AMENDED IN SENATE APRIL 22, 2015

# SENATE BILL No. 637

**Introduced by Senator Allen**

February 27, 2015

An act to *amend Section 5653 of the Fish and Game Code, and to* add Section 13172.5 to the Water Code, relating to ~~water quality~~ *dredging*.

LEGISLATIVE COUNSEL'S DIGEST

SB 637, as amended, Allen. ~~Water quality: suction~~ *Suction* dredge mining: permits.

Existing law prohibits the use of any vacuum or suction dredge equipment by any person in any river, stream, or lake of this state without a permit issued by the Department of Fish and Wildlife. *Existing law requires the department to issue a permit, if the department determines that the use of a vacuum or suction dredge will not be deleterious to fish, upon the payment of a specified fee.*

*This bill would instead require the department to issue a permit if the department determines that the use does not cause any significant effects on fish and wildlife and would authorize the department to adjust the specified fee to an amount sufficient to cover all reasonable costs of the department in regulating suction dredging activities.*

Under existing law, the State Water Resources Control Board and the California regional water quality control boards prescribe waste discharge requirements in accordance with the federal Clean Water Act and the Porter-Cologne Water Quality Control Act (state act). The state act, with certain exceptions, requires a waste discharger to file certain information with the appropriate regional board and to pay an annual fee. The state act additionally requires a person, before discharging

mining waste, to submit to the regional board a report on the physical and chemical characteristics of the waste that could affect its potential to cause pollution or contamination and a report that evaluates the potential of the mining waste discharge to produce acid mine drainage, the discharge or leaching of heavy metals, or the release of other hazardous substances.

This bill would require, by July 1, 2017, the State Water Resources Control board to establish a permitting process for suction dredge mining and related mining activities in rivers and streams in the state, consistent with requirements of the state act. The bill would require that the regulations, at a minimum, address cumulative and water quality impacts of specified issues. A person who violates these regulations would be liable for an unspecified penalty. The bill would provide that the state board is not prohibited from adopting regulations that would prohibit suction dredge mining, if the state board makes a certain finding relating to water quality objectives, to the extent consistent with federal law. The bill would prohibit these provisions from affecting any other law, including the California Environmental Quality Act and specified provisions relating to streambed alteration requirements.

*The bill would specify that a suction dredge contains any of specified components for purposes of permits issued by the Department of Fish and Wildlife and for purposes of the permitting process established by the state board.*

Vote: majority. Appropriation: no. Fiscal committee: yes. State-mandated local program: no.

*The people of the State of California do enact as follows:*

*SECTION 1. Section 5653 of the Fish and Game Code is amended to read:*

5653. (a) The use of ~~any~~ *a* vacuum or suction dredge equipment by ~~any~~ *a* person in ~~any~~ *a* river, stream, or lake of this state is prohibited, except as authorized under a permit issued to that person by the department in compliance with the regulations adopted pursuant to Section 5653.9. Before ~~any~~ *a* person uses ~~any~~ *a* vacuum or suction dredge equipment in ~~any~~ *a* river, stream, or lake of this state, that person shall submit an application for a permit for a vacuum or suction dredge to the department, specifying the type and size of equipment to be used and other information as the department may require.

(b) Under the regulations adopted pursuant to Section 5653.9, the department shall designate waters or areas wherein vacuum or suction dredges may be used pursuant to a permit, waters or areas closed to those dredges, the maximum size of those dredges that may be used, and the time of year when those dredges may be used. If the department determines, pursuant to the regulations adopted pursuant to Section 5653.9, that the ~~operation will not be deleterious to fish~~ *use of a vacuum or suction dredge does not cause any significant effects to fish and wildlife*, it shall issue a permit to the applicant. If ~~any~~ *a* person operates ~~any~~ equipment other than that authorized by the permit or conducts the operation in any waters or area or at any time that is not authorized by the permit, or if any person conducts the operation without securing the permit, that person is guilty of a misdemeanor.

