False Bay Creek (San Juan Island, WA)
Freshwater Fish and their Prey:
Significant Contaminants and their Sources

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September 2010

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Summary

Water quality suitability for the restoration of Pacific salmon in False Bay Creek was assessed by a combination of field measurements (temperature, conductivity, pH and dissolved oxygen); colorimetric determination of nutrient loading (nitrates, ammonia, and phosphates); ELISA immunoassays of tissue extracts from fish and invertebrate prey for polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), and widely used pyrethroid pesticides; and ESI mass spectrometry of nonyl phenols (NPs) in fish and invertebrate tissue as a measure of exposure to nonionic surfactants.

A number of water quality issues were identified that are attributable to negligible summer instream flows, use of the riparian corridor as cattle pasture, outdoor use of spray pesticides, and untreated runoff from county roads. A relatively small reach of the stream just above the tidal prism persists after instream flows cease in early summer, impounded by numerous upstream ponds. Water in the surviving reach warms and becomes hypoxic within weeks, and the diversity and abundance of fish and potential invertebrate prey for fish falls sharply by mid-summer. Ammonia was also a threshold concern at levels close to, and sometimes exceeding one part per million.

Three Spined Sticklebacks (*Gasterostreus aculeatus*) and Reticulated Sculpins (*Cottus perplexus*) were collected from the stream for the determination of toxic loading by ELISA and mass spectrometry. All target contaminants were present in fish at levels of potential biological effects with mean concentrations of 2.0 parts per billion (2.0 micrograms per kilogram) PCBs, 4.1 parts per billion pyrethroid pesticides, 15 parts per billion NPs, and 29 parts per billion PAHs. Toxic loading of invertebrates was twice the loading of fish. Of particular concern is the NP loading, which is high enough to result in loss of fertility in salmonid eggs.

Restored summer instream flows, more heavily vegetated riparian corridors, more prudent management and treatment of runoff from county roads, reduced outdoor use of pyrethroid pesticides, and reduced use of all pesticides and herbicides emulsified with nonionic surfactants for use in hose sprayers, are recommended actions to improve water quality in False Bay Creek and increase the likelihood of successful salmonid restoration.

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R. Barsh, J. Bell, E. Blaine, G. Ellis, and S. Iverson

False Bay Creek drains the largest watershed in the San Juan Islands, representing nearly one-half of the land mass of San Juan Island. It rises in the San Juan Ridge, which includes Cady Mountain and Mount Dallas, and flows south through the broad, shallow San Juan Valley, the islands' oldest agricultural area with farms dating to the 1850s. The head of the valley is distinguished by low bedrock knolls and was known to early setters as The Oak Prairie. According to naturalist C.B.R. Kennerly, who explored the valley in 1859, Garry oaks extended for a square mile between the ridge and the level wetland soils that comprise the southern two-thirds of the valley.

The historical False Bay watershed can be divided into three eco-regions based on early observations and evidence from contemporary soils and vegetation. Multiple small streams in narrow ravines, interrupted by small shallow wetlands, characterized the upper reaches of the watershed in the ridge. This topography may still be seen on the south side of Cady Mountain, south of West Valley Road, where a mile of original riparian corridor remains undeveloped. The midsection of the watershed—including the Oak Prairie—was a mosaic of seasonal wetlands, meadows, and wooded knolls. From thence to the sea at False Bay, there was a continuous broad mid-valley vernal pool with braided channels. A number of different salmonid populations may have used these habitats, from land-locked cutthroat and rainbows in the ridge reaches, to seasonal chum and coho spawners entering from False Bay. No corroborative historical records exist, however.

Post-Contact agriculture realigned the watershed profoundly.[1] As elsewhere in the San Juan Islands, farmers ditched and drained seasonal wetlands for livestock and haying. Deep straight ditches replaced braided shallow stream channels: less suitable for salmon, although old-timers do recall salmon in the big ditch that forms the lowest reach of False Bay Creek, within the last 20-50 years. Ditches and draining altered habitat structure, but water delivery to the valley from upstream continued with little change until mid-century logging of the ridge led to construction of a series of fire ponds in the headwaters. Ponds proliferated further as part of residential growth of the watershed since the 1970s. Today, headwaters streams are strings of dug ponds without control structures that fill in late fall and winter, overflow in spring, and withhold water from the stream in summer.

An additional factor is growing diversion of water from Trout Lake by the Town of Friday Harbor. Trout Lake is part of the Mount Dallas tributary of False Bay Creek.

Summer flows in the valley today are negligible. However, freshwater fish persist in the roughly 500 meters of shaded stream channel immediately above the tidal prism

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[1] Evidence of pre-Contact camas cultivation has not yet been documented but is likely, based on similarities between the lower False Bay watershed and the Davis Bay (Lopez) watershed, which Kennerly reported as cleared and burnt by Coast Salish peoples presumably for their camas gardens.

(on the cover of this report), which remains flooded year-round, and they venture as far upstream as Bailer Hill Road when stream conditions permit. These surviving freshwater fish populations and their food web are the focus of the present study, as indicators of the conditions that may be confronted by enhanced or re-introduced salmon populations. We have investigated toxic contaminants as well as limiting factors such as nutrients, oxygen, temperature, and acidity in this small downstream fish refuge.

Contemporary land-use patterns define the potential for water quality problems in the lower stream. The headwaters and middle reaches of False Bay Creek are residential, mainly dispersed individual households, as well as some clusters such as Erickson Lake. The lower valley is agricultural, mainly cattle and hayfields. Major roads cross the False Bay system: West Valley Road and Beaverton Valley Road each crosses tributaries of the creek at several places, and Bailer Hill Road crosses the main steam.

