2012 Survey of Trihalomethanes in Finfish Collected Near the Outfalls of Domestic Wastewater Treatment Facilities

Florida Department of Environmental Protection

Bureau of Laboratories
Division of Environmental Assessment and Restoration

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Executive Summary

During the summer and early fall of 2012, the Florida Department of Environmental Protection's (FDEPs) Bureau of Laboratories conducted investigations into the presence of trihalomethanes (THMs), a class of disinfection by-products, in fish that were caught near the outfalls of domestic wastewater treatment facilities. The study had three specific goals:

1. Document the presence of THMs in the edible fillets from a variety of marine/estuarine and freshwater fishes collected near the outfalls of domestic wastewater treatment plants. Those data were compared to data from fishes collected at marine and freshwater background sites where no known sources of THMs were present;
2. Evaluate the effect of sample treatment on THM levels in the edible tissue. One commonly employed method to preserve concentrations of contaminants in biological media is to freeze tissues as soon as possible after collection. However, most recreationally-caught fish are not immediately frozen when caught; placing the fish or their fillets on wet ice is a common practice. The study examined whether significant losses of THMs occur when fish are not immediately frozen; conversely, since ice may contain THMs, the study was also designed to evaluate whether storage on ice might contribute to THMs in the tissue.
3. Evaluate the role of cooking on THM levels in edible fish tissue. The null hypothesis that cooking would not reduce or eliminate THMs from the edible tissue was tested;

Thirteen (13) domestic wastewater facilities were identified for sampling. Additionally, one freshwater and two marine background sites were selected for fish collection. The background sites were located far from any known discharges or other sources of THMs. At each facility, fish were harvested for the analysis of THMs in the edible muscle tissue near the wastewater discharges. Additionally, samples were collected for the analysis of THMs and sucralose from each facility's effluent and from the surface water at a point near where fish were harvested.

Concentrations of individual THMs (chloroform, bromoform, bromodichloromethane and dibromochloromethane) found in edible fillets from fish caught near domestic wastewater discharges varied considerably. Levels of some THMs in individual fish fillets exceeded 100 µg/Kg. Neither wet ice preservation of fish following capture nor cooking had any appreciable effect on the concentration of THMs in edible fillet portions.

Introduction

Trihalomethanes (THMs) are a class of chemical compounds often produced as by-products of disinfection from the treatment of potable or non-potable water. These compounds are produced by the reaction of chlorine and bromine with organic matter present in the water being treated. The four major disinfection by-products are chloroform, bromoform, bromodichloromethane and dibromochloromethane.

Wastewater discharges can contain elevated levels of THMs. By rule, THMs are allowed to be present in drinking water up to 80 µg/L (as total THMs). In addition to the THM load reaching domestic wastewater treatment facilities as influent, many facilities use chlorine to disinfect treated effluent prior to discharge, potentially producing additional THMs.

Despite efforts to limit the generation and discharge of THMs, many domestic wastewater treatment facilities have difficulties controlling these compounds in their discharge. In a pollutant discharge survey conducted in 2011-12 by the Bureau of Laboratories, it was estimated that more than 10% of Florida's domestic wastewater treatment facilities that discharge to State surface waters may have difficulties complying (at the point of discharge) with Florida's existing surface water quality criteria for THMs. That percentage would likely increase if the fish consumption rate used to derive human health-based water quality criteria is increased.

Water quality criteria derived for the protection of human health are dependent, to a large extent, on the degree to which each regulated chemical compound is concentrated or accumulated in the edible tissues of aquatic life (e.g., fish and shellfish). For THMs, there is scant reliable data on field or laboratory measured bioaccumulation or bioconcentration factors. In such cases, criteria are derived primarily from other information (such as the octanol:water partition coefficient) and adjusted for the lipid content of edible fish and shellfish. The bioaccumulation factors (BAFs) for THMs are quite low (3.75) when compared to BAFs of other organic compounds, which can be in the thousands to tens of thousands. Low BAFs indicate that THMs have a lesser potential to accumulate in biological tissues than many other organic contaminants.

Due to the lack of field-measured data on THMs in aquatic biota and their prevalence in some wastewater outfalls, the Bureau of Laboratories engaged in a study to determine whether there exists any real risk of THM exposure to humans by consuming fish caught near wastewater outfalls. The study focused on facilities discharging a range of THMs to estuarine and freshwater systems and had three specific goals:

1. Document the presence of THMs in the edible fillets from a variety of marine/estuarine and freshwater fishes collected near the outfalls of domestic wastewater treatment plants. Those data were compared to data from fishes collected at marine and freshwater background sites where no known sources of THMs were present;
2. Evaluate the effect of sample treatment on THM levels in the edible tissue. One commonly employed method to preserve concentrations of contaminants in biological media is to freeze tissues as soon as possible after collection. However, most recreationally-caught fish are not immediately frozen when caught; placing the fish or their fillets on wet ice is a common practice. The study examined whether significant losses of THMs occur when fish are not immediately frozen; conversely, since ice may contain THMs, the study was also designed to evaluate whether ice could contribute to THMs in the tissue.

3. Evaluate the role of cooking on THM levels in edible fish tissue. The null hypothesis that cooking would not reduce or eliminate THMs from the edible tissue was tested;

Materials and Methods

Facility Selection

In order to address the goals of the study enumerated above, facilities were selected that met the following criteria:

- Had measureable THMs present in the discharge during previous sampling;
- Had a discharge to a waterbody of sufficiently small size or with minimal mixing such that the discharge could be identified within the receiving waterbody where fish might reside. The intent was to capture fish within the influence of the discharge;
- Had easy access to the discharge point and the waterbody near the discharge;
- Had a reasonable potential for successfully harvesting of fish near the outfall (by hook/line, electro-shocking or netting/seining);

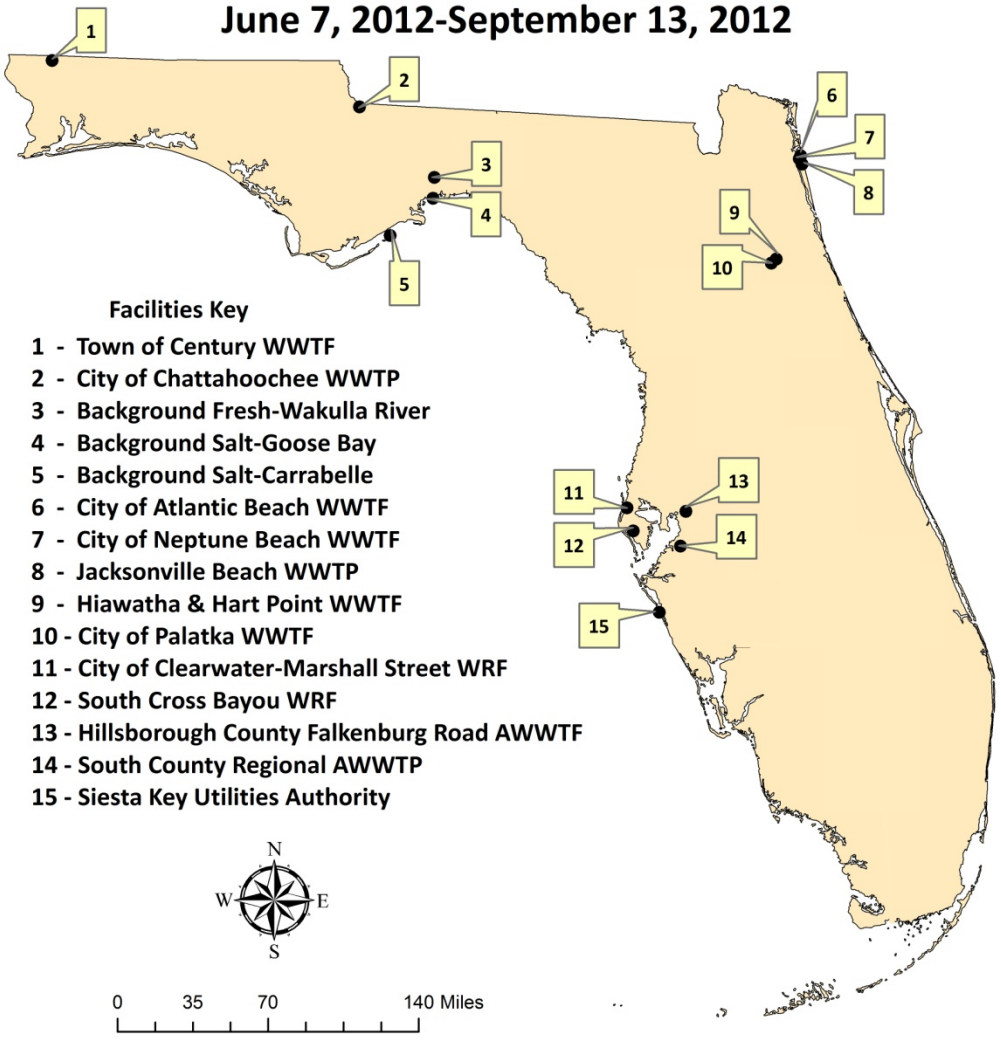
Using the criteria described above, thirteen (13) domestic wastewater facilities were identified for sampling. Additionally, one freshwater and two marine background sites were selected for fish collection. The background sites were located far from any known discharges or other sources of THMs. The facility locations and background sites are listed in Table 1 and depicted on the map below (Figure 1).

