Toward Toxicological Interpretation of Diffusive Gradients in Thin Films in Marine Waters Impacted by Copper

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Introduction

Diffusive Gradient in Thin-film (DGT), a passive sampling method, has the potential to better quantify the bioavailable fraction of Cu in seawater when determining toxicological endpoints. In the present study, laboratory exposure of DGTs and *M. galloprovincialis* larvae at varying levels of dissolved organic carbon (DOC) and Cu were performed to resolve the degree to which mimicry of toxicity-buffering occurs in passive sampler quantification. The resulting toxicological endpoints are then used to assess in-progress field studies in the context of water quality criteria. The field data presented here were collected in the Puget Sound Naval Shipyard (PSNS) under Project ENVVEST; an environmental monitoring program being conducted for Naval Base Kitsap, in the Puget Sound receiving waters of Sinclair and Dyes Inlets.

In DGT theory, diffusional flux through an agarose cross-linked polyacrylamide gel allows for temperature dependent diffusion of both inorganic and organic labile complexes to a Chelex resin through an open-pore system (Fig 1). In complex natural systems, binding selectivity by the DGT resin layer is dependent upon competition effects as well as binding site equilibria [1], and the affinity of the resin has the ability to dissociate and retain weakly complexed Cu ions, while DOC bound Cu remains predominately inert. Therefore, C_{DGT} values have to potential to more accurately represent the potential for biological effects; making their use highly applicable to protection of the most sensitive life stages in species sensitivity distributions (e.g. mussel embryos), which are the current basis of aquatic life criteria.

Methods

Determination of C_{DGT} Cu EC50 — Seawater was collected from Sequim Bay, WA; a source which is historically low in ambient trace metals. Individual 2L samples of seawater were spiked with Cu [as Cu(II)SO_4], and DOC derived from Suwannee River Natural Organic Matter (SRNOM) 2R101N (IHSS, St. Paul, MN), at ranges centered on expected *M. galloprovincialis* EC50 values and the natural DOC range in seawater, based on a screening study and observations previously reported [2,3].

DGTs were purchased from DGT® Research (Lancaster, England) where they are commercially available in the LSNM-NP (loaded, solution deployment type, Chelex100, APA, polyethersulphone) model for measurement of cations in solution. Two DGTs were suspended in each 2L test solution by monofilament and placed on an orbital table in the environmental chamber that housed *M. galloprovincialis* embryo test vials.

Median effective concentrations were determined by exposing *M. galloprovincialis* embryos to seawater aliquots from the individual 2L test preparations, and one laboratory control seawater (LCS) from San Diego Bay for 48 hours as prescribed in USEPA 1995 [4]. Evaluation of the number of surviving larvae that developed normally (D-shaped, prodissoconch I stage) relative to the number of total embryos initially added to each vial was assessed (% normal alive endpoint), while the proportion of normally developed larvae relative to the number of larvae counted (% normal development) was also calculated. Because these endpoints were essentially identical, the data reported herein are for % normal development. The % normally developed larvae from the multi-concentration tests were used to calculate EC50s using measured dissolved Cu and C_{DGT} Cu with the toxicity statistics program CETIS™ (Tidepool Scientific).

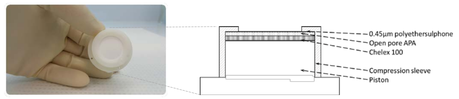

Figure 1. Components of a DGT passive sampler.

Field demonstration of C_{DGT} capture — Targeted campaigns were operationally defined as wet (December 2016 and March 2017) and dry (August 2017) season events, and correlated with conventional water quality monitoring efforts at PSNS. The approach to deployment was polypropylene cages suspended from floating piers, held at ~1m in the water column by non-metallic weights (Fig 2). This design allows the sampler face protection from large debris. DGTs were inverted to the bottoms of the cage lids to minimize exposure to photosynthetically active radiation (biofouling potential). Temperature was continuously logged at each station via HOBO Pendant® and averaged per time period. The data presented here is an exposure integration of 72 hours, the midpoint of *M. galloprovincialis* and *S. purpuratus* embryo-larval development toxicity test specifications [4], which potentially more effectively represents the impact of bioavailable metals [5,6].

