lower than g_{en} (Fig 7), indicating nutrient limitation
232 on the net growth rates. Both g_{en} and g_{en}' were comparable due to no significant difference between the two (Welch's t -
233 test). These results imply that g_{en} of all size-fractionated chlorophyll balances the microzooplankton grazing mortality
234 with the maximum growth. Particularly for the nano-fractionated chlorophyll, the net growth rates were slightly low due
235 to the mortality rates by microzooplankton grazing exceeded the maximum growth rates.

236

237 4 Discussion

238 The Kuroshio Current impinges on numerous shallow ridges with small islands and seamounts in the Tokara
239 Strait. Several studies have pointed out that those steep topographic features stir and modify the water column through

240 upwelling (Hasegawa et al., 2004, 2008) and turbulent mixing (Tsutsumi et al., 2017; Nagai et al., 2017). Comparing with
241 the turbulent nitrate fluxes among the **previous** study sites, the fluxes observed in the Tokara Strait of the Kuroshio Current
242 were one order higher than those reported in the Kuroshio Extension front (Kaneko et al., 2012, 2013; Nagai et al., 2017),
243 much greater than those at other oceanic sites, and equivalent to those at coastal sites (Cyr et al., 2015). The turbulent
244 nitrate flux in the downstream Kuroshio Current where was close to the Tokara Strait was similar magnitude to our
245 estimates (Nagai et al., 2019). Since the Kuroshio Current steadily runs in the Tokara Strait, such nutrient supply induced
246 by turbulence diffusivity is considered as one of mechanisms that phytoplankton productivity is enhanced even under
247 oligotrophic Kuroshio.

248 In spite of the large turbulent nitrate flux ($O: 1 \text{ mmol m}^{-2} \text{ d}^{-1}$), the chlorophyll *a* concentrations in the Tokara
249 Strait of the Kuroshio Current were as low as the values reported from the neighboring Kuroshio (Kobari et al., 2018,
250 2019) and oceanic sites in the North Pacific Ocean (Calbet and Landry, 2004). Based on the gradient enrichment
251 experiments, standing stocks and their growth rates of all size-fractionated phytoplankton increased at the nitrate
252 enrichments above $0.5 \mu\text{mol L}^{-1}$ that were equivalent to the observed turbulent nitrate flux. These results suggest that
253 phytoplankton standing stocks and growths are stimulated by the magnitude of the observed turbulent nitrate flux. In the
254 global comparisons, microzooplankton reveal a significant grazing impact on phytoplankton, particularly in oceanic sites
255 (Calbet and Landry, 2004). Microzooplankton standing stocks in the Kuroshio Current at the Tokara Strait were lower
256 than those on the continental shelf of the ECS (Chen et al., 2003) **and might be removed by mesozooplankton predation**
257 **(Kobari et al., 2019). These results expected** low microzooplankton grazing on phytoplankton. However, the dilution
258 experiments demonstrated that phytoplankton mortality by microzooplankton grazing was significantly high and
259 equivalent to 41 to 122% of maximum growth rates of phytoplankton based on the ratio of the mortality rate to the

260 maximum growth rates for total chlorophyll *a* (Table 2). Indeed, phytoplankton net growth likely balances
261 microzooplankton grazing mortality with phytoplankton maximum growth, particularly for nano-fractionated
262 phytoplankton (Fig. 7). These results from the simultaneously conducted experiments suggest that phytoplankton standing
263 stocks are stimulated by turbulent nitrate flux and then quickly removed by microzooplankton grazing, particularly for
264 nanophytoplankton. Taking into account for the size range of prey for ciliates (Pierce and Turner, 1992) and copepod
265 nauplii (Uye and Kasahara, 1983), microzooplankton grazing would be a major reason why phytoplankton do not attain
266 high growth rates and standing stocks, even under the high potential growth and sensitive to nutrient enrichments. Thereby,
267 the rapid transfer of the elevated phytoplankton production to microzooplankton might be a possible mechanism of the
268 low chlorophyll even under the large turbulent nitrate flux in the Kuroshio Current.

269 The standing stocks and growth rates of all micro-sized heterotrophs were relatively higher at the larger nitrate
270 enrichments, but the increasing patterns were less clear than those of phytoplankton. This difference was probably due to
271 the large variations in these micro-heterotrophs standing stocks among stations (Table 1) and slower growth than
272 phytoplankton. Indeed, such unclear pattern was remarkable for copepod nauplii representing their slower growth rate,
273 less abundance in the bottle and large individual body mass. On the other hand, “intra-guild” predation within micro-
274 heterotrophs community might be another explanation on the less clear pattern of their standing stocks and growth rates.
275 Growth rates of copepod nauplii were always higher than those of naked ciliates, especially under no or less nitrate supply.
276 The ratio of mean equivalent spherical diameter of body mass between copepod nauplii (88 μm) and naked ciliates (16
277 μm) was estimated to be 5:1 and much different from to the predator-prey mass ratio (i.e., 18:1, Hansen et al., 1994).
278 Thus, such intraguild predation of copepod nauplii on naked ciliates would not happen in the bottles. More importantly
279 to no or less clear pattern of the growth of micro-heterotrophs, the results from the simultaneously conducted experiments

280 imply that phytoplankton productivity is stimulated by the turbulent nitrate flux and rapidly grazed by microzooplankton
281 but standing stocks and growths of micro-heterotrophs are not elevated during 3 days in the Kuroshio Current. Increase
282 of micro-heterotrophs standing stocks and their trophic transfer to mesozooplankton might be found in the further
283 downstream of the Kuroshio Current.