We identified five potential concerns based on the hydrology and land-use history of the False Bay watershed:

1. High ammonia-low oxygen summer water conditions due to a lack of instream flow and nutrient-rich runoff from pastures.
2. High polycyclic aromatic hydrocarbon (PAH) loading of the aquatic food web from road runoff and farm machinery.
3. High pyrethroid pesticide loading of the aquatic food web from outdoor home lawn and commercial applications, in particular to control carpenter ants.[2]
4. Alkyl phenyl ethoxylates (APEOs) added to a wide variety of spray pesticides and herbicides as wetting agents are endocrine disrupting compounds and may pose greater risks to aquatic ecosystems than the declared active ingredients in outdoor products.
5. Polychlorinated biphenyls (PCBs) used in the islands' electrical supply grid to the 1980s are so persistent that a significant legacy signal may be expected in island freshwater ecosystems. Some atmospheric deposition may be expected as well (Demers et al. 2007; Oyama et al. 2004). The combined signal should be relatively small compared with marine waters of Puget Sound, however.

Previous research had shown that pyrethroid pesticides are widely distributed in the islands' freshwater ecosystems (Barsh et al. 2008), and that downstream sediments in False Bay Creek are moderately toxic to *Vibrio fischeri* (Barsh 2009).

Spring 2010 hand-seining of the stream in a shaded bend immediately above the tidal prism and farther upstream at Bailer Hill Road found large numbers of Three Spined Sticklebacks (*Gasterostreus aculeatus*) at both sites, young of the year as well as adults. Small numbers of reticulated sculpins (*Cottus perplexus*) were also seen at the lower site, including some early post-larval examples. We concluded that these two fish species are residents and sufficiently abundant to sustain the level of sampling required by this study.

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[2] Bifenthrin—one of the pyrethroid species most toxic to fish, and most persistent in the environment, is the active ingredient in the carpenter ant products currently most popular in the San Juan Islands. It is common to spray foundations of buildings repeatedly each year to manage ant infestations.

Chum salmon (*Oncorhynchus keta*) fry were also briefly observed in the lower stream but were not sampled.

Methods

Freshwater fish were collected from the lower ("Green Bend") and upper ("Bailer Hill" stream by hand seine and dip net with the assistance of James Fletcher (Wild Fish Conservancy). A total of 11 age 1+ freshwater sculpins (*Cottus perplexus*) and 21 age 1+ sticklebacks (*Gasterostreus aculeatus*) were collected by this means in May to July 2010. Invertebrates (crustaceans, aquatic insects, aquatic insect larvae, mollusks) were collected during the same period with a 160-micron plankton net fitted with a 20 cm wide collar for 25-meter-long timed quantitative tows. Plankton tows nearly always yielded 0+ age fish: a total of 29 early post-larval sculpins and 85 juvenile sticklebacks retrieved from tows to prepare five composite samples of age 0+ fish. A single 58 mm juvenile Starry Flounder (*Platicthys stellatus*) was also found in a tow sample and has been included in this study.

Invertebrates were combined in composite samples of 8 to 44 animals collected on the same date. Animal specimens were briefly rinsed in Milli-Q water, then dried, and frozen until use. Extractions were carried out within 30 days of collection.

Field measurements of temperature, conductivity, pH and dissolved oxygen were made with a 556 MPS multi-parameter electronic logger (YSI Inc., Yellow Springs, OH). Turbidity was measured with a Micro TPI nephelometer (HF Scientific, Ft. Myers, FL).

Nitrate-nitrogen was determined by the cadmium reduction method; ammonia by Nesslerization; and inorganic phosphates by the ascorbic acid reduction method; with the Smart2 spectrophotometer (LaMotte Inc., Chestertown, MD).

PCBs, PAHs and pyrethroid pesticides were determined by ELISA immunoassays (Abraxis LLC, Warminster, PA; Strategic Diagnostics, Inc., Newark, DE) using magnetic particle formats. Animal specimens were extracted using a matrix solid-phase dispersion (MSPD) method (Barker 2007; Zhao et al. 1999). Specimens were first thawed, crushed, and ground in a glazed ceramic mortar. A sub-sample of 0.2g of ground tissue was then combined with 0.75g of Na_2SO_4 and 2.0g of 40-63 micron mesh silica gel and loaded into a 10-mL syringe tipped with a 22-µm cellulose acetate membrane syringe filter on top of another 2.0g of silica, separated by 1.5-cm qualitative paper filter discs. Pestanal™ grade methanol was then added to the syringe in three equal 2.5 mL aliquots over a period of 20 minutes. The plunger was then inserted into the syringe barrel and depressed slowly and firmly, eluting 1-2 mL of extract, which was refrigerated until use.

ELISA tests were carried out in batches of 12-20 specimens. Each batch included positive and negative controls, an instrument blank and four standard solutions of varying concentrations of the target analytes to provide a calibration curve. Any anomalies in the calibration curve or results of the controls led to rejection of the batch and re-testing.

Exposure of fish to Alkyl Phenyl Ethoxylates (APEOs) was investigated by ESI mass spectrometry using a Finnegan Duo LC/MS tuned for the detection of Nonyl Phenol (NP), a relatively stable APEO metabolic degradation product. LC/MS tuning conditions and calibration spectrograms are available on request.

We prepared fish tissue extracts for mass spectrometry by adapting the preferred method of Gadzala-Kopciuch et al. (2008). The initial sample of approximately 3 grams was ground in a ceramic mortar with 10 grams of aluminum oxide conditioned with MS-grade acetonitrile (ACN), then packed into a 20 mL glass syringe mounted with a 0.45µm GMF syringe filter, coupled in turn to a C18 Solid Phase System Cartridge (500mg/4mL) pre-conditioned with 10 mL of ACN followed by 3 mL of Milli-Q water.