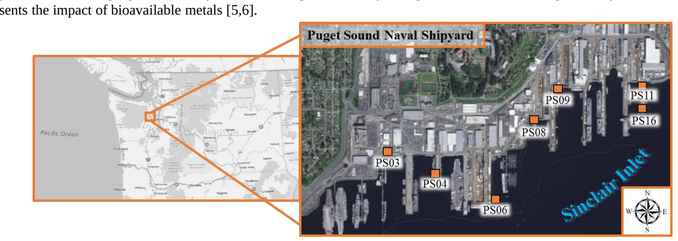

Figure 2. Passive sampler deployment locations for NBK monitoring program that fall within the PSNS (Sinclair Inlet).

C_{DGT} Calculation — The basis for converting the mass of metal accumulated by the resin to the concentration in solution as measured by DGT (C_{DGT}) is the classical equation given in Zhang and Davidson 1995 [7],

$$C_{DGT} = [\frac{c_e(V_g+V_e)}{f_e}]\Delta g/D_G A t$$

where C_e is the concentration metal eluted from the resin, V_g is the volume of the resin, V_e is the volume of HNO_3, f_e is the elution efficiency, Δg is the thickness of the diffusional path, D_G is the temperature dependent diffusion coefficient, A is the area of the polyethersulphone face exposed to seawater, and t is the deployment time. The LSNM-NP contained a 0.40mm Chelex layer, a 0.78mm APA and 0.14mm polyethersulphone layer with an exposure area of 3.14cm².

Results of laboratory determination of C_{DGT} Cu EC50

Dissolved Cu EC50 values (Fig 3) calculated in the present study are in agreement with Arnold et al (2010) [3], and follow a similar trend as presented by Chadwick et al (2008) [8], across the tested range of DOC concentration; validating the co-measured C_{DGT} values also presented in Figure 3. The proportion normal endpoints, over that DOC range, spanned from 4.8 to 11.5µg/L C_{DGT} Cu, indicating that the difference in diffusional residence time between *M. galloprovincialis* and DGT did allow for detectable unequal dissociation of weakly bound Cu-DOM complexes, but favorably, the range of EC50 determined by this method is greatly reduced in comparison to that of Cu_{DISS}. Based on this result, the model $[0.861(DOC) + 4.89]$ may be used in conjunction with C_{DGT} Cu for determination of the *M. galloprovincialis* toxicological endpoints; conversely, is extensible to normalize the EC50 to 4.8µg/L C_{DGT} Cu for toxicological monitoring where DOC levels are unknown.

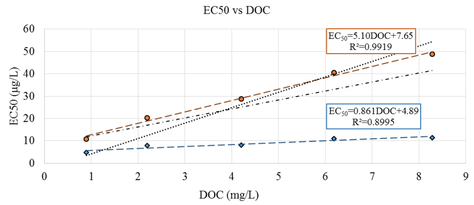

Figure 3. EC50 of *M. galloprovincialis*, over the expected range natural of DOC concentrations in seawater, as Cu_{DISS} (circles) and C_{DGT} Cu (diamonds) proportion normal endpoints. The dash-dot line represents the Arnold et al (2010) Cu_{DISS} EC50 model; the dotted line, Chadwick et al (2008).

Multiple regression analysis was used to test if the spike levels significantly predicted lability degree to the DGTs. The results of the regression indicated the two analytes explained 90.6% of the variance ($_{F(2,32)} = 153$, $p < .001$). Individually, both Cu_{DISS} ($t_{33} = 17.4$, $p<.001$) and DOC concentration ($t_{33} = -5.43$, $p<.001$), were found to significantly predict DGT uptake. This agreement allowed for creation of a training set by which to compare future datasets (Fig. 4).

