

# Nutrient pulses driven by internal solitary waves enhance heterotrophic bacterial growth in the South China Sea

Tzong-Yueh Chen,<sup>1†</sup> Jen-Hua Tai,<sup>1†</sup> Chia-Ying Ko,<sup>1</sup> Chih-hao Hsieh,<sup>2</sup> Chung-Chi Chen,<sup>3</sup> Nianzhi Jiao,<sup>4</sup> Hong-Bin Liu<sup>5</sup> and Fuh-Kwo Shiah<sup>1,2,6\*</sup>

<sup>1</sup>Research Center for Environmental Changes, Academia Sinica, Taipei, Taiwan.

<sup>2</sup>Institute of Oceanography, National Taiwan University, Taipei, Taiwan.

<sup>3</sup>Department of Life Science, National Taiwan Normal University, Taipei, Taiwan.

<sup>4</sup>State Key Lab of Marine Environmental Science, Xiamen University, Xiamen, China.

<sup>5</sup>Division of Life Sciences, The Hong Kong University of Science and Technology, Hong Kong, China.

<sup>6</sup>Institute of Marine Environment and Ecology, National Taiwan Ocean University, Keelung, Taiwan.

## Summary

**This study demonstrated the potential effects of internal waves (IWs) on heterotrophic bacterial activities for the first time. Nine anchored studies were conducted from 2009–2012 in the South China Sea areas with different physical conditions, i.e. areas subjected to elevation IWs, to depression IWs, and to weak/no IWs. The latter two areas were treated as the Control sites. Field survey results indicated that within the euphotic zone, the minima of the depth-averaged bacterial production (IBP;  $\sim 1.0 \text{ mgC m}^{-3} \text{ d}^{-1}$ ) and growth rate ( $\text{IB}\mu$ ;  $\sim 0.1 \text{ d}^{-1}$ ) at all sites were similar. Except for one case, the maxima of IBP ( $6\text{--}12 \text{ mgC m}^{-3} \text{ d}^{-1}$ ) and  $\text{IB}\mu$  ( $0.55\text{--}1.13 \text{ d}^{-1}$ ) of the elevation IWs areas were  $\sim$ fivefolds higher than those of the Control sites (IBP  $1.7\text{--}2.1 \text{ mgC m}^{-3} \text{ d}^{-1}$ ;  $\text{IB}\mu$   $0.13\text{--}0.24 \text{ d}^{-1}$ ). Replicate surveys conducted at the north-western area of the Dongsha atoll during spring-to-neap (NW1 survey) and neap-to-spring (NW2 survey) tide periods showed a great contrast to each other. Low variation and averages of  $\text{IB}\mu$  in NW1 survey were similar to those of the**

**Control sites, while those in NW2 were similar to the other elevation IWs sites with larger variation and higher averages of  $\text{IB}\mu$ . This finding suggests that bacterial activities may be a function of the lunar fortnightly (14-day) cycle. Enrichment experiments suggested more directly that the limiting inorganic nutrients introduced by the elevation waves (EIWs) may contribute a higher  $\text{IB}\mu$  within the euphotic zone.**

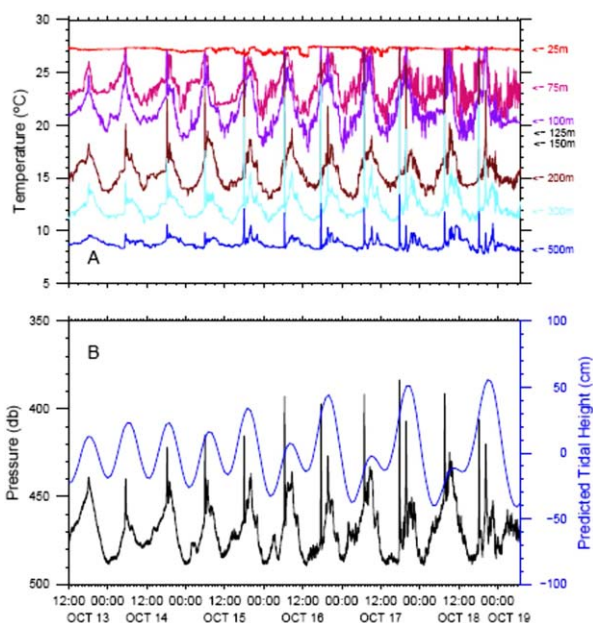
## Introduction

South China Sea (i.e. SCS) is one of the few marine systems possessing significant internal solitary waves (i.e. IWs; Chang *et al.*, 2006; Jan *et al.*, 2008; Alford *et al.*, 2010 and citations therein). While propagating westward from the Luzon Strait to the SCS-shelf, IWs take the form of depression waves moving downward in a deep-water region, and elevation waves (i.e. EIWs) moving upward in a shallow-water region with bottom depths  $<$ ca. 2X of the mixed layer depths (i.e. 100 m in the SCS; Liu *et al.*, 1998). According to Pineda (1999) and Sharples and colleagues (2001), IWs profoundly impact nonlinear cross-shelf transport processes and the enhancement of vertical mixing in shelf seas. From a biological perspective, this phenomenon implies that the supply of 'new nutrient' via EIWs processes should affect plankton biomasses and/or activities in shallow-water areas.

Exactly how IWs affect phytoplankton has received considerable attention since the early 1970s (Haury *et al.*, 1983; Stevens *et al.*, 2012 and citations therein). However, the extent to which IWs may affect heterotrophic bacterial activities have not addressed until recently. In the SCS, Wang and colleagues (2007) attributed the high chlorophyll-a concentration observed at the north-western section off the Dongsha atoll to the extra nutrient availability induced by the occurrence of EIWS in this area (Lynett and Liu, 2002; Chao *et al.*, 2006). In a satellite image study, Pan and colleagues (2012) indicated that biomass/production of phytoplankton in the EIWs area of the SCS exceeded the areas without EIWs. We speculate that in the EIWs area, heterotrophic bacterioplankton biomass/activity

Received 1 May, 2014; revised 25 November, 2015; accepted 17 February, 2016. \*For correspondence. E-mail fkshiah@rcec.sinica.edu.tw; Tel. +886-2-2653-9885 #271; Fax. +886-2-2789-3234.

<sup>†</sup>These authors equally important as the first author.



**Fig. 1.** Moored buoy data showing the temporal changes of (A) water temperatures at various depths and (B) pressure (black line) at 500 m depth and the predicted tidal height (blue line) derived from the OSU tidal model (Egbert and Erofeeva, 2002). The temperatures at 125 and 150 m depths in panel (A) were not shown for better resolution.

might be elevated, owing to either higher primary production or inorganic nutrient inputs (see below), or both.

Heterotrophic bacterioplankton (i.e. bacteria) are single cellular prokaryotes that feed on dissolved organic matter. Bacteria are generally viewed as decomposers (i.e. nutrient regenerators) in aquatic ecosystems. However, according to investigations carried out in the 1980s (Currie and Kalff, 1984a,b), in oligotrophic environments, bacteria could function as nutrient assimilators and compete with phytoplankton for limiting inorganic nutrients (Azam, 1998; Kirchman, 2000; Thingstad *et al.*, 2005 and citations therein). Therefore, the availability of inor-

**Table 2.** The correlation coefficients of temperature vs. individual depth measurements<sup>a</sup> of the nine surveys.

	P	Chl-a	DOC	BB	BP	B <sub>μ</sub>	
	μMP	mgChl-a m <sup>-3</sup>	μMC	mgC m <sup>-3</sup>	mgC m <sup>-3</sup> d <sup>-1</sup>	d <sup>-1</sup>	Sampling Size
NW1	-0.95		+0.38	+0.58			48
NW2	-0.93		+0.51	+0.72			78
ES1	-0.97	-0.39		+0.45			54
ES2	-0.92		+0.44	+0.46			45
SS1	-0.99	+0.51		+0.52	+0.75		54
SS2	-0.95		+0.50				45
<b>ED</b>	-0.95		+0.60	+0.68			53
<b>ST1</b>	-0.96		+0.45	+0.55			56
<b>ST2</b>	-0.99	+0.86	+0.69	+0.95			28

Blank with no value indicated insignificant at  $P=0.01$  level.

a. BB, BP and B<sub>μ</sub> represent bacterial biomass, production and specific growth rate (=BP/BB). See Table 1 for abbreviations of the rest

ganic nutrients might eventually affect bacterial activity and, ultimately the dynamics (accumulation vs. depletion) of dissolved organic carbon (DOC; Thingstad *et al.*, 1997; Lai *et al.*, 2014 and citations therein), which has an inventory equal to that of CO<sub>2</sub> in the atmosphere (Benner *et al.*, 1992; Hedges, 1992).

