SUPPLEMENTARY MATERIALS FOR

THE PIXIE

A LOW-COST, OPEN-SOURCE, MULTICHANNEL IN SITU FLUOROMETER APPLIED TO DYE-TRACING IN HALIFAX HARBOR

By Kyle Park, Dariia Atamanchuk, Aaron MacNeill, and Vincent Sieben

https://doi.org/10.5670/oceanog.2025.309

CROSS-SPECTRAL SENSITIVITY

Once both channels were calibrated, a further cross-spectral sensitivity test was performed. The rhodamine water tracer (RWT) channel was allowed to run in a mid-concentration, temperature-controlled chlorophyll-*a* (Chl-a) standard, and the Chl-a channel was allowed to run in a mid-concentration, temperature-controlled RWT standard. Further, both channels collected data from a blank (0 ppb RWT, 0 ppb Chl-a) standard of 90% acetone. These data, when juxtaposed with data from the appropriate channels, evaluates the cross-sensitivity between the two channels, or the amount of interference one measurement receives (e.g., RWT) in the presence of the other fluorophore (e.g., Chl-a). Though in principle the direct measurement of a mixture of known concentrations of both fluorophores would be valuable, the interaction between RWT and acetone would confound this direct approach.

The calibrated channels were then used to measure three additional standards: one blank in 90% acetone, one Chl-a in 90% acetone, and one RWT in deionized water, at 20°C. The blank and Chl-a standard data is repurposed from the 0 ppb and 40 ppb calibration setpoints at 20°C. In each case, the RWT channel was allowed to measure after the calibration measurements were complete. The RWT standard was obtained by titrating concentrated RWT dye until the RWT channel's raw signal level was comparable to that observed on the Chl-a channel during Chl-a calibration, or 35.2 ppb RWT. The raw data were processed in the same way as during calibration, whereby 300 points of raw data collected at an effective rate of 1 sample per second were averaged to obtain one data point. The resulting data from each of the three standards are plotted together (see Figure S1). The results do not show any meaningful cross-sensitivity between the RWT and Chl-a channels (e.g., RWT measured in the Chl-a standard is not significantly different from the blank result). This can be explained by the careful selection of excitation LEDs and the use of high-density emission (OD4) and excitation (OD6) optical filters; both the light that can excite the unintended fluorophore and the detected light emitted from the unintended fluorophore are attenuated, by a factor of $10e^{-4}$ and $10e^{-6}$, respectively. If a given application can tolerate more cross sensitivity than evidenced here, cost savings could be realized by reducing the quality of the excitation filter or omitting it entirely, though the performance of that PIXIE configuration would need to be evaluated separately.

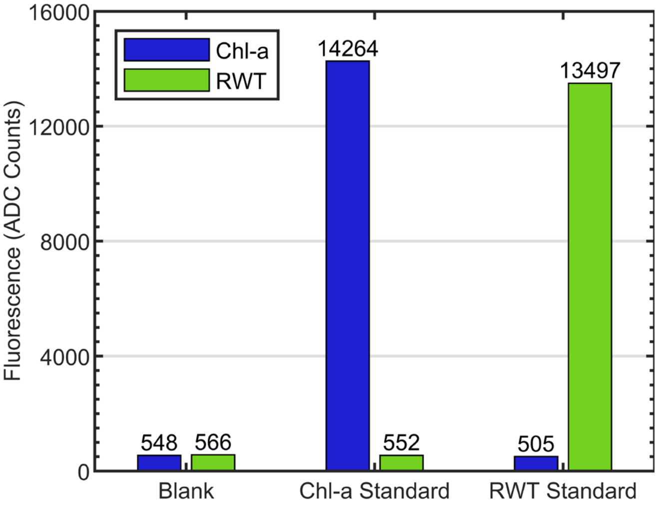

FIGURE S1. Cross-spectral sensitivity test results for the PIXIE Chlorophyll a (Chl-a) and rhodamine water tracer (RWT) channels. Data were collected from Blank (pure solvent, 90% acetone), Chl-a (in 90% acetone), and RWT (in deionized water) standards at 20°C. The blank and RWT data were collected using the same method as in calibrations. The Chl-a blank and standard data are the 0 ppb and 40 ppb calibration points, respectively, whereas the RWT standard was titrated in to reach a comparable point in the fluorometer's range (35.2 ppb).

RHODAMINE PROFILE COMPARISON

Near each drop point, the ecoCTD (Dever et al., 2020) equipped with a Cyclops-7F rhodamine fluorometer (Turner Designs) performed horizontal transects of the harbor. This provided horizontal and vertical distributions of RWT against which the bottle samples, and PIXIE, could be compared. To further validate the performance of the PIXIE, the August 10, 2023, profile at Station 3 can be compared to the nearest ecoCTD transect, occurring just after the Niskin bottle samples were collected.

The ecoCTD and the PIXIE simultaneously measured the surface RWT concentration at Station 2, allowing for its Cyclops 7F fluorometer to be two-point calibrated against the 217.8 ppb bottle sample at 18°C. The use of fixed-temperature calibration curves for both fluorometers allowed for a more direct comparison of the two devices' raw fluorescence measurements without introducing the uncertainty and time responses of their corresponding temperature sensors.

