

Responses of phytoplankton communities to internal waves in oligotrophic oceans

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Abstract

Nutrients associated with internal waves are known to perturb phytoplankton communities in oligotrophic oceans, but details of the relevant processes and mechanisms are unclear. Here we report insights about the impacts of internal waves on the phytoplankton community based on 154-hour time-series of observations in an oligotrophic basin of the South China Sea. We found that the temporal variations of phytoplankton communities in the upper, middle, and lower layers of the euphotic zone differ. We demonstrated that these changes probably resulted from the perturbation caused by internal waves. These results suggest that the structure of the phytoplankton community in oligotrophic oceans is best described by a three-layer system at steady state and that the perturbation caused by internal waves helps to reveal this structure. We believe that the paradigm of this three-layer structure will provide a new theoretical framework for the study of phytoplankton-based biogeochemical processes in oligotrophic oceans.

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1 **Responses of phytoplankton communities to internal waves**
2 **in oligotrophic oceans**

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22 **Key Points:**

- 23 • We provided strong evidence that there is internal wave activity at the
24 oligotrophic basin of the South China Sea
- 25 • The temporal variations of phytoplankton communities in the upper, middle, and
26 lower layers of the euphotic zone differ
- 27 • The perturbation caused by internal waves made the three-layer structure of the
28 phytoplankton community in oligotrophic oceans apparent

30 **Abstract**

31 Nutrients associated with internal waves are known to perturb phytoplankton
32 communities in oligotrophic oceans, but details of the relevant processes and
33 mechanisms are unclear. Here we report insights about the impacts of internal waves
34 on the phytoplankton community based on 154-hour time-series of observations in an
35 oligotrophic basin of the South China Sea. We found that the temporal variations of
36 phytoplankton communities in the upper, middle, and lower layers of the euphotic
37 zone differ. We demonstrated that these changes probably resulted from the
38 perturbation caused by internal waves. These results suggest that the structure of the
39 phytoplankton community in oligotrophic oceans is best described by a three-layer
40 system at steady state and that the perturbation caused by internal waves helps to
41 reveal this structure. We believe that the paradigm of this three-layer structure will
42 provide a new theoretical framework for the study of phytoplankton-based
43 biogeochemical processes in oligotrophic oceans.

44

45 **Plain Language Summary**

46 Internal waves are a widespread phenomenon in stratified oceans. They are
47 especially important in oligotrophic oceans because they can provide the dominant
48 source of nutrient supply to the euphotic zone. This process is expected to enhance the
49 phytoplankton biomass and primary productivity and change the phytoplankton
50 community, but details of the relevant processes and mechanisms are still unclear.
51 Based on results from satellite image, 154 hours of time series observations, and
52 model output, we provided strong evidence that there is internal wave activity at the
53 SEATS station in the South China Sea. The patterns of change of the phytoplankton
54 community in the upper, middle, and lower layers of the euphotic zone differed during
55 our observations. We demonstrated that these changes were resulted from the
56 perturbation caused by internal waves. We therefore hypothesized that the structure of
57 the phytoplankton community in the euphotic zone of oligotrophic oceans is best
58 described by a three-layer structure at steady state. The perturbation caused by
59 internal waves made this structure apparent because the factors that limited
60 phytoplankton growth differed in the three layers. This novel concept will facilitate
61 estimates of marine primary production and enhance understanding of the global
62 carbon cycle.

63

64 **Introduction**

65 Internal waves are widely present in stratified oceans and play an important role
66 in promoting the movement of water masses and the exchange of nutrients and
67 materials (Garwood et al., 2020; Whalen et al., 2020). In oligotrophic oceans, mixing
68 driven by internal waves can dominate the supply of nutrients to the euphotic zone
69 (Tuerena et al., 2019), which in turn stimulates phytoplankton primary productivity
70 (Lucas et al., 2011). Studies of how the phytoplankton community responds to
71 internal waves have concentrated mainly on upwelling regions, where nutrients are
72 relatively abundant (Ma et al., 2020; Omand et al., 2011; Villamaña et al., 2017).
73 There have been few similar studies in oligotrophic oceans.