Bacteria in the field can grow at rates ranging from hours in estuaries/coast to weeks in oligotrophic oceans. As is generally accepted, biomass normalized (or specific) growth rate is more or less independent of top-down control (i.e. protozoan grazing and viral lyses) processes, and is affected mainly by bottom-up control (i.e. organic substrate/inorganic nutrient supply) and temperature (Ducklow and Shiah, 1993; Azam, 1998). EIWs and, thus, the inorganic nutrient pulses brought up to surface waters occur within a tidal cycle (Liu *et al.*, 1998). Based on the biophysical temporal characteristics of bacterial growth rate and IWs, anchored sampling over several tidal cycles and nutrient enrichment experiments are performed in this study.

**Table 1.** The ranges and averages ( $\pm$ standard deviations; std) of individual depth measurements of temperature (T), phosphate (P), chlorophyll-a (Chl-a) and dissolved organic carbon (DOC) concentrations of the nine anchored studies.

	T (°C)	P (μMP)	Chl-a (mgChl-a m <sup>-3</sup> )	DOC (μMC)
Exp	Range; Ave $\pm$ std	Range; Ave $\pm$ std	Range; Ave $\pm$ std	Range; Ave $\pm$ std
NW1	20.0–28.6; 24.6 $\pm$ 2.3	< 0.01–0.54; 0.16 $\pm$ 0.18	0.05–1.06; 0.38 $\pm$ 0.27	53–99; 76 $\pm$ 11
NW2	19.2–28.3; 24.5 $\pm$ 2.5	< 0.01–0.74; 0.17 $\pm$ 0.20	0.05–0.74; 0.22 $\pm$ 0.18	56–147; 82 $\pm$ 17
ES1	22.8–26.6; 25.9 $\pm$ 0.8	< 0.01–0.31; 0.08 $\pm$ 0.07	0.05–0.31; 0.20 $\pm$ 0.07	53–106; 78 $\pm$ 11
ES2	21.5–26.6; 25.8 $\pm$ 1.3	< 0.01–0.37; 0.06 $\pm$ 0.08	0.07–0.27; 0.20 $\pm$ 0.08	65–104; 79 $\pm$ 9
SS1	18.2–26.5; 24.5 $\pm$ 2.0	0.02–0.78; 0.20 $\pm$ 0.20	0.09–0.60; 0.31 $\pm$ 0.11	52–101; 72 $\pm$ 14
SS2	22.8–26.4; 25.8 $\pm$ 0.7	0.02–0.34; 0.08 $\pm$ 0.07	0.19–0.51; 0.32 $\pm$ 0.17	59–84; 71 $\pm$ 7
<b>ED<sup>a</sup></b>	19.6–27.5; 25.5 $\pm$ 2.1	< 0.01–0.70; 0.15 $\pm$ 0.20	0.02–1.03; 0.18 $\pm$ 0.20	49–129; 85 $\pm$ 25
<b>ST1<sup>a</sup></b>	18.1–29.4; 23.8 $\pm$ 4.0	< 0.01–0.97; 0.26 $\pm$ 0.35	0.02–0.52; 0.14 $\pm$ 0.12	60–119; 80 $\pm$ 25
<b>ST2<sup>a</sup></b>	20.5–25.0; 24.0 $\pm$ 1.2	0.13–0.48; 0.24 $\pm$ 0.13	0.01–0.67; 0.38 $\pm$ 0.20	52–75; 62 $\pm$ 6

a. The Control sites.

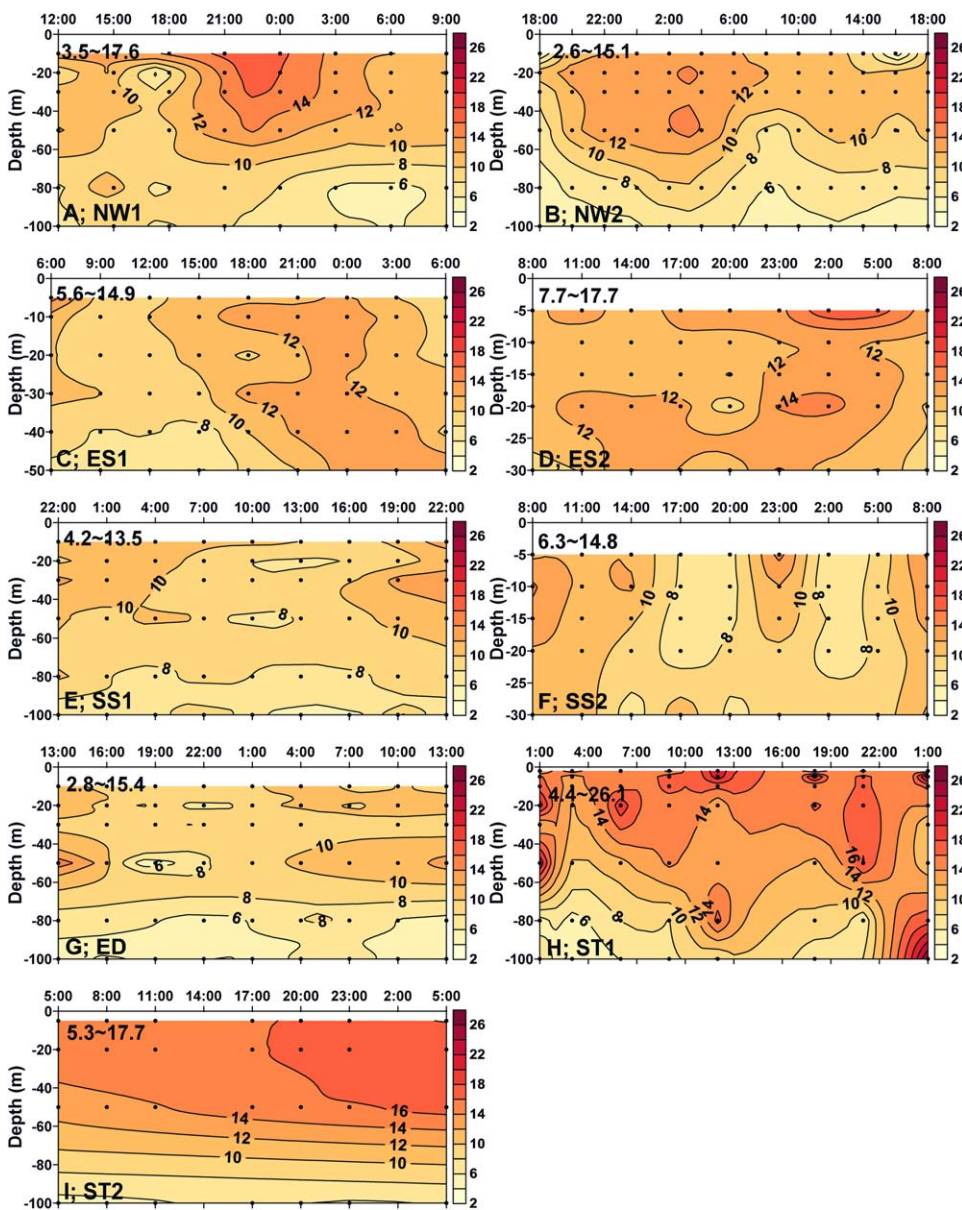
## Results

### Moored buoy data

The buoy data revealed that the IWs significantly impacted water temperature variation throughout the upper 500 m (Fig. 1). A sudden increase of temperatures ca. 8°C at depths >300 m was common. The physical properties of the IWs of two different time-scales were noticed. Over one tidal cycle, strong IWs occurred mainly during the flood-tide period; IWs could be seen during the ebb-tide period yet with much weaker amplitudes. In term of fortnightly cycle, the amplitudes of IWs intensified from neap to spring tide period and weakened from spring to neap tide period.

### Measurements for vertical profiles

Temperature ranged 18–29°C with averages of 24–26°C (Table 1) among the nine studies. Note that temperatures <20°C were only observed at sapling depth of 100 m. Model derived/calculated tidal heights indicated two tidal types: diurnal tide for the ST1 and ST2 surveys (Fig. 5H and I) and semi-diurnal tide (Fig. 5A–G) for the rest surveys. Phosphate concentrations (Table 1) were low in the surface waters and increased with depth at a range from <0.01 (detection limit) to 0.97  $\mu\text{MP}$ ; the concentrations showed strong negative correlations with temperature in all of the surveys ( $r > -0.90$ ,  $P < 0.01$ ; Table 2). Chlorophyll-a concentrations varied >100-fold



**Fig. 2.** Bacterial biomass ( $\text{mgC m}^{-3}$ ) profiles of the nine anchored studies. Upper X axis indicates time of the day. Numerical at the upper-left of each panel indicates the range. All panels are in the same colour scale. Solid dots indicated sampling time and depth.

among surveys ( $< 0.01$ – $1.06$  mgChl-a  $m^{-3}$ ; Table 1) with lower concentrations at the surface waters and subsurface chlorophyll maxima ( $0.27$ – $1.06$  mgChl-a  $m^{-3}$ ) locating at depths of 30–60 m. Chlorophyll-a readings were neither correlated with temperature (Table 2) nor with phosphate ( $P > 0.01$ ). DOC concentrations varied  $>$  threefold ranging from 49 to 147  $\mu$ MC. Eight out of nine surveys showed higher DOC readings generally appeared in the surface then decreased with depth as inferred from the positive correlation with temperature (Table 2).

