Special Study Proposal: Contaminants of Emerging Concern (CECs) in Urban Stormwater

Summary: Preliminary results from a 2016 RMP Special Study that scanned Bay water samples for contaminants via non-targeted analysis suggest that stormwater has the potential to contain significant levels of potentially harmful contaminants. An independent effort to probe stormwater-related Coho salmon aquatic toxicity in the Puget Sound region has led to development of a targeted list of key CECs in urban stormwater, which includes contaminants derived from sources such as vehicle tires and urban use pesticides. As part of a West Coast screening effort using this new, targeted analyte list, we propose analyzing stormwater samples collected from urban watersheds discharging to San Francisco Bay.

Three additional classes of emerging contaminants were identified in recent RMP reports and ECWG discussions as critical stormwater data needs: per- and polyfluoroalkyl substances (PFASs), phosphate flame retardants, and ethoxylated surfactants. A minimum two-year study is proposed to provide an intensive and pioneering examination of CECs in urban stormwater. The first year would include site selection and pilot sample collection and analysis for all four CEC classes, and the second year would focus on collecting a greater number of samples for this Bay Area-wide screening study.

Estimated Cost: $447,000 (Year 1 $180,000; Year 2 $267,000; possible Year 3 $0)
Oversight Group: ECWG and SPLWG
Proposed by: Rebecca Sutton (SFEI), Ed Kolodziej (University of Washington), Chris Higgins (Colorado School of Mines), Da Chen (Jinan University), Lee Ferguson (Duke University)

PROPOSED DELIVERABLES AND TIMELINE

Deliverable	Due Date
Task 1. Site selection and reconnaissance, in coordination with SFEI stormwater and STLS teams; development of sampling protocol	Summer 2018
Task 2. Pilot field collection of stormwater samples	Fall – Winter 2018
Task 3. Laboratory analysis of pilot samples; review of data to improve protocols and analytical methods	Spring 2019
Task 4. Site selection and reconnaissance, in coordination with SFEI stormwater and STLS teams; dissemination of sampling protocols to partners recruited to assist in sample collection	Summer 2019
Task 5. Field collection of stormwater samples	Fall 2019 – Spring 2020
Task 6. Laboratory analysis of samples	Summer 2020
Task 7. Review and analysis of data	Fall – Winter 2020
Task 8. Draft manuscripts and summary for managers for ECWG meeting	Spring 2021
Task 9. Final manuscripts and summary for managers	Fall 2021

Background

An important element of the RMP's CEC Strategy is the application of non-targeted methods to identify unexpected contaminants that merit further monitoring (Sutton et al. 2017). In 2016, the RMP funded a special study to use a type of non-targeted analysis to examine Bay water samples collected from three sites influenced by three different pathways: effluent, stormwater, and agricultural runoff.

Preliminary findings from this study, presented at both the ECWG meeting (Ferguson et al. 2017) and the RMP Annual Meeting (Sun et al. 2017) last year, indicate that water samples from the stormwater-influenced site, San Leandro Bay, contained a broad array of unique contaminants with strong signals suggesting higher concentrations. Contaminants identified with high confidence include 1,3-diphenylguanidine (DPG), a rubber vulcanization agent derived from vehicle tires, as well as ε-caprolactam, used to make the nylon polymers found in tires and many other products. The European Chemicals Agency has established predicted no effect concentrations (PNEC) for DPG of 30 µg/L in freshwater and 3 µg/L in marine waters (ECHA 2018). While the non-targeted analysis provides only qualitative data, the high relative strength of the DPG signal in San Leandro Bay suggests that this contaminant has the potential to be present at concentrations similar to these PNECs.

These findings indicate that stormwater is a pathway by which unique contaminants from vehicles and roadways make their way to tributaries and near-shore Bay environments. An additional factor contributing to a special interest in emerging contaminants from stormwater is that, unlike wastewater, this pathway generally receives no treatment. As a result, limited degradation or trapping of contaminants occurs prior to their discharge to the Bay. Furthermore, CEC investigations to date in the RMP and elsewhere have focused primarily on wastewater, and CECs in stormwater have received relatively little attention.