284 There is increasing information that turbulence-induced nutrient fluxes have been suggested to promote
285 phytoplankton growth in the open ocean (Kaneko et al., 2013; Nagai et al., 2017, 2019), however, no experimental
286 documentation is available for response of phytoplankton community to the nutrient supply or of subsequent trophic
287 transfer in a planktonic food web. In the tropical and subtropical oceans, microzooplankton grazing has been thought to
288 be a major source of phytoplankton mortality and has been shown to account for more than 75% of phytoplankton daily
289 growth (Calbet and Landry, 2004). Furthermore, strong trophic linkages are well known between microbes and metazoans
290 through microzooplankton (Calbet and Landry, 1999; Calbet et al., 2001; Calbet and Saiz, 2005; Kobari et al., 2010). Our
291 study has provided the first experimental evidence that phytoplankton standing stocks and growths are stimulated by
292 turbulent nutrient fluxes and rapidly grazed by microzooplankton. These results imply a possibility that biological
293 productivity is underestimated by apparent low nutrients and low phytoplankton biomass in the Kuroshio. Because strong
294 turbulence amplified by the Kuroshio Current, phytoplankton productivity stimulated by the nutrient flux and rapid
295 trophic transfer to microzooplankton are likely to happen in the Tokara Strait and the downstream, we propose that
296 unobservable biological productivity in the Kuroshio is sustained by these rapid and systematic trophodynamics. Such
297 unobservable biological production elevated by the rapid and systematic trophodynamics may provide good food
298 availability for the vulnerable stages of foraging fishes around the Kuroshio and thus explain a part of the Kuroshio
299 Paradox.

300

301 **Data Availability Statement:**

302 All relevant data are shown in the paper as tables and figure.

303

304 **Author Contributions**

305 T. Kobari, DH and NY conceived and designed the oceanographic observations and experiments. DH, HN, AN,
306 ET, TM, TN performed the oceanographic observations and turbulence measurements. T. Kobari, TH, T. Kanayama and
307 FK performed the onboard experiments. T. Kobari, TH, T. Kanayama, FK, NY, KS analyzed the samples and data of the
308 onboard experiments. DH and TT analyzed the data of oceanographic observations and turbulence measurements. T.
309 Kobari, GK, HN and XG organized the research cruises.

310

311 **Competing interests:**

312 The authors declare no competing and conflict interests.

313

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317

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321 Ecosystem Dynamics for Sustainable Fisheries).

322

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434 **Table 1** Information on locations and environmental conditions at the stations conducted the gradient enrichment (EXP_a)
 435 and dilution experiments (EXP_b) in the ECS-Kuroshio. Depth: sampling depth (m) of water samples for each experiment.
 436 WT: mean water temperature during the experiments (°C). NUTS₀: nutrients concentrations (μmol L⁻¹) at the beginning
 437 of each experiment. CHL₀: Chlorophyll *a* concentration (μgCHL L⁻¹) at the beginning of the experiments. MiZ₀: micro-
 438 heterotrophs standing stocks at the beginning of each experiment (μgC L⁻¹). DL: below the detection limit.
 439

Station	Location		Date	Year	Depth	WT	NUTS ₀		CHL ₀	MiZ ₀
	Longitude	Latitude					NO ₃ +NO ₂	PO ₄		
EXP _a										
C02	30°11'N	129°41.0'E	13 Nov	2016	68	26.1	DL	0.02	0.34	0.19
C03	29°50'N	129°08.4'E	13 Nov	2016	75	26.2	DL	0.01	0.41	0.27
F01	29°53'N	129°22.4'E	14 Nov	2016	81	25.1	0.21	0.04	0.35	0.15
G01	29°51'N	129°57.2'E	14 Nov	2016	91	26.1	0.26	0.07	0.44	0.12
K02	29°34'N	128°26.3'E	12 Nov	2017	50	25.6	0.18	DL	0.31	0.23
K05	30°06'N	130°11.9'E	14 Nov	2017	105	24.8	0.57	0.02	0.52	0.79
K08	30°24'N	131°23.6'E	15 Nov	2017	115	25.5	1.82	0.12	0.15	0.34
K11	31°24'N	132°29.2'E	16 Nov	2017	90	25.0	0.16	DL	0.27	0.55
EXP _b										
A05a	30°10'N	129°17.5'E	3 Nov	2017	13	25.5	0.10	0.03	0.23	0.12
A05b	30°10'N	129°17.5'E	7 Nov	2017	95	25.5	DL	DL	0.16	0.15
A05c	30°11'N	129°17.2'E	7 Nov	2017	34	25.3	0.02	0.01	0.24	0.05
A06a	30°00'N	129°15.1'E	3 Nov	2017	12	25.4	DL	0.02	0.16	0.13
A06b	30°00'N	129°15.0'E	7 Nov	2017	110	25.7	1.61	0.11	0.14	0.04
A08a	29°19'N	129°09.4'E	6 Nov	2017	76	25.6	DL	0.02	0.29	0.22
A08b	29°26'N	129°12.4'E	6 Nov	2017	71	25.6	0.03	0.01	0.21	0.17
A09a	29°09'N	129°00.0'E	6 Nov	2017	105	25.6	0.11	0.02	0.20	0.15

440 **Table 2** Phytoplankton growth rate (d^{-1}) derived from the gradient enrichment experiments (**EXP_a**) in the ECS-Kuroshio.
 441 Enriched nitrate concentrations ($\mu\text{mol L}^{-1}$) are shown at the top of each column. A and B: duplicate bottles. Pico:
 442 chlorophyll smaller than 2 μm . Nano: chlorophyll between 2 and 11 μm . Micro: chlorophyll larger than 11 μm .