Bacterial biomass (Fig. 2) varied  $\sim 10$ -fold ( $2.6$ – $26$  mgC  $m^{-3}$ ) with higher readings in the surface waters and then decreased with depth which resulted in positive correlations with temperature (Table 2). Bacterial production ( $0.09$ – $15.94$  mgC  $m^{-3} d^{-1}$ ) was low ( $< 5$  mgC  $m^{-3} d^{-1}$ ) in the surveys of NW1 (Fig. 3A) and the Control sites (i.e. ED, ST1 and ST2; Fig. 3E–G). The vertical structures of production in the remaining five surveys (i.e. NW2, ES1, ES2, SS1 and SS2) were much more heterogeneous than in the above-mentioned four surveys. Notably, higher production readings ( $> 5$  mgC  $m^{-3} d^{-1}$ ) throughout the water column occurred at the mid sampling period of the NW2 survey (Fig. 3B), and at the early and late sampling periods of the ES1, ES2, SS1 and SS2 surveys (Fig. 3C and D). Bacterial (specific) growth rates (Fig. 4) varied 100-fold with a range of  $0.02$ – $2.01$   $d^{-1}$ . In general, the tempo-spatial depth contours of the growth rates of all surveys followed the patterns of their production counterparts ( $r = +0.87$ – $+0.92$ ,  $P < 0.01$ ). Namely, growth rates were homogeneously low in the surveys of the Control sites and highly heterogeneous at the EIWs area with the exception of NW1. Neither production nor growth rates of bacteria showed correlation with temperature/depth (Table 2).

#### Diel patterns of bacterial measurements

Depth-averaged bacterial biomass (i.e. IBB) in all of the surveys showed a similar pattern with higher readings occurring at night time (Fig. 5A–F and H–I) except for the ED station (Fig. 5G), which had no clear pattern. In all of the surveys, depth-averaged bacteria specific growth rate (i.e.  $IB_{\mu}$ ) was highly correlated with depth-averaged bacterial production (i.e. IBP;  $r = +0.88$ – $0.92$ ,  $P < 0.01$ ). Consequentially, the temporal changes of  $IB_{\mu}$  were used to illustrate the following bacterial activity.

Notably, the  $IB_{\mu}$  did not appear to have a pattern with light-dark cycle. In the EIWs surveys, a higher  $IB_{\mu}$  occurred at day time in NW2 (Fig. 5B), while that of SS1 (Fig. 5E) and SS2 (Fig. 5F) was recorded mainly at night time. The light-dark patterns of the  $IB_{\mu}$  for the remaining three EIWs surveys (Fig. 5A and C–D) were ambiguous. For the Control sites (Fig. 5G–I), although varied within a

rather narrow range of  $0.1$ – $0.2$   $d^{-1}$ , most if not all of the higher  $IB_{\mu}$  values occurred at night time.

In term of  $IB_{\mu}$ -tidal heights relationships, for the EIWs surveys, two-thirds showed higher  $IB_{\mu}$  readings during flood-tide, while the remaining one-thirds showed a higher  $IB_{\mu}$  during ebb-tide (Fig. 5A–F). For the Control sites,  $IB_{\mu}$  showed a negative trend with tidal heights at ED station (Fig. 5G), while that of ST1 (Fig. 5H) and ST2 (Fig. 5I) surveys showed no trend with tidal heights.

#### Spatial comparison

Figure 6 schematically depicts the minima, maxima and means of the depth-averaged measurements of all surveys. For the concentrations of phosphate (Fig. 6A), chlorophyll-a (Fig. 6B) and dissolved organic carbon (Fig. 6C), the Control sites and EIWs surveys did not appear to have patterns in terms of ranges and means. The means of IBB (Fig. 6D) at ST1 and ST2 stations ( $> 12$  mgC  $m^{-3}$ ) were  $> 20\%$  higher than those of other stations, which had a mean value  $\leq 10$  mgC  $m^{-3}$ . The minima of IBP (Fig. 6E) and  $IB_{\mu}$  (Fig. 6F) in all surveys were around  $\sim 1.0$  mgC  $m^{-3} d^{-1}$  and  $0.1$   $d^{-1}$  respectively. However, with one exception (i.e. NW1), the maximal IBP ( $> 10$  mgC  $m^{-3} d^{-1}$ ) and  $IB_{\mu}$  ( $> 1.0$   $d^{-1}$ ) in the EIWs area were  $\sim$ fivefold higher than those recorded from the Control sites, which had IBP and  $IB_{\mu}$  values of  $\sim 2$  mgC  $m^{-3} d^{-1}$  and  $\sim 0.2$   $d^{-1}$  respectively.

Statistical (ANOVA) analysis indicated that the surveys did not differ in the means of IBP (and  $IB_{\mu}$ ) except for NW2 ( $IBP = 3.7 \pm 3.2$ ;  $IB_{\mu} = 0.44 \pm 0.39$ ) which was significantly higher than those of the Control sites and NW1. The means of IBP (and  $IB_{\mu}$ ) of ES1, ESS2, SS1 and SS2 did not differ from those of the Control sites and NW1, mainly due to large variances of these four studies. The coefficients of variance ( $CV = \text{standard deviation}/\text{mean} \times 100\%$ ) of the IBP in the Control sites and NW1 ranged from 23–37%, which is much smaller than those of the remaining surveys (57–125%). The CV analysis results for  $IB_{\mu}$  were the same as those for IBP.

#### Enrichment experiments

Combining 8 treatments (i.e. control, C, N, P... and C+N+P) and then comparing their percentages of increase of bacterial production identified what substrate (i.e. the black bold Italic values of Table 3) limited bacterial activity at any given time point. Twelve out of the 15 cases yielded satisfactory results. The three confusing cases (red bold values in Table 3) could be discarded since their percentage increase in the values was only 2–4% higher than the critical point of 20%.

The results of the enrichment experiments indicated that bacterial growth rates were constantly but differentially