74 The euphotic zone of oligotrophic oceans can be divided into a nutrient-depleted
75 layer (NDL) above the nutricline and a light-limited layer or nutrient-replete layer
76 (NRL) below the NDL (Du et al., 2017; Dugdale, 1967). However, the transition
77 region between limitation by light or nutrients in oligotrophic oceans is usually
78 characterized by a deep chlorophyll maximum layer (DCML), where light and
79 nutrients are co-limiting and the rates of growth and loss of phytoplankton are usually
80 in dynamic equilibrium (Chen et al., 2013; Guo et al., 2014; Landry et al., 2011b). In
81 the euphotic zone below the DCML, grazing exceeds phytoplankton growth (Landry
82 et al., 2011a). These scenarios suggest that the phytoplankton community in the
83 euphotic zone of oligotrophic oceans may be characterized by a three-layer structure
84 under steady state conditions: the upper portion of the NDL, where phytoplankton
85 growth is strictly limited by nutrients; a middle layer, the DCML, where light and
86 nutrients are co-limiting and phytoplankton gains and losses are in a dynamic
87 equilibrium; and a light-limited region below the DCML, the NRL, where nutrients
88 are abundant and the grazing pressure is the greatest. We hypothesized that if this

89 system were perturbed by an influx of allochthonous nutrients from the bottom of the
90 euphotic zone by internal waves, the differences of the physiological conditions of the
91 phytoplankton in each layer would cause their responses to differ in ways that would
92 clearly reveal the three-layer structure.

93 To test this hypothesis, we used 154 hours of data collected from the SouthEast
94 Asia Time-series Study (SEATS, 116°E, 18°N) station during a cruise in the northern
95 South China Sea (SCS) during the summer of 2014. The northern SCS basin is a
96 typical oligotrophic ocean, and the SEATS station, located in the center of the
97 northern SCS basin, is a site often used to study the biogeochemical characteristics of
98 oligotrophic oceans (Wong et al., 2007). The Luzon Strait, which borders the SCS on
99 the east, generates energetic internal waves that radiate both eastward and westward.
100 The energy of these internal waves is concentrated mainly in internal tides (Lin et al.,
101 2020), i.e., internal waves of tidal frequency. In the SCS, the energy fluxes of
102 semidiurnal internal tides propagate mainly toward the northwest (Zhao, 2014; 2020).
103 These onshore, radiating, semidiurnal internal tides finally evolve into nonlinear
104 internal solitary waves and dissipate on the continental shelf. In contrast, diurnal
105 internal tides radiate toward the southwest and mainly affect the center of the SCS
106 (Zhao, 2020), as shown in Figure 1a. The northern SCS basin is therefore an area into
107 which diurnal, internal waves from the Luzon Strait radiate and is an ideal region to
108 test our hypothesis.

109 **Data and Methods**

110 **Sampling and Measurements**

111 Field observations were conducted onboard the R/V *Shiyan 1* and lasted from
112 10:30 on 23 August to 20:30 on 29 August at the SEATS station (Figure 1a). Water

113 temperature, salinity, and pressure were recorded during each cast with a SeaBird
114 model SBE9/11 conductivity-temperature-depth (CTD) recorder. A total of 40 CTD
115 casts were conducted at different time intervals, including at least every 6 hours from
116 02:00 on 25 August to 22:00 on 27 August. Seawater samples for nutrient analyses
117 were collected from depths of 5, 25, 50, 75, and 100 m on the first cast. The
118 concentrations of nitrate plus nitrite (NO_x , $\mu\text{mol L}^{-1}$) were measured with a
119 QUAATRO nutrient analyzer.

120 Seawater samples for phytoplankton pigment analyses were collected from the
121 same depths sampled for nutrient concentrations every 6 hours from 02:00 on 25
122 August to 22:00 on 27 August. Thirteen marker pigments were quantified by high
123 performance liquid chromatography (HPLC) following the modified method of
124 Furuya et al. (2003). We then used the CHEMTAX program (Mackey et al., 1996) to
125 calculate the relative contributions of 9 phytoplankton groups to the total Chlorophyll
126 *a* (TChl *a*, the sum of Chl *a* and Divinyl Chl *a*). These procedures have previously
127 been reported in detail (Ma et al., 2020; Xiao et al., 2018) and are described briefly in
128 Text S1.

129 **Field incubation experiments**

130 To determine the nitrate assimilation rate (ρNO_3^-), new production (NP), and
131 gross primary production (GPP), water samples were collected in acid-cleaned bottles
132 from depths of 3, 17, 33, 55, and 110 m, corresponding to light intensities of about
133 66%, 50%, 25%, 10%, and 1% of the surface irradiance, respectively. ρNO_3^- was
134 measured daily following the method of Dugdale and Wilkerson (1986). Incubation
135 procedures and subsequent calculations of the ρNO_3^- have previously been reported
136 in detail (Ma et al., 2020) and are described in Text S2.

137 Seawater samples of 10 L were collected from depths of 5 m, 25 m, 50 m, 75 m,

138 and 110 m during the second day (24 August) to conduct dilution experiments for the
139 measurement of phytoplankton growth rates (μ) and microzooplankton grazing rates
140 (m) (Landry and Hassett, 1982; Landry et al., 2008) (Text S3).