To extract, 18 mL of ACN was forced slowly through the syringe. The expressed liquid was then evaporated in a warm water bath (40-50^{o}C) under a stream of nitrogen, and the residue was reconstituted in 100 µL of Pestanal™ grade methanol before cleanup using a second C18 cartridge conditioned with ACN and fitted with a syringe plunger for gentle pressure. We determined that most of the co-extracted compounds responsible for masking or suppressing the NP signal are eluted in the first few drops expressed through the C18 cartridge, followed by several drops of NP-enriched methanol.

Results

<u>Nutrients</u>

Table 1 summarizes general stream conditions and nutrient loading at the Green Bend study site based upon 10 sequential summer samples.

Table 1. Water quality in False Bay Creek, Summer 2010

Parameter	*Units*	*Means*		*Season maximum*	*Season minimum*
		May-June	*July-August*		
Temperature	^{o}C	19.69	20.97	24.56	17.42
Conductivity	mS	22.15	22.69	39.43	6.13
Turbidity	NTU	6.57	8.13	9.72	3.40
Acidity	pH	6.28	6.70	5.42	7.05
Nitrate-nitrogen	ppm	0.03	0.28	0.41	0.00
Ammonia-nitrogen	ppm	0.83	0.64	1.44	0.34
Phosphates	ppm	0.32	0.50	1.09	0.05
Dissolved oxygen	ppm	3.35	2.05	4.39	0.84

Summer water temperatures were somewhat high for salmonids, although coastal cutthroat (*Oncorhynchus clarki clarki*) have been observed in San Juan County streams at temperatures as high as 19^{o}C, and even higher temperatures in deep ponds (Barsh 2010). A greater concern is low dissolved oxygen levels. Most fish show signs of distress below 5 ppm (mg/L) oxygen, and oxygen demand increases with water temperature and activity. Salmonids have not been found in San Juan County streams with summer oxygen levels below 5 ppm although sticklebacks appeared to have greater tolerance for hypoxic stream conditions (Barsh 2010). Sticklebacks were indeed present—and reproducing—at Green Bend throughout summer 2010. However, a dead 2+ stickleback and a dead sculpin were observed on the stream bottom on June 15, and the sticklebacks observed in August were predominantly young of the year. Larger adult sticklebacks with greater oxygen demand may have relocated downstream to the estuary or nearshore to avoid suffocation. Table 2 (below) suggests that some invertebrates may also have suffered from hypoxia.

Nitrogen and phosphorus loads observed in summer 2010 were comparable to the loads we have previously observed in other agricultural areas of the county. Ammonia is toxic to fish at levels varying by species but generally within the range of 0.2 to 2.0 ppm. Salmonids are reportedly particularly susceptible to ammonia. Livestock are the primary source of elevated ammonia in the islands, and cattle are grazed on pastures immediately upstream of Bailer Hill Road in the False Bay watershed. Much of the stream channel is accessible to livestock, grazed, and lacks significant woody buffer areas.

Accumulation of nutrients over the course of the summer is not surprising in view of low to zero instream flows that leave the Green Bend reach static with declining water depth; and the absence of significant aquatic vascular vegetation on the stream bottom. Numerous rafts of colonial micro-algae were observed floating at Green Bend in August, suggesting borderline eutrophic conditions. Again, additional vegetation buffering along the agricultural reaches of the stream seems warranted.

Food web composition

Table 2 summarizes results of plankton tows and kick-net sampling of the Green Bend study site by month. A single plus sign indicates that the taxon was present; two or more indicate that the taxon was abundant (over 10 individuals per tow) or very abundant (over 100 individuals per tow). Shaded boxes indicate the month of peak reproduction for each taxon inferred from the proportion of egg-bearing adults or juveniles in samples.

Table 2: Aquatic invertebrate community in False Bay Creek, 2010

Taxa	*Species*	*May*	*June*	*July*	*August*
Isopods	2	++	++	+++	+++
Gammarid amphipods	1	+	+	+	+
Daphnids (Cladocerans)	1	+	+		
Calanoid copepods	3	+			
Cyclopoid copepods	1	+			
Corixidae (Coleoptera)	2	+	+	+	++
Other aquatic Coleoptera	2	+	+		
Culcidae (Diptera) larvae	2	++	+		
Chironomidae (Diptera) larvae	1	+	+		
Tipulidae (Diptera) larvae	1		+		
Other Dipteran larvae	4	+	+		
Plecoptera nymphs	1	+	+		
Trichoptera larvae	1	+			
Gerridae (Hemiptera)	1		+		
Thysanoptera larvae	1	+			
Collembola	1	+			
Gastropod adults/veligers	2	+	+	+	+
Total taxa	27	15	12	4	4

Other than adult gastropods and the largest Corixidae (diving beetles), all of these aquatic taxa are potential prey for fish. Isopods were by far the most abundant with more than a hundred taken in each of three summer plankton tows. Diptera—chiefly Culcidae (mosquito) larvae—were second in abundance. Most taxa reproduced in early summer at times when water conditions were comparatively good. Many taxa were no longer found in the stream in mid-to-late summer. Some insects had presumably pupated and assumed terrestrial habits e.g. the Chironomidae (midges) and Plecoptera (stoneflies). Crustaceans such as copepods and daphnids had probably left their egg sacs in the stream bottom mud and died. Invertebrate diversity declined sharply between June and July, at the same time that temperatures peaked and oxygen levels fell to their lowest levels of the season.