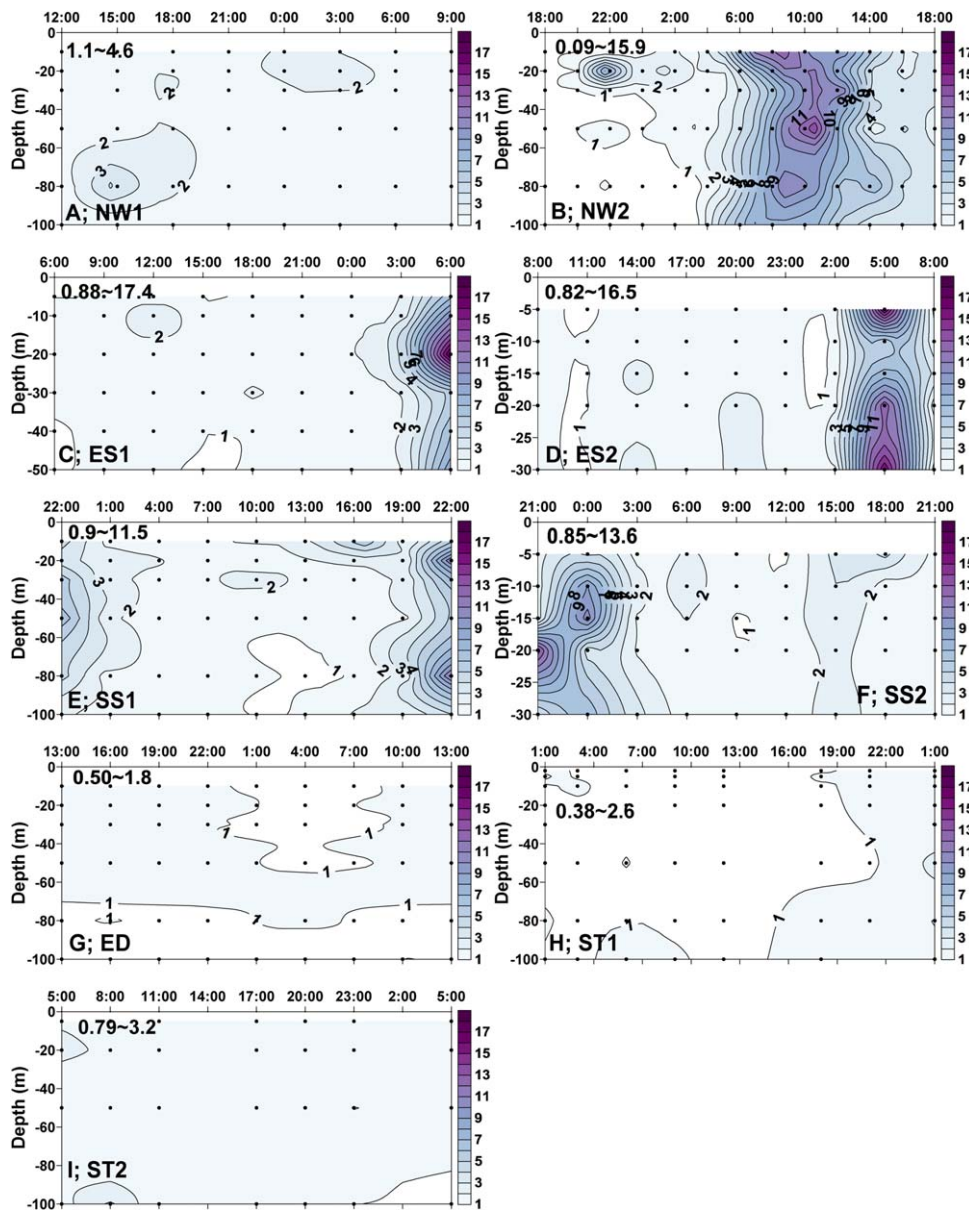


Fig. 3. The same as Fig. 2, but for bacterial production ( $\text{mgC m}^{-3} \text{d}^{-1}$ ).

limited by organic carbon or minerals or the combinations of organic carbon plus minerals. For example, in the NW1 study (Fig. 7A), growth rates were enhanced sequentially by C-, P- and P-addition at the first three sampling points, then switched to N- and CN-limitation afterwards. Similar phenomenon occurred in the NW2 study (Fig. 7B). Both studies revealed a general trend implying that P-limitation occurred whenever ambient phosphate concentrations were  $< 0.1 \mu\text{MP}$ , and C-limitation occurred whenever ambient dissolved organic carbon concentrations were  $< 75$  (in NW1 study) or  $< 70 \mu\text{MC}$  (in NW2 study). Owing to the absence of ammonium concentrations in these two studies, the threshold concentration for N-limitation was unknown.

## Discussion and conclusions

As is well recognized, temperature and substrate supply (i.e. bottom-up control processes) are the two potential factors in controlling the variance of bacterial growth rate in the field (Ducklow and Shiah 1993; Hoch and Kirchman 1993; Shiah *et al.*, 2003 and citations therein). The studies Shiah and colleagues (2001, 2003) suggested that  $20^\circ\text{C}$  appeared to be the optimal temperature for bacteria living in temperate shelf-seas. The temperatures in the upper water-column all exceeded  $20^\circ\text{C}$  (Table 1) except at the depth of 100 m. Therefore, this study concluded that temperature might play a less important role in regulating bacterial growth rates in the upper water-column. Inhibitory