Table 1 – Facility and Background Sampling Location Information

Facility Name	Receiving Water	Latitude (North)	Longitude (West)	NPDES #
Background-Wakulla River	Fresh	30° 12' 47.34"	84° 15' 39.60"	-
Background-Carrabelle	Marine	29° 49' 11.42"	84° 36' 2.41"	-
Background-Goose Bay	Marine	30° 4' 16.39"	84° 16' 13.37"	-
City of Atlantic Beach WWTF*	Marine	30° 20' 7.84"	81° 24' 31.75"	FL0038776
City of Chattahoochee WWTP	Fresh	30° 41' 12.01"	84° 50' 47.36"	FL0027669
City of Clearwater-Marshall Street WRF**	Marine	27° 58' 52.82"	82° 47' 14.35"	FL0021857
City of Neptune Beach WWTF*	Marine	30° 18' 56.09"	81° 25' 12.25"	FL0020427
City of Palatka WWTF	Fresh	29° 36' 56.95"	81° 39' 4.43"	FL0040061
Hiawatha & Hart Point WWTF	Fresh	29° 38' 32.96"	81° 36' 48.56"	FL0043389
Hillsborough County Falkenburg Road AWWTF	Marine	27° 57' 10.62"	82° 20' 22.49"	FL0040614
Jacksonville Beach WWTP*	Marine	30° 16' 43.10"	81° 23' 54.20"	FL0020231
Siesta Key Utilities Authority	Marine	27° 16' 33.85"	82° 33' 3.24"	FL0025755
South County Regional AWWTP	Marine	27° 43' 13.55"	82° 23' 2.26"	FL0028061
South Cross Bayou WRF	Marine	27° 49' 33.24"	82° 44' 31.92"	FL0040436
Town of Century WWTF	Fresh	30° 57' 53.96"	87° 15' 25.96"	FL0032468

* There was only one combined discharge for these three facilities;
** Fish collected near this facility were used to validate analytical and field collection methods; however, due to difficulties capturing fish near the outfall, the site was not used to examine THMs following wet ice storage or cooking ;

Figure 1
Human Health Criteria
Fish Tissue Sampling Facility Locations
June 7, 2012-September 13, 2012



Sample Collection

The goal of the study was to capture up to twelve (12) fish from an area under the influence of the facilities' discharges. Upon capture, the fish were processed and enough of the edible, skin-free muscle tissue was removed to fill (to the extent possible) a 60-ml wide-mouth glass jar with a Teflon-lined lid. That aliquot of muscle tissue (fillet) was frozen immediately on dry ice and labeled 'Dry Ice'. The remainder of the fish was labeled, placed in a plastic bag, and stored on wet ice for approximately four hours in a closed cooler. Because THMs are relatively volatile, an assumption was made that the plastic bags would present a minimal barrier for either losses or amendments of THMs to the fish. The use of plastic bags allowed for proper labeling and segregation of similarly sized fish of the same species that were caught at different times.

At the end of the four hour waiting period, two more aliquots of fillets were taken from the fish stored on wet ice. Those aliquots were placed in separate 60-ml jars as described above. One of those two aliquots (labeled 'Wet Ice') was to assess losses or amendments to the THM burden after storage on wet ice relative to the aliquot frozen immediately. The other aliquot (labeled 'Cook') was used for testing for loss of THMs upon cooking. Both the jars containing the 'Wet Ice' and 'Cook' aliquots were immediately frozen on dry ice for transport back to the laboratory.

In addition to the fish tissue, a surface water grab sample was collected 0.5 meters below the surface in the vicinity of the outfall where fish were caught. The water sample was analyzed for THMs and sucralose using protocols outlined in Standard Operating Procedures (SOPs) published by the Florida Department of Environmental Protection (http://www.dep.state.fl.us/water/sas/sop/index.htm). Conductivity, temperature, pH, and dissolved oxygen were measured in-situ using either a YSI 650 Multiparameter Display System or Professional Plus portable field meter with appropriate sondes. Facility effluent grab samples were collected at, or prior to, the point of discharge using the sampling protocols referenced above. Field blanks were collected for THMs and sucralose at all sites and trip blanks for THMs were transported to and from the field. All aqueous samples were placed in coolers on wet ice immediately following collection for transportation to the laboratory.

Sucralose, an artificial sweetener, is highly soluble in water, resistant to degradation and not effectively removed by metabolic or wastewater treatment processes. Analytical methods are available to measure sucralose at low parts-per-trillion concentration. These characteristics make sucralose an excellent tracer for the presence of wastewater. Samples were analyzed for sucralose as an aid to assess the presence of diluted effluent at each location where fish were collected.

Sample Analysis

Upon receipt by the laboratory, each aliquot of fish fillet was frozen at minus twenty centigrade (-20° C) until preparation and analysis could be performed. All water samples (including blanks) were stored at a nominal temperature of 4° C (0° – 6° C).

For the analysis of THMs in raw fish fillets, frozen tissue samples were shaved with a razor or dermatome tool into thin, nearly transparent slices. One (1) gram of the frozen shavings was transferred to a septa sealed 40 ml VOA vial along with 5 ml of deionized water, one drop of Anti-Foam Agent A and a small magnetic stir bar. All quality control samples (method blanks, matrix spikes, replicates and laboratory control samples) were treated in a similar fashion. The vials containing tissue samples and quality control samples were agitated on a shaker table at 1500 rpm for 30 minutes. The magnetic stir bar aided in breaking the tissue up and creating an ultra-fine suspension of particles. The samples were subsequently analyzed by heated purge and trap, gas chromatography/mass spectroscopy (P&T-GCMS) using a Tekmar Atomx P&T system and an Agilent 7890 gas chromatography system coupled with an Agilent 5975 mass spectrometer. All surrogates and internal standards were added prior to the analysis. Details on the full analytical method may be found at: ftp://ftp.dep.state.fl.us/pub/labs/lds/sops/5734.pdf. Instrument and purge settings are at: ftp://ftp.dep.state.fl.us/pub/labs/lds/sops/5735.pdf.

Cooked fish fillet portions were also analyzed for THMs as described above. The cooking procedure followed the microwave cooking recommendations cited in NOAA Technical Memorandum NOS OR&R9 'Guidance on Sensory Testing and Monitoring of Seafood for Presence of Petroleum Taint Following an Oil Spill, http://archive.orr.noaa.gov/book_shelf/964_seafood.pdf, page 47, Table 2. The cooking procedure was performed in a Milestone Ethos Microwave Digestion Labstation equipped with a temperature probe to control the heating cycle. For cooking, fifteen (15) grams of frozen tissue was placed into a clean, Teflon microwave digestion vessel and ramped to 71.1° C (160° F) over 8 minutes and held at that temperature for an additional 8 minutes. After cooling to room temperature (~ 15 minutes) the tissue was either weighed out for immediate preparation and analysis or refrozen until preparation and analysis could be performed. Details of the cooking procedure are published with the analytical procedure (ftp://ftp.dep.state.fl.us/pub/labs/lds/sops/5734.pdf).

Aqueous samples (surface water, effluent and field/trip blanks) were analyzed for THMs using EPA Method 624; sucralose was analyzed using an Applied Biosystems API 3200 high pressure liquid chromatography with tandem mass spectroscopy detection following solid phase extraction on a Waters Oasis HLB column. Details of sample preparation and analysis can be found at: ftp://ftp.dep.state.fl.us/pub/labs/lds/sops/5663.pdf and ftp://ftp.dep.state.fl.us/pub/labs/lds/sops/5673.pdf. All quality control checks (laboratory control samples, matrix spikes, replicates, method blanks, etc.) were

performed as described in the Bureau of Laboratories standard operating procedures and quality manual (http://www.dep.state.fl.us/labs/library/lab_sops.htm).

Results and Discussion

Table 2 gives the species of wild fish collected from background sites and from the vicinity of the domestic wastewater discharges sampled in this study. The majority of fish harvested were collected by seine or trawl nets with the assistance of the Florida Fish and Wildlife Conservation Commission, although some were caught using hook and line (which was used predominately at the background sites) and electro-shocking. While every effort was made to collect fish from the influence of the discharge, there was no knowledge of the actual exposure captured fish might have had to the wastewater. A water sample was collected from the general location where the fish were harvested; however that sample may not adequately represent the concentration of THMs to which the captured fish were actually exposed. Additionally, no information on the duration of exposure to THMs by harvested fish may be gleaned from this study. To adequately assess exposure, controlled dosing studies would be needed. As stated earlier, one goal of this study was merely to document the THM concentrations found in wild fish caught in the vicinity of domestic wastewater treatment discharges.

Concentrations of individual THMs (chloroform, bromoform, bromodichloromethane and dibromochloromethane) found in edible fillets from fish caught near domestic wastewater discharges are depicted in Figures 2a – d below. Box-and-whisker plots depict the range of the THMs found in in edible tissues following three different treatments: 1) tissue placed immediately on dry ice after fish harvesting; 2) tissue collected from fish that were stored on wet ice for four (4) hours following harvesting; and 3) cooked tissue from fish that were stored on wet ice for four hours after harvesting. Also shown is the facility THM effluent concentration (on the upper 'x' axis), the THM instream concentration at a location close to where fish were harvested (on the lower 'x' axis) and the number of fish harvested at each site. Note that one of the sites where fish were harvested was located near the combined discharge from three different facilities; therefore a single discrete effluent concentration was not depicted for that site. Instead, that site is labeled 'Combo' on the charts and the effluent THM values are provided in the upper right margin of the figures (2a – d). Data from background sites are not shown in Figure 2; all tissue and water concentrations from the three background sites were below analytical detection limits for THMs.

Several observations and conclusions may be drawn from these box-and-whisker plots. First, THMs can accumulate in edible fish tissue to surprisingly high concentrations. Individual fish collected at one facility had tissue concentrations of dibromochloromethane in excess of 100 µg/Kg. Amongst the fish with measureable THM concentrations in tissues, chloroform generally appeared to accumulate to the least extent. For example, at the facility where eleven (11) fish were captured, the chloroform concentration measured in the effluent and surface

water was comparable to that of bromodichloromethane and dibromochloromethane. Even in the absence of exposure information, the effluent and surface water data suggest that fish at that site are likely exposed to nearly the same level of all three THMs. However, the amount of chloroform in fish tissue was much less than concentrations observed for the other two disinfection by-products (i.e., tissue values for chloroform were roughly one half to one quarter of the tissue concentrations found for other two THMs). Whether this observation stems from mechanisms causing a different exposure to chloroform or to characteristics of the individual THMs causing different uptake/metabolism/depuration rates is unknown.

Other observations are related to the THM concentrations themselves. The concentrations of THMs in fish tissues are not closely linked to either the measured surface water concentrations or the effluent concentrations. That observation is not surprising since the exposure of fish to the discharge was not controlled. The surface water sample represents a snapshot of THM concentrations at a single point in time and space; the sampling design was not intended to reflect exposure concentrations. Additionally, there is little or no relationship between effluent concentrations and the surface water concentrations. Differences in available dilution of the wastewater upon discharge and differences in distance from the discharge to the location where fish were caught (which is where the surface water was sampled) explain the lack of relationship amongst the effluent and surface water measurements.

There were not enough individuals of each fish species captured to say anything definitive about the propensity for one species to accumulate a greater or lesser THM burden than another. However as an observational note only, fish such as mullet that tended to congregate at wastewater outfalls generally had higher concentrations of THMs in their flesh than did more wide ranging, predatory fish.