141 **Simulation of internal tides**

142 The characteristics and influence of internal waves at the SEATS station were
143 analyzed using the 26-year-coherent satellite altimeter results of Zhao (2020) (Figure
144 1a) based on a new mapping technique that combines plane wave analysis with 2-D
145 band-pass filtering. The output from the MITgcm LLC4320, a state-of-the-art
146 submesoscale-permitting global ocean circulation model, was also used to analyze the
147 characteristics of internal wave at the SEATS station. The genesis and propagation of
148 internal waves could therefore be seen from the altimeter results, while their
149 structures in the ocean interior were revealed by the LLC4320.

150 The LLC4320 provides one of the most high-resolution and advanced
151 hydrodynamic outputs at present, with a horizontal grid spacing of $1/48^\circ$ and 90
152 vertical levels (Forget et al., 2015; Torres et al., 2018). The model outputs included
153 sea level height, potential temperature, salinity, and velocity, for a period of 14
154 months from 13 September 2011 to 15 November 2012 at intervals of 1 h. The LLC
155 output is available from the ECCO project (<https://data.nas.nasa.gov/ecco/data.php>).
156 Because there were no simulation results during our observation period, we first
157 determined the relationship between internal waves and barotropic tides from the
158 model output at the SEATS station, and then we used that relationship and barotropic
159 tide data to predict the characteristics of internal waves at SEATS during our
160 observations. Tidal information was obtained from the regional tidal solution of the
161 OSU TPXO Tide Models using Tide Model Driver (TMD) Matlab Toolbox
162 (<https://www.esr.org/research/polar-tide-models/tmd-software/>).

163 **Results**

164 **Internal tides from observations and numerical simulations**

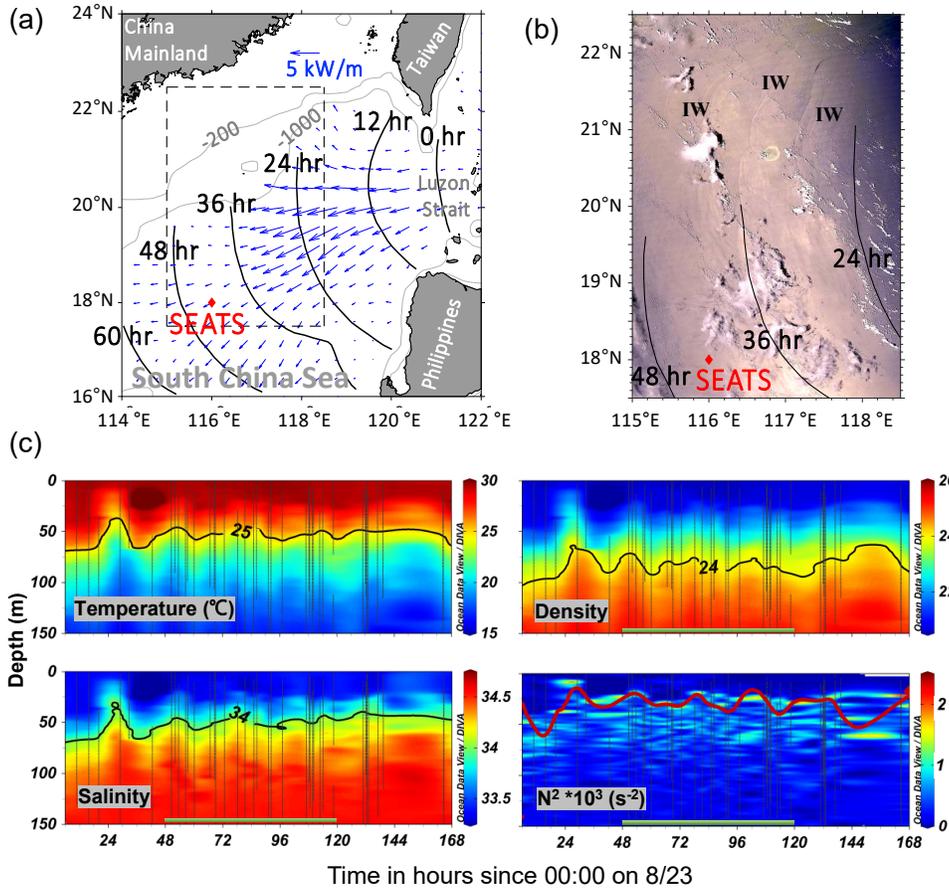
165 The influence of internal waves at the SEATS station was revealed by satellite
166 and field observations (Figure 1), as well as results of the simulations. The
167 26-year-coherent satellite altimeter results indicated that internal waves at SEATS
168 were mainly diurnal internal tides. After their generation in the Luzon Strait, the
169 diurnal, internal tides propagated southwestward, as shown by the energy fluxes and
170 phases of the internal tides in Figure 1a. It took about 1.5–2 days for these waves to
171 reach SEATS from the Luzon Strait.