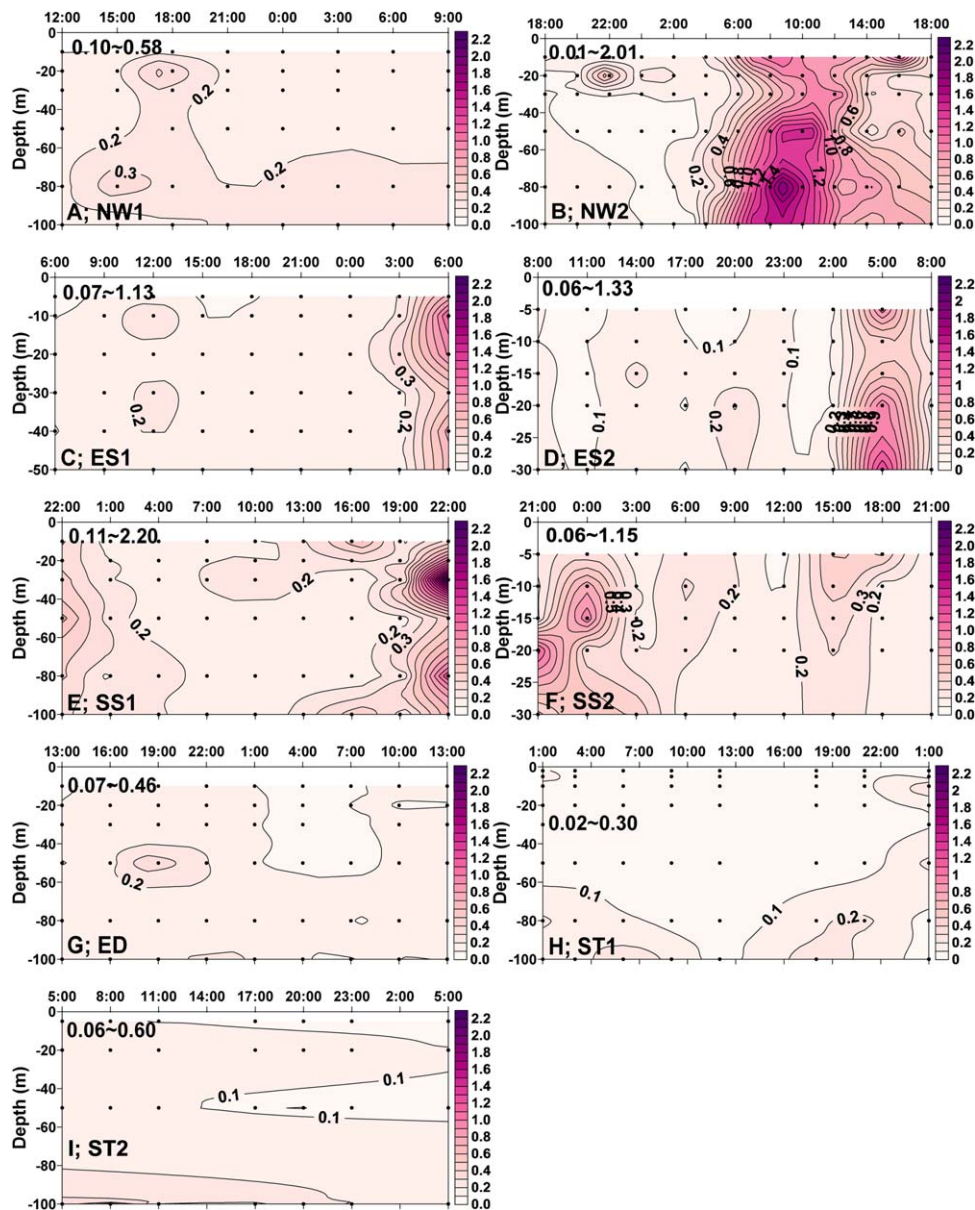


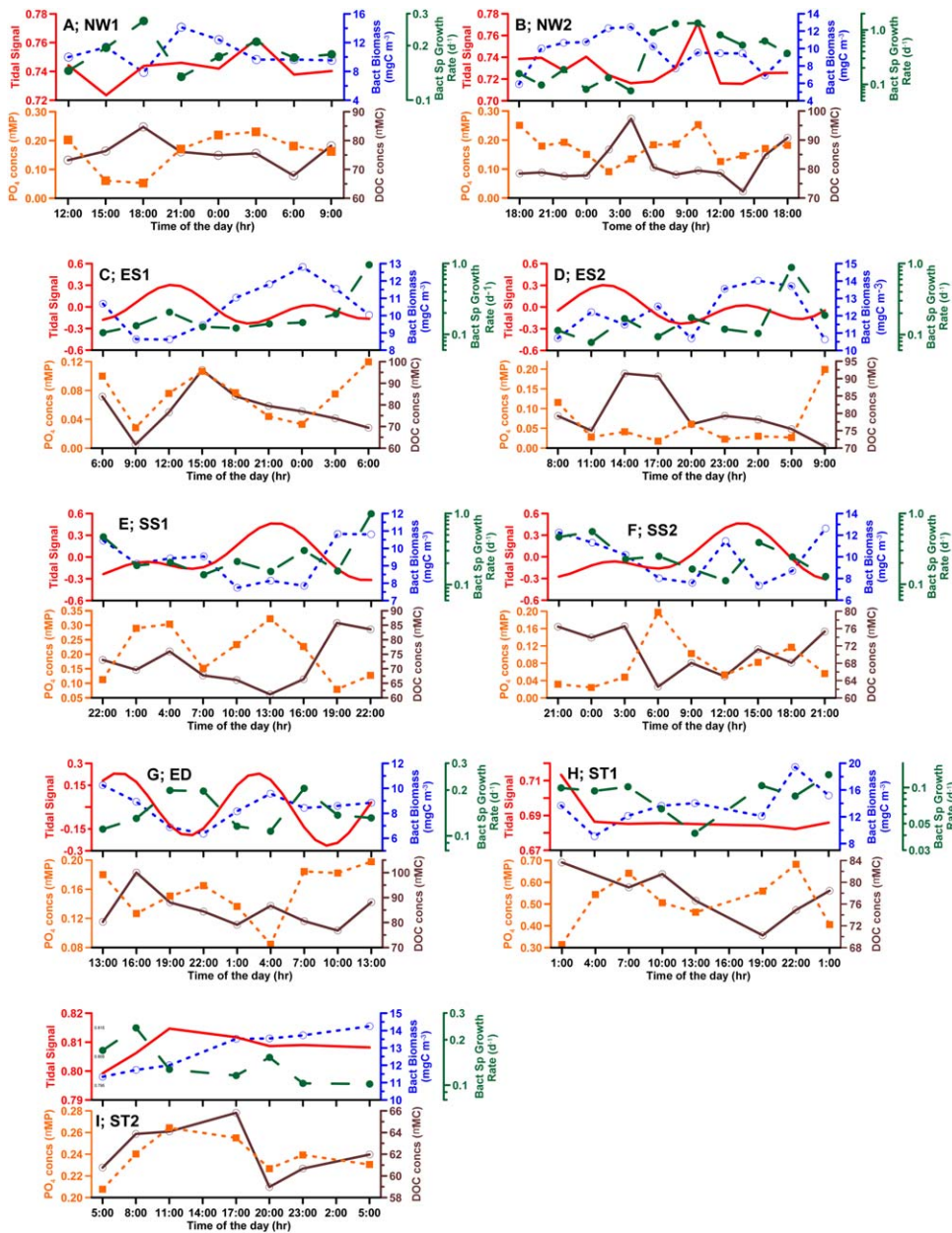
Fig. 4. The same as Fig. 2, but for bacterial (specific) growth rates ( $\text{d}^{-1}$ ).

effects caused by low ( $< 20^{\circ}\text{C}$ ) temperature could be more and more substantial in deep-water areas.

In terms of the substrate, bacteria require organic matter and inorganic nutrients (i.e. DIN) for growth. Potentially, these chemicals inside Dongsha atoll (i.e. the Atoll) might be exported through tidal movements and fuel bacterial growth at the four sampling stations (i.e. ES1, ES2, SS1 and SS2) located outside the Atoll. The hydrological characteristics inside the Atoll are quite unique in terms of tidal movement and the sea water chemistry. The radius, area and average depth of the Atoll are about 25 Km, 500  $\text{Km}^2$  and  $< 10$  m respectively. The major conduits for water

exchange are the northern and southern navigation channels (Fig. 1). Water flowed into the Atoll through the northern and southern navigation channels during the flood tide. During the ebb-tide, water flowed out mainly through the northern navigation channel and then bifurcated to the north and south (YH Wang, personal communication; yhwang@nysu.edu.tw). Therefore, the likelihood of massive water exchange between the Atoll and our four near-shore stations (i.e. ES1, ES2, SS1 and SS2) may have been quite low.

Liang (2012; Table 2 in that study) indicated that the sea-water inside the Atoll was characterized by high nitrate

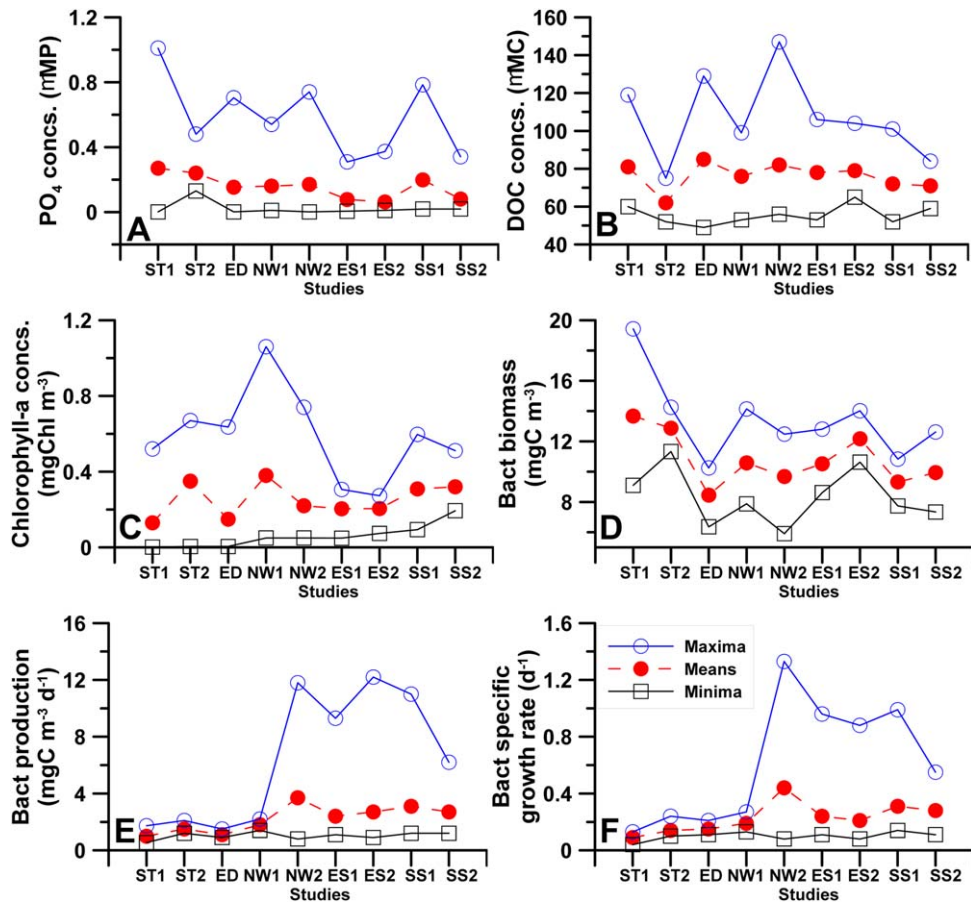


**Fig. 5.** Diel changes of tidal heights, depth-averaged bacterial biomass, bacterial specific growth rate, phosphate, and DOC concentrations derived from the nine anchored studies. Black bars indicate night-time (6 PM–6 AM). Note the five Y axes are in different scales for better resolution.

(1.63–2.58  $\mu\text{MN}$ ) yet extremely low phosphate (0.06–0.11  $\mu\text{MP}$ ) concentrations; in addition, the N:P molar ratios (29:1–42:1) were threefold higher than that of bacteria (N:P = 9:1; Kirchman, 2000). The results of manipulation experiments (Liang, 2012; fig. 12 in that study) also indicated that B $\mu$  inside the Atoll was P-limited in the summer and autumn; in addition, DOC limitation occurred in the winter despite a quite high ambient DOC concentration (176–266  $\mu\text{MC}$ ). This high DOC might largely originate from the seasonal death and the detritus decay of the sea grasses in the winter (HJ Lin, personal communication, hjlin@dragon.nchu.edu.tw), which was refractory for bacteria (Liang, 2012). While even assuming that the seawater exchange occurred, the chemicals that originated from the

Atoll were poor in quality for bacterial growth at the four near-shore stations. Moreover, the total volume of seawater inside the Atoll is markedly smaller than that of surrounding oligotrophic SCS seawaters (Lai *et al.*, 2014). Any substance carried in the outflows from the Atoll could be quickly diluted.

The direct physic-chemical environment changes (e.g. nutrient inputs) when there is a wave overturning and mixing, which occurs generally quite irregularly, or when there is a dissipation of the waves in shallow waters. That is, IWs can only inject nutrients in upper layers if there is some dissipation and turbulent mixing. The abrupt changes of the sea-bottom topography (i.e. becomes shallow) surrounding the Atoll (particularly the east and south



**Fig. 6.** The ranges (minima and maxima) and means of the depth-averaged measurements derived from the nine diel studies. Red dash squares indicate the control studies.

**Table 3.** The percentage of increase (%) of bacterial specific growth rate (BGR;  $d^{-1}$ ) of the enriched treatments<sup>a</sup> conducted in the NW1 and NW2 studies.