Figure S2 presents the transect path (see Figure S2a) and its downcast RWT profiles in a waterfall plot (see Figure S2b). The waterfall plot uses 12 ppb increments to compare the PIXIE's RWT profiles to the ecoCTD's Cyclops-7F RWT fluorometer profiles. The solid curves in Figure S2b represent downcast whereas the dot-dashed curves represent upcast profiles. The two devices show reasonable agreement in maximum observed concentration at ~10 ppb. The ecoCTD shows self-consistency across its casts. As the distance between Station 3 and the nearest transect profile is shorter (48 m) than the distance between the first and last transect profiles, the concordance between the PIXIE and ecoCTD profiles suggests the PIXIE's profile is a valid representation of the concentration in Station 3's water column, although both sets of measurements fall short of the 15.7 ppb surface Niskin bottle sample.

Both the PIXIE and the ecoCTD's Cyclops-7F show inconsistencies between downcasts and upcasts in Figure S2b. This inconsistency is commonly observed among in situ fluorometers and related devices (Briggs et al., 2011; Cetinic et al., 2012), with proposed mechanisms including pressure and temperature hysteresis (Shigemitsu et al., 2020), the order of operations (enter sample volume before sampling on upcast, enter sample volume after sampling on downcast), and device orientation, which allows differential sunlight interference (Shigemitsu et al., 2020) between casts. Qualitatively, the PIXIE demonstrates good self-consistency with regards to Figure S2b compared to the Cyclops-7F but nevertheless demonstrates cast discrepancy as described. This may be due to an element of the PIXIE's optical design or designed ambient light rejection ability and warrants a focused future examination.

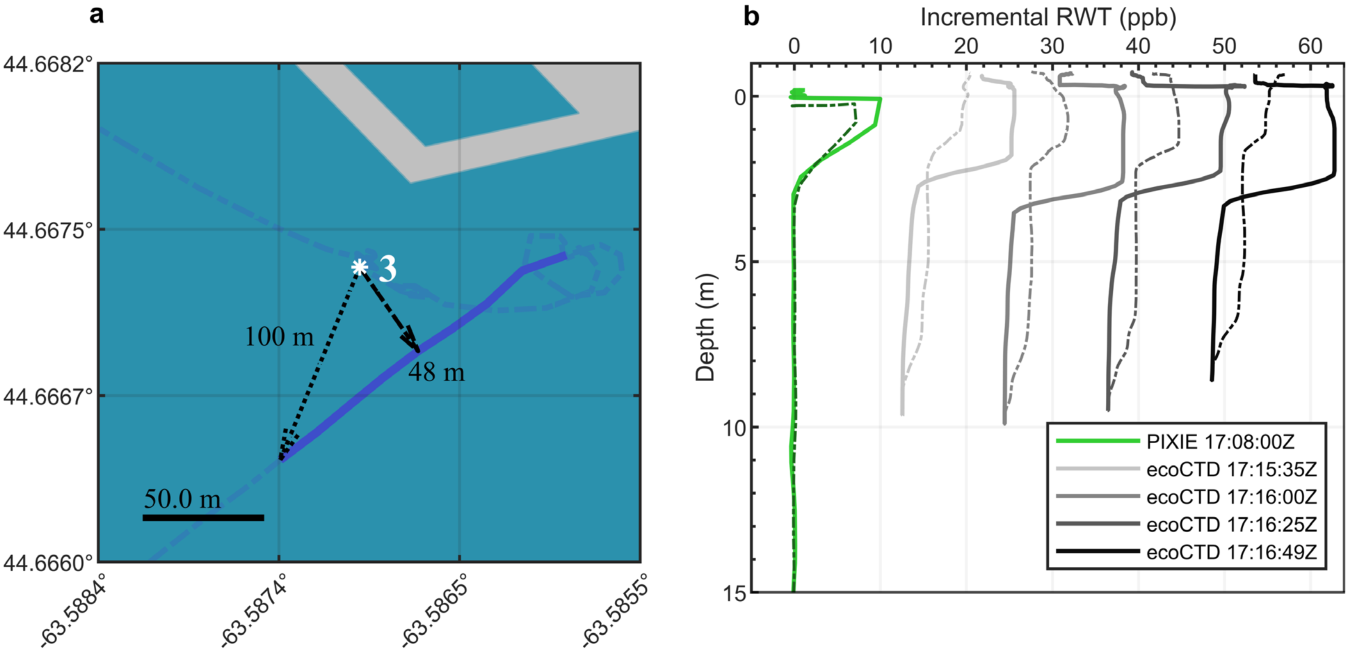

FIGURE S2. GPS locations of Station 3 and Eastcom's path on August 10, 2023. The solid purple line illustrates the path of the harbor transect nearest to Station 3. The faded dot-dashed path represents the vessel's course before and after the transect. Position vectors indicate the nearest and furthest distances from Station 3 to the transect. (b) Waterfall plot, in 12 ppb increments, of vertical profiles captured at (PIXIE) and near (ecoCTD) Station 3 on August10 at indicated UTC times. Solid curves indicate downcasts, dot-dashed curves indicate upcasts.