172 As expected, direct evidence of the presence of internal waves during our field
173 observations was provided by a MODIS true color image obtained at 05:25 on 28
174 August 2014 (Figure 1b). In this image, which has a spatial resolution of 250 m,
175 surface imprints of internal waves are apparent near the Dongsha Atoll (117°E,
176 20.6°N). The signature was clear because of the nonlinear steepening of the internal
177 wave during shoaling. The spatial distribution of the fronts of these internal waves
178 was consistent with the phase of the K_1 internal tide (Figure 1b). The implication is
179 that internal waves were present and influencing our field measurements. Moreover,
180 field hydrographic observations revealed synchronous, diurnal fluctuations in the
181 time-series of temperature, salinity, and density profiles as well as the depth of
182 maximum buoyancy frequency (N^2) (Figure 1c). This synchrony provided further
183 support that diurnal internal tides impacted conditions at the SEATS station during
184 our observation period.

185 To overcome the limitation of the sampling frequency in our observations, we
186 used the LLC4320 simulation results to show the characteristics of internal waves at
187 the SEATS station (Figure S1). Consistent with our observation and analysis, the

188 simulation results showed periodic fluctuations of isopycnals in the upper 300 m
189 throughout August 2012 (Figure S1c), an indication of diurnal internal tides. The
190 barotropic tides in the Luzon Strait and at SEATS were basically in phase during
191 August 2012 (Figure S1a,b), but the largest-amplitude internal waves at SEATS
192 lagged the spring tide in the Luzon Strait by 2–3 days (Figure S1). This time lag is
193 consistent with the propagation time of internal waves from the Luzon Strait to
194 SEATS (Figure 1a). We could therefore use the barotropic tidal phase to predict the
195 internal tides at SEATS. Because the barotropic tides were similar in August 2014 and
196 August 2012 and because our observations covered the period from the spring tide
197 before 25 August to the neap tide on 27 August (Figure S2), the amplitude of the
198 spring tide during our observations was presumably as much as ~30 m (Figure S1c).
199 This conclusion is consistent with our hydrographic observations (Figure 1c).

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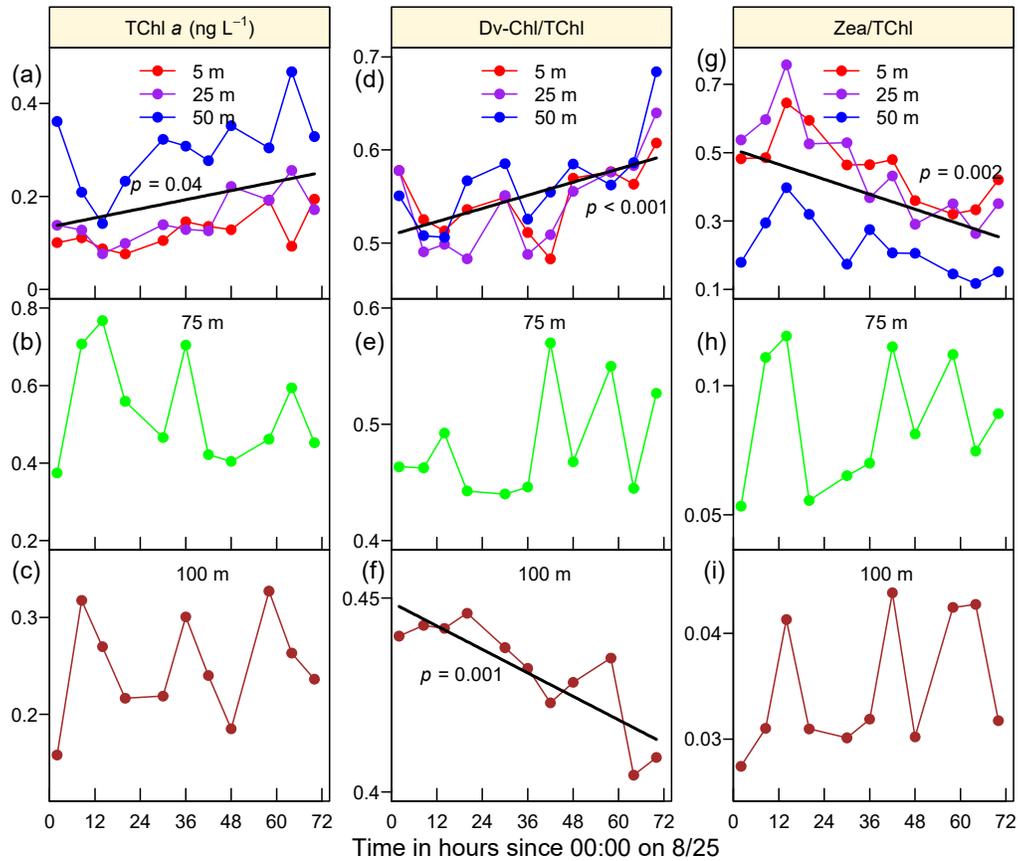
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202 **Figure 1.** Field-sampling station in the South China Sea basin in summer 2014 and its
 203 hydrographic characteristics. (a) Location of the SEATS station (red diamond). Blue
 204 arrows show depth-integrated energy fluxes and black contours show propagating
 205 time of the K_1 internal tide from the Luzon Strait, based on the results of Zhao (2020).
 206 (b) MODIS true color image taken at 05:25 on 28 August 2014, with the propagating
 207 time of K_1 internal tide from the Luzon Strait. (c) Temporal variations of
 208 hydrographic parameters at SEATS, where N^2 is the squared buoyancy frequency, and
 209 the red solid line indicates the depth of maximum N^2 .