Exps	Samp Pointb	BGR of the Controls	C	N	P	C+N	C+P	N+P	C+N+P
NW1	1	0.145	<sup>c</sup> <b>80 ± 8%</b>			82 ± 9%	89 ± 15%		83 ± 9%
	2	0.194			<b>102 ± 19%</b>		108 ± 10%	117 ± 15%	114 ± 14%
	3	0.274			<b>93 ± 11%</b>		96 ± 14%	90 ± 12%	91 ± 13%
	4	0.135		<b>84 ± 17%</b>		89 ± 12%		81 ± 14%	83 ± 11%
	5	0.210				<b>79 ± 10%</b>			82 ± 9%
	6	0.171			<sup>d</sup> 24 ± 7%	<b>49 ± 11%</b>			47 ± 12%
	7	0.179				<b>88 ± 10%</b>			84 ± 12%
	8	0.145		<b>97 ± 17%</b>		99 ± 11%		92 ± 14%	98 ± 8%
NW2	1	0.157	<b>63 ± 8%</b>			62 ± 9%	69 ± 10%		63 ± 9%
	3	0.186	<b>89 ± 9%</b>		<sup>d</sup> 22 ± 8%	92 ± 8%	99 ± 12%		93 ± 8%
	5	0.132			<b>113 ± 10%</b>		116 ± 16%	110 ± 11%	111 ± 14%
	7	0.899				<b>59 ± 8%</b>			52 ± 9%
	9	1.334				<b>48 ± 10%</b>			47 ± 12%
	11	0.524				<b>98 ± 11%</b>			94 ± 14%
	13	0.371		<b>77 ± 13%</b>	<sup>d</sup> 20 ± 6%	79 ± 10%		72 ± 11%	78 ± 10%

Blank with no value indicated that value of the percentage of increase was <20%.

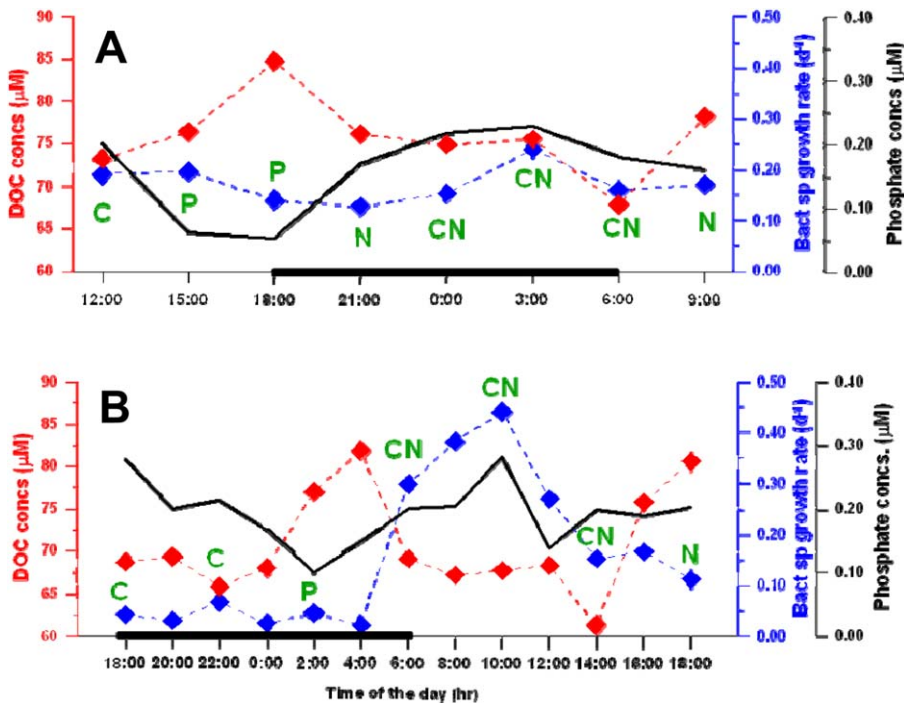
a. treatments C, N, P, C+N, C+P, N+P and C+N+P represented the addition of glucose, ammonium, phosphate, glucose plus ammonium, glucose plus phosphate, ammonium plus phosphate and glucose plus ammonium plus phosphate respectively.

b. See the X axes of Fig. 7 for the time of the sampling point.

c. Value in black bold italic indicated the type of substrate limitation after evaluation.

d. Value was considered ineffective since the value was just above the critical point of 20%.





**Fig. 7.** Results of the nutrient enrichments conducted in the (A) NW1 and (B) NW2 studies. Capital letters C (glucose addition), N (ammonium addition) and P (phosphate addition) indicate the treatments are at least 20% higher than the control at that time point. CN, co-limitation by C and N. Black bars indicate night-time.

sides) may provide physical conditions for the IWs dissipation and turbulent mixing.

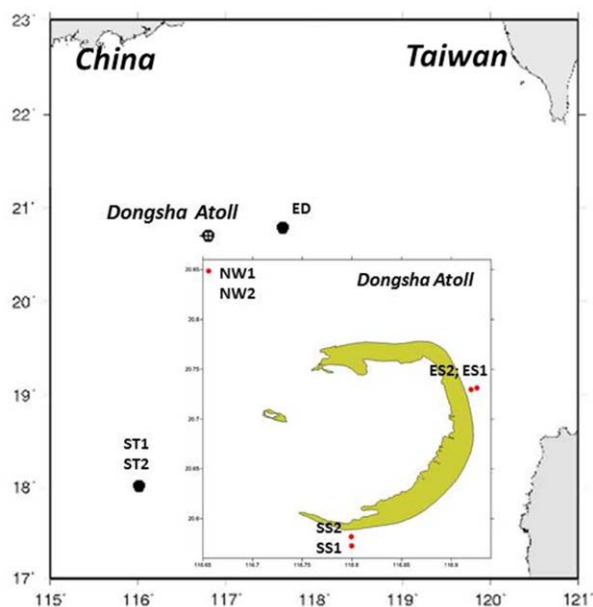
As mentioned earlier, despite its deep water depth (~250m), EIWs may have affected the NW station, owing to wave-wave or wave-topography interactions (Lynett and Liu, 2002; Chao *et al.*, 2006). The contrasting patterns of NW1 (spring-to-neap period) and NW2 (neap-to-spring period) suggested the importance of the fortnightly cycle in affecting bacterial growth rates. According to Fig. 1, the amplitude of IWs increased from neap to spring tide, which echoed the tidal condition of NW2 study. It is deduced that in the NW2 study, a greater amplitude of the EIWs resulted in a higher supply of DIN from the deep-waters and a greater bacterial growth rate within the euphotic zone. In contrast, the strength of EIWs of the NW1 survey was greatly reduced since investigation was conducted during the spring-to neap tide period. Low variation and low average of bacterial growth in NW1 due to low nutrient supply were expected.

Moored buoy data (Fig. 1) suggested that EIWs occurred mainly during the flood-tide period. Eight of the 12 semi-diurnal tidal cycles exhibited higher bacterial growth rates during the flood-tide period (Fig. 5A–F). This phenomenon highlighted the possibility that the DIN introduced by the EIWs could stimulate bacterial growth rate (Currie and Kalf, 1984a,b; Shiah *et al.*, 1997; Kirchman, 2000; Vadstein, 2000; Thingstad *et al.*, 2005). This possibility was verified by the results of the enrichment experiments (Fig. 7), indicating that the single addition of P or N could enhance bacterial growth rate. However, four

cases still exhibited low bacterial growth rates during the flood-tide period, i.e. the first two sampling points of ES1 and ES2 as well as the 5th and 6th sampling points of SS1 and SS2 (Fig. 5C–F). Exactly why this phenomena occurred remains unclear.

Results of the enrichment experiments indicated that bacterial growth could be differentially limited by either DIN or organic carbon or a combination. Additionally, bacteria appeared to be consistently at a status of starvation (Fast and Famine coexistence). That is, oligotrophic bacteria are conceived of as those never invited to a feast; their properties should include microbial adaptations to uninterrupted nutrient/substrate limitation (Poindexter, 1981). Interestingly but not surprisingly, the substrate limitation in these two experiments (Fig. 7) followed *Liebig's Law* (or *the Law of Minimum*; van der Ploeg *et al.*, 1999). This law states that biological growth is controlled by the scarcest or least available resource (limiting factor). According to our results, substrate X (either in single form or combination) acted as the limiting factor for bacterial; growth when its ambient concentration was the lowest.