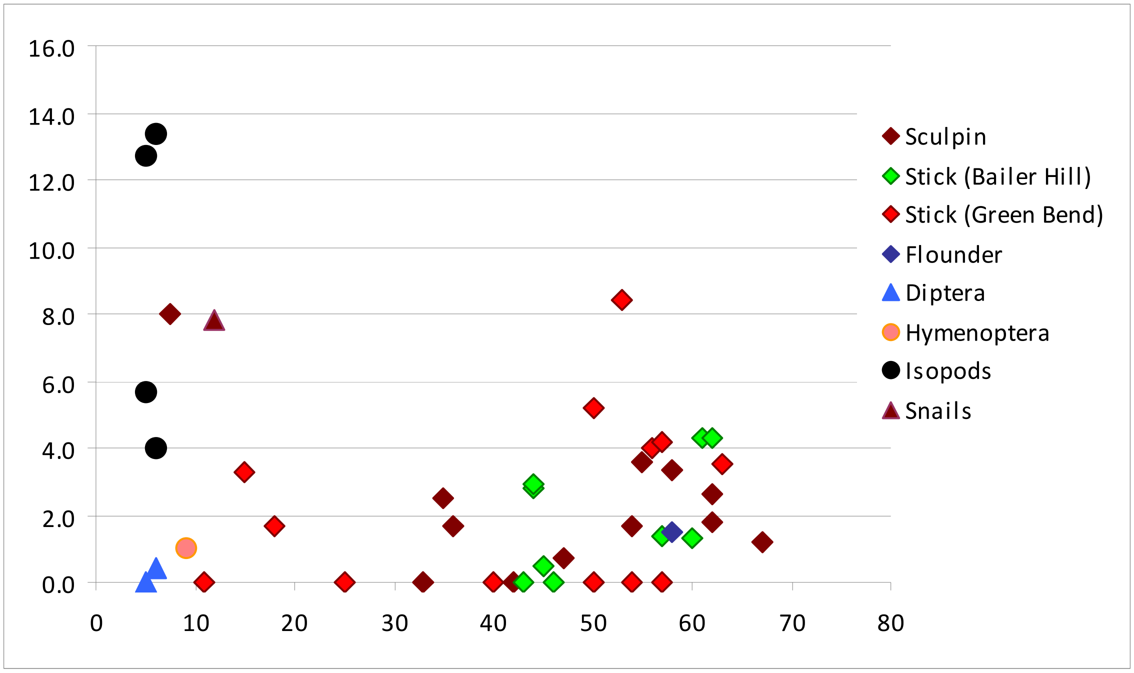
Legacy toxics (PCBs)

Figure 1 shows concentrations of PCBs measured by ELISA in False Bay fish and invertebrates, by the length of each animal. Length is used here as a proxy for age within taxa: a 65 mm stickleback is generally older than a 35-mm stickleback, and therefore may have accumulated greater toxic loads from its environment. Animals under 10 mm are all invertebrates and potential prey.

Mean PCB loading of False Bay Creek fish was 2.0 parts per billion (ppb); mean loading of the invertebrates was 5.6 ppb. There was no statistically significant difference between the loading of fish collected at Green Bend and Bailer Hill, although the range of PCB loading was somewhat higher at Green Bend, the site farther downstream. Nor was there any statistically significant difference between PCB loading of sticklebacks and the sculpins collected from the same location. The highest concentrations observed (over 10 ppm) were in two composite samples of isopods.

Figure 1: PCBs in False Bay fish and invertebrates, 2010

Concentration in parts per billion by size of animal in millimeters



Toxic loading was not a function of the size of fish. This is not surprising, as the sticklebacks in the sample were probably all less than two years old, and differed little in the duration of their exposure to PCBs in False Bay Creek water and sediments. Loading varied considerably at the individual level, regardless of size, suggesting that the primary factor determining uptake of PCBs is the varied diet of individual fish: as seen in Figure 1 there was considerable variation in the loading of invertebrate prey species.

Current-source toxics (PAHs, pyrethroid pesticides)

Unlike PCBs, which are no longer used or discharged in the islands and should no longer be increasing in freshwater habitats, pyrethroid pesticides continue to be applied to gardens, lawns, and (for carpenter ant and termite control) to the foundations of homes—and PAHs from incomplete combustion of petroleum fuels and lubricants continue to run off from roads, driveways and parking areas. Accumulation of these toxics in fish and in the environment may continue, at least for the more persistent pyrethroids e.g. Bifenthrin, the most popular choice for ant control, and for the heavier and less volatile PAHs, which are relatively stable under ambient conditions.

Figure 2 shows results of ELISA testing of False Bay Creek fish and invertebrates for pyrethroid pesticides in relation to the size of the animals. Mean concentration in fish was 4.1 parts per billion; mean concentration in invertebrates was 8.37 ppb. As was seen in the case of PCBs, toxic loading of invertebrates was roughly twice the toxic loading of fish. No statistically significant differences were found between Green Bend and Bailer Hill fish, or between fish species; nor was toxic loading of fish a function of size.