210

211 **Vertical and temporal distributions of phytoplankton biomass and community**
212 **composition**

213 The TChl *a* concentration initially increased and then decreased with depth, and
214 the DCML appeared at 75 m (Figure S4). The dominant phytoplankton groups were
215 mainly *Prochlorococcus* and *Synechococcus* above the DCML and *Prochlorococcus*
216 and Haptophytes_8 below the DCML. During the three-day observation period, there
217 were significant changes of the concentrations of TChl *a* and the relative abundances
218 of the main phytoplankton groups. The patterns of change varied between depths
219 (Figure 2). The concentration of TChl *a* in the upper 50 m increased significantly over
220 time (Figure 2a), but there was no obvious temporal trend at 75 m and 100 m (Figure
221 2b, c). The changes of the phytoplankton community were attributable mainly to
222 significant changes in the relative abundances of *Prochlorococcus* and *Synechococcus*
223 during the observation period. The relative abundance of *Prochlorococcus* (quantified
224 by the ratio of its marker pigment divinyl chlorophyll *a* to TChl *a*, i.e., Dv-Chl/TChl)
225 increased significantly in the upper 50 m (Figure 2d) and decreased significantly at
226 100 m (Figure 2d, e), but there was no obvious change at 75 m (Figure 2f). The
227 relative abundance of *Synechococcus* (represented by the ratio of its marker pigment
228 zeaxanthin to TChl *a*, Zea/TChl) decreased significantly in the upper 50 m (Figure 2g),
229 but there was no obvious trend at 75 m and 100 m (Figure 2h, i).
230



231

232 **Figure 2.** Temporal variations of phytoplankton biomass and community composition
 233 in different layers at the SEATS station. (a–c) TChl *a*; (d–f) The relative abundance of
 234 *Prochlorococcus* (represented by the ratio of its marker pigment divinyl chlorophyll *a*
 235 to TChl *a*, Dv-Chl/TChl); (g–i) The relative abundance of *Synechococcus*
 236 (represented by the ratio of its marker pigment zeaxanthin to TChl *a*, Zea/TChl).

237

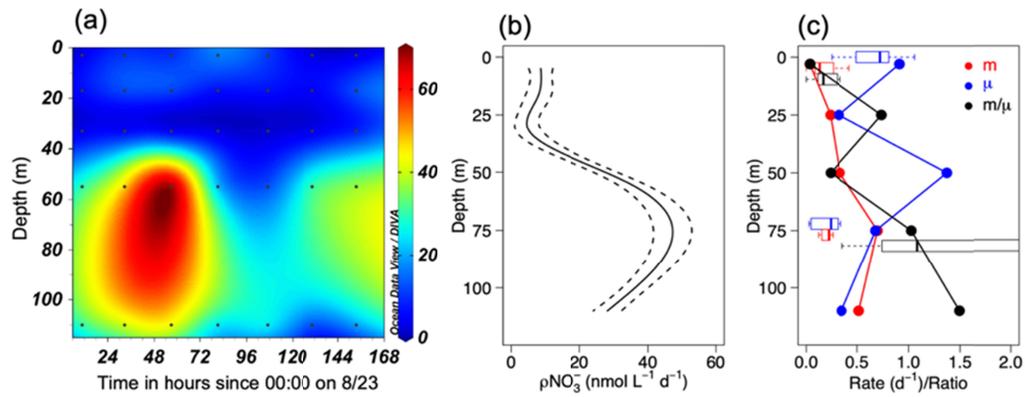
238

239 **Process rates and production**

240 The ρNO_3^- underwent obvious changes during the 7-day observation period.
241 High values at 55 m and 110 m were particularly noteworthy on the second day (24
242 August) and third day (25 August) (Figure 3a), which corresponded to the time when
243 the uplift of the thermocline, halocline, and pycnocline occurred (Figure 1c). The
244 value of ρNO_3^- was highest at 55 m, extremely low in the upper 33 m, and
245 intermediate at 110 m (Figure 3b). The ρNO_3^- estimated with a generalized additive
246 model (GAMs, Text S4) reached a maximum at a depth of about 75 m (Figure 3b).
247 New production (NP) estimated from the ρNO_3^- was significantly lower than gross
248 primary production (GPP) above 75 m, but the gap gradually narrowed at greater
249 depths (Figure S5).