Stormwater-derived contaminants have been an especially high concern and research focus in the Puget Sound region, where adult Coho salmon (*Oncorhynchus kisutch*) in Puget Sound streams experience acute toxicity via pre-spawn mortality following exposure to urban runoff (Du et al. 2017). This response is not correlated with conventional water chemistry parameters including temperature, dissolved oxygen, and suspended solids; disease; spawner conditions; or exposure to monitored pesticides, metals, or polycyclic aromatic hydrocarbons (Scholz et al. 2011).

In an effort to identify the potential cause of this acute toxicity in the Puget Sound area, non-targeted analysis of stormwater and tissues from runoff-exposed fish has resulted in the identification of a number of unique contaminants with sources specific to vehicle traffic. One example is hexa(methoxymethyl)melamine (HMMM), a component of tire resin, which can occur in highway runoff at concentrations exceeding 10 µg/L (Kolodziej, unpublished data). More recent research indicates that aqueous leachates from automobile tires can induce acute toxicity in Coho salmon, leading to a focus on understanding the risks of this pollutant source to salmonids and other aquatic organisms. In addition to the acute effects, related ecotoxicology research suggests that stormwater exposure can induce altered growth, decreased immune function, impaired lateral line development, and cardiotoxicity in salmonids (McIntyre et al. 2016; Young et al. 2018), suggesting that a suite of adverse

sublethal impacts derived from stormwater exposures are important aspects of water quality in urbanized areas.

A direct outcome from these non-targeted analytical efforts was the development, by Dr. Kolodziej, of a list of target analytes to assess the stormwater pathway as major contaminant inputs. While there are a number of targeted CEC lists designed around the influence of wastewater (e.g., focused on pharmaceuticals and other compounds typically disposed of down the drain), this is the first major effort to develop a CEC list targeting the influence of urban runoff in aquatic habitats with a concerted analytical effort.

The RMP has the opportunity to take part in a West Coast-wide screening effort, analyzing Bay Area stormwater using this new list of targeted CECs derived from vehicular sources, urban use pesticides, and other ubiquitous urban contaminants. While the endangered Coho salmon, the focus of the Puget Sound research effort, are now absent from tributaries discharging to the Bay, steelhead (*Oncorhynchus mykiss*), a threatened species, are observed in some Bay streams (e.g., Guadalupe River, Alameda Creek).

In addition to this newly developed list of urban stormwater CECs, three more classes of emerging contaminants have been identified in recent RMP documents and ECWG discussion as critical data gaps for stormwater.

Per- and polyfluoroalkyl substances (PFASs) – PFOS, PFOA, and other long-chain perfluorocarboxylates are classified as Moderate Concerns for the Bay, while other PFASs are considered Possible Concerns. A conceptual model of sources of PFASs to stormwater includes outdoor textiles, plastic items, paints, and urban litter (e.g., food packaging), as well as industrial products such as fire-fighting foams. Atmospheric deposition is also possible. The RMP's draft PFAS Synthesis and Strategy (Sedlak et al. 2017) reviewed two studies of stormwater that have been conducted in the Bay Area: a seven site study conducted in Water Year 2010, and a 10 site study conducted in Water Year 2011. A relatively small number of PFASs were monitored; in addition, the watersheds monitored were not specifically selected to provide representative data for these contaminants in the Bay Area. The PFAS Synthesis and Strategy recommends stormwater monitoring as an RMP priority for future work.

Phosphate flame retardants – At present, alternative flame retardants are generally considered Possible Concerns for San Francisco Bay. A conceptual model of sources of these contaminants to stormwater includes outdoor products such as construction and building materials, as well as volatilization from a far broader assortment of consumer goods to the air followed by deposition to urban streams. Samples collected during two storms (Water Year 2014) at two Bay Area stormwater sites indicate the presence of phosphate flame retardants at concentrations generally comparable to those found in wastewater (Sutton et al. in prep). A draft RMP report that reviews available data for this class of CECs recommends stormwater monitoring as a priority for the RMP (Lin and Sutton 2018).

Ethoxylated surfactants – Ethoxylated surfactants include alkylphenol ethoxylates (classified as Moderate Concerns for the Bay), as well as alcohol ethoxylates and others. A conceptual model of sources of ethoxylated surfactants to stormwater includes outdoor use and automotive cleaners, lubricants and other fluids, as well as pesticides, plastics, paints, and many other products. The non-targeted analysis of San Francisco Bay sites described

previously also identified a number of ethoxylated surfactants at with strong signals in the stormwater-influenced site, San Leandro Bay (Ferguson et al. 2017; Sun et al. 2017).