(c) ~~The~~ *(1) Except as provided in paragraph (2), the* department shall issue a permit upon the payment, in the case of a resident, of a base fee of twenty-five dollars ($25), as adjusted under Section 713, when an onsite investigation of the project size is not deemed necessary by the department, and a base fee of one hundred thirty dollars ($130), as adjusted under Section 713, when the department deems that an onsite investigation is necessary. ~~In~~ *Except as provided in paragraph (2), in* the case of a nonresident, the base fee shall be one hundred dollars ($100), as adjusted under Section 713, when an onsite investigation is not deemed necessary, and a base fee of two hundred twenty dollars ($220), as adjusted under Section 713, when an onsite investigation is deemed necessary.

*(2) The department may adjust the base fees for a permit described in this subdivision to an amount sufficient to cover all reasonable costs of the department in regulating suction dredging activities.*

(d) It is unlawful to possess a vacuum or suction dredge in areas, or in or within 100 yards of waters, that are closed to the use of vacuum or suction dredges.

*(e) For purposes of this section and Section 5653.1, a suction dredge contains any of the following:*

*(1) A hose that vacuums sediment from a river, stream, or lake.*

*(2) A motorized pump.*

*(3) A motorized sluice box.*

~~SECTION 1.~~

*SEC. 2.* Section 13172.5 is added to the Water Code, to read:

13172.5. (a) On or before July 1, 2017, the state board shall establish by regulation a permitting process for suction dredge mining and related mining activities in rivers and streams in the state. The regulations shall be consistent with the requirements of this division and, at a minimum, address cumulative and water quality impacts of each of the following:

(1) Mercury loading to downstream reaches of rivers and streams affected by suction dredge mining.

(2) Methylmercury formation in water bodies.

(3) Bioaccumulation of mercury in aquatic organisms.

(b) A person who violates a regulation adopted pursuant to this section shall be liable in the amount of ____ ($____).

(c) Nothing in subdivision (a) shall prohibit the state board from adopting regulations that prohibit suction dredge mining if the state board finds that prohibition is necessary to regulate waste discharges that violate or impair water quality objectives or other criteria under this division, to the extent consistent with federal law. In making this determination, the state board may consider, but is not limited to, soil types, fueling and refueling activities, and horsepower limitations.

(d) This section does not affect any other law, including the California Environmental Quality Act (Division 13 (commencing with Section 21000) of the Public Resources Code) and the Department of Fish and Wildlife's streambed alteration requirements described in Chapter 6 (commencing with Section 1600) of the Fish and Game Code.

*(e) For purposes of this section, a suction dredge contains any of the following:*

*(1) A hose that vacuums sediment from a river, stream, or lake.*

*(2) A motorized pump.*

*(3) A motorized sluice box.*

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**Senate Environmental Quality Committee**
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Dear Committee Members and Staff,

As a liaison working in coordination with numerous interested non-profit organizations, businesses, politicians, members of the scientific community, and individuals, I hereby oppose SB-637 of 2015 as written and amended. Please include this letter, my comments, and provided documentation for consideration when preparing the legislative analysis and in hearing this bill.

Previously, I submitted my written and verbal statements of opposition, comments, and supporting documentation to the Natural Resources a Water Committee, which shall be forwarded to this committee.

Furthermore, I wish to lodge my objection in the strongest manner regarding the amendments to the bill introduced in the analysis for NR&W, which would drastically increase the scope of the originally proposed bill:

- Amendment 1 significantly alters the criteria from that which is “deleterious to fish” — the causing of harm to fish, to “does not cause any significant effects to fish and wildlife”, a much less clear and more broad definition which could be construed to disallow activities which may have significant positive impacts.

- Redefining a suction dredge per Amendment 2 is absurd on its face — it would cause sluices, rocker boxes, water pumps, and culvert maintenance equipment to be defined as “suction dredges”, which they clearly are not.

- Amendment 3 proposes to give DFG “explicit authority to set suction dredge mining fees by regulation to fully cover all program costs”, despite their clear statement that they do not in fact have the appropriate regulatory and permitting authority or premacy to administer such a program.

**National Pollution Discharge Elimination System — 40 CFR §122.2 Definitions.**

...

*Discharge* when used without qualification means the “discharge of a pollutant.”