250 The phytoplankton growth rates and microzooplankton grazing rates agreed well
251 with previous averages in the SCS (Figure 3c). In general, the growth rate exceeded
252 the grazing rate above the DCML, the two rates were basically in balance at the
253 DCML, and the growth rate was less than the grazing rate below the DCML. The
254 grazing pressure (the ratio of grazing rate to growth rate, m/μ) increased from the
255 surface to 110 m.

256



258

263 **Figure 3.** (a) Temporal variations of nitrate assimilation rate; (b) The vertical profile
 264 of nitrate assimilation rate was fitted by generalized additive models (GAMs); (c)
 265 Vertical profiles of phytoplankton growth rate, microzooplankton grazing rate, and
 266 grazing pressure, where solid dots and lines were results obtained in this study and
 267 boxes were results obtained from Chen et al. (2013).

264

264 **Discussion**

265 We found that the temporal trends of TChl *a* and the dominant phytoplankton
266 groups differed above, within, and below the DCML during our observation period
267 (Figure 2). The differences between these trends suggested that the phytoplankton
268 communities in these three layers were regulated by different environmental
269 mechanisms. The results therefore supported our hypothesis that the structure of the
270 phytoplankton community in oligotrophic oceans differs in the NDL, DCML, and
271 NRL. Under steady-state conditions, the boundary between the DCML and NRL is
272 not apparent (Figure S4), and the two-layer structure described by Dugdale (1967)
273 and Du et al. (2017) adequately describes the phytoplankton community. However,
274 under unstable conditions or when the system is perturbed, the difference between the
275 DCML and NRL becomes apparent (Figure 2) and a three-layer structure analogous to
276 what has been described by Landry et al. (2011a) emerges. We then asked whether
277 internal waves might be responsible for this perturbation at SEATS.

278 The impact of internal waves on phytoplankton is mainly the result of an
279 increase in the supply of allochthonous nutrients (Garrett, 2003; Sharples et al., 2009).
280 These nutrients would have been taken up by phytoplankton if there had been
281 adequate light in the lower layers of the euphotic zone. The ρNO_3^- in the lower
282 euphotic zone (50–110 m) was higher during 23–25 August than afterward (Figure
283 3a), and 23–25 August was when the spring tides occurred (Figure S2). The
284 implication is that strong internal wave activity during the spring tides enhanced the
285 influx of nutrients from the nutricline into the lower layers of the euphotic zone.
286 There is usually a time lag between the uptake of nutrients and the increase of
287 phytoplankton chlorophyll (Ma et al., 2020; Sharples et al., 2007; Wang et al., 2007).
288 Changes of the phytoplankton community would therefore have become apparent

289 after the spring tides. If nitrate were added at the base of the euphotic zone,
290 *Prochlorococcus* would lose its competitive advantage because most *Prochlorococcus*
291 ecotypes lack the ability to take up nitrate (Bouman et al., 2006), and other
292 phytoplankton species with the ability to take up nitrate would benefit. This line of
293 reasoning could well explain the significant decrease in the relative abundance of
294 *Prochlorococcus* at 100 m (Figure 2f). However, the grazing pressure of
295 microzooplankton on phytoplankton was relatively high at 100 m (Figure 3c),
296 consistent with previous observations (Landry et al., 2011a), and the growth of
297 phytoplankton was severely light limited at that depth (Figure 3c). The result was that
298 the grazing of microzooplankton constrained the accumulation of phytoplankton
299 biomass, and hence there was no increase of TChl *a* (Figure 2c). In the DCML (75 m),
300 the allochthonous nutrients supplied by internal waves led to high phytoplankton
301 growth rates due to relatively favorable irradiances and nutrient concentrations, but
302 the high rate of microzooplankton grazing balanced the high growth rates (Figure 3c).
303 Therefore, although there were diurnal fluctuations of the TChl *a* and the relative
304 abundances of dominant groups in the DCML, there was no obvious trend in the
305 composition or biomass of the phytoplankton community in the DCML during the 3
306 days of observation (Figure 2b, e, h). The microzooplankton were likely consumed by
307 mesozooplankton (Fonda Umani et al., 2005), which can move vertically (Hays,
308 2003), and their upward migrations might therefore have resulted in excretion of
309 ammonium into the NDL (King et al., 1987; Webb and Johannes, 1967). We did not
310 directly determine ammonium concentrations and ammonium uptake rates, but the
311 reduction of the NP/GPP ratio in the upper layers (Figure S5) was an indication that
312 recycled ammonium became relatively more important than nitrate as a source of
313 inorganic nitrogen. The fact that most *Prochlorococcus* can use the ammonium