Figure 2: Pyrethroids in False Bay fish and invertebrates, 2010

Concentration in parts per billion by size of animal in millimeters

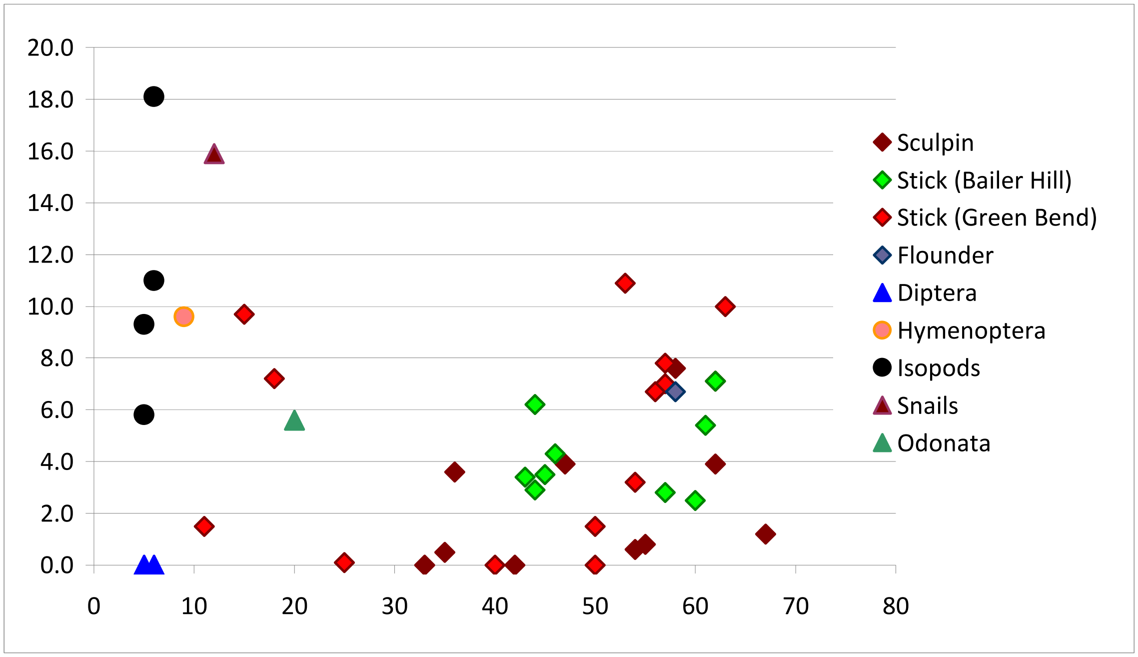
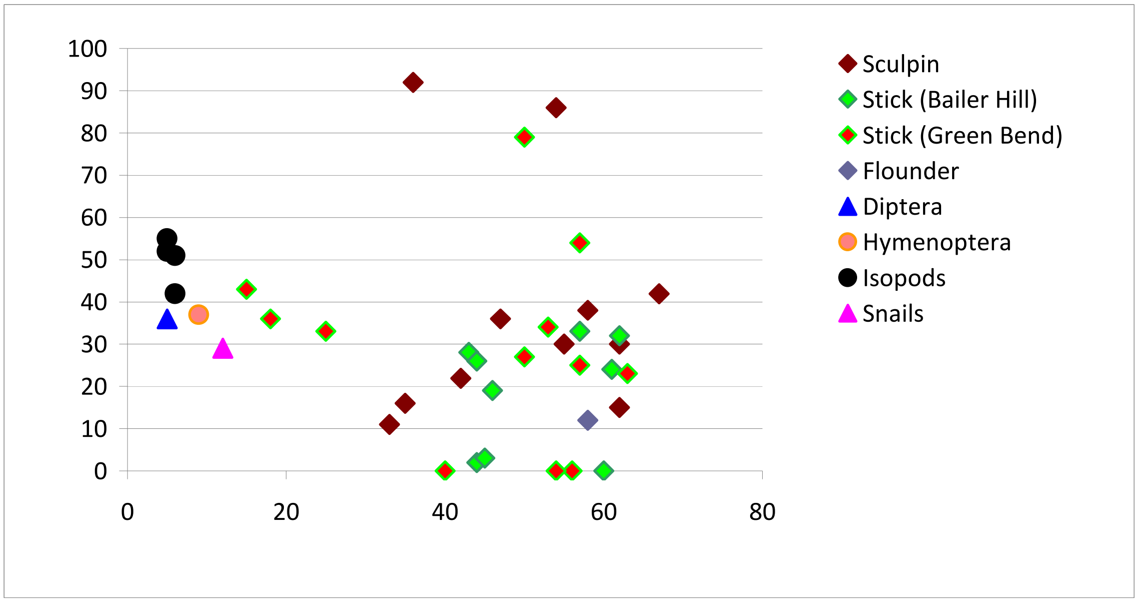


Figure 3: PAHs in False Bay fish and invertebrates, 2010

Concentration in parts per billion by size of animal in millimeters



ELISA results for PAHs (Figure 3) were considerably greater than either PCBs or pyrethroid pesticides (Figure 4), with mean PAH concentrations of 29 ppb for fish, and 43 ppb for invertebrates. Toxic loading of invertebrates was once again about twice the toxic loading observed in fish, and differences between fish species, collection sites, and size classes of fish were not statistically significant.