Lastly, Figure 2 provides evidence that the treatment of fish following capture had little or no influence on measured THM tissue concentrations. Tissue samples frozen immediately on-site were intended to reflect, to the extent possible, the THM burden at the time of capture. The box-and-whisker plots show that THM levels in fish stored on ice for 4 hours following capture were not significantly different from concentrations in the tissue frozen immediately on dry ice. THMs appear to have been unaffected by storage of the fish on ice prior to filleting.

As noted earlier, sucralose was monitored in the surface water as a conservative tracer of wastewater. Figures 3a and 3b depict sucralose versus total THM concentrations measured in facility effluents and in the surface waters where fish were captured. There were only three facilities that had detected values for all four THM components in the effluent sample and in the instream sample. With such a small number of known values, a quantitative relationship between the instream sucralose concentration and total THM values cannot be estimated. While sucralose can serve as a conservative tracer of wastewater dilution, the effluent and instream sampling was not timed to capture the same batch of wastewater. Therefore, the data were not sufficient to determine whether THM concentrations present in surface waters were

affected only by available dilution or whether volatilization or other processes might significantly affect concentrations in receiving waterbodies near the point of discharge.

Another goal of this study was to test the hypothesis that cooking would not reduce or eliminate THMs that accumulate in edible fish muscle tissue following exposure to domestic wastewater discharges. Figures 4 a – d show the relationship between THMs measured in fish muscle tissue that had been kept on wet ice for four hours after collection and the THM concentration found in similarly stored fillet portions after cooking using the NOAA protocol referenced above. These data show remarkable consistency in the cooked and uncooked fillet portions, especially considering that different portions of muscle tissue were collected, stored and analyzed separately for the test of this hypothesis. These data suggest that changes in tissue concentrations resulting from this method of cooking are minimal or virtually insignificant. Because of the wide THM concentration ranges in the cooked and uncooked tissue, the data were also log-log transformed (Figures 5 a – d). For the log transformed data, the slope of the regression curves for all four THMs is not significantly different from one (1), suggesting that cooking had no statistically significant ($\rho<0.05$) effect on the tissue concentration. For the untransformed data, cooking had no statistically significant influence on dibromochloromethane tissue concentrations. That particular disinfection by-product was observed at the highest concentrations in fish collected in this study. Table 3 gives the 95% confidence intervals around the slope estimate for regression curves derived from the cooked and uncooked tissue data set. Where the confidence interval range includes the number one (1.0), the slope of the regression curve is not significantly different from one and the null hypothesis that cooking has no influence on THM concentrations in fish tissue should be accepted.

Table 3 – Cooked versus Uncooked Data Regression Analysis
95% Confidence Intervals (C.I.) around the slope of regression curves

THM	**Data Treatment**	**Lower 95% C.I.**	**Upper 95% C.I.**
Chloroform	None	0.64	0.95
	Log-log Transform	0.74	1.09
Bromoform	None	1.12	1.86
	Log-log Transform	0.90	1.36
Bromodichloromethane	None	0.69	0.94
	Log-log Transform	0.81	1.02
Dibromochloromethane	None	0.69	1.16
	Log-log Transform	0.82	1.12

Highlighting indicates acceptance of the null hypothesis that cooking has no statistically significant effect on fish tissue THM concentrations;

It should be pointed out that this study only evaluated one method of cooking fish. There are many other cooking methods that might conceivably yield different losses of THMs. Nevertheless, fish tissue cooked with the NOAA procedure resulted in well done, flaky meat

similar to what might be produced by some other cooking methods. Photographs of the cooking process and cooked tissue are shown in Figure 6. The goal of this study was not to examine various cooking methods but to determine the extent to which THMs were lost when fish were cooked to an edible consistency.

Conclusions

This study demonstrated that THMs can accumulate in the edible fillets of wild fish when they are directly exposed to domestic wastewaters. Depending on the conditions under which fish might be exposed, THMs can accumulate to surprisingly high concentrations in the fillet (exceeding 100 µg/Kg for total THMs). THMs which accumulate in fish are not lost to any appreciable extent through normal handling practices or by cooking fillets to an edible consistency.

Acknowledgements

The collection of fish for this study was made possible with the assistance and cooperation of the Florida Fish and Wildlife Conversation Commission (FWCC). The Bureau of Laboratories wishes to thank the many FWCC personnel who helped support this project.

Table 2 – Species of Fish Harvested Near Wastewater Outfalls

Facility Name	Species of Fish Harvested
Background Fresh-Wakulla River	Bass
	Blue Gill
Background Salt-Carrabelle	Sand Trout
Background Salt-Goose Bay	Jack Crevalle
	Lady Fish
	Sea Trout
City of Atlantic Beach WWTF* City of Neptune Beach WWTF* Jacksonville Beach WWTP*	Mullet
	Sheepshead
	Tilapia
City of Chattahoochee WWTP	Bass
	Mullet
	Sucker
	Sunfish
City of Clearwater-Marshall Street WRF**	Mullet
City of Palatka WWTF	Bass
	Crappie
	Sunfish
	Crappie
Hiawatha & Hart Point WWTF	Bass
	Flounder
	Sunfish
	Tilapia
Hillsborough County Falkenburg Road AWWTF	Ladyfish
Siesta Key Utilities Authority	Drum
	Mullet
	Pinfish
	Sheepshead
South County Regional AWWTP	Ladyfish
	Mullet
	Snook
	Striped Mojara
	Tilapia
South Cross Bayou WRF	Mullet
	Sheepshead
	White Mullet
Town of Century WWTF	Brim
	Carp
	Mullet
	Sucker

Figure 2a – Chloroform found in Edible Fish Tissue Collected near Facility Outfalls

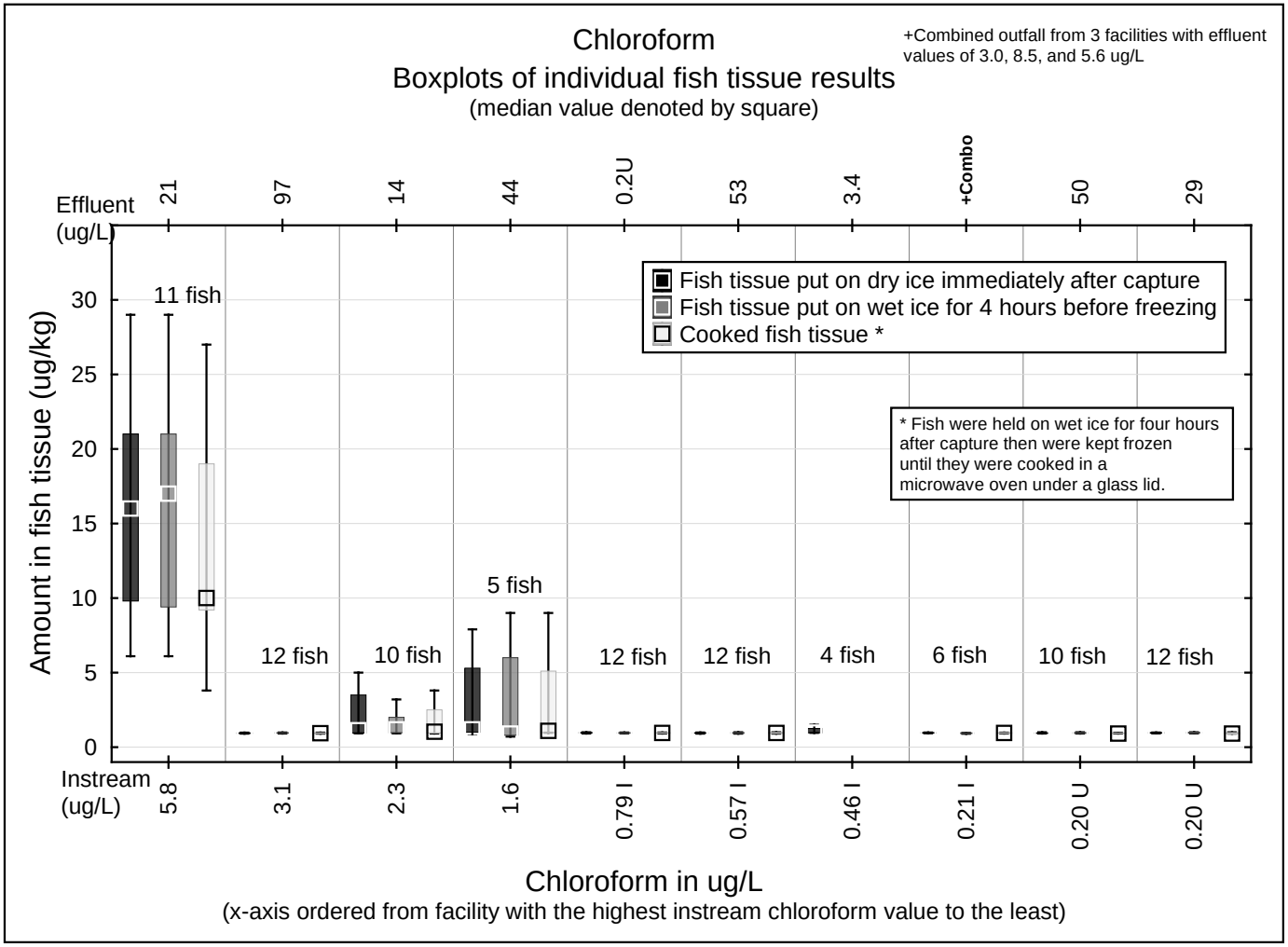


Figure 2b – Bromoform found in Edible Fish Tissue Collected near Facility Outfalls