At the spring ECWG meeting (April 12-13, 2018), stakeholders and experts expressed strong support for a broad screening of stormwater for all four groups of CECs mentioned above. At the spring SPLWG meeting (May 23, 2018), stakeholders and experts expressed general support as well. The budget was increased to provide additional funding for sample collection, as recommended by the SPLWG.

The SPLWG also noted that the study might need to be extended for a third year to enable collection of all targeted samples. At present, the yearly budget allocations and deliverables schedules in this proposal are designed for a two-year study, with the understanding that if insufficient samples are collected in the first two years, funds would be carried over to complete the study during the third year, with deliverable deadlines extended accordingly.

Study Objectives and Applicable RMP Management Questions

Table 1. Study objectives and questions relevant to RMP ECWG management questions

Management Question	Study Objective	Example Information Application
1) Which CECs have the potential to adversely impact beneficial uses in San Francisco Bay?	Compare new occurrence data for stormwater CECs with toxicity information reported in the scientific literature. Evaluate future monitoring needs and toxicity data gaps.	Do any stormwater CECs merit additional monitoring in the Bay or a specific classification in the tiered risk framework? What are the potential risks of these CECs? Is a need for management actions indicated?
2) What are the sources, pathways and loadings leading to the presence of individual CECs or groups of CECs in the Bay?	Compare concentrations observed at different sites in the Bay Area to glean possible insights regarding the influence of sources or land use types. Compare Bay Area concentrations to other measurements of other urban areas.	What are the key sources or land uses that are associated with idividual CECs or CEC classes in stormwater?
3) What are the physical, chemical, and biological processes that may affect the transport and fate of individual CECs or groups of CECs in the Bay?	N/A	

4) Have the concentrations of individual CECs or groups of CECs increased or decreased in the Bay?	Compare concentrations with previous monitoring data for a limited number of analytes.	The data from this study can establish baseline data for stormwater CECs in the Bay Area. Instructive comparisons are possible for a subset of analytes previously examined in Bay Area stormwater, though robust trends cannot be inferred due to data limitations.
5) Are the concentrations of individual CECs or groups of CECs predicted to increase or decrease in the future?	N/A	
6) What are the effects of management actions?	N/A	

Approach

Stormwater Sample Collection

For this screening effort, two phases are recommended. Pilot sample collection will occur in fall and winter of 2018, and a larger monitoring effort, supplemented with field work by stakeholder partners, will occur fall through spring of 2019/2020. If insufficient samples are collected within two years, extension to a third year is possible.

Site selection will occur prior to each of these sample collection campaigns, in consultation with the stormwater loading team at SFEI, the RMP's Small Tributaries Loading Strategy (STLS) team, and the California Department of Pesticide Regulation (DPR). Sites will be selected based on multiple factors including: 1) greater relative urban land use in the watershed, with an emphasis on proximity to roadways; 2) unique land uses associated with potential contaminant sources, such as airports; and 3) reduced sample collection costs due to existing sample collection underway as part of other studies. Site selection will be informed by the conceptual models of potential sources of the CECs to stormwater, with sites located in proximity to these sources being of particular interest. Two tiers of sites will be identified, a top tier of highest priority that is tied to exploring specific characteristics of the CECs, and a lower tier of sites for which there is more flexibility and therefore a greater ability to leverage other stormwater monitoring activities.

During pilot sample collection, 10-20 samples (including field blank and duplicate samples) will be collected. Samples will consist of grabs or composites collected into pre-cleaned amber glass containers. Composites collected using an ISCO pump during the rising hydrograph of a storm are preferred (though not required) for use with the new stormwater CECs analyte list developed by Dr. Kolodziej. For the other types of contaminants, use of the ISCO pump may lead to procedural contamination. For these contaminants, one or more grab samples will be collected at each site, and may be combined in the analytical laboratory to produce a composite.

Particular focus will be placed on capturing the first fall flush at one or more sites of interest, with storm size criteria to be developed in consultation with STLS and RMP experts. At least

one site will be revisited during a later storm as an initial means of assessing variability. QA/QC samples collected will include at least one field duplicate and one field blank. A greater number of samples (e.g., 20) will be collected during the pilot phase if a number of storms pass through the region, in case sample collection proves difficult during the next phase due to fewer storms.