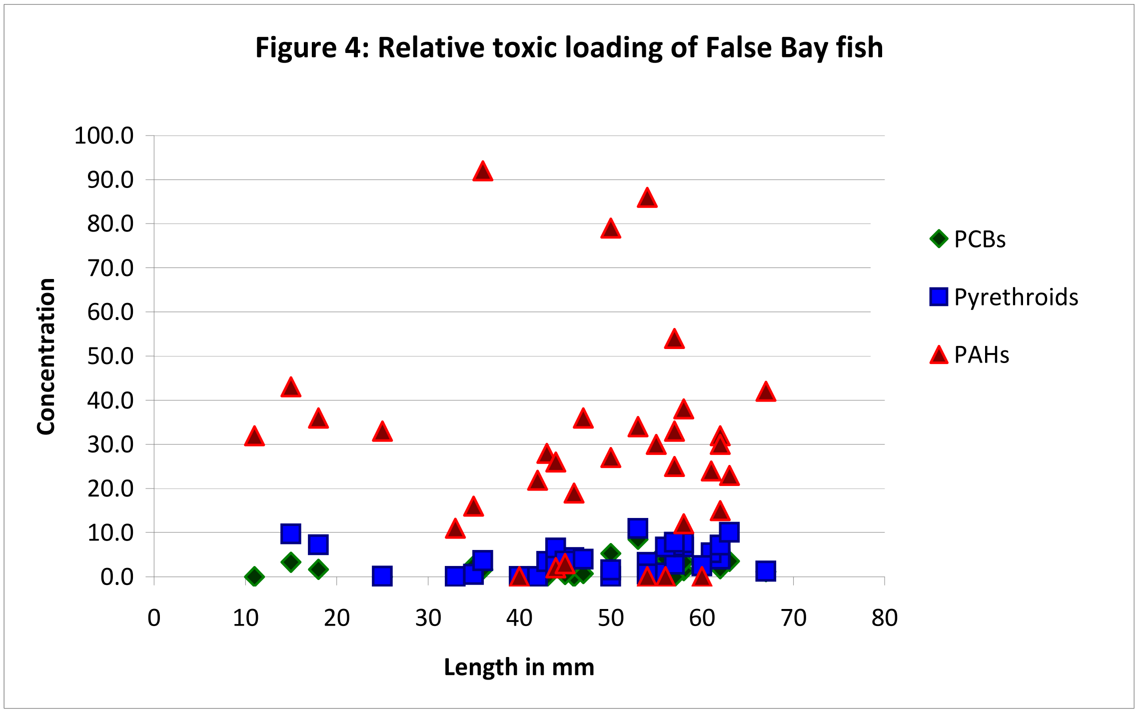
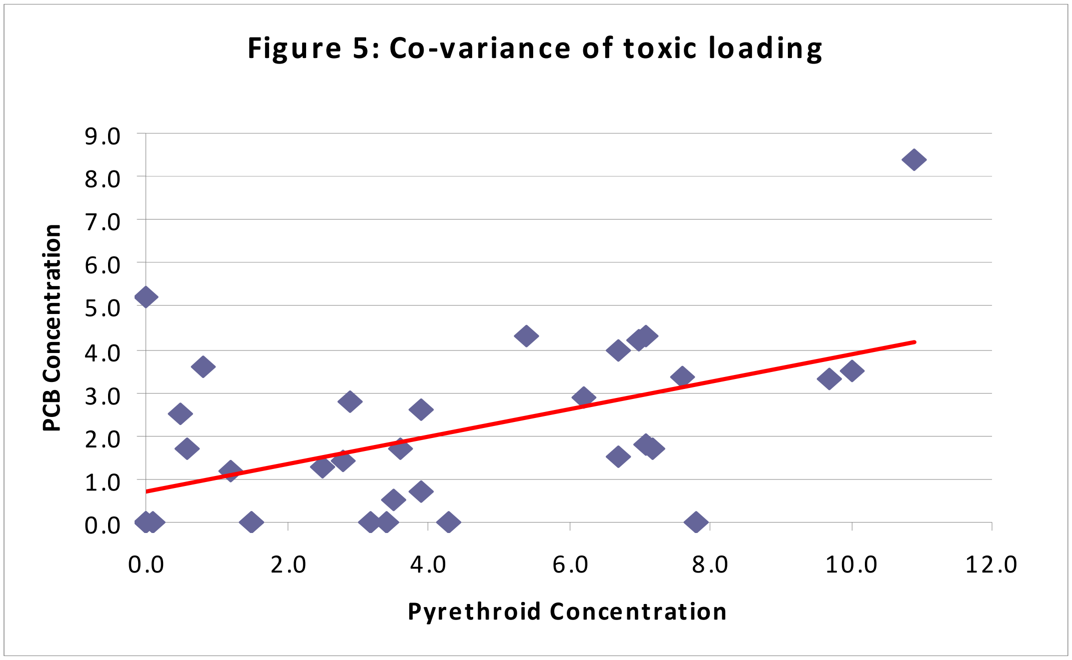
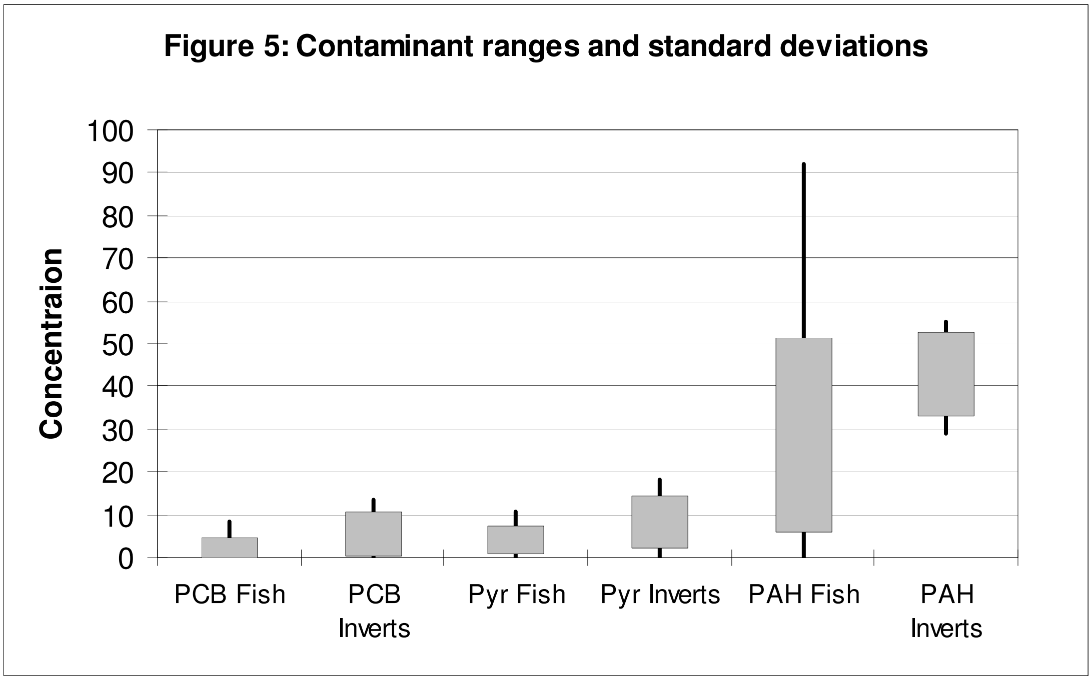