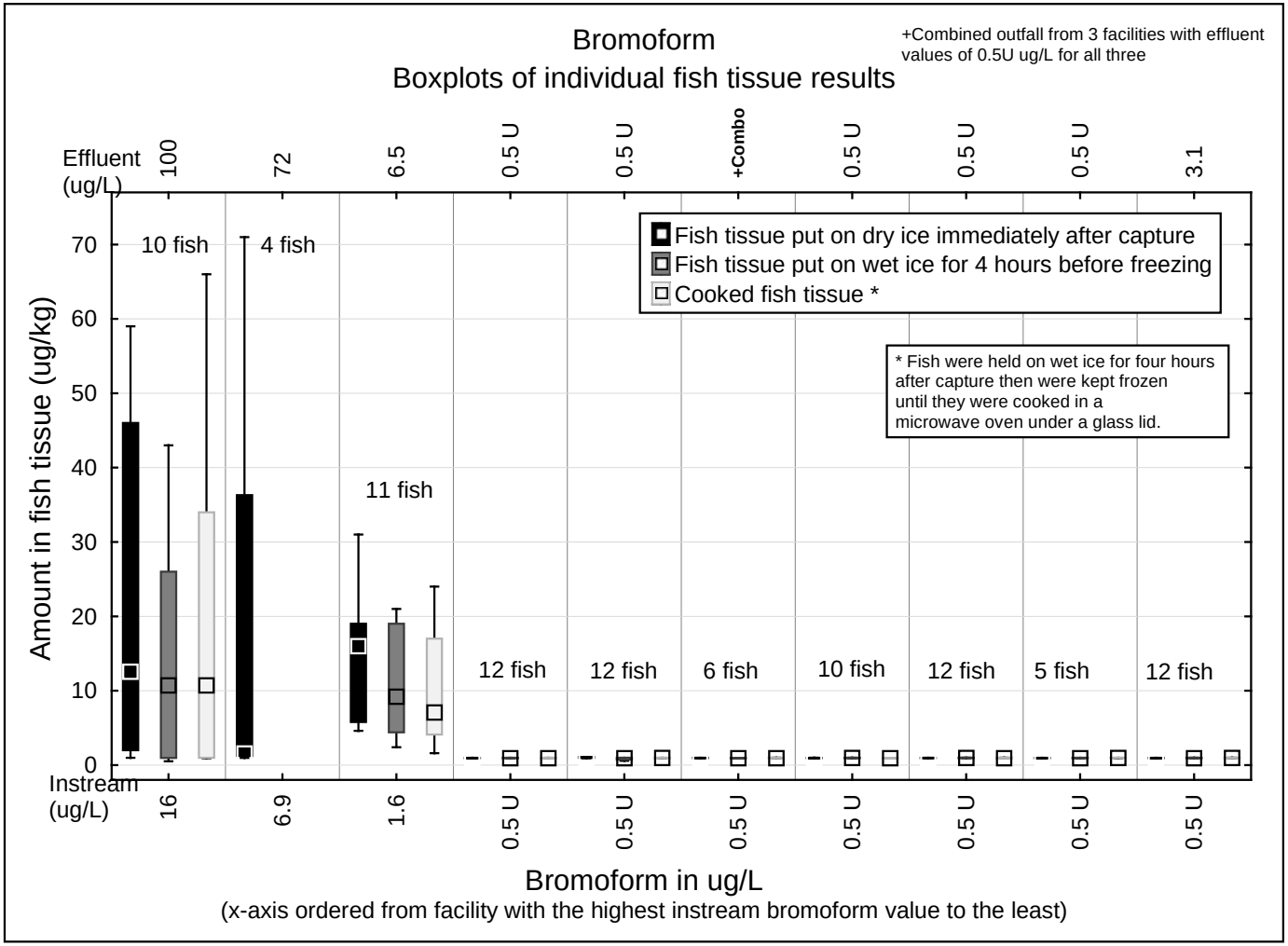


Figure 2c – Bromodichloromethane found in Edible Fish Tissue Collected near Facility Outfalls

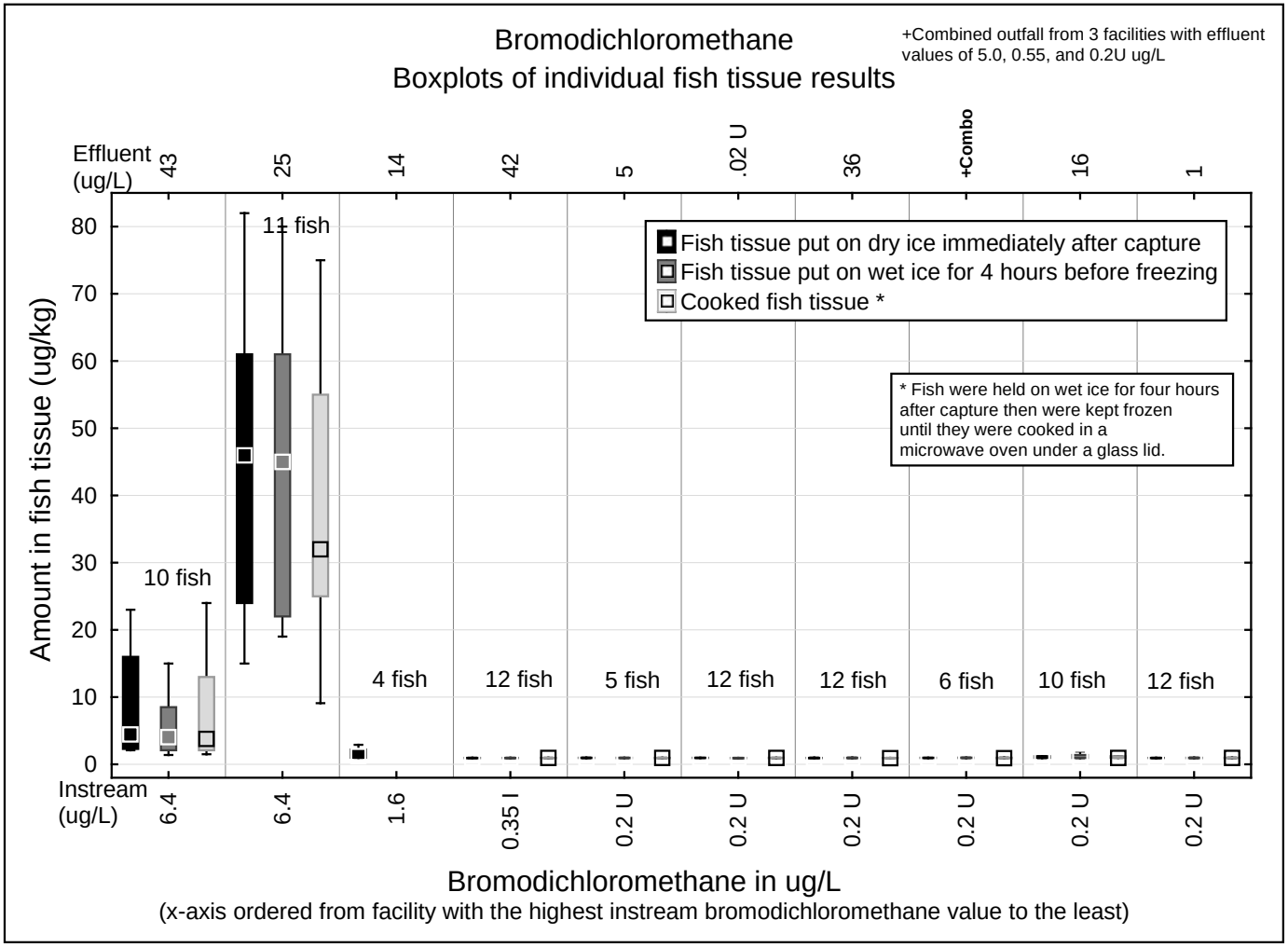


Figure 2d – Dibromochloromethane found in Edible Fish Tissue Collected near Facility Outfalls