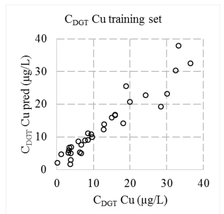

Figure 4. Multiple regression prediction of C_{DGT} Cu using DOC and Cu_{DISS} variables. The regression model will assist in validation of DGT toxicological mimicry under varying DOC source influence during ongoing testing.

A preliminary determination of a Criterion Maximum Concentration (CMC) C_{DGT} Cu of 2.4µg/L allowed for in-progress field studies to place DGT results in context of water quality criteria. This pilot criteria concentration allows for conservative toxicological dose (based on most sensitive life stage of a single EC50 test) to be quantified by setting C_{DGT} EC50 at 0.89mg/L DOC equal to the FAC.

Summary of laboratory determination of C_{DGT} Cu EC50

- The DGT passive samplers have successfully mimicked the highly protective effects afforded to *M. galloprovincialis* by Cu-DOC kinetics within a margin of error that is acceptable for conservative water quality objective monitoring at PSNS.
- The results provide preliminary EC50s ranging from 4.8 to 11.5µg/L as C_{DGT} Cu over the span of 0.892-8.28mg/L DOC.
- C_{DGT} Cu EC50 is recommended to be conservatively viewed as 4.8µg/L when DOC concentrations are unknown.
- A preliminary determination of a CMC C_{DGT} Cu of 2.4µg/L may allowed for in-progress field studies to interpret DGT results in the context of water quality criteria.
- Future research should incorporate naturally sourced seawater, naturally ranging in DOC concentration, to gauge fitment of these preliminary toxicological thresholds.
- Eventual implementation of this monitoring method by regulatory compliance programs potentially allows for datasets that better represent biological effects at relevant scales, by pulse integration, as opposed to single-point grab sampling.
- Project ENVVEST is committed to the use of leading-edge methodology for quantification of analytes of concern.

Results of 3-day DGT deployments at PSNS

The Pacific Northwest National Laboratories Marine Science Laboratory (MSL), as the environmental chemistry branch of Project ENVVEST, has performed 7 DGT monitoring campaigns at PSNS and the surrounding Inlets from 2016-2018, over varying time scales. The three campaigns summarized here represent the stations within PSNS that were monitored via 3-day DGT deployments (Fig 5). These deployments represent integration of pulse inputs at time scales relevant to the toxicological endpoints given by the laboratory derived C_{DGT} Cu EC50 [4,5,6].

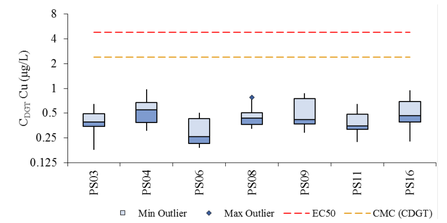

Figure 5. C_{DGT} Cu, integrated over 3 days, of seven monitored stations within the PSNS deployed during 3 campaigns (Dec 2016, Mar 2017 & Aug 2017) in relation to the laboratory obtained EC50, and preliminary C_{DGT} Cu CMC (n=18 per station). The DOC range was 0.644-2.45mg/L.

Stations were selected based on proximity to storm/ dry-dock effluent points, which contribute to a proposed National Pollutant Discharge Elimination System (NPDES) mixing zone. In conjunction with NPDES limits, calculated as the maximum daily load of total recoverable metals at the end of pipe, with the aim of maintaining the watershed at levels below dissolved chronic aquatic life criteria, C_{DGT} provides a measure of the labile fraction, which represents the resulting free ion in the receiving waters and provides realization of end of pipe limit effects with greater accuracy than dissolved fraction measurements. An example of C_{DGT} Cu monitoring is provided as Figure 6, which also demonstrates that the cause of the maximum outlier in Figure 5 is likely a precipitation related pulse.