Alternatively, the 'new nutrients' injected to the upper water-column by EIWs might affect bacterial growth indirectly. That is, phytoplankton biomass/production was stimulated first, followed by the fueling of a higher bacterial growth by algal exudates. Interestingly, algal biomass seemed to be unaffected by the 'new nutrients' brought by EIWs. Averaged chlorophyll-a concentrations (Table 2) recorded at the Control (0.14–0.38 mgChl m<sup>-3</sup>) and EIWs



**Fig. 8.** Map of the South China Sea showing the sampling stations and Dongsha atoll.

sites ( $0.20\text{--}0.38\text{ mgChl m}^{-3}$ ) were in the same range. Actual mechanisms for this could be many but cannot be explored here due to the lack of essential data, such as phytoplankton growth rate, zooplankton abundance/grazing rate and others.

As to the Control sites where the DIN inputs from deep-water were none or only minor ones, their  $IB_{\mu}$  (Fig. 6F) varied within a very narrow range ( $0.1\text{--}0.2\text{ d}^{-1}$ ) and showed no relationship with any physical/chemical/biological factors except for the ED station conducted in the depression IWs area, where its  $IB_{\mu}$  showed a negative trend with tidal heights (Fig. 5G). In contrast to stations of NW, SS, ES and ST, there was no replicate observation for

ED station, making it difficult to determine whether the phenomenon was a random or regular one.

In conclusion, this study demonstrates for the first time that elevation internal solitary waves in the SCS could enhance the bacterial rate parameters (i.e. production and growth rates). The 'new nutrients' injected to the upper water-column might directly boost bacteria activity. According to our results, the affected areas of the elevation IW are quite heterogeneous environments for bacterial growth. Any snap-shot type of sampling without considering tidal phase and the fortnightly cycle may be quite misleading. Internal solitary waves in the SCS is a basin-wide phenomenon, which potentially impact autotrophic (e.g. primary production) and heterotrophic (e.g. community respiration) processes. Thus, system organic C-budget are mostly unknown and worthy for further investigation. The issues of the composition of the bacterial communities at these sites; the rates of injection (by EIWs) and microbial consumption of the 'new nutrients' within the eu-photic zone await further investigation.

## Experimental procedures

### Study site and sampling

From 2009–2012, nine anchored studies were conducted in 5 cruises at 7 stations at the northern SCS (Fig. 8; Table 4). The NW station was located at the north-west  $\sim 53\text{ Km}$  off Dongsha atoll (i.e. the Atoll). According to Lynett and Liu (2002) and Chao and colleagues (2006), this area was subjected to EIWs through the so-called 'wave-wave' or 'wave-topography' interactions, despite the fact that its bottom depth was  $\sim 250\text{m}$ . Two surveys were performed at the NW station in 2009 (i.e. NW1) and 2010 (i.e. NW2). Table 4 indicated that these two studies were performed at different periods, in term of lunar fortnightly (14-day) tidal cycle. The NW1 study was conducted during spring-to-neap or full-moon to new-moon period (i.e. 3 days after spring tide); while NW2 was performed during the

**Table 4.** General information for the stations and the nine anchored studies.

Exps	Bot depth (m) and distance from the Atoll (Km)	Physical characteristics	Cruise number & Study period	Tide information
NW1	250; 52.86	Deep station with elevated IWs <sup>a</sup>	OR3-CR1379 11–12 June, 2009	3 days after spring-tide (June 8 <sup>th</sup> )
NW2	250; 52.86	Deep station with elevated IWs <sup>a</sup>	OR1-CR929 8–10 June, 2010	6 days before spring-tide (June 14 <sup>th</sup> )
ES1	60; 60.21	Shallow station with elevated IWs	OR1-CR1015 14–15 October, 2012	Fig. 1 of this study
ES2	40; 59.65	Shallow station with elevated IWs	OR1-CR1015 14–15 October, 2012	Fig. 1 of this study
SS1	110; 39.85	Shallow station with elevated IWs	OR1-CR1015 16–17 October, 2012	Fig. 1 of this study
SS2	40; 40.18	Shallow station with elevated IWs	OR1-CR1015 16–17 October, 2012	Fig. 1 of this study
ED	1,000; 129.16	Deep-water station with depression IWs	OR1-CR1015 13–14 October, 2012	Fig. 1 of this study
ST1	$\sim 3,800$ ; $> 500$	Deep-water station with weak/no IWs <sup>a</sup>	OR1-CR944 13–15 October, 2010	Diurnal tide
ST2	$\sim 3,800$ ; $> 500$	Deep-water station with weak/no IWs <sup>a</sup>	OR1-CR988 23–25 December, 2011	Diurnal tide

Surveys ED, ST1 and ST2 were treated as the control sites.

**a.** Data sources: Liu and colleagues (1998); Lynett and Liu (2002) and Chao and colleagues (2006).

neap-to-spring or new-moon to full-moon period (i.e. 6 days before the spring tide (Table 4). The long-term ADCP data reported by Lien and colleagues (2005) indicated a positive correlation between the tidal height in Luzon Strait and the vertical velocity of IW. Therefore, the amplitude of IW and thus the supply of inorganic nutrient supply were primarily modulated in a fortnightly cycle.

The ST station (18 °N, 116 °E; aka. the South-East Asia Time Series station) is located at the centre of the northern SCS with a bottom depth > 3800 m. No evidence has suggested that IWs occur in this area (Liu *et al.*, 1998). The ST station was visited twice in 2010 (i.e. ST1) and 2011 (i.e. ST2). In 2012, investigations were conducted at stations at the east (i.e. ES1 and ES2) and the south (i.e. SS1 and SS2) off the Atoll. ES1, ES2, SS1 and SS2 were shallow-water stations with bottom depths of 60, 40, 110 and 40 m respectively (Table 4). The signal of IWs was detected by deploying a moored buoy at station ED (Fig. 8; bottom depths ~1000 m) to record temperature changes at 9 depths in the upper 500 m at a rate of 5 min. The tides of ED, ES1, ES2, SS1 and SS2 studies were then simulated by the Oregon State University (Egbert and Erofeeva, 2002) tidal model with the moored buoy data. Meanwhile, those of NW1, NW2, ST1 and ST2 were calculated as the reciprocal of the temperature reading at the depth of 25 m of that given study.

Water-column sampling was performed every 2 or 3 h. For stations with bottom depths > 100 m, water samples were taken within the upper 100 m by 20 l Go-Flo bottles from 6 depths. For shallow-water stations, water sampling at six depths was performed from the surface (2 m depth) to a depth of ~10 m above the bottom. Meanwhile, profiles of temperature, salinity, fluorescence and underwater PAR (photosynthetic available radiance) were recorded by sensors attached to CTD rosette (General Oceanic Model 1015; General Oceanics, Miami, FL, USA).

#### *Inorganic nutrients and chlorophyll-a concentrations*

Inorganic nutrients (nitrate and phosphate) and chlorophyll-a concentrations were determined according to the methods of Parsons and colleagues (1984). Complete nitrate data were unavailable since the water samples of NW1 survey were lost due to a preservation problem. However, an adequate nitrate-phosphate correlation ( $r = +0.90$ ,  $n = 415$ ,  $P < 0.001$ ) was observed for the remaining surveys. Thus, the changes in inorganic nutrients were illustrated using phosphate.

#### *Bacterial biomass and production*

Bacterial activity and abundance were determined by <sup>3</sup>H-thymidine incorporation (Fuhrman and Azam, 1982) and epifluorescence microscopy (Hobbie *et al.*, 1977) respectively. After the injection of <sup>3</sup>H-thymidine, water samples were incubated at *in situ* temperature for 2 h. Bacterial biomass and production in C units were derived with a thymidine and a carbon conversion factor of  $1.18 \times 10^{18}$  cell mole<sup>-1</sup> and  $2 \times 10^{-14}$  gC cell<sup>-1</sup> respectively. Moreover, bacterial specific growth rate was calculated by dividing production with biomass. Details of bacterial parameters can be found in Shiah and colleagues (2003).

#### *Nutrient enrichment experiments*

Two experiments were conducted in the NW1 and NW2 surveys. Water samples at a depth of 10 m were collected along with nutrient enrichment experiments. The samples were filtered through 3.0 µm polycarbonate filter to remove bacteria grazers (i.e. flagellates and ciliates) and large planktons. Next, 3.0 µm filtrates were assigned to 8 treatments (as describe below) that were incubated in triplicate 250 ml polycarbonate bottles at ambient temperature for 1 h. Glucose (i.e. C), NH<sub>4</sub>Cl (i.e. N) and NaH<sub>2</sub>PO<sub>4</sub> (i.e. P) were used as added substrates. The 8 treatments were un-amended control, C enriched, N enriched, P enriched, C+N enriched, C+P enriched, N+P enriched, and C+N+P enriched. The final concentrations of glucose, N and P were 50 µMC, 0.5 µMN and 0.25 µMP respectively. Bacterial production and biomass were determined upon completion of incubation. Effective enrichment was defined when the bacterial (specific) growth rate reading of the treatment was at least 20% higher than that of the control (Shiah *et al.*, 2001).