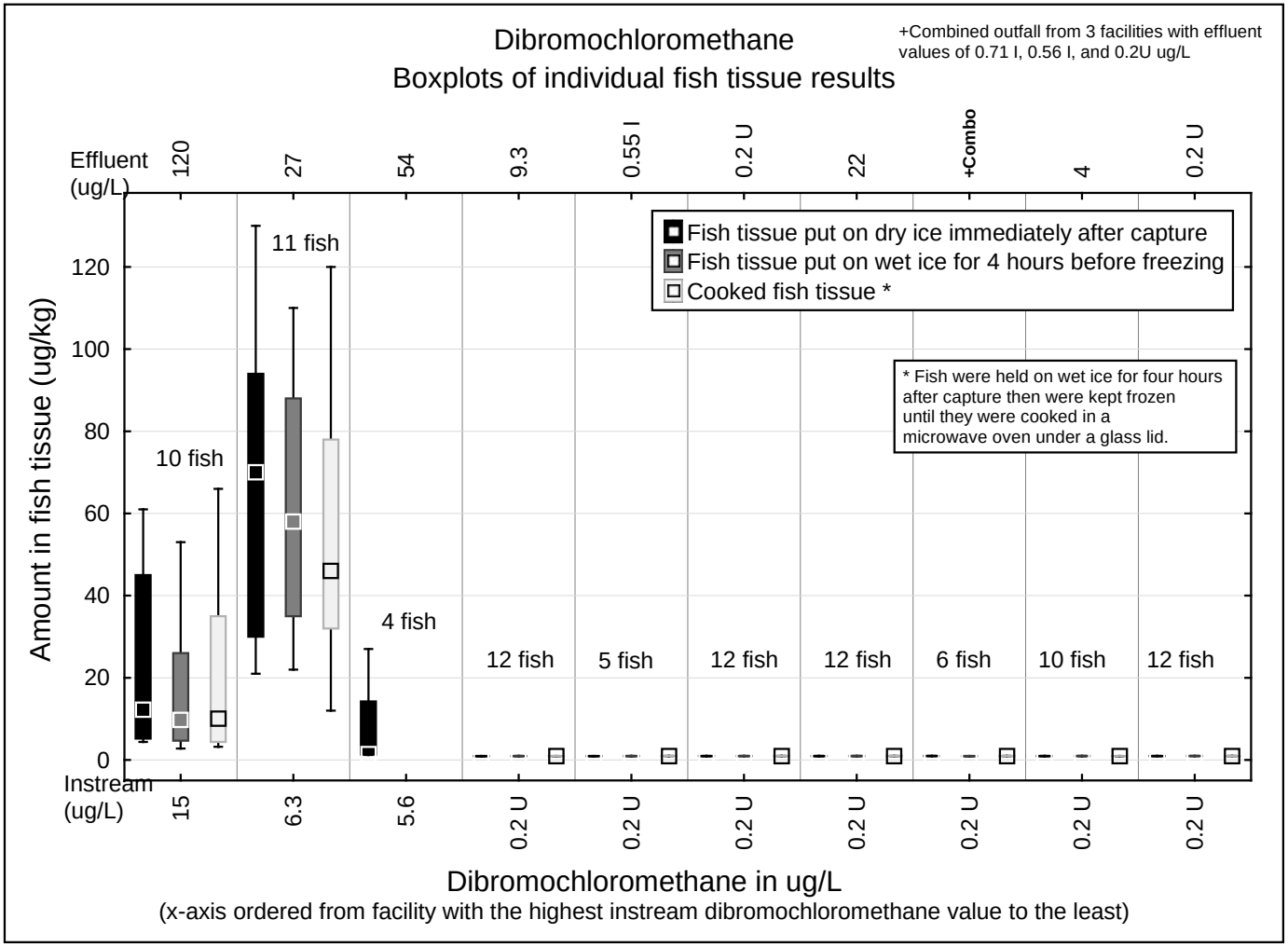


Figure 3a – Sucralose and Total THMs found in Surface Water Samples

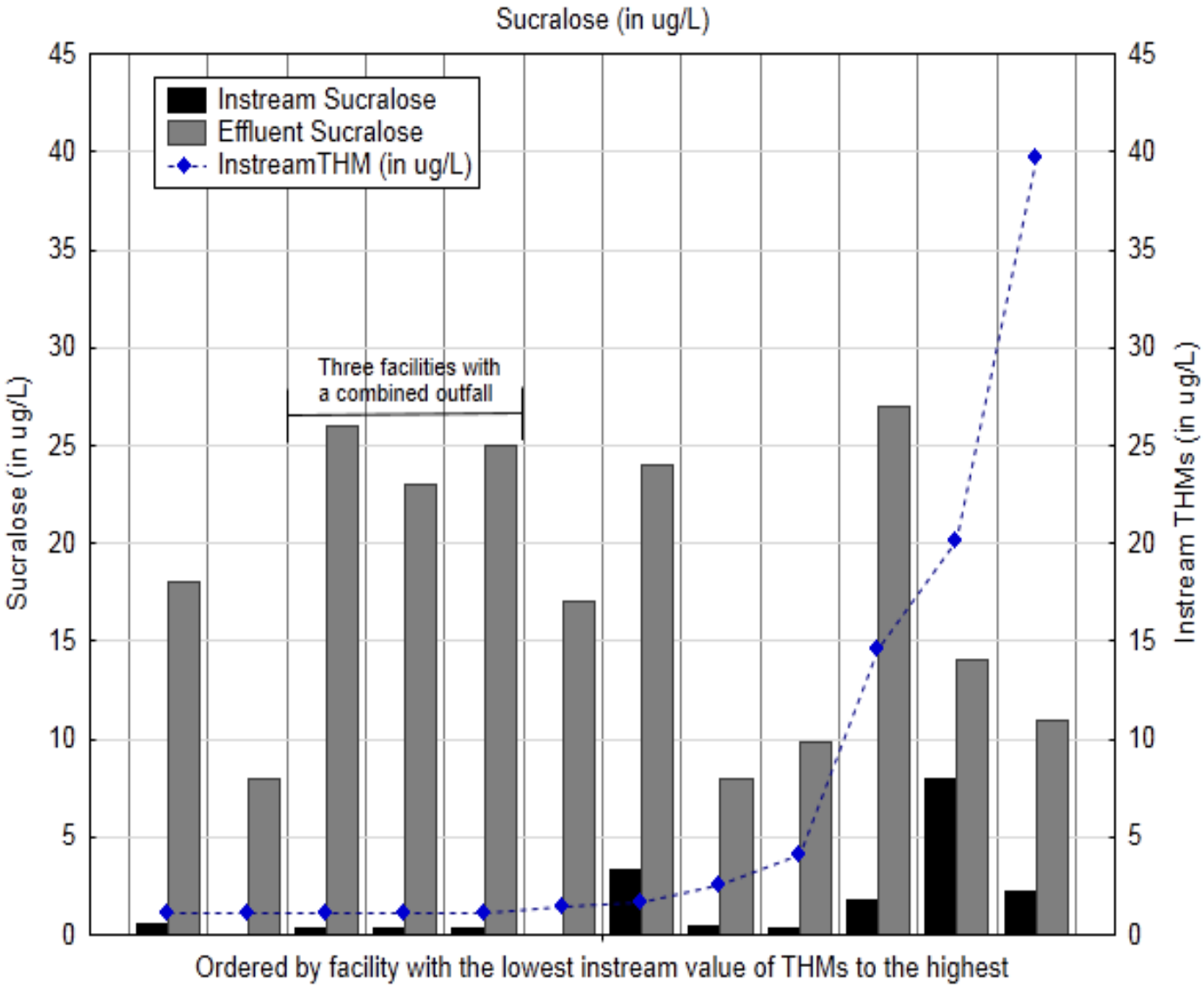


Figure 3b – Sucralose and Total THMs found in Surface Water Samples

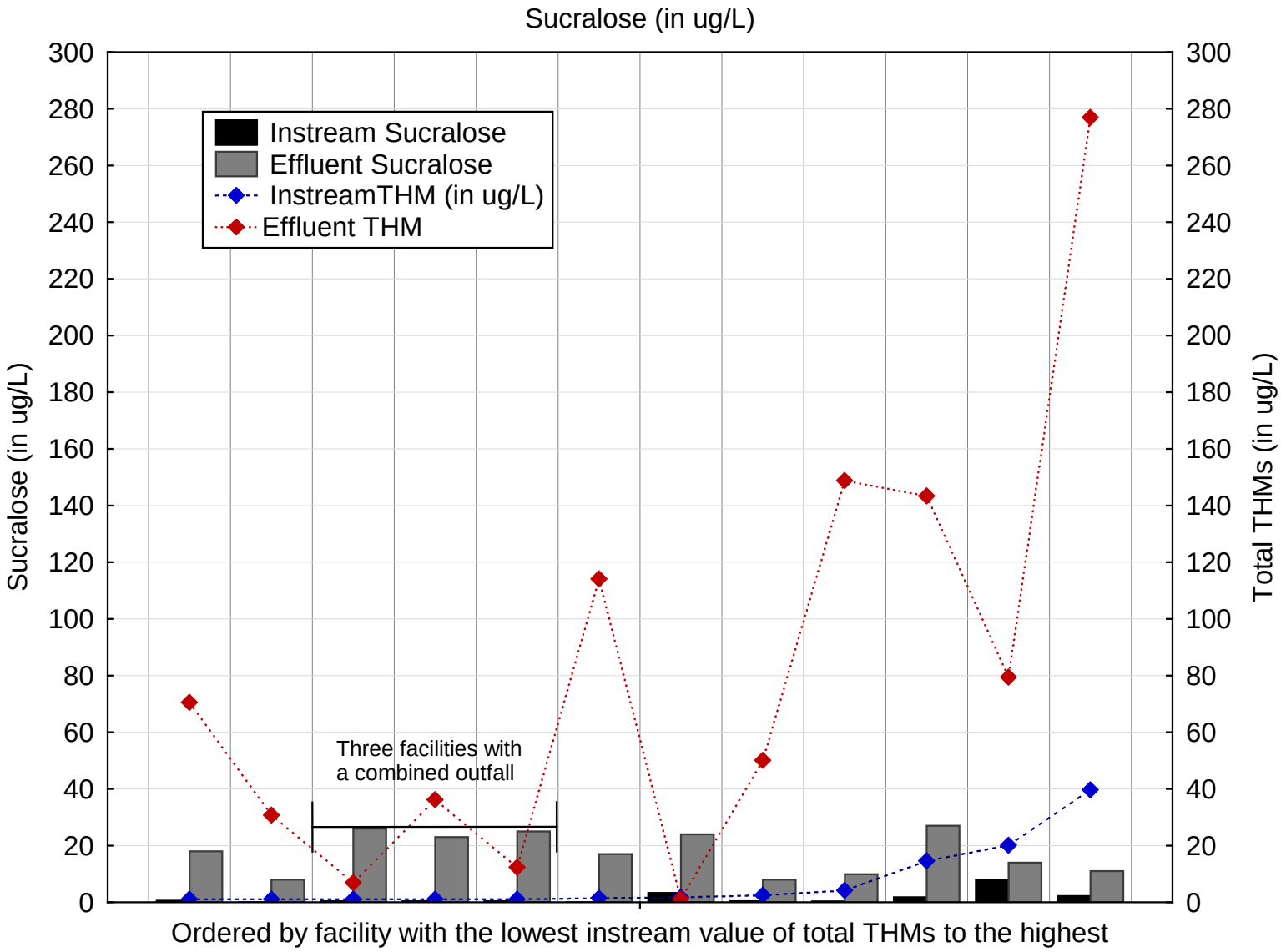


Figure 4a – Effect of Cooking on Chloroform Concentrations in Edible Fish Tissue

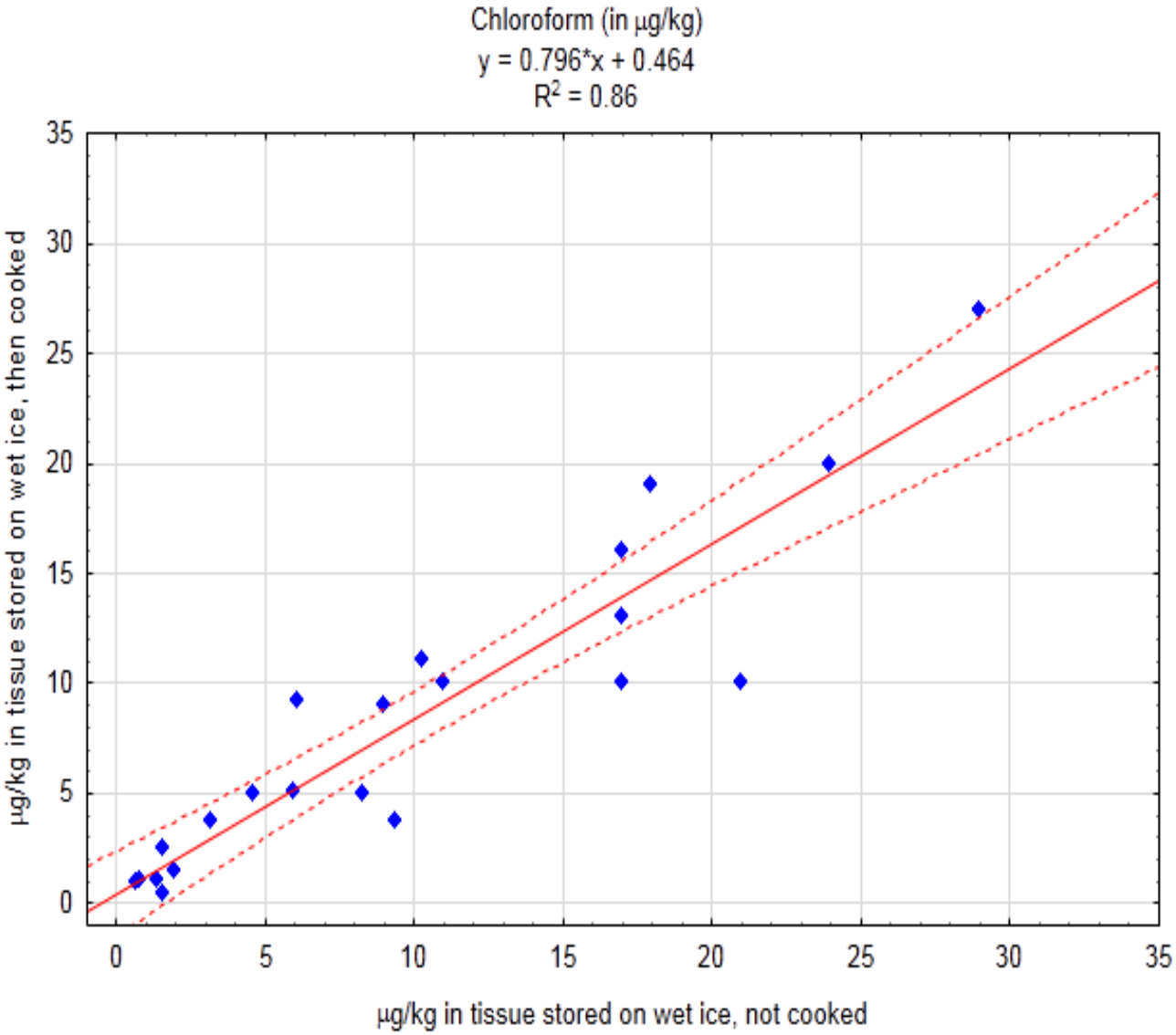