*Discharge of a pollutant* means:

(a) Any addition of any “pollutant” or combination of pollutants to “waters of the United States” from any “point source,” or

(b) Any addition of any pollutant or combination of pollutants to the waters of the “contiguous zone” or the ocean from any point source other than a vessel or other floating craft which is being used as a means of transportation.

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**Porter-Cologne Water Quality Control Act § 13263.3. Legislative findings; definitions**

...

(b)(1) For the purposes of this section, “pollution prevention” means any action that causes a net reduction in the use or generation of a hazardous substance or other pollutant that is discharged into water and includes any of the following:

...

(c) For the purposes of this section, “discharger” means any entity required to obtain a national pollutant discharge elimination system (NPDES) permit pursuant to the Clean Water Act (33 U.S.C. Sec. 1251 et seq.), or any entity subject to the pretreatment program as defined in Part 403 (commencing with Section 403.1) of Subchapter N of Chapter 1 of Part 403 of Title 40 of the Code of Federal Regulations.

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This bill misconstrues the “incidental fallback” of “indigenous sediments” in substantially the same location as they are removed for processing by a suction dredge as a “waste discharge”, despite there being no “addition” under the Clean Water Act, and in fact generally resulting in a “net reduction [...] of a hazardous substance...”, as explicitly encouraged by the Porter-Cologne Water Quality Control Act. It is thus counterproductive to the purpose of achieving long-term water quality improvements through the reduction of mercury, lead, and other heavy metals — not to mention removal of other anthropogenic waste and trash — with little or no additional costs to taxpayers, individuals, or businesses, and minimal impact to fish, wildlife, and the ecosystem.

Sincerely,

Chris A. Giorgi

## Comments on Text of SB637:

*(Note: I am not a lawyer.)*

The first sentence of (a) conflicts with the concept of equal application of the law by singling out mining activities, while ignoring other uses of the same techniques which have equal or greater impact.

The second sentence of (a) states that “The regulations shall be consistent with the requirements of this division…”, however no actual requirements are articulated anywhere within the text. The list of impacts to address states no goals or criteria of any sort.

Impact (1) fails to identify what is meant by loading, nor why it is a concern — a dredge removes mercury and thus reduces total loading. Seasonal high-water events frequently transport the entire sediment bed (including the mercury and gold) a long distance downstream, while sediment processed through a suction dredge has the vast majority of the heavy materials selectively removed, and the remaining material is re-deposited in the same area from which it was extracted, minus most of the mercury.

Impact (2) is a naturally occurring process which takes place faster in warm, stagnant, and anoxic conditions, commonly found in reservoirs and the delta where non-mining dredging activities are common, but occur rarely in most areas where suction dredge mining is prevalent.

Impact (3) is a much greater concern in areas where mercury enters the food-chain in vapor form than liquid. Consider that nearly 30% of air pollution impacting the San Francisco Bay Area originates from coal-powered (mercury, lead, and radioisotope laden) industries in China and travels here on the winds in less than a week. In aquatic environments, if elements such as selenium are present in sufficient quantities, they can chemically bind with mercury to reduce its bioavailability and apparent toxicity to organisms.

The undefined statutory fine under (b) fails to consider the nature, severity, or impact of a purported violation of yet undefined regulations.

The classification of fallback of dredged material under (c) as waste discharge has already been litigated and found invalid. Prohibition is against federal law, which contradicts the clause internally. Many criteria given are unrelated to the activity of dredging itself, and are already covered under existing law. Clause allows specifically for unlimited construction regarding criteria which may be used as a basis for prohibition, regardless of relevance or impact.

Clause (d) provides additional evidence of failure of the equal application of the law.