Station	0		0.05		0.15		0.5		0.75		1.5		5	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Micro														
C02	-0.108	-0.116	-0.089	-0.082	0.019	-0.073	0.470	0.426	0.422	0.441	0.686	0.798	0.796	0.556
C03	-0.116	-0.118	-0.073	-0.078	-0.004	-0.008	0.453	0.426	0.588	0.706	0.780	0.892	0.862	0.906
F01	0.150	0.159	0.332	0.277	0.282	0.344	0.445	0.495	0.511	0.497	0.490	0.385	0.372	0.467
G01	0.062	0.051	0.135	0.089	0.163	0.108	0.438	0.477	0.795	0.736	0.828	0.969	0.861	0.781
K02	-0.305	-0.282	-0.205	-0.265	-0.113	-0.305	0.264	0.295	0.119	0.097	0.422	0.652	0.831	0.669
K05	-0.147	0.027	0.007	-0.053	0.037	0.084	0.329	0.176	0.263	0.168	0.645	0.716	0.792	0.701
K08	0.348	0.266	0.350	0.315	0.333	0.407	0.361	0.185	0.448	0.416	0.377	0.468	0.403	0.417
K11	-0.062	-0.036	-0.105	-0.092	0.043	-0.081	0.193	0.179	0.514	0.390	0.765	0.730	0.469	0.558
Nano														
C02	-0.479	-0.260	-0.208	-0.409	-0.297	-0.345	-0.050	0.144	0.173	0.151	0.249	0.333	0.330	0.264
C03	-0.275	-0.261	-0.211	-0.257	-0.080	-0.206	0.113	0.031	0.247	0.192	0.363	0.355	0.288	0.256
F01	-0.244	-0.154	-0.286	-0.092	-0.025	0.101	0.182	0.050	0.148	0.039	0.015	0.056	0.104	0.105
G01	-0.304	-0.172	-0.313	-0.189	-0.165	-0.117	-0.063	-0.178	0.100	0.001	0.286	0.325	0.369	0.053
K02	-0.321	-0.149	-0.384	-0.152	0.022	0.035	0.223	0.251	-0.027	-0.135	0.433	0.229	0.559	0.523
K05	-0.389	-0.318	-0.680	-0.546	-0.267	-0.394	-0.484	-0.248	-0.407	-0.458	0.053	-0.034	0.102	0.196
K08	0.353	0.244	0.508	0.472	0.455	0.436	0.406	0.397	0.473	0.369	0.408	0.546	0.380	0.384
K11	-0.138	-0.088	-0.257	-0.243	-0.134	-0.293	0.073	0.026	0.175	0.201	0.296	0.312	0.434	0.501
Pico														
C02	-0.383	-0.188	-0.186	-0.199	-0.119	-0.162	0.188	0.143	0.162	0.241	0.257	0.291	0.377	0.205
C03	-0.202	-0.258	-0.259	-0.282	-0.143	-0.160	0.017	-0.019	0.148	0.191	0.194	0.248	0.230	0.300
F01	-0.071	-0.091	-0.054	-0.032	0.050	0.129	0.205	0.144	0.216	0.141	0.170	0.134	0.031	0.172
G01	0.019	-0.061	0.051	-0.032	0.019	0.008	0.156	0.162	0.323	0.188	0.338	0.308	0.344	0.366
K02	-0.245	-0.253	-0.257	-0.275	-0.243	-0.230	-0.046	0.010	-0.067	-0.101	0.065	-0.030	0.203	0.089
K05	-0.087	0.031	0.014	-0.027	0.103	0.157	0.057	0.261	0.130	0.339	0.316	0.255	0.368	0.404
K08	0.032	0.055	-0.013	0.228	0.262	0.201	0.240	0.069	0.262	0.281	0.177	0.284	0.222	0.327
K11	-0.197	-0.216	-0.194	-0.146	-0.046	-0.071	-0.005	0.033	0.163	0.076	0.236	0.049	0.092	0.179

443

444 **Table 3** Parameters derived from the dilution experiments (EXP_b) in the ECS-Kuroshio. g_{max} : maximum growth rate (d^{-1}). m : mortality rate by microzooplankton grazing (d^{-1}). g_o : net
 445 growth rate measured in the non-enriched and non-diluted bottles (d^{-1}). g_{en} : net growth rate measured in the enriched and non-diluted bottles (d^{-1}). r^2 : coefficient of determination
 446 defined from the linear regression of the apparent growth rate of total chlorophyll *a* concentrations against dilution factors. p : p-value. Pico: chlorophyll smaller than 2 μm . Nano:
 447 chlorophyll between 2 and 11 μm . Micro: chlorophyll larger than 11 μm . Total: total chlorophyll from pico- to micro.