Figure 4b – Effect of Cooking on Bromoform Concentrations in Edible Fish Tissue

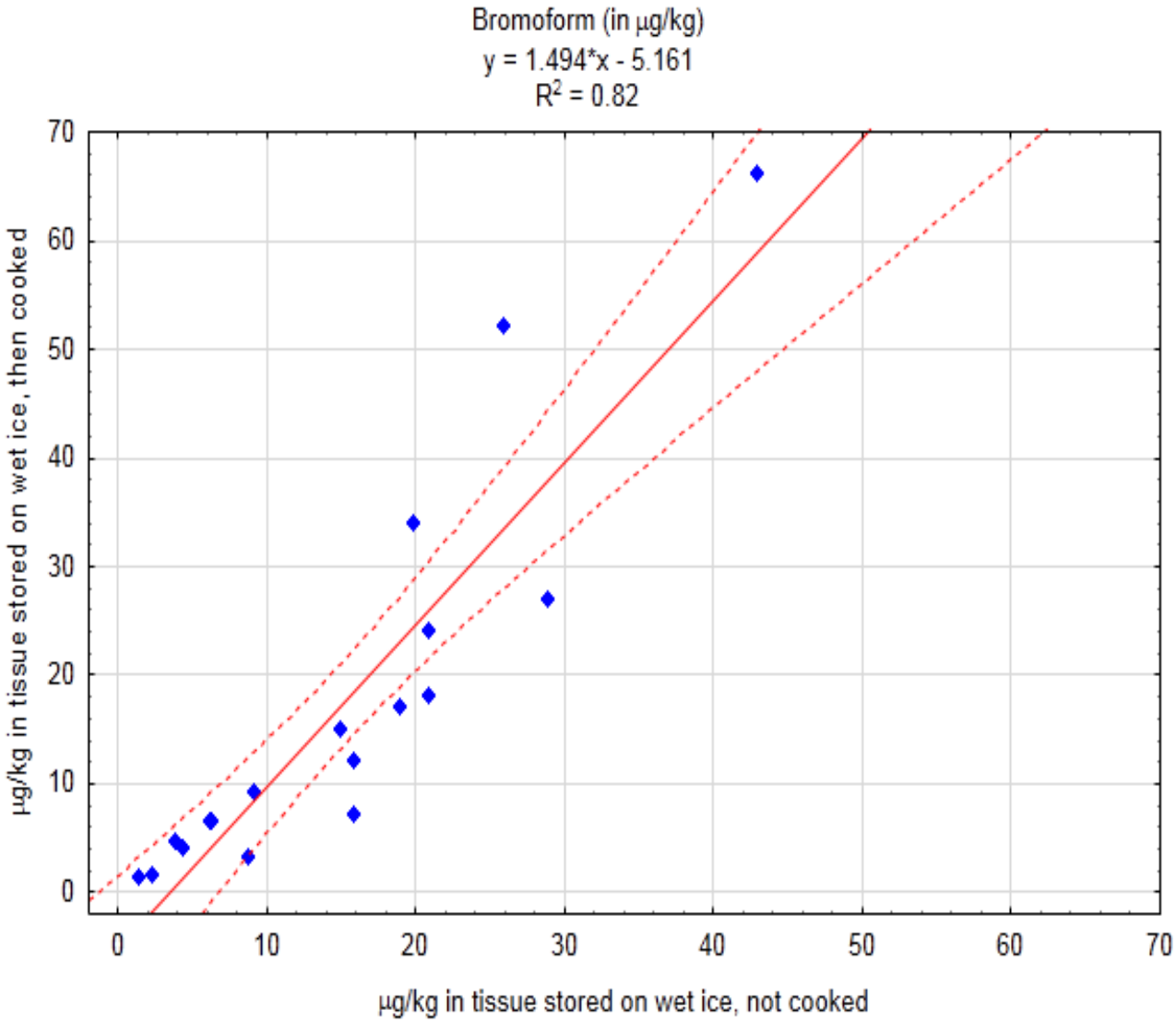


Figure 4c – Effect of Cooking on Bromodichloromethane Concentrations in Edible Fish Tissue

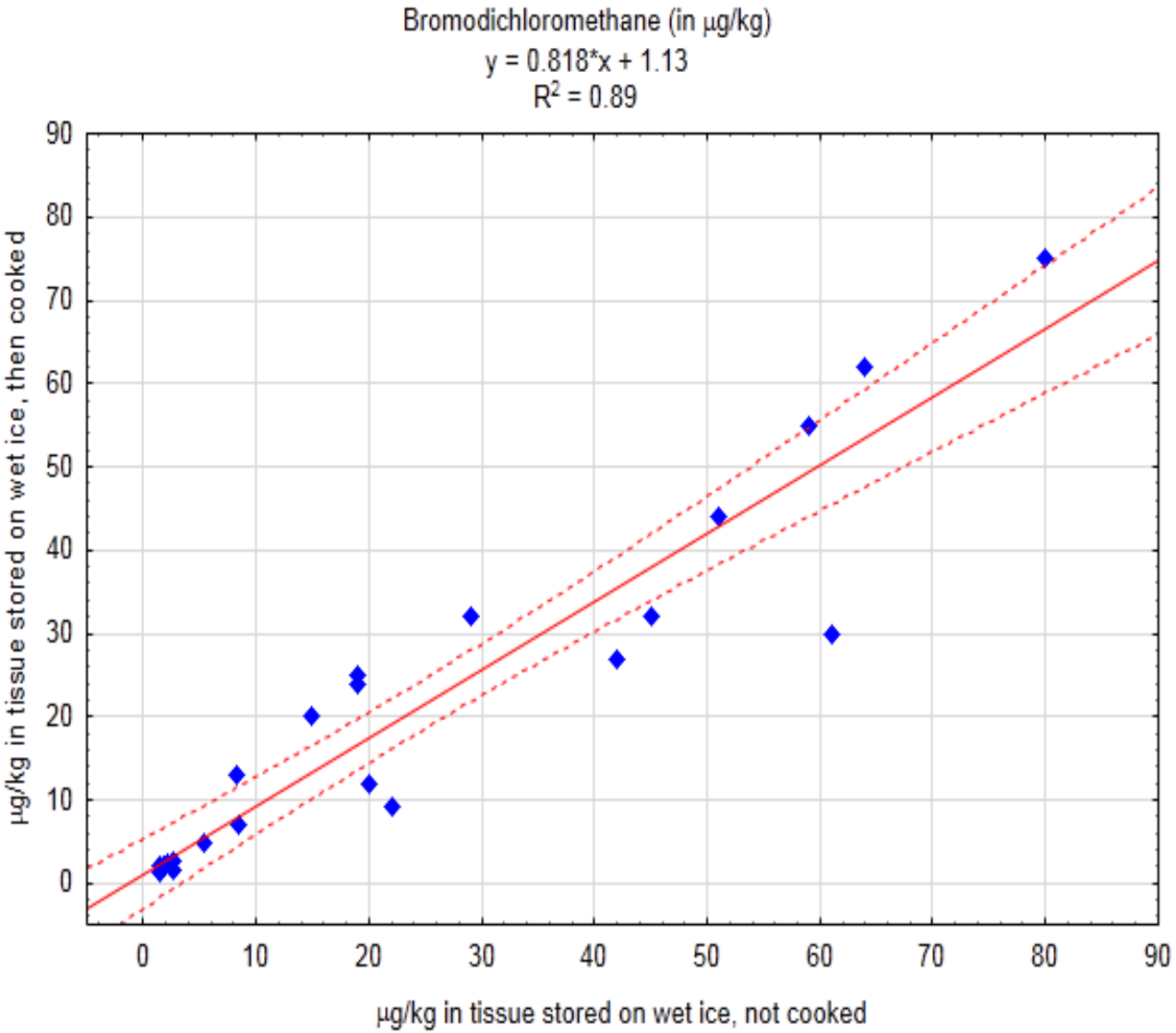


Figure 4d – Effect of Cooking on Dibromochloromethane Concentrations in Edible Fish Tissue