Review of preliminary data from this pilot effort and further consultation with local stormwater experts in 2019 will support refined sample collection protocols and site selection for the larger monitoring effort to occur fall 2019 – spring 2020. This more comprehensive sample collection effort is expected to produce 30-40 samples (including field blank and duplicate samples), in part through in-kind assistance from interested stormwater agencies and the leveraging of existing monitoring activities by the RMP and agencies such as DPR. Training and equipment will be provided to these sample collection partners. If insufficient samples are collected within two years, extension to a third year is possible.

Chemical Analysis

A total of 50 stormwater samples (including field duplicates and field blanks) will be characterized over a minimum of two years for four sets of CECs, by four different academic laboratories with specialized expertise.

Stormwater CECs: Unfiltered samples will be analyzed by the Kolodziej laboratory (University of Washington) with a newly developed, targeted analytical method using multi-residue solid phase extraction (SPE) and liquid chromatography with tandem mass spectroscopy (LC-MS/MS). Approximately 35 compounds will be monitored, including pharmaceuticals, pesticides, and several vehicle-specific analytes such as DPG and HMMM. A description of the analytes is provided as a separate attachment. This suite of representative tracers for urban runoff includes a broad range of contaminants with different physical-chemical parameters (e.g., various chemical functionalities, wide range of polarities and biodegradation potential). The compounds were selected to represent three primary urban sources: residential use, roadways, and wastewater.

PFASs: Unfiltered samples will be analyzed by the Higgins laboratory (Colorado School of Mines) using quadrupole time-of-flight mass spectrometry (LC-Q-ToF-MS). The samples will be extracted and cleaned up using established protocols for the analysis of PFASs in soils and sediments (McGuire et al. 2014; Barzen-Hanson et al. 2017). Each sample will be split, with one aliquot being subjected to the TOP assay (oxidation followed by LC-QToF-MS; Houtz and Sedlak, 2012) and the other aliquot being directly analyzed by LC-QToF-MS. The stormwater extracts will be injected and separated on a C18 column prior to analysis by both ESI+ and ESI- LC-QToF-MS. Quantitative analysis will be performed on 45 PFASs, including different perfluoroalkanoic acids, perfluoroalkane sulfonates, perfluoroalkane sulfonamides, fluorotelomer sulfonates, and fluorotelomer alkanoic acids. This list includes PFASs on the UCMR3 list along with many others.

Phosphate Flame Retardants: Both dissolved and particulate phase samples will be analyzed by the Chen laboratory of Jinan University. Samples will be extracted in the U.S. by a partner laboratory, then shipped to China, where Dr. Chen will characterize contaminants within the aqueous and solid phases using highly sensitive liquid chromatography–triple quadrupole

mass spectrometry (LC-QQQ-MS/MS) based analysis methods (Chen et al. 2012; Chu et al. 2011). Limits of detection are typically in the range of 0.1 ppb. Dr. Chen has agreed to undertake method development to add recently identified phosphate flame retardants, including isopropylated and tert-butylated triarylphosphate esters (ITPs and TBPPs; Phillips et al. 2017), to his extensive list of target analytes.

Ethoxylated Surfactants: Stormwater samples will be analyzed for ethoxylated surfactants by the Ferguson laboratory of Duke University, using a method to be developed. The matrix is likely to be total water, and the analyte list is expected to include the following surfactant families: nonylphenol ethoxylates, octylphenol ethoxylates, and C12, C14, and C16 alcohol ethoxylates. Analytes for each family will include compounds with a broad range of ethoxylate chains. Isotopically labeled standards are only available for a few of these analytes; however, the uncertainty associated with quantitation was deemed acceptable by the ECWG for screening purposes.

Data Interpretation

We anticipate most of these contaminants will be widely observed in urban areas but have lower concentrations in non-urban areas. Therefore, the screening data will be evaluated based on land use type. Specific indicators of source types, such as road density, will be used for an initial investigation into key sources or land uses associated with these CECs.

Results for the Bay Area will also be compared to levels observed in other urban regions. In particular, Dr. Kolodziej is coordinating a West Coast wide sampling effort, which is likely to include southern California, the Portland area, and the Seattle/Puget Sound area.