314 excreted by zooplankton better than other phytoplankton such as *Synechococcus*
315 (Berube et al., 2015; Moore et al., 2002) may explain why the growth of
316 *Prochlorococcus* exceeded that of other phytoplankton in the upper 50 m (Figure 2d).
317 The phytoplankton in the NDL were not light-limited, and because the grazing
318 pressure of microzooplankton was low (Figure 3c), grazing could not control the
319 accumulation of phytoplankton biomass. This line of reasoning could explain the
320 increasing trend of TChl *a* in the NDL (Figure 2a). In summary, the likely impact of
321 internal waves was basically consistent with the different responses that revealed the
322 three-layer structure of the phytoplankton community.

323 To further test the internal wave hypothesis, we assumed that all the nitrate taken
324 up by phytoplankton within the 3 days (25–27 August) was converted into
325 phytoplankton biomass. We then compared the theoretical accumulation of
326 phytoplankton biomass in terms of chlorophyll *a* with the observed maximum change
327 of chlorophyll within the 3 days to determine whether the biomass supported by
328 nitrate equaled the change of total biomass. The results showed that the theoretical
329 accumulation of chlorophyll *a* was significantly lower than the observed maximum
330 change of chlorophyll *a* in the upper 50 m. The opposite was true at 100 m, and at the
331 DCML the two values were basically the same (Figure S6). The implication is that
332 even if the phytoplankton biomass produced from nitrate uptake accumulated
333 monotonically during the 3 days, the estimated net accumulation was still
334 significantly lower than the observed changes of chlorophyll in the upper 50 m. The
335 implication is that nitrate was not the only nitrogen source in the upper 50 m and
336 underscores the importance of ammonium nitrogen in controlling the dynamics of the
337 phytoplankton community in the NDL. In contrast, the fact that the theoretical
338 accumulation of chlorophyll *a* was significantly higher than the observed maximum

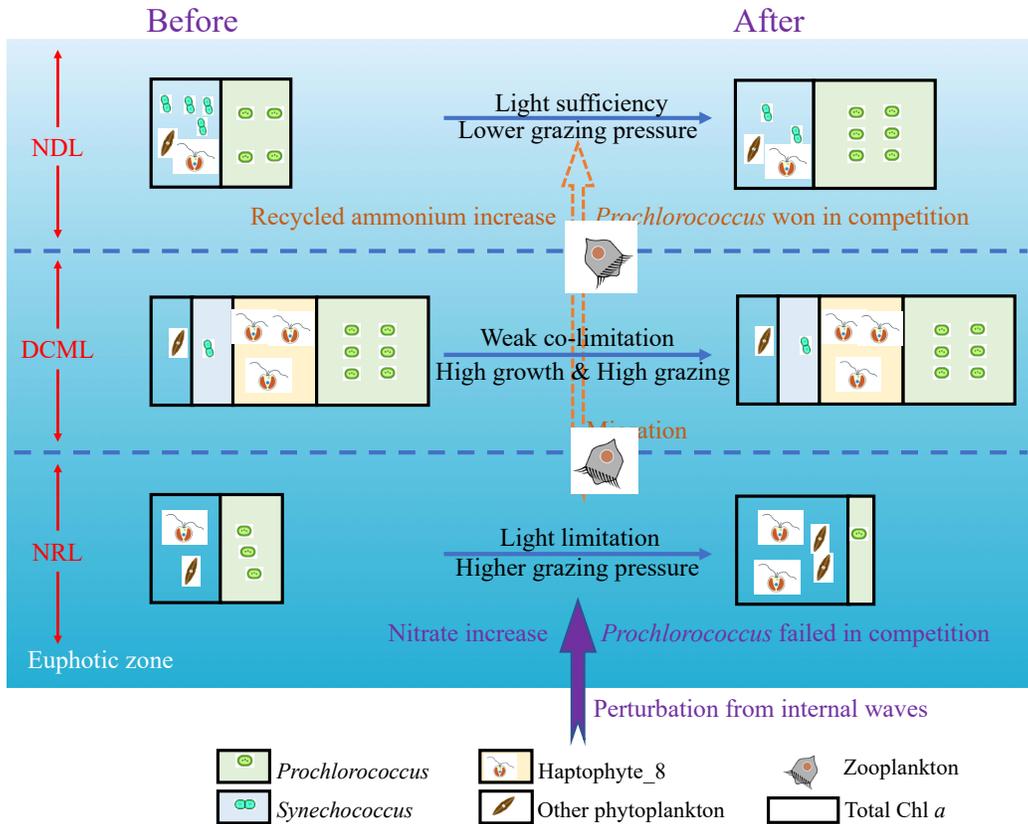
339 change of chlorophyll *a* at 100 m implies that there was a large loss of phytoplankton
340 biomass produced from nitrate uptake. This conclusion is consistent with the intense
341 grazing pressure from microzooplankton in the NRL (Figure 3c). The agreement
342 between the theoretical and observed changes of chlorophyll *a* concentrations in the
343 DCML was consistent with the dynamic balance between phytoplankton growth and
344 microzooplankton grazing in this layer (Figure 3c).