#### *Data management and statistical analysis*

The bulk properties of measured variables were compared in terms of tempo-spatial (horizontal) variation by deriving depth-averaged values through means of dividing the integrated (trapezoidal method) values by the deepest sampling depth. Statistical analysis was performed using SPSS® V12.0 software (IBM, Armonk, NY, USA).

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#### **References**

- Alford, M.H., Lien, R.C., Simmons, H., Klymak, J., Ramp, S., Yang, Y.J., *et al.* (2010) Speed and evolution of nonlinear internal waves transiting the South China Sea. *J Phys Oceanogr* **40**: 1338–1355.
- Azam, F. (1998) Microbial control of oceanic carbon flux: the Plot thickens. *Science* **280**: 694–696.
- Benner, R., Pakulski, J.D., McCarthy, M., Hedges, J.I., and Hatcher, P.G. (1992) Bulk chemical characteristics of dissolved organic matter in the ocean. *Science* **225**: 1561–1564.
- Chang, M.H., Lien, R.C., Tang, T.Y., Asaro, E.A.D., and Yang, Y.J. (2006) Energy flux of nonlinear internal waves in northern South China Sea. *Geophys Res Lett* **33**: L03607. doi: 10.1029/2005GL025196.
- Chao, S.Y., Shaw, P.T., Hsu, M.K., and Yang, Y.J. (2006) Reflection and diffraction of internal solitary waves by a circular island. *J Oceanogr* **62**: 811–823.
- Currie, D., and Kalff, J. (1984a) The relative importance of bacterioplankton and phytoplankton in phosphorus uptake in freshwater. *Limnol Oceanogr* **29**: 311–321.

- Currie, D., and Kalff, J. (1984b) Can bacteria outcompete phytoplankton for phosphorus? *Microb Ecol* **10**: 205–216.
- Ducklow, H.W., and Shiah, F.K. (1993) Bacterial production in estuaries. In *Aquatic Microbiology: An Ecological Approach*. Ford, T. (ed). Boston, MA, USA: Blackwell Sci. Publ. pp 261–287.
- Egbert, G.D., and Erofeeva, S.Y. (2002) Efficient inverse modeling of barotropic ocean tides. *J Atmos Oceanic Technol* **19**: 183–204.
- Fuhrman, J.A., and Azam, F. (1982) Thymidine incorporation as a measurement of heterotrophic bacterioplankton production in marine surface waters: evaluation and field results. *Mar Biol* **66**: 109–120.
- Haury, L.R., Wiebe, P.H., Orr, M.H., and Briscoe, M.G. (1983) Tidally generated high-frequency internal wave packets and their effects on plankton in Massachusetts Bay. *J Mar Res* **41**: 65–112.
- Hedges, J.I. (1992) Global biogeochemical cycles: progress and problems. *Mar Chem* **39**: 67–93.
- Hobbie, J.E., Daley, R.J., and Jasper, S. (1977) Use of nucleopore filters for counting bacteria by fluorescence microscopy. *Appl Environ Microbiol* **33**: 1225–1228.
- Hoch, M.P., and Kirchman, D.L. (1993) Seasonal and inter-annual variability in bacterial production and biomass in a temperate estuary. *Mar Ecol Prog Ser* **98**: 283–295.
- Jan, S., Lien, R.C., and Ting, C.H. (2008) Numerical study of baroclinic tides in Luzon Strait. *J Oceanogr* **64**: 789–802.
- Kirchman, D.L. (2000) Uptake and regeneration of inorganic nutrients by marine heterotrophic bacteria. In *Microbial Ecology of the Oceans*. Kirchman, D.L. (ed). Wiley-Liss. Hoboken, NJ, USA, pp. 261–288.
- Lai, C.C., Fu, Y.W., Liu, H.B., Kuo, H.Y., Wang, K.W., Lin, C.H., et al. (2014) Distinct bacterial production–DOC–primary production relationships and implications for biogenic C-cycling in the South China Sea shelf. *Biogeochemistry* **11**: 147–156.
- Liang, C.H. (2012) Tempo-spatial variation of heterotrophic bacterioplankton production and its growth controlling factor in Dong-sha Atoll. Master Thesis. Keelung, Taiwan: National Taiwan Ocean University.
- Lien, R.C., Tang, T.Y., Chang, M.H., and D'Asaro, E.A. (2005) Energy of nonlinear internal waves in the South China Sea. *Geophys Res Lett* **32**: L05615.
- Liu, A.K., Chang, Y.S., Hsu, M.K., and Liang, N.K. (1998) Evolution of nonlinear internal waves in the East and South China Seas. *J Geophys Res* **103**: 7995–8008.
- Lynett, P.J., and Liu, P.L.F. (2002) A two-dimensional, depth-integrated model for internal wave propagation over variable bathymetry. *Wave Motion* **36**: 221–240.
- Nagata, T., and Kirchman, D.L. (1992) Release of dissolved organic matter by heterotrophic protozoa: implications for microbial foodwebs. *Arch Hydrobiol* **35**: 99–109.
- Pan, X.J., Wong, G.T.F., Shiah, F.K., and Ho, T.Y. (2012) Enhancement of biological productivity in the life phases of internal waves: observations in the northern South China Sea. *J Oceanogr* **68**: 427–437.
- Parsons, T.R., Maita, Y., and Lalli, C.M. (1984) *A Manual of Chemical and Biological Methods for Seawater Analysis*. New York, NY, USA: Pergamon.
- Pineda, J. (1999) Circulation and larval distribution in internal tidal bore warm front. *Limnol Oceanogr* **44**: 1400–1414.
- van der Ploeg, R.R., Böhm, W., and Kirkham, M.B. (1999) On the origin of the theory of mineral nutrition of plants and the Law of the Minimum. *Soil Sci Soc Am J* **63**: 1055–1062.
- Poindexter, J.S. (1981) Oligotrophy: fast and famine existence. In *Advances in Microbial Ecology*, Vol 5. Alexander, M. (ed). New York, NY, USA: Plenum Press, pp. 63–89.
- Sharples, J., Moore, C.M., and Abraham, E.R. (2001) Internal tide dissipation, vertical mixing and nitrate flux at the shelf edge of NE New Zealand. *J Geophys Res* **106**: 14069–14081.
- Shiah, F.K., Kao, S.-J., and Liu, K.-K. (1997) Bacterial production in the western equatorial Pacific: implications of inorganic nutrient effects on dissolved organic carbon accumulation and consumption. *Bull Mar Sci* **62**: 795–808.
- Shiah, F.K., Chen, T.Y., Gong, G.C., Chen, C.C., Chiang, K.P., and Hung, J.J. (2001) Differential coupling of bacterial and primary production in mesotrophic and oligotrophic systems of the East China Sea. *Aquat Microb Ecol* **23**: 273–282.
- Shiah, F.K., Gong, G.C., and Chen, C.C. (2003) Seasonal and spatial variation of bacterial production in the continental shelf of the East China Sea: a synthesis of controlling mechanisms and potential roles in carbon cycling. *Deep-Sea Res II* **50**: 1295–1309.
- Stevens, C.L., Sutton, P.J.H., Law, C.S. (2012) Internal waves downstream of Norfolk Ridge, western Pacific, and their biophysical implications. *Limnol Oceanogr* **57**: 897–911.
- Thingstad, T.F., Hagstrom, A., and Rassoulzadegan, F. (1997) Accumulation of degradable DOC in surface waters: is it caused by a malfunctioning microbial loop? *Limnol Oceanogr* **42**: 398–404.
- Thingstad, T.F., Krom, M.D., Mantoura, R.F.C., Flaten, G.A.F., Groom, S., Herut, B., et al. (2005) Nature of phosphorus limitation in the ultraoligotrophic eastern Mediterranean. *Science* **309**: 1068–1071.
- Vadstein, O.A. (2000) Heterotrophic, planktonic bacteria and cycling of phosphorus: phosphorus requirements, competitive ability, and food web interactions. In *Advances in Microbial Ecology*, Vol 16. Schink, B. (ed). New York, NY, USA: Kluwer Academic/Plenum Publishers, pp. 115–167.
- Wang, Y.H., Dai, C.F., and Chen, Y.Y. (2007) Physical and ecological processes of internal waves on an isolated reef ecosystem in the South China Sea. *Geophys Res Lett* **34**: L18609. doi:10.1029/2007GL030658.