448
 449

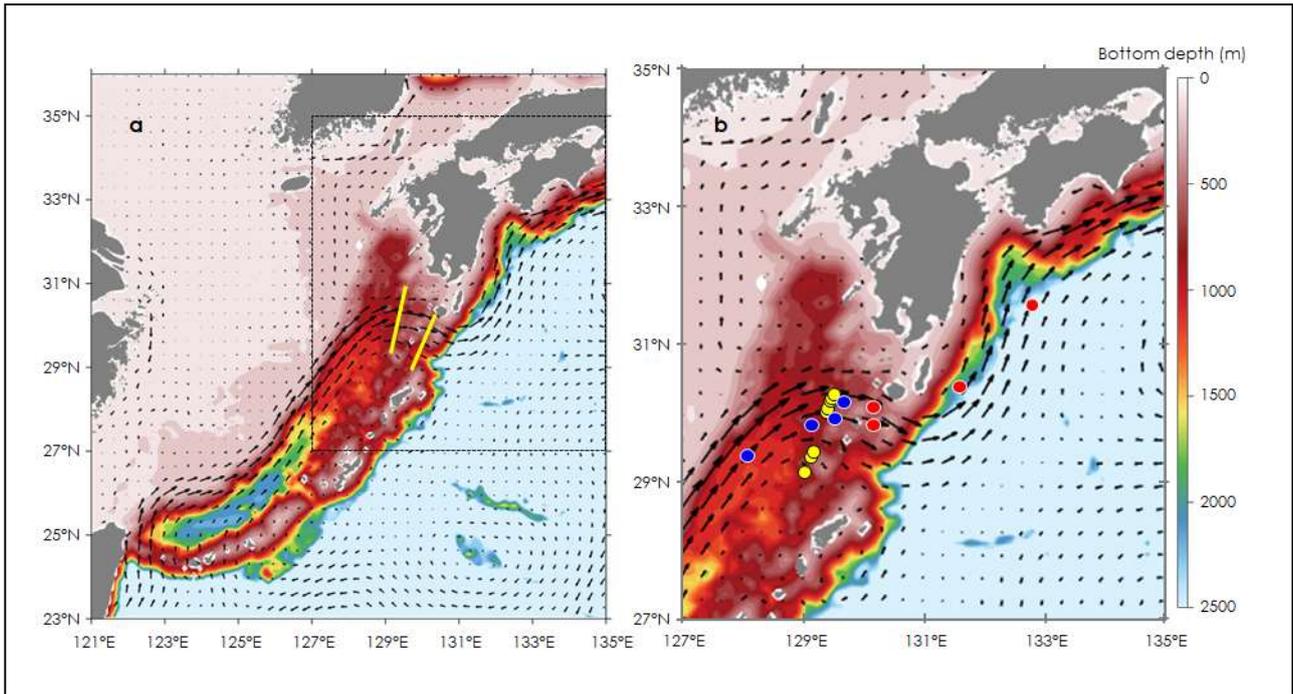
Station	Pico				Nano				Micro				Total				r^2	p
	g_{max}	m	g_o	g_{en}	g_{max}	m	g_o	g_{en}	g_{max}	m	g_o	g_{en}	g_{max}	m	g_o	g_{en}		
A05a	0.283	0.887	0.415	0.681	1.181	1.345	-0.267	0.181	0.913	0.962	0.059	0.045	1.059	0.619	0.199	0.492	0.757	<0.01
A05b	0.931	1.106	-0.109	0.279	1.354	1.050	-0.505	-0.239	0.477	0.583	-0.030	0.107	1.073	1.051	-0.232	0.113	0.901	<0.01
A05c	0.501	0.647	-0.025	0.190	1.298	1.192	-0.183	-0.066	0.313	0.500	-0.269	0.201	0.828	0.752	-0.074	0.122	0.875	<0.01
A06a	0.179	0.814	0.440	0.646	0.865	1.270	0.247	0.341	0.232	0.597	-0.315	0.339	0.941	0.381	0.347	0.550	0.541	<0.01
A06b	0.648	-0.398	-0.869	-1.020	0.947	0.247	-0.789	-0.629	-0.118	-0.037	-0.038	0.065	-0.052	0.711	-0.735	-0.714	0.750	<0.01
A08a	0.434	0.458	-0.097	0.035	1.448	1.289	-0.072	-0.150	0.401	0.564	-0.537	0.181	0.765	0.775	-0.113	0.009	0.856	<0.01
A08b	0.370	0.846	-0.040	0.509	0.652	1.068	-0.259	0.430	0.553	1.122	-0.620	0.529	0.937	0.471	-0.123	0.488	0.693	<0.01
A09a	0.488	0.417	-0.399	-0.026	0.894	0.734	-0.182	-0.082	0.353	0.022	-0.474	-0.235	0.526	0.640	-0.324	-0.052	0.760	<0.01

450 **Table 4** Parameters derived from relationship of phytoplankton growth rates against logarithmically transformed
 451 concentrations of enriched nitrate **in the gradient enrichment experiments (EXP_a)**. Slope: sensitivity of phytoplankton
 452 growth rate to logarithmically transformed concentrations of enriched nitrate. Intercept: growth potential at the low nitrate
 453 concentration. r^2 : coefficient of determination defined from the linear regression of growth rate of size-fractionated
 454 chlorophyll *a* concentrations against logarithmically transformed concentrations of enriched nitrate. Pico: chlorophyll
 455 smaller than 2 μm . Nano: chlorophyll between 2 and 11 μm . Micro: chlorophyll larger than 11 μm .

456

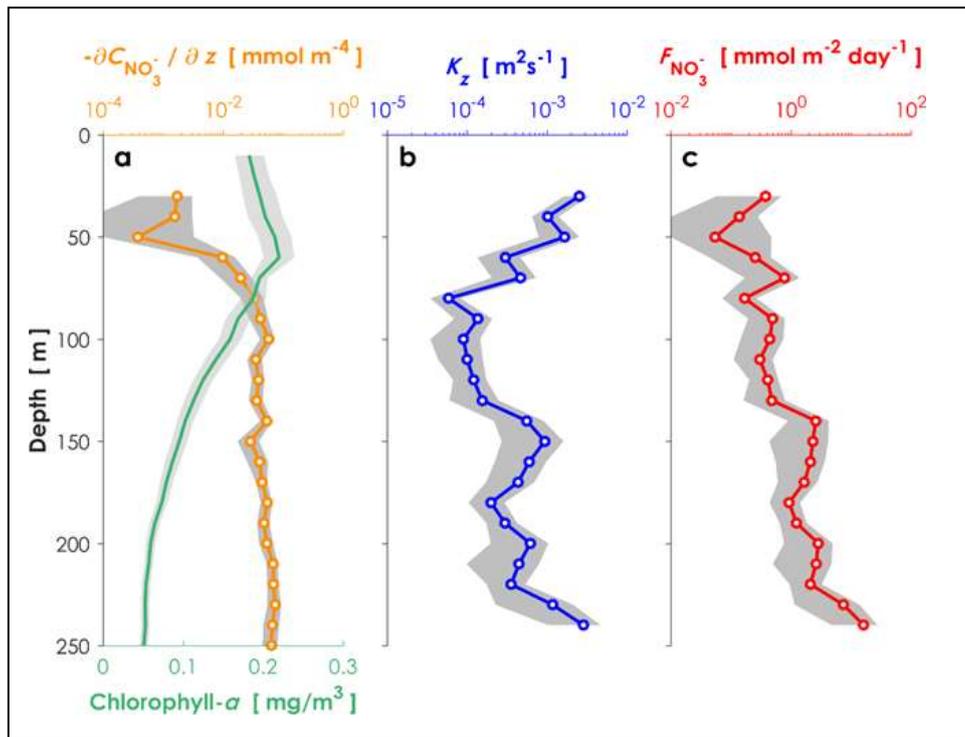
457

Station	Pico			Nano			Micro		
	Slope	Intercept	r^2	Slope	Intercept	r^2	Slope	Intercept	r^2
C02	0.281	0.178	0.848	0.370	0.131	0.831	0.458	0.492	0.846
C03	0.295	0.121	0.922	0.308	0.177	0.830	0.560	0.611	0.914
F01	0.074	0.129	0.317	0.120	0.067	0.420	0.077	0.430	0.368
G01	0.203	0.243	0.866	0.272	0.085	0.688	0.448	0.657	0.817
K02	0.213	-0.014	0.883	0.364	0.233	0.726	0.531	0.353	0.872
K05	0.188	0.251	0.772	0.355	-0.165	0.729	0.419	0.439	0.843
K08	0.070	0.231	0.242	-0.038	0.426	0.213	0.045	0.386	0.162
K11	0.167	0.077	0.750	0.394	0.201	0.943	0.403	0.409	0.744