345 Other environmental changes that occurred during our observation period
346 included an anticyclonic warm eddy (Figure S7) and rain that occurred for a short
347 time on 26 and 27 August. The impact of the warm eddy was obviously untenable
348 because there was little chance for a warm eddy to fuel phytoplankton growth in the
349 upper waters of the SCS basin both vertically and horizontally (Huang et al., 2010;
350 Wang et al., 2018). The rainy weather could have been associated with an increased
351 flux of allochthonous nutrients from the atmosphere or a decrease in light intensity.
352 We did not measure any parameter associated with atmospheric deposition, but a
353 significant increase in the flux of nutrients from the atmosphere would have increased
354 the nutrient concentrations in the upper water column and would inevitably have led
355 to an increase of ρNO_3^- in the NDL, which is typically N-limited in the SCS (Du et al.,
356 2017; Savarino et al., 2007). However, the fact that ρNO_3^- at shallow depths did not
357 change significantly during our 7-day observation period (Figure 3a) was inconsistent
358 with the atmospheric deposition hypothesis. Because of the rainy weather, the PAR at
359 13:00 was indeed significantly lower on 26 August than on other observation dates
360 (Figure S3). A decrease of light intensity could reduce photoinhibition of
361 *Prochlorococcus* at the surface (Flombaum et al., 2013; Xiao et al., 2019; Xie et al.,
362 2018). The effect of a decrease of light intensity on *Prochlorococcus* would therefore
363 have differed between the surface and subsurface. The fact that the changes of

364 *Prochlorococcus* abundance were almost the same at the surface and at 25 m and 50
365 m was therefore inconsistent with the photoinhibition scenario (Figure 2d–f). We
366 therefore concluded that both the warm eddy and the brief period of rainy weather
367 were probably not the main factors that caused the three-layer structure of the
368 phytoplankton community to become apparent.

369 **Conclusions and Implications**

370 This study provided strong evidence that the perturbation caused by internal
371 waves made it apparent that the structure of the phytoplankton community in the
372 euphotic zone of South China Sea was best described by a three-layer structure at
373 steady state because the factors that limited phytoplankton growth differed in the three
374 layers (Figure 4). It has been a common assumption that the DCML is an ecologically
375 important feature of stratified oceans since the seminal paper by Yentsch (1965).
376 Primary production in the DCML accounts for 30–70% of water column production,
377 and this layer also accounts for the largest proportion of new production (Cai et al.,
378 2015; Lee Chen et al., 2008). Production in the NRL is much lower because of severe
379 light limitation (Figure S5), but its contribution to particle export flux can be high if
380 grazing rates are high (Landry et al., 2011a). High grazing rates in the NRL and
381 DCML support high rates of regenerated production in the NDL, which would
382 enhance carbon export through the microbial carbon pump (Jiao et al., 2010). The
383 implication is that the concept of a three-layer euphotic zone will facilitate estimates
384 of marine primary production and enhance understanding of the global carbon cycle.



385

386 **Figure 4.** The three-layer structure of the phytoplankton community in the euphotic
 387 zone of oligotrophic oceans. In steady state, phytoplankton are restricted by nutrient
 388 limitation in the upper nutrient-depleted layer (NDL), by light in the lower
 389 nutrient-replete layer (NRL), and by both nutrients and light in the middle deep
 390 chlorophyll maximum layer (DCML). When there is a perturbation caused by internal
 391 waves, uplifted nitrate stimulates the phytoplankton groups other than
 392 *Prochlorococcus* in the NRL, and because of light limitation, phytoplankton biomass
 393 is held in check by microzooplankton grazing. The result is a decreased relative
 394 abundance of *Prochlorococcus* and unchanged TChl *a*. In the DCML, weak
 395 co-limitation by nutrients and light results in high nitrate assimilation rates and high
 396 growth rates, but the increased phytoplankton growth rates are balanced by high
 397 grazing rates. The result is a stable, high TChl *a* concentration. In the NDL,
 398 *Prochlorococcus* benefits from ammonium regenerated by zooplankton that migrate
 399 from below. The result is an increased relative abundance of *Prochlorococcus* and
 400 increase of TChl *a*.
 401

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412

413 **Data Availability Statement**

414 Physical-biochemical data collected onboard used in this study can be found in
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