

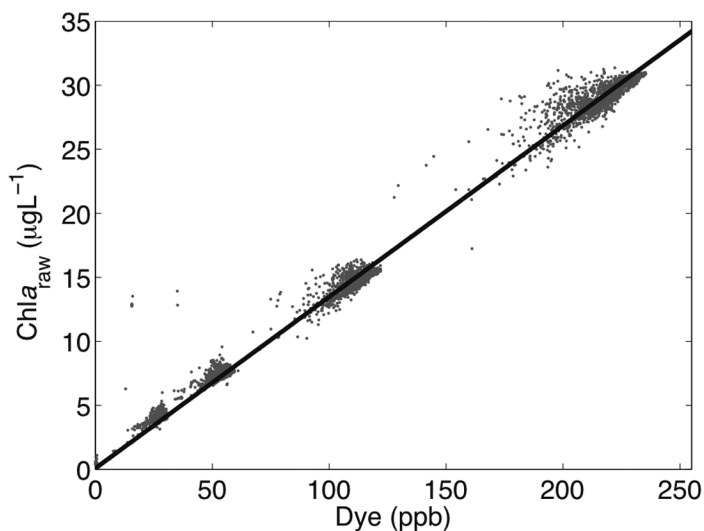
# Influence of bubbles and sand on chlorophyll-a fluorescence measurements in the surfzone

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## Appendix 1

### A: Effect of Rhodamine-WT dye on $Chl a_{raw}$

During several days of the field experiment, Rhodamine-WT dye was released in the surfzone. Lab experiments were performed to assess the effect of fluorescent dye on  $Chl a_{raw}$  (mechanism C, Fig. 1C), and the viability of using  $Chl a_{raw}$  fluorometer data during this time period. We filled 15-L buckets with seawater (nominal 2  $\mu m$  filtered) of low  $Chl a_{raw}$  concentration (0.6  $\mu g L^{-1}$ ) and varying dye concentrations (25, 55, 110, and 220 ppb). Significant errors in  $Chl a_{raw}$  occurred when dye was present (Fig. A1). Rhodamine-WT fluoresces at a broad range of wavelengths (Wilson et al. 1986), leading to some fraction of dye fluorescence detected as  $Chl a_{raw}$  (mechanism C).  $Chl a_{raw}$  increased linearly with dye concentration, giving  $Chl a_{raw}$  as large as 30  $\mu g L^{-1}$ . The regression slope between  $Chl a_{raw}$  and dye is  $0.13 \pm 0.01 \mu g L^{-1} ppb^{-1}$ , and the percent variance described by the fit is high ( $r^2 > 0.9$ ).



**Fig. A1.**  $Chl a_{raw}$  with  $Chl a_{true} = 0.6 \mu g L^{-1}$  versus Rhodamine-WT dye concentration (gray points). The black line is the linear fit (slope =  $0.13 \pm 0.01 \mu g L^{-1} ppb^{-1}$ ,  $r^2 < 0.9$   $P < 0.001$ ).

### B: Effect of backscattered ambient sunlight on $Chl a_{raw}$

Ambient sunlight in the water column may enhance  $Chl a_{raw}$  (mechanism D, Fig. 1D). On a cloudless sunny day, SCUBA divers holding downward-facing (vertical) ECO Triplet fluorometers took three measurements of  $Chl a_{raw}$  in an area of direct sunlight and three in an adjacent (10 m distant) shady area under the Scripps pier (La Jolla, CA).  $Chl a_{raw}$  was measured at 0.2, 2, and 4 m below the surface (in 6 m total water depth). The mean and standard deviation of  $Chl a_{raw}$  was calculated at each level (Table B1). Over a 30-min period, three subsurface positions were sampled at the two horizontal locations (light, shade), alternating between light and shade every 2 min.