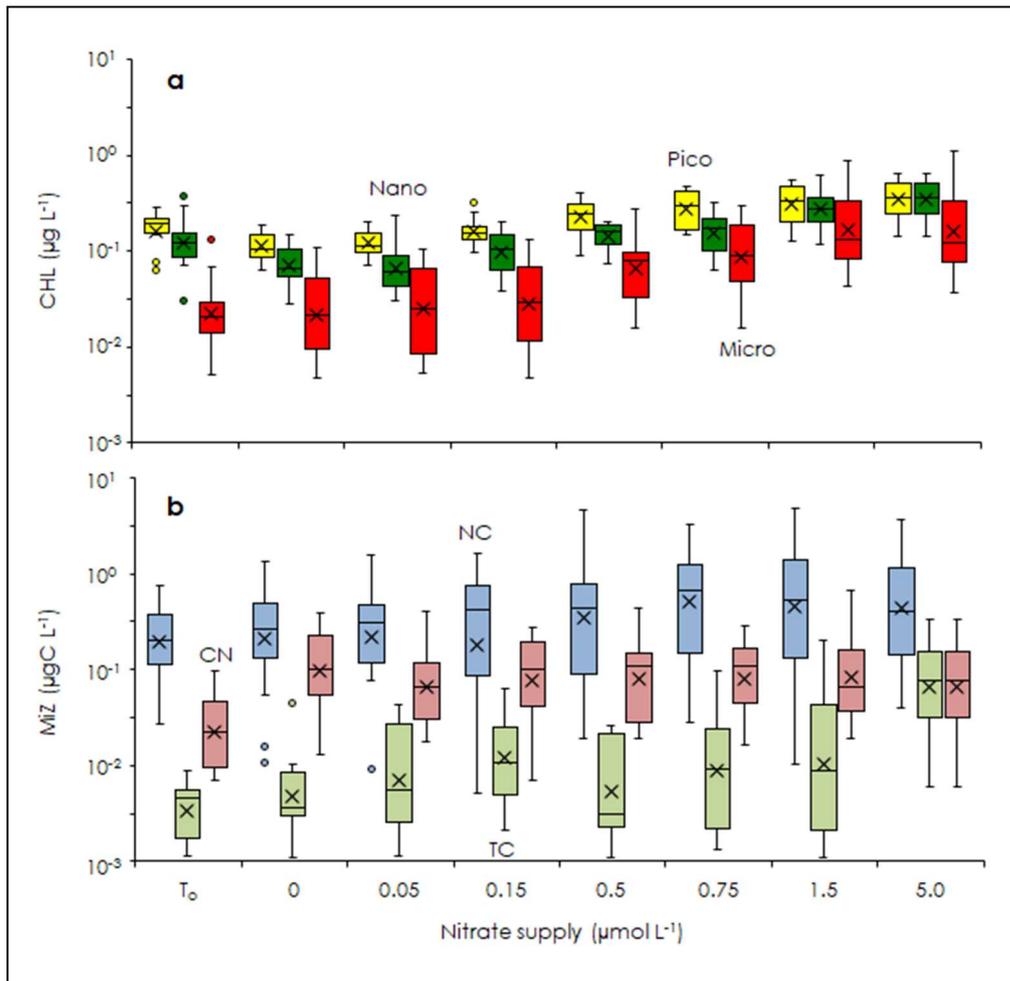
458

459 **Figure 1** Locations for oceanographic observations and onboard experiments in the Kuroshio Current of the East China
 460 Sea (ECS-Kuroshio). **(a)** Oceanographic observations by Deep SUNA V2 and TurboMAP-L (yellow lines). **(b)** Onboard
 461 experiments for phytoplankton and microzooplankton growth (EXP_a: red and blue circles) and for microzooplankton
 462 grazing (EXP_b: yellow circles). EXP_a are conducted in the upstream (blue circles) and downstream Kuroshio (red circles)
 463 in the Tokara Strait. Current directions and velocities (arrows) are shown as monthly means during November 2016.
 464 Bottom depth (m) is indicated as colored contours.