Levels in Bay Area stormwater will also be compared to available toxicity thresholds. Findings may highlight concerns, data gaps, and the need for further research.

Budget

Budget Justification

The budget is outlined for a two-year study. If insufficient stormwater samples are collected within two years, funding from the second year may be carried over into a third year, to support sample collection, data quality assurance and upload, and review and reporting.

Planning and Stakeholder Engagement Costs

In consultation with RMP and STLS stormwater experts, we will establish a study design and basic sampling and analysis plan that includes site selection criteria, a tiered list of potential sites, and storm criteria. Study design established in advance of the pilot sampling effort is likely to be refined prior to the second phase of sampling, based on experience in the field and initial laboratory analyses. Study design discussions and preliminary data reports will require regular participation in monthly calls with the STLS team.

Stormwater agencies will be recruited to provide in-kind assistance through sample collection during the second wet season, to establish a more comprehensive and representative dataset for this initial screening. Training and equipment will be provided to

assure sample collection goes smoothly. At the conclusion of the study, SFEI staff will craft a presentation of findings for stormwater agencies.

Field Costs
This special study proposal includes up to $144,000 devoted to stormwater sample collection (site selection and reconnaissance, permit applications, development of sample collection protocols, and field work). Sample collection will occur over two wet seasons.

Every effort will be made to minimize field costs through leveraging existing stormwater monitoring activities of the RMP and the California Department of Pesticide Regulation (DPR). DPR plans to monitor three Bay Area stormwater sites for pesticides, and may be able to collect stormwater samples for RMP studies.

Data Management Costs
Data services will include quality assurance and upload to CEDEN.

Analysis and Reporting Costs
Preliminary results will be reported to and reviewed by ECWG, STLS, and SPLWG. Preparation of draft manuscripts for publication in a peer-reviewed journal (stormwater-themed special issue) would generally be led by the analytical partners; RMP scientists may be lead authors of one of the manuscripts, and coauthors of others. After the manuscripts are complete, RMP staff will produce a summary document for managers that describes the results and their implications for stakeholders.

Laboratory Costs
Each laboratory is receiving a budget sufficient to refine method development and analyze 50 samples. Laboratory QA/QC samples will be analyzed at no charge, while field blanks and field duplicates will be considered part of the 50 samples charged to the RMP.

Table 2. 2019 CECs in Stormwater budget

Expense	Estimated Hours	Estimated Cost
Labor - Year 1		
Study Design, Stakeholder Engagement	130	18,000
Stormwater Sample Collection	444	67,500
Labor - Year 2*		
Study Design, Stakeholder Engagement	170	25,000
Stormwater Sample Collection	516	76,500
Data Technical Services		43,000
Analysis and Reporting	370	60,000
Subcontracts		
Stormwater CECs: Kolodziej, U. Washington		35,000
PFASs: Higgins, Colorado School of Mines		30,000
Phosphate Flame Retardants: Chen, Jinan U.		35,000
Ethoxylated Surfactants: Ferguson, Duke U.		30,000
Direct Costs		
Equipment		3,000
Travel		4,000
Shipping		20,000
Grand Total		447,000

*If insufficient samples are collected in the first two years, a third year of sample collection may be necessary. In this situation, Labor activities specified in Year 2, including Data Technical Services and Analysis and Reporting, will be performed in Year 3, using leftover Year 2 funds.

Reporting

Deliverables will include: a) draft manuscripts[1] that serve as RMP technical reports, due spring 2021; b) a summary for managers describing the results and their implications, due spring 2021; and c) additions to other RMP publications such as the Pulse. If the study is extended to a third year, deadlines for reports will likewise be extended by one year (to 2022).

References

Barzen-Hanson KA, Davis SE, Kleber M, Field JA. 2017. Sorption of fluorotelomer sulfonates, fluorotelomer sulfonamido betaines, and a fluorotelomer sulfonamido amine in National Foam Aqueous Film-Forming Foam to soil. Environ Sci Technol 51(21):12394-12404.

Chen D, Letcher RJ, Chu S. 2012. Determination of non-halogenated, chlorinated and brominated organophosphate flame retardants in herring gull eggs based on liquid chromatography – tandem quadrupole mass spectrometry. J Chromatogr A 1220: 169-174.