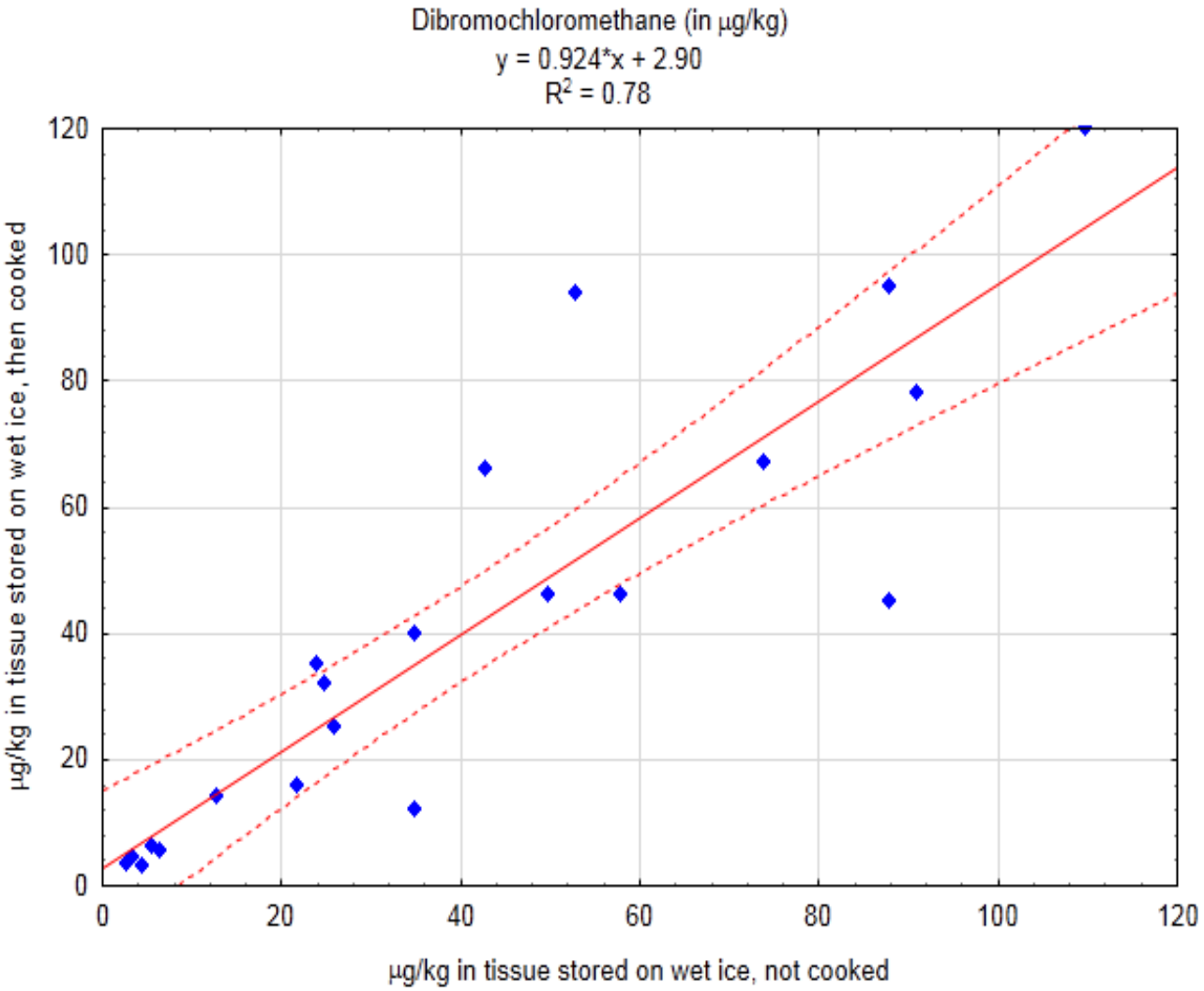


Figure 5a – Effect of Cooking on Chloroform Concentrations in Edible Fish Tissue
(log-log Transformed Data)

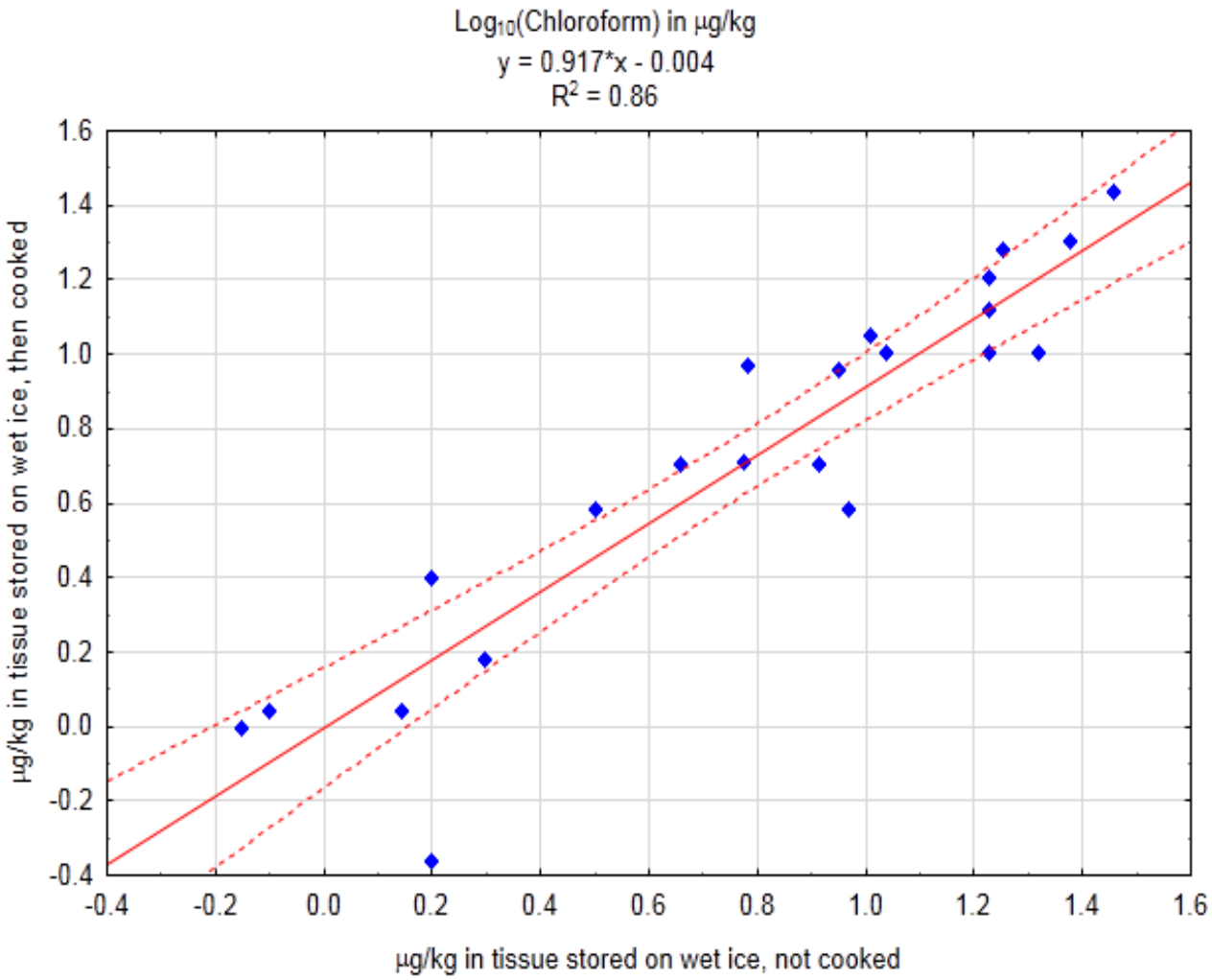


Figure 5b – Effect of Cooking on Bromoform Concentrations in Edible Fish Tissue
(log-log Transformed Data)

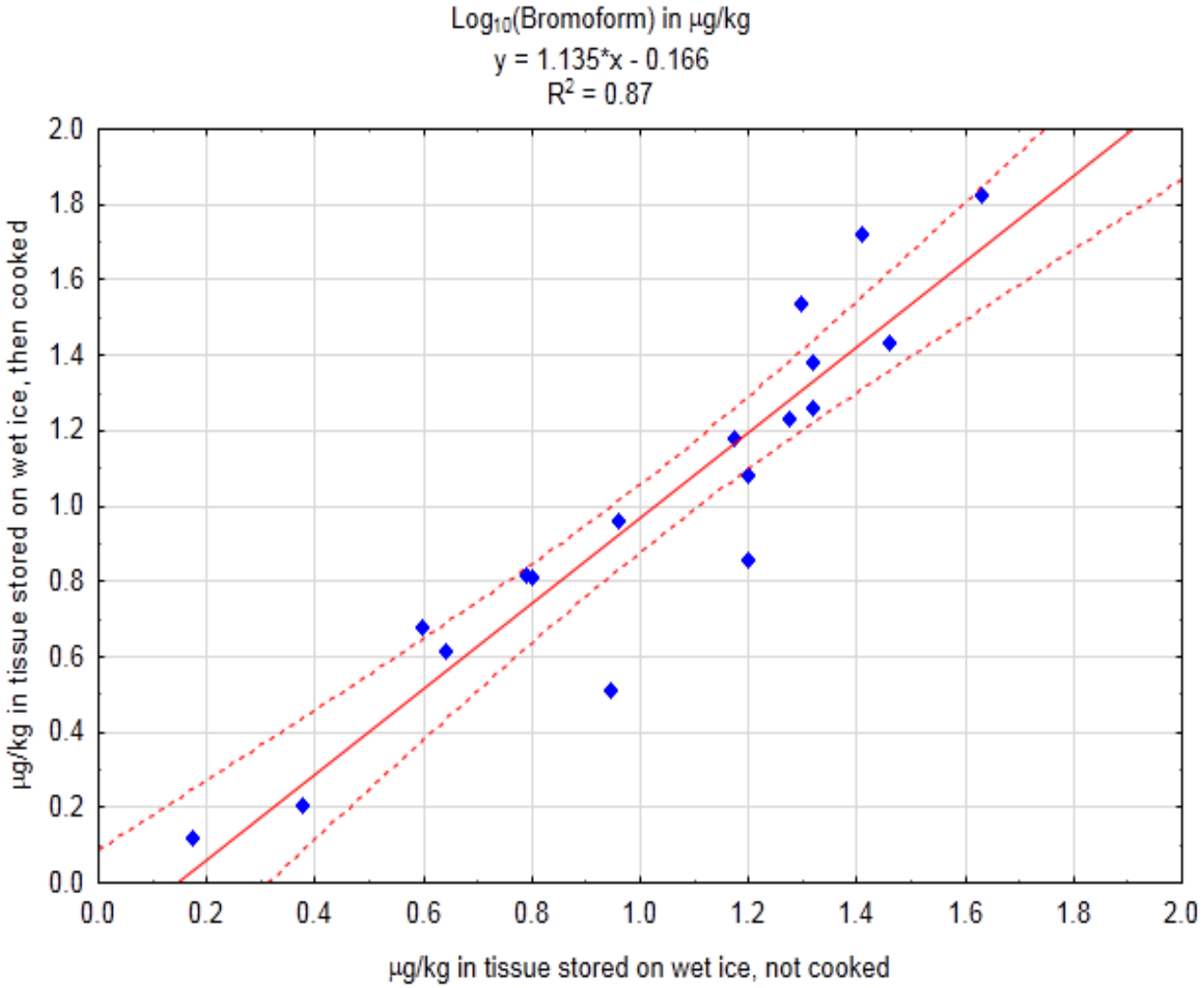


Figure 5c – Effect of Cooking on Bromodichloromethane Concentrations in Edible Fish Tissue (log-log Transformed Data)