Figure 4: Relative toxic loading of False Bay fish

The consistent ratio of invertebrate to fish loading, and lack of site, size or species differentiation among fish, suggests that processes of toxic uptake for PCBs, PAHs, and pyrethroid pesticides in False Bay Creek were similar. While there is co-variance of PCB and pyrethroid pesticide loads at the individual level (Figure 5), consistent with similar underlying processes, the relationship is not very strong (r^2=0.28).



PAH loads do not co-vary with PCB or pyrethroid pesticide loads, moreover. The reason for this is individual variation in toxic loads, especially for PAHs (Figure 6).



Emerging issues (APEOs)

Measuring Nonyl Phenol in animal tissue, as opposed to water or sediment, poses considerable technical challenges. Animal tissue is fatty. Like many other contaminants, NPs are lipophilic and accumulate in fatty tissues. Separating NPs from fats in extraction is difficult but necessary: fats do not ionize easily, can accumulate on the ionization wire in a mass spectrometer resulting in frequent shut-downs for cleaning, and can ‘mask’ the NP signal in a cloud of similar-sized ions. ELISA methods could avoid these difficulties but are not yet available for NPs.

At the same time, all cleanup methods lose some of the original sample, and poor recovery rates make it impossible to ‘see’ very small concentrations of the target analyte (Gadzala-Kopciuch et al. 2008). Recovery rates are estimated by spiking tissue extracts with known quantities of the target analyte; cleaning up the extracts; and measuring post-cleanup concentrations of the target analyte. By this means we estimated that our method for cleaning up fish tissue extracts has a recovery rate of approximately 50 percent. At a limit of detection of roughly 1 part per billion for our ESI mass spectrometer, this means that the smallest concentration of NP we can reliably determine in fish tissue is 2 ppb.

An additional difficulty is the initial quantity of tissue required. Every step of the extraction and cleanup process involves some loss of sample in separatory columns and filters. For a final volume of 1 mL, sufficient for four replicate measurements at a LOD of 2 ppb, we found by experiment that we required several grams of fish tissue. Young-of-the-year sticklebacks tend to weight less than 0.2 grams. It was accordingly necessary to begin with a composite sample, rather than extracting NPs from individual fish.

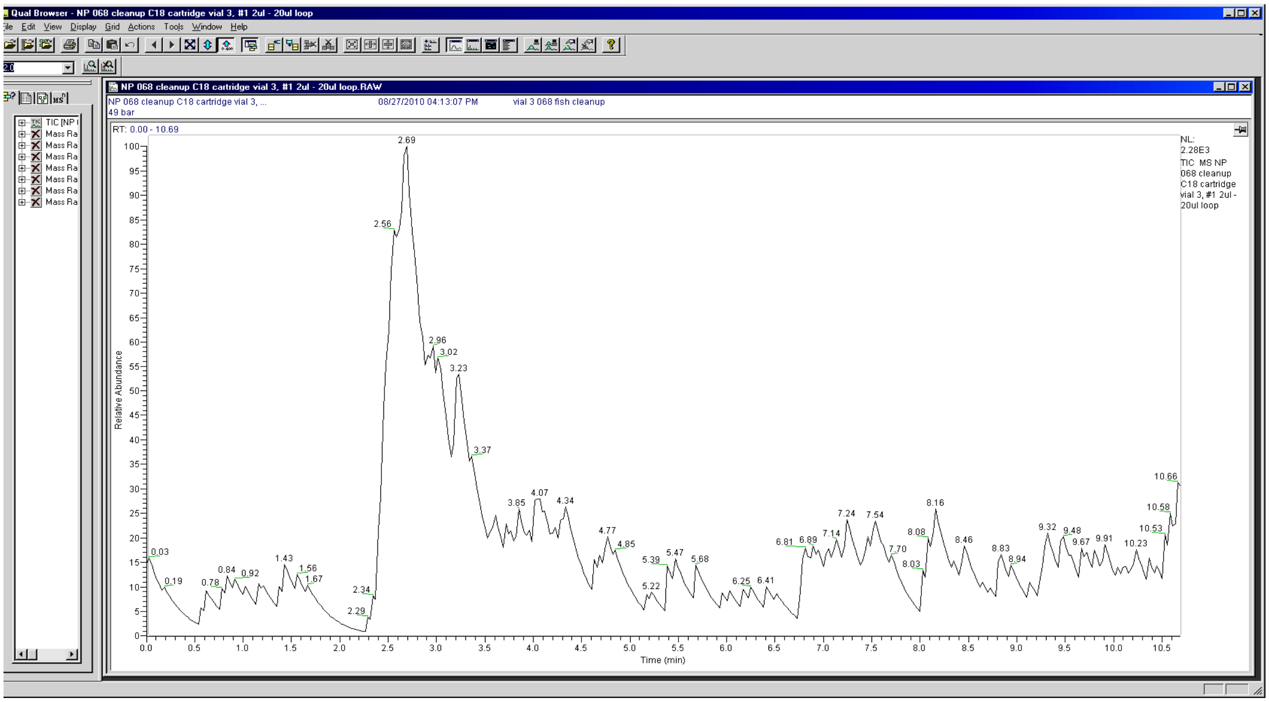
A single composite sample of 24 age 0+ sticklebacks was homogenized, extracted and cleaned up as described under Methods, above. The final step produced a succession of 2 μL fractions. Each fraction was ‘shot’ in turn to ascertain where the NP signal was strongest (highest signal-to-noise ratio). The third fraction displayed a distinct peak for a Product Ion Mass of 219.2 at the appropriate elution time 2.54 minutes (Figure 7).