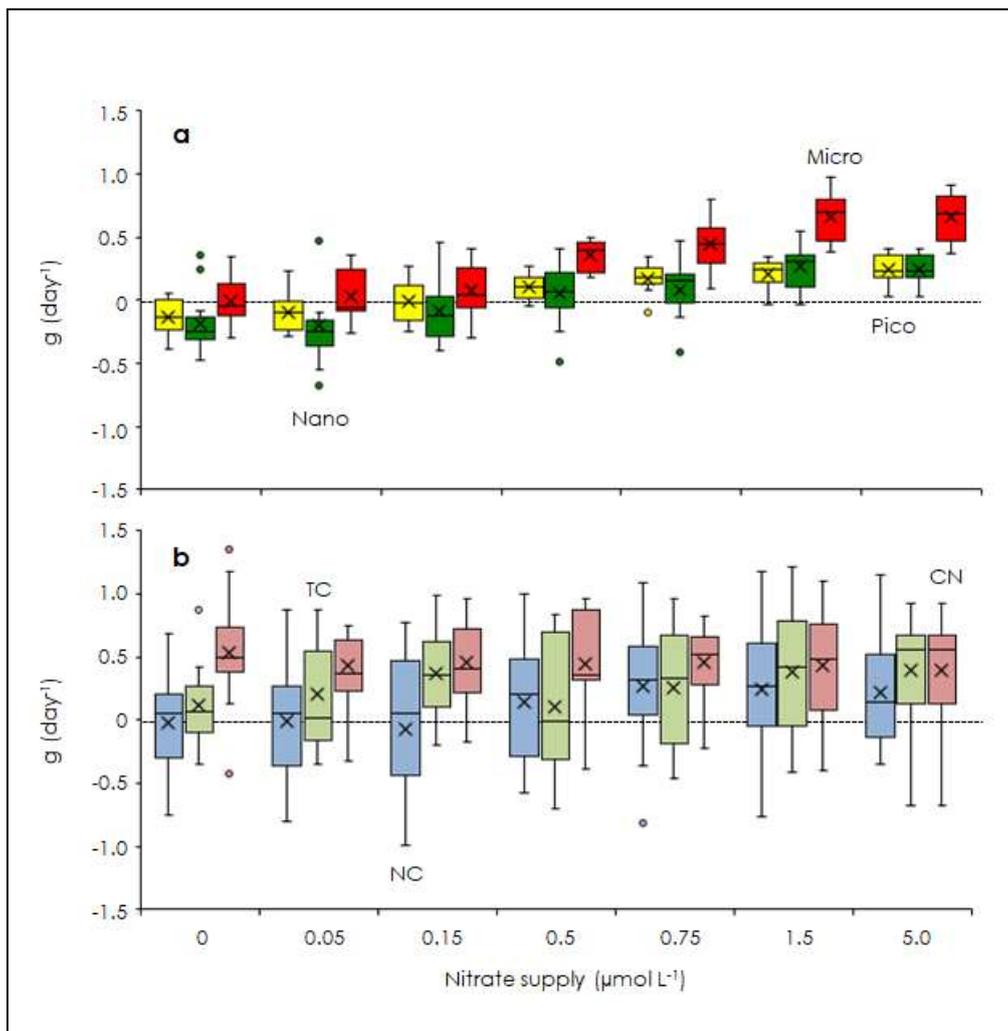
465

466 **Figure 2** Vertical profiles of environmental conditions in the Kuroshio Current. **(a)** Nitrate **gradient curve** (orange) and
 467 chlorophyll *a* concentrations (green) measured with a nitrate sensor (Deep SUNA V2) attached to an SBE-9plus CTD
 468 system. **(b)** Turbulent diffusivity measured with a TurboMAP-L (blue). **(c)** Calculated turbulent nitrate fluxes (red) in the
 469 ECS-Kuroshio. The shaded areas are the 95 percent confidence intervals obtained by a bootstrap process.



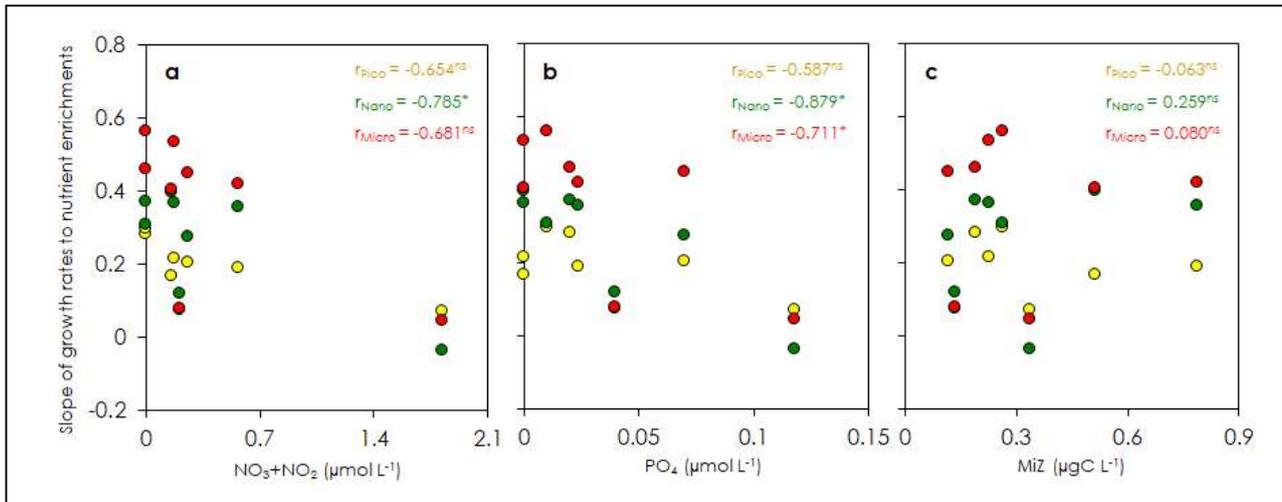
470

471 **Figure 3** Changes in phytoplankton and **micro-sized heterotrophs** standing stocks during the gradient enrichment
 472 experiments (EXP_a). **(a)** Size-fractionated chlorophyll *a* concentrations (CHL). **(b)** **Micro-heterotrophs** standing stocks
 473 (MiZ). T₀: at the beginning of the gradient enrichment experiments. 0: no enrichment. 0.05 to 5.0 µmol L⁻¹: enrichment.
 474 Box-and-whisker diagram at each nitrate concentrations was compiled with the results conducted at the 8 stations. Box
 475 represents first (bottom), second (bar) and third (top) quartiles, and cross marks are the average values. Whiskers indicate
 476 minimum and maximum values, and circles are outliers. Pico: chlorophyll smaller than 2 µm (yellow). Nano: chlorophyll
 477 between 2 and 11 µm (green). Micro: chlorophyll larger than 11 µm (red). NC: naked ciliates (light blue). TC: tintinnid
 478 ciliates (light green). CN: copepod nauplii (light pink).