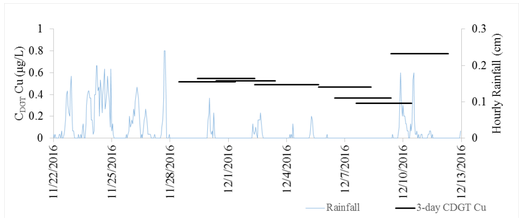

Figure 6. C_{DGT} Cu, pulse capture at PSNS station PS08 during the Dec 2016 campaign, indicating a precipitation induced spike in labile Cu occurred between 12/9 and 12/12/2016.

Figure 7 illustrates the disconnect between the EPA's 2016 draft version of the marine biotic ligand model (MBLM ver. 0.6.2.39) and the passive sampling results as a fraction of their CMCs, as determined by grab sampling that coincided with the passive sampler deployments. This highlights the variability in recorded water quality that occurs when single point-in-time samples are collected, as opposed to integrated pulse values, as well as variable CMC outcomes predicated by variability in study endpoints.

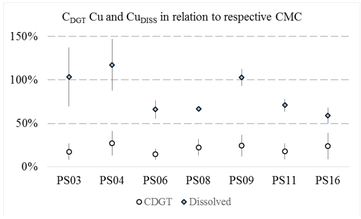

Figure 7. Cu levels in respect to toxicological endpoints by two methods (1) C_{DGT} versus the 0.892mg/L DOC level derived CMC, and (2) Cu_{DISS} versus the MBLM derived CMC.

Summary of DGT monitoring at PSNS

- C_{DGT} Cu values from PSNS campaigns have demonstrated protection of beneficial uses to Sinclair Inlet, resulting from implemented best management practices.
- DGT passive sampling allows capture of precipitation induced pulses of Cu into the marine environment, and integrates over a timescale which potentially more effectively represents the impact of bioavailable metals.
- Disagreement between bioavailability predictions shows the benefit in using time integrated values for assessing water quality; this is especially relevant to wet season monitoring where rapid fluctuations occur.

References
1. Davison, W. (Ed.). (2016). Diffusive Gradients in Thin-films for Environmental Measurements. Cambridge University Press.
2. Arnold, W. R., Cotsifas, J. S., & Corneillie, K. M. (2006). Validation and update of a model used to predict copper toxicity to the marine bivalve Mytilus sp. Environmental Toxicology, 21(1), 65-70.
3. Arnold, W., Cotsifas, J. S., Ogle, R. S., DePalma, S. G., & Smith, D. S. (2010). A comparison of the copper sensitivity of six invertebrate species in ambient salt water of varying dissolved organic matter concentrations. Environmental toxicology and chemistry, 29(2), 311-319.
4. U.S. Environmental Protection Agency (1995). Short-term methods for estimating the chronic toxicity of effluents and receiving waters to west coast marine and estuarine organisms. EPA/600/R- 95/136. National Exposure Research Laboratory, Cincinnati, OH.
5. Angel, B. M., Simpson, S. L., Chariton, A. A., Stauber, J. L., & Jolley, D. F. (2015). Time-averaged copper concentrations from continuous exposures predicts pulsed exposure toxicity to the marine diatom, Phaeodactylum tricornutum: importance of uptake and elimination. Aquatic Toxicology, 164, 1-9.
6. Angel, B. M., Simpson, S. L., Granger, E., Goodwyn, K., & Jolley, D. F. (2017). Time-averaged concentrations are effective for predicting chronic toxicity of varying copper pulse exposures for two freshwater green algae species. Environmental Pollution, 230, 787-797.
7. Zhang, H., & Davison, W. (1995). Performance characteristics of diffusion gradients in thin films for the in situ measurement of trace metals in aqueous solution. Analytical chemistry, 67(19), 3391-3400.
8. Chadwick, D. B., Rivera-Duarte, I., Rosen, G., Wang, P., Santore, R. C., Ryan, A. C., & Choi, W. (2008). Demonstration of an integrated compliance model for predicting copper fate and effects in DoD harbors (No. SPAWAR/CP-TR-1973). SPAWAR Pacific San Diego CA.

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