Chu S, Chen D, Letcher RJ. 2011. Dicationic ion-pairing of phosphoric acid diesters post-liquid chromatography and subsequent determination by electrospray positive ionization-tandem mass spectrometry. J Chromatogr A 1218(44): 8083-8088.

Du B, Lofton JM, Peter KT, Gipe AD, James CA, McIntyre JK, Scholz NL, Baker JE, Kolodziej EP. 2017. Development of suspect and non-target screening methods for detection of organic contaminants in highway runoff and fish tissue with high-resolution time-of-flight mass spectrometry. Environ Sci Process Impacts 19(9):1185-1196.

ECHA (European Chemicals Agency). 2018. Brief Profile: 1,3-diphenylguanidine. https://echa.europa.eu/brief-profile/-/briefprofile/100.002.730

Ferguson PL, Overdahl KE, DeStefano N, Sun J, Sutton R. 2017. Application of Non-targeted Analysis Approaches to Assess Organic Micropollutants in the San Francisco Bay. Emerging Contaminants Workgroup Meeting, Regional Monitoring Program for Water Quality in San Francisco Bay. Richmond, CA.

Houtz E, Sedlak D. 2012. Oxidative conversion as a means of detecting precursors to perfluoroalkyl acids in urban runoff. Environ Sci Technol 46(17):9342-9349.

Lin D, Sutton R. 2018. Alternative Flame Retardants in San Francisco Bay: Synthesis and Strategy. Draft Report. San Francisco Estuary Institute, Richmond, CA.

[1] The draft manuscript will be distributed to RMP stakeholders for review by email, not published on the website, so as to not jeopardize publication of the manuscript in a peer-reviewed journal.

McGuire ME, Schaefer C, Richards T, Backe WJ, Field JA, Houtz E, Sedlak DL, Guelfo JL, Wunsch A, Higgins CP. 2014. Evidence of remediation-induced alteration of subsurface poly- and perfluoroalkyl substance distribution at a former firefighter training area. Environ Sci Technol 48(12):6644-6652.

McIntyre J.K., Edmunds R.C., Redig M.G., Murdock E.M., Davis J.W., Incardona J.P., Stark J.D., Scholz, N.L. 2016. Confirmation of Stormwater Bioretention Treatment Effectiveness Using Molecular Indicators of Cardiovascular Toxicity in Developing Fish. Environmental Science and Technology, 50(3) 1561-1569.

Phillips, A.L., Hammel, S.C., Konstantinov, A., Stapleton, H.M., 2017. Characterization of individual isopropylated and tert-butylated triarylphosphate (ITP and TBPP) isomers in several commercial flame retardant mixtures and house dust standard reference material SRM 2585. 51(22):13443-13449.

Scholz NL, Myers MS, McCarthy SG, Labenia JS, McIntyre JK, et al. (2011) Recurrent Die-Offs of Adult Coho Salmon Returning to Spawn in Puget Sound Lowland Urban Streams. PLoS ONE 6(12): e28013. doi:10.1371/journal.pone.0028013

Sedlak M, Sutton R, Wong A, Lin D. 2017. Per and Polyfluoroalkyl Substances (PFASs) in San Francisco Bay: Synthesis and Strategy. Draft Report. San Francisco Estuary Institute, Richmond, CA.

Sun J, Sutton R, Ferguson L, Overdahl K. 2017. Water Quality Monitoring 2.0: Using Non-Targeted Analysis to Find What We Have Been Missing. Annual Meeting of the Regional Monitoring Program for Water Quality for San Francisco Bay. Berkeley, CA. http://www.sfei.org/projects/rmp-annual-meeting

Sutton R, Sedlak M, Sun J, Lin D. 2017. Contaminants of Emerging Concern in San Francisco Bay: A Strategy for Future Investigations. 2017 Revision. SFEI Contribution 815. San Francisco Estuary Institute, Richmond, CA. http://www.sfei.org/documents/contaminants-emerging-concern-san-francisco-bay-strategy-future-investigations-2017

Sutton, R., Chen, D., Sun, J., Sedlak, M., Greig, D., in prep. Characterization of Brominated, Chlorinated, and Phosphate Flame Retardants in an Urban Estuary.

Young, A, Kochenkov, V., Mcintyre, J.K., Stark, J.D., Coffin, A.B. 2018. Urban stormwater runoff negatively impacts lateral line development in larval zebrafish and salmon embryos. Scientific Reports, In press.