The ratio  $Chl a_{raw}$  (sun) to  $Chl a_{raw}$  (shade) was significantly different from unity (two-tailed  $t$  test) at 0.2 m below the surface. Here, sunlight induced a  $Chl a_{raw}$  enhancement of  $21 \pm 6\%$  over the  $Chl a_{raw}$  measured in the shade. The sun/shade bias decreased with depth below the surface as the ambient sunlight intensity, particularly red wavelengths, decayed. At 2 and 4 m below the surface, the ratio was not significantly different from unity (two-tailed  $t$  test). Decreases in sun-shaded fluorescence are not due to physiological adaptation to high light conditions (e.g., nonphotochemical suppression, NPQ; Falkowski and Raven 1997, Muller et al. 2001), as NPQ increases  $Chl a$  fluorescence in low irradiance. Instead, the measured surface sun/shade bias was likely created by scattered light from the broad sunlight spectrum entering the fluorescence detectors (mechanism D, Fig. 1D).

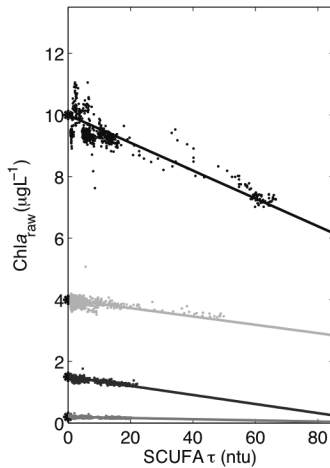
**Table B1.** Depth below the mean surface, mean  $Chl a_{sun}/Chl a_{shade}$ , standard deviation (SD) of  $Chl a_{sun}/Chl a_{shade}$ .

Depth, m	$Chl a_{sun}/Chl a_{shade}$	SD	$t$ test	$P$
0.2	1.21	0.06	1	<0.001
2	0.98	0.11	0	0.07
4	1.02	0.13	0	0.09

Two-tailed  $t$  test (if  $t$  test = 1, mean is significantly different than unity at the 5% confidence limit).

### C: Effect of bubbles on Chl*a*<sub>raw</sub> from a single-channel fluorometer

The effect of bubble-generated  $\tau$  on Chl*a*<sub>raw</sub> was explored in the laboratory with a single-channel WET Labs WETStar flow-through Chl*a* fluorometer (460/695 nm excitation/emission wavelengths, 0–150  $\mu\text{g L}^{-1}$  range). Turbidity was measured by a Turner SCUFA instrument with a flow-through cap (545 nm wavelength, 0.03–100 ntu range; www.turnerdesigns.com). The Chl*a* sensor, enclosed in the WETStar housing, was not contaminated by the  $\tau$  emitter enclosed inside the SCUFA. Turbidity and Chl*a*<sub>raw</sub> were sampled at 5 Hz. Tests were performed with Chl*a*<sub>true</sub> = 0.2, 1.5, 4, and 10  $\mu\text{g L}^{-1}$ . As with the ECO Triplet, the WETStar Chl*a*<sub>raw</sub> was linearly correlated with  $\tau$  (Fig. C1), and the  $\tau$ -Chl*a*<sub>raw</sub> regression slope  $\gamma$  (Eq. 1) depended linearly on Chl*a*<sub>true</sub>, consistent with  $\alpha = 0$  in Eq. 3. The SCUFA and ECO Triplet  $\tau$  sensors have different optics and thus different responses to bubbles and sand, precluding quantitative comparison of the  $\gamma$ -Chl*a*<sub>true</sub> relationship (i.e.,  $\beta$ ) between the two instrument pairs. However, the results suggest that the model (Eq. 2) may apply broadly to single-channel, multi-channel, and flow-through fluorometers.



**Fig. C1.** WETStar Chl*a*<sub>raw</sub> versus bubble-induced turbidity ( $\tau$ ) in seawater with four known Chl*a*<sub>true</sub> concentrations (black asterisks on vertical axis corresponding to 10, 4, 1.5, and 0.2  $\mu\text{g L}^{-1}$ ). Solid lines are linear fits with intercept set equal to Chl*a*<sub>true</sub>. The fraction of variance described by each fit (in ascending Chl*a*<sub>true</sub> order) is  $r^2 = 0.77, 0.35, 0.44, 0.10$ , respectively. The  $r^2$  in bold type correspond to  $P < 0.001$ .