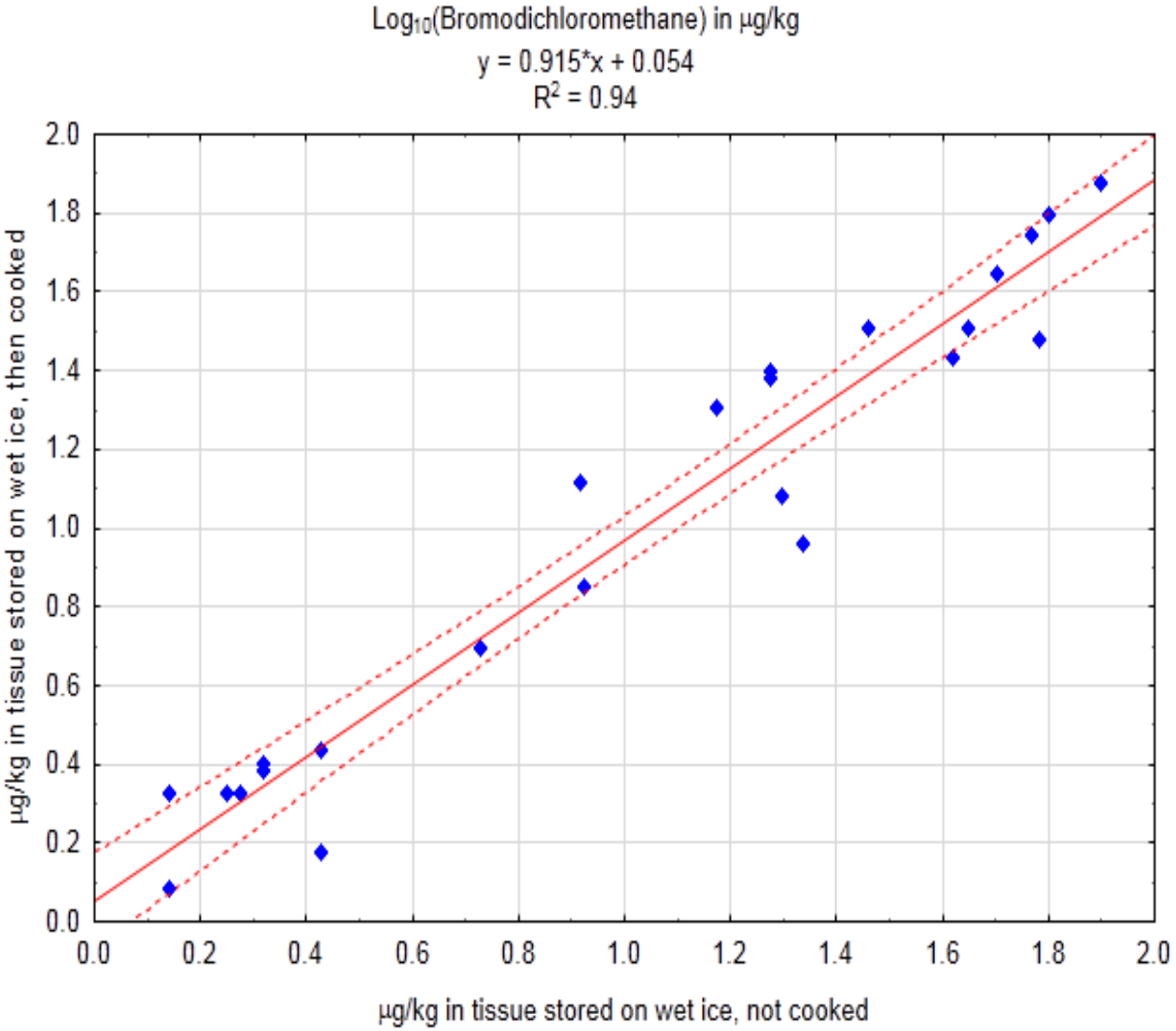


Figure 5d – Effect of Cooking on Dibromochloromethane Concentrations in Edible Fish Tissue (log-log Transformed Data)

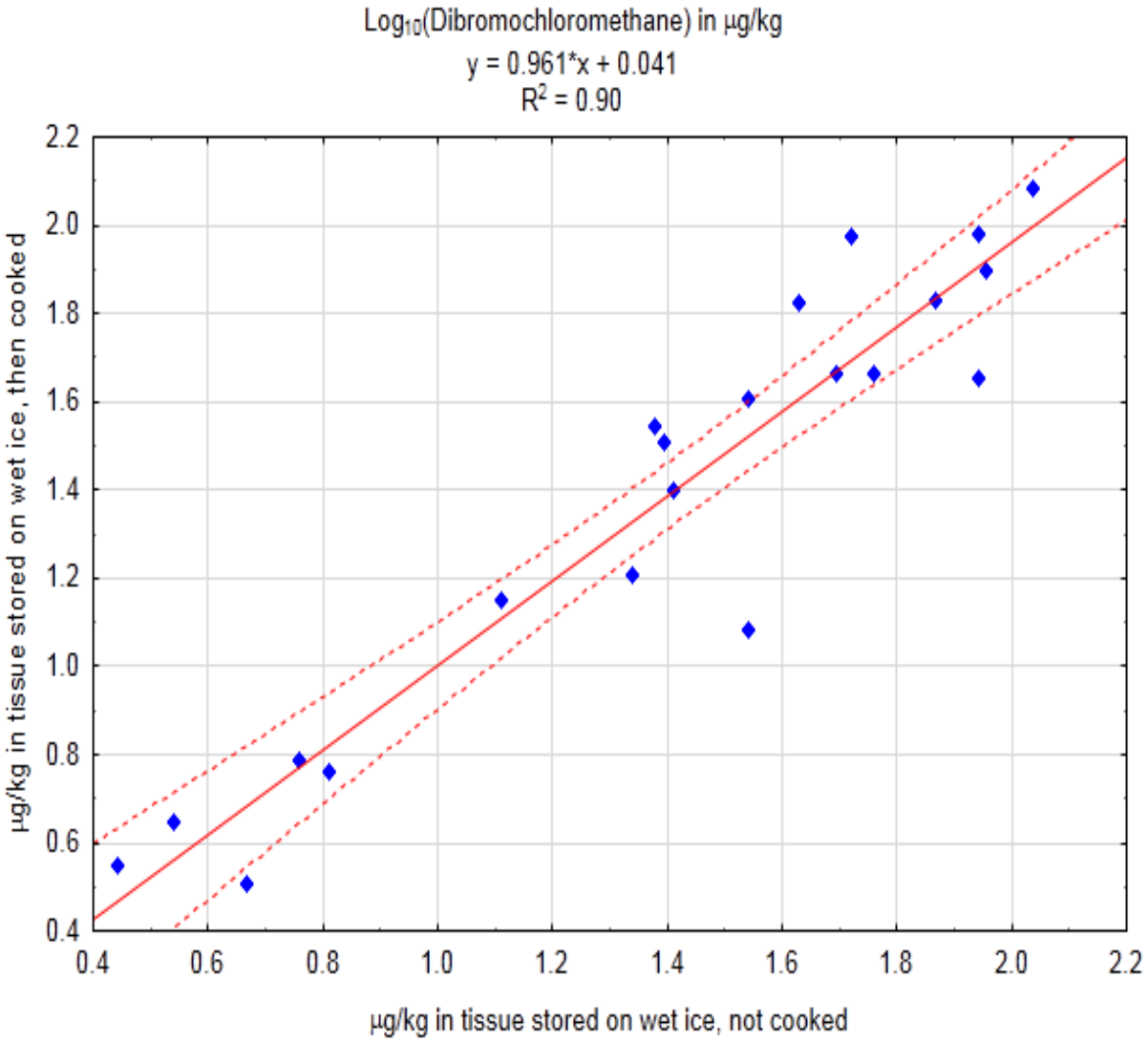
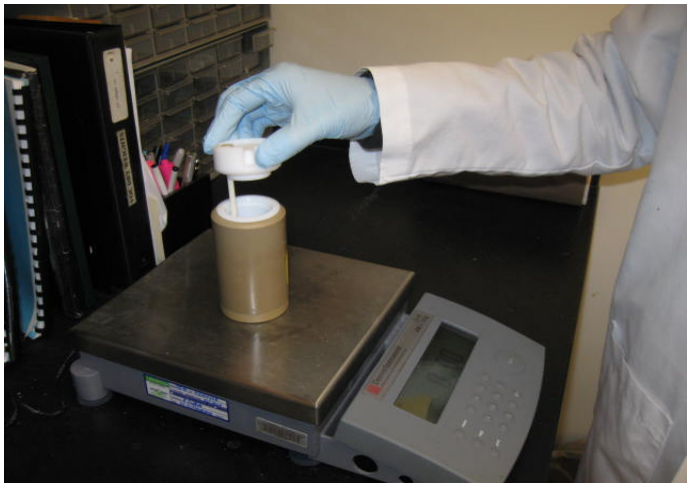


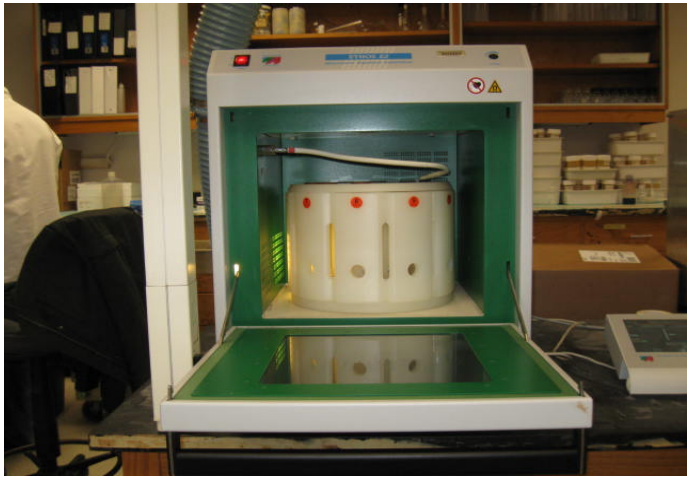
Figure 6 – Photos of the Fish Tissue Cooking Process

Frozen, raw tissue samples from the field



Sample measured into a microwave digestion vessel

Vessels loaded into a microwave carousel



Carousel inserted into the microwave cavity

Figure 6 Continued

Cooked, flaky fish tissue

Homogenized tissue ready for analysis