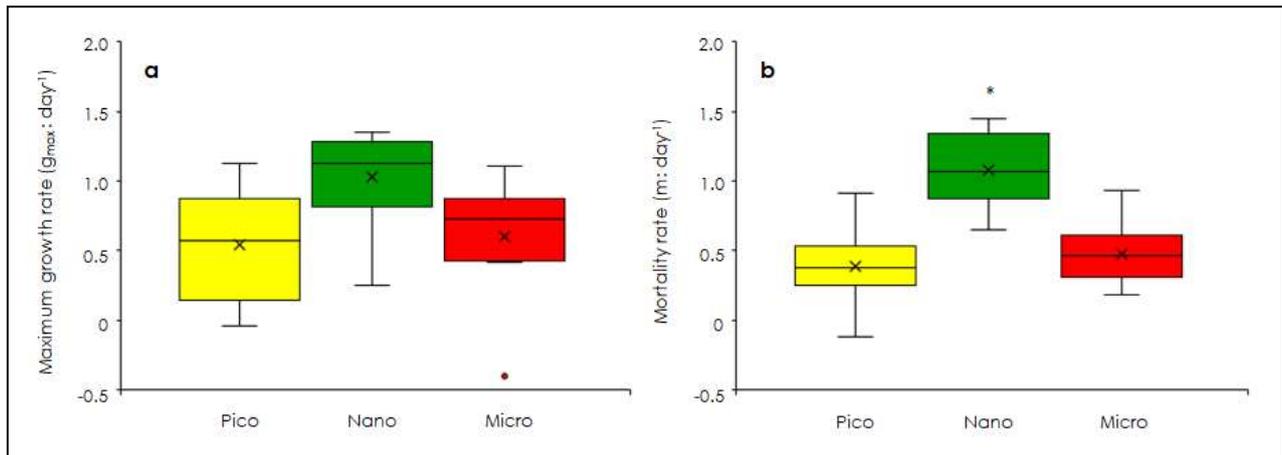
479

480 **Figure 4** Changes in phytoplankton and **micro-sized heterotrophs** growth rates in response to nitrate enrichments in the
 481 gradient enrichment experiments (**EXP_a**). **(a)** Growth rates ($g: d^{-1}$) of size-fractionated chlorophyll. **(b)** **Micro-**
 482 **heterotrophs** growth rates ($g: d^{-1}$). 0: no enrichment. 0.05 to 5.0 $\mu mol L^{-1}$: enrichment. Box-and-whisker diagram at each
 483 nitrate concentration is based on the results conducted at the eight stations. The symbols have the same meaning as in
 484 Figure 3.



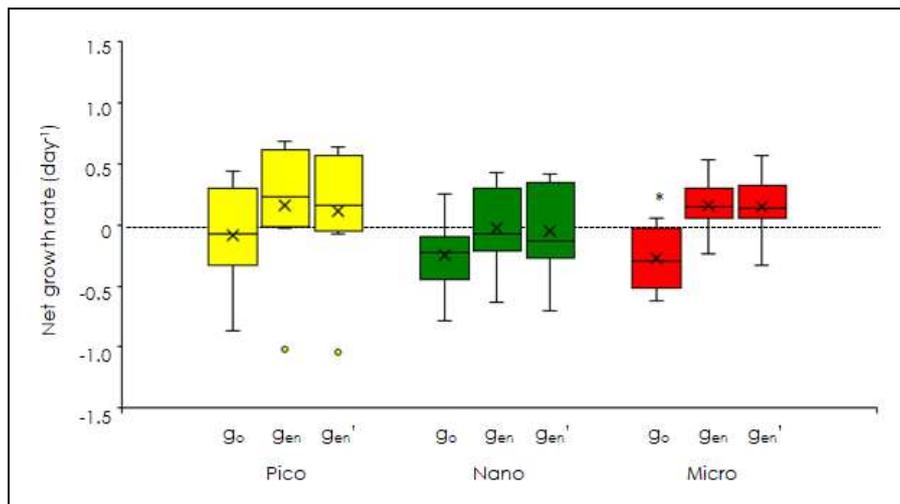
485

486 **Figure 5** Correlation of the regression slopes of phytoplankton growth rates to nutrients concentrations and micro-sized
 487 heterotrophs biomass at the beginning of the gradient enrichment experiments (EXP_a). (a) Regression slopes of the size-
 488 fractionated phytoplankton growth versus the concentrations of nitrate (NO_3) plus nitrite (NO_2). (b) Regression slopes of
 489 the size-fractionated phytoplankton growth versus the phosphate concentrations (PO_4). (c) Regression slopes of the size-
 490 fractionated phytoplankton growth versus the micro-heterotrophs biomass (MiZ). r: Pearson correlation coefficient. Pico:
 491 chlorophyll smaller than 2 μm . Nano: chlorophyll between 2 and 11 μm . Micro: chlorophyll larger than 11 μm . *: $p < 0.05$.
 492 ns: no significant.