The response factor was determined using standard solutions of NP of 25, 50, 75, and 100 ppb, and the observed NP peak was computed as 6.5 ppb on column, and 15 ppb. after correction for the incomplete recovery of NPs during cleanup of the original tissue extract. This likely underestimate actual body loads of NP in the False Bay Creek fish since our method probably did not extract 100 percent of the NP from homogenized fish tissue.

Our previous study of San Juan County surface waters and fresh water sediments (Barsh et al. 2008) estimated total non-ionic surfactant loads by the CTAS method, which had a Limit of Detection (LOD) of 1 part per million (ppm). Ambient loads of non-ionic surfactants by this method ranged as high as 12.6 ppm with a mean of 1.27 ppm. We had concluded that the CTAS method was not acceptably precise or reliable, however. While the present study found only 15 ppb in freshwater fish—one percent of the mean result of CTAS testing of water and sediments—it should be borne in mind that mass spectrometry is not only more reliable and sensitive than CTAS colorimetry, but more specific, focused on a single metabolic breakdown byproduct of only one family on non-ionic surfactants:

Alkyl Phenyl Ethoxylates. CTAS reacts with all families of non-ionic surfactants, as well as cross-reacting with many of the historically more widely used anionic surfactants, such as phosphonates and sulfonates.

Figure 7: Mass spectrogram of False Bay Creek stickleback tissue



Non-ionic surfactants reportedly degrade relatively quickly in natural waters, and NPs are lipophilic, hence likely to accumulate in sediments. Uptake and retention of NPs by fish may result in low body loads compared to NP concentrations in runoff water (the fresh signal) or even in sediments (longer term accumulation).

In any case, 15 ppb is not trivial from a physiological perspective, especially in 0+ age fish with developing gonads. Chronic exposure to concentrations as low as 1 ppb can affect the physiology of salmonids (Burkhart-Holm et al. 2000). Lech et al. (1996) have experimentally determined the EC50 of NP for Rainbow trout (*Oncorhynchus mykiss*) to be 14.4 ppb, and the EC100 to be approximately 45 ppb. In other words, half of the salmonids tested displayed estrogenic effects at 14.4 ppb, and all of them were affected at 45 ppb. Schwaiger et al. (2002) found that exposure of adult Rainbow trout to 10 ppb of NP resulted in less viable eggs.

Discussion and Recommendations

From the perspective of salmonid restoration, the portion of False Bay Creek that retains water in summer at the present time is very warm and hypoxic by midsummer and consequently unsuitable for most fish species. Ammonia concentrations in excess of one part per million (ppm), presumably due to runoff from upstream cattle pastures, also pose a potential hazard to fish.

Adverse effects can also be expected from observed pyrethroid pesticide loads in freshwater fish and invertebrates averaging 4.1 parts per billion and PAH loads averaging 29 parts per billion (ppb).

This study is the first confirmation of measurable, biologically significant loading of freshwater biota in the San Juan Islands with nonionic surfactants. Fish tissue loads of 15 parts per billion Nonyl Phenol (NP) observed in a single composite sample of juvenile sticklebacks would be sufficient to cause physiological stress and loss of egg viability in salmonids.

In summary, chemical conditions in False Bay Creek are barriers to the successful restoration of historical salmonid populations, and should be addressed as part of plans to improve connectivity and habitat quality. Priority should be given to:

- Modifying control structures on upstream lakes and ponds to provide a minimum of 0.25 cfs summer instream flow and ensure adequate oxygen (Barsh 2010);
- Constructing wider, denser, and more effective woody riparian buffers, especially in working pastures above Bailer Hill Road, to reduce nutrient loads (in particular, ammonia), moderate summer water temperatures, and sequester contaminants;
- Ensuring that all county roads are equipped with appropriate vegetated ditches that drain to natural or constructed wetlands sufficient in soil depth and vegetated areas to impound and degrade motor oils, motor fuels, road tars and asphalts;
- Reducing outdoor home and garden use of products containing pyrethroids within the False Bay watershed, in particular the pyrethroid species that are most toxic to salmonids (as well as persistent in the environment) such as Bifenthrin, Cyfluthrin, Cyhalothrin, Esfenvalerate and Deltamethrin that have LC50s for salmonids of one part per billion or less;
- Encouraging homeowners and outdoor professionals to avoid products that employ nonionic surfactants as emulsifiers or wetting agents. This includes pesticides and herbicides that are sold in hose sprayers, or as concentrates to be mixed with water for use with a hose sprayer.

Acknowledgments

Funding for this study was provided by the Washington Water Trust under a grant from the Washington State Salmon Recovery Funding Board. Research was conducted at the University of Washington's Friday Harbor Laboratories, Friday Harbor, WA..

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