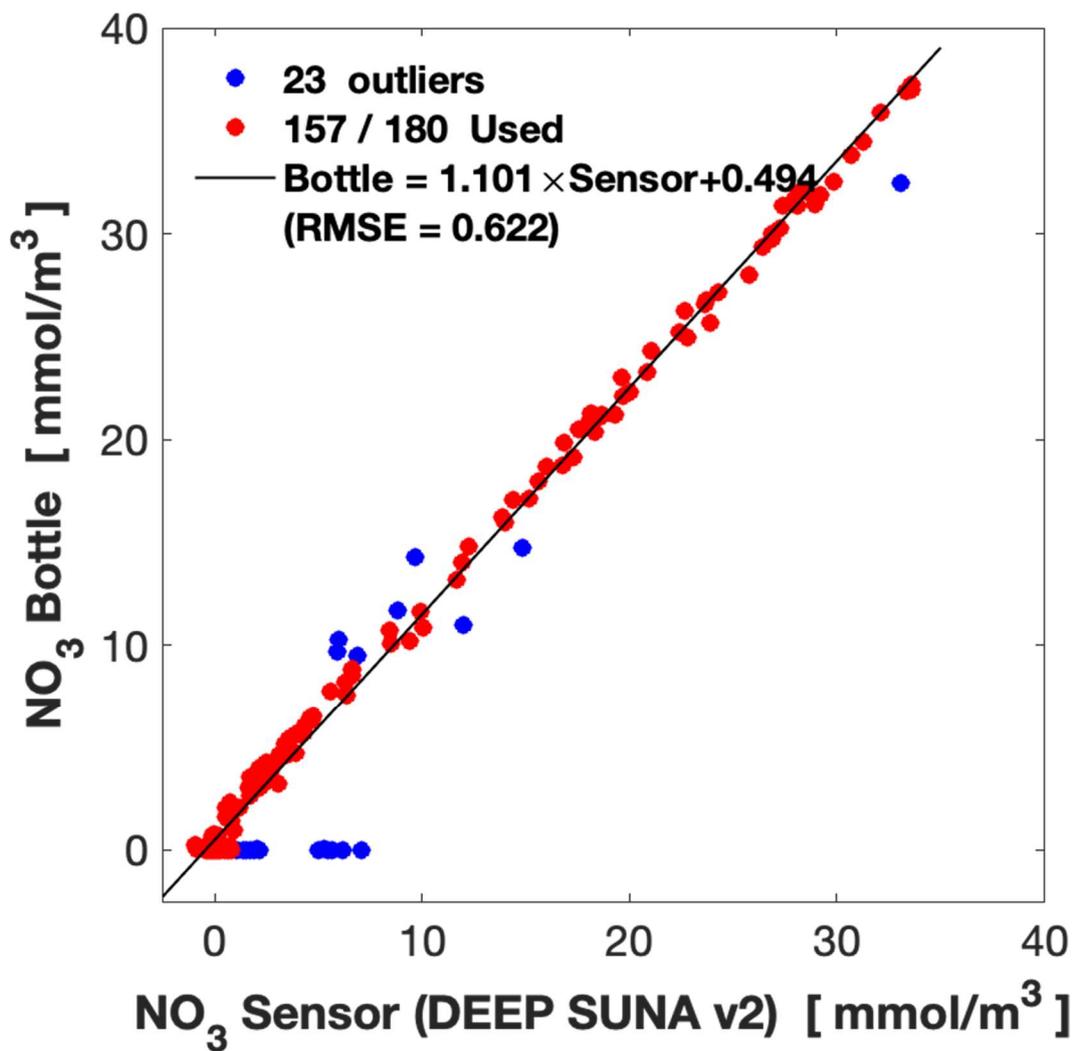
493

494 **Figure 6** Comparisons of phytoplankton growth and mortality rates among the three size-fractionated chlorophyll derived
 495 from the dilution experiments (EXP_b). **(a)** Maximum growth rates (g_{max}). **(b)** Mortality rates by microzooplankton grazing.
 496 Box-and-whisker diagram at each nitrate concentrations was compiled with the results conducted at the 8 stations. Box
 497 represents first (bottom), second (bar) and third (top) quartiles, and cross marks are the average values. Whiskers indicate
 498 minimum and maximum values, and circles are outliers. Asterisk means significant difference among the three size-
 499 fractions (ANOVA+Tukey, $p < 0.05$). Pico: chlorophyll smaller than 2 μm . Nano: chlorophyll between 2 and 11 μm . Micro:
 500 chlorophyll larger than 11 μm .



501

502 **Figure 7** Comparisons of phytoplankton net growth **derived from the dilution experiments (EXP_b)** among the three
 503 different methods. g₀: Observed net growth rates without enrichment in the non-diluted bottles. g_{en}: Observed net growth
 504 rates with enrichment in the non-diluted bottles. g_{en'}: Estimated net growth rates subtracting the mortality rates (m) from
 505 the maximum growth rates (g_{max}). Box-and-whisker diagram at each nitrate concentrations was compiled with the results
 506 conducted at the 8 stations. Asterisk means significant difference between g₀ and g_{en} (Welch's t-test, p < 0.05). The symbols
 507 have the same meaning as in Figure 6.

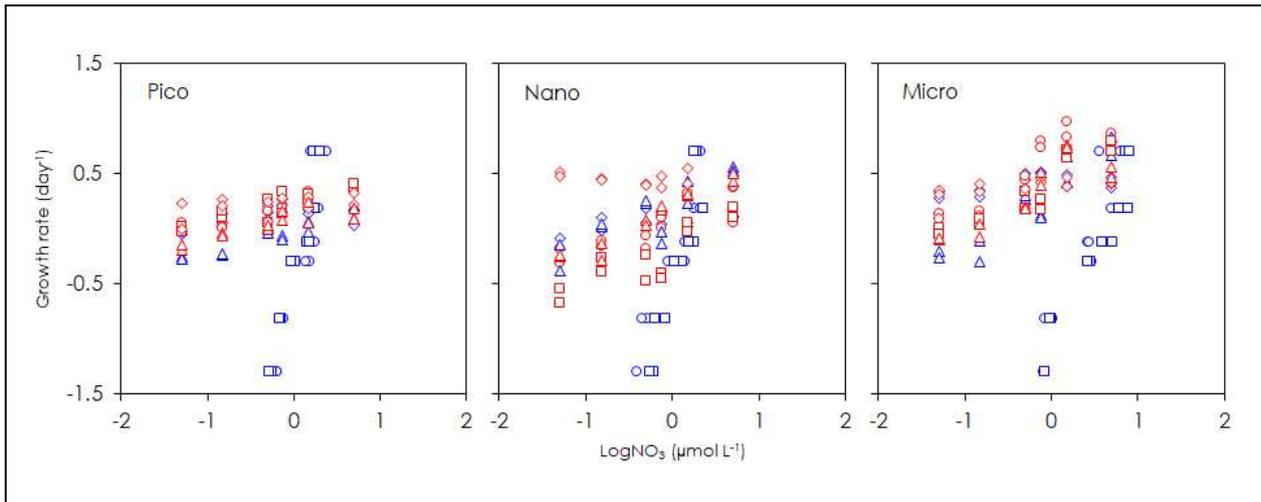


508

509

510 **Supplement Figure 1** In situ nitrate measurements by Deep SUNA V2 plotted against the laboratory water analysis
 511 results from bottle sampled water in KG1515. For obtaining the regression line used for the sensor calibration, we
 512 excluded outlier data in which the absolute value of the difference between the data and regression line exceeded 2.2
 513 times the RMSE.

514



515

516 **Supplement Figure 2** Relationship of phytoplankton growth rates to logarithmically transformed concentrations of
517 enriched nitrate. Blue and red circles mean the stations in the upstream and downstream Kuroshio in the Tokara Strait,
518 respectively. Pico: chlorophyll smaller than 2 μm. Nano: chlorophyll between 2 and 11 μm. Micro: chlorophyll larger
519 than 11 μm.

520