## Further Comments Regarding the Transport, Methylation, and Bioaccumulation of Mercury:

- A properly operating dredge is usually more than 90% efficient at removing elemental mercury (in fact, state and federal studies show actual efficiency near 98%) , and will generally recover at least 99% after 2 passes; furthermore, given even huge quantities:

  Removing 90% of the original quantity leaves 10%
  Removing 90% of the remaining 10% leaves 1%
  Removing 90% of the remaining 1% leaves 0.1%
  Removing 90% of the remaining 0.1% leaves 0.01%
  Removing 90% of the remaining 0.01% leaves 0.001%
  Removing 90% of the remaining 0.001% leaves 0.0001%

  Thus, after 6 passes through a dredge between a concentrated point source in a stream or river and a downstream reservoir, up to 99.9999% of original mercury is reclaimed, leaving only one ounce for every 1,000,000 ounces of elemental mercury contained in sediments processed, and recovering 999,999 ounces. Consider that each seasonal high water event move significantly more material than all the dredgers on a river could move in an entire season; the entire quantity of mercury thus mobilized moves downstream unimpeded, unlike when material is processed through a dredge and the mercury captured.
- Methylmercury is rapidly broken down by exposure to UV light, which can only penetrate to shallow depths in water; a sluice box and the area of dispersion in the water a short distance beyond its end provide just such an environment; methylmercury formation preferentially takes place in deep, compacted, anoxic sediments, which dredging breaks up and oxygenates.
- Since dredging necessarily results in a reduction of total elemental mercury through direct removal, and reduction of methylmercury through photochemical degradation and inhibiting formation, the net effect of consistent dredging on the bioaccumulation of mercury is a potentially significant overall reduction, especially where such operations are undertaken upstream of lakes, reservoirs, deltas, floodplains, or coastal shallows.

See US Supreme Court case: LOS ANGELES COUNTY FLOOD CONTROL DISTRICT v NATURAL RESOURCES DEFENSE COUNCIL, INC. — “Pot of Soup” analogy by Justice Ruth Bader Ginsburg.

# 10 Essential Mercury Facts

1. **The Food and Drug Administration writes that its dietary mercury guidelines were "established to limit consumers' methyl mercury exposure to levels 10 times lower than the lowest levels associated with adverse effects."** Americans who consume twice as much mercury as the FDA recommends are still protected by a 500-percent cushion. The same generous safety margin applies to the Environmental Protection Agency's mercury "Reference Dose." And the Centers for Disease Control and Prevention reports that zero percent of American children exceed the EPA's hyper-cautionary guideline.

2. **The U.S. government's Institute of Medicine (a division of the National Academies of Science) warned in a major 2006 report that a "spillover effect" from one-size-fits-all fish warnings could deny most consumers the health benefits of seafood consumption.** This report demonstrates a severe disagreement between serious scientists and activists who demand "warning" signs (aimed at *all* consumers) on grocery-store fish counters.

3. **There are no scientifically documented cases of Americans developing mercury poisoning from eating commercially available fish.** The only documented cases in the medical literature are from Japan in the 1950s and 1960s, following a massive industrial spill of mercury into fishing waters. Mercury levels today (in both fish and people) are nowhere near the levels measured during this tragic episode.

4. **The federal government's mercury-in-fish recommendations are based largely on a single study whose participants were exposed to mercury by eating whale meat—not fish.** The study was conducted in Denmark's Faroe Islands. Unlike fish, whale meat is contaminated with a variety of pollutants, so isolating mercury's effects is practically impossible. In 2004 the lead Faroe researcher acknowledged in *The Boston Herald* that "fish consumption does not harm Faroese children … the fish consumption most likely is beneficial to their health."

5. **A twelve-year study conducted in the Seychelles Islands (in the Indian Ocean) recently found *no negative health effects* from exposure to mercury through heavy fish consumption.** On average, people in the Seychelles eat between 12 and 14 fish meals every week, and the mercury levels measured in the island natives are higher than those measured in the United States. But they suffered no ill effects from mercury in fish, and they received a significant health benefit from eating fish in the first place.

6. **In February 2007, *The Lancet* (the United Kingdom's most prestigious medical journal) published U.S. government-funded research demonstrating a clear health *benefit* to children whose mothers ate large amounts of fish while pregnant.** Researchers wrote that they could find "no evidence to lend support to the warnings of the U.S. advisory that pregnant women should limit their seafood consumption." Of the more than 9,000 pregnant women in this study, those who ate the most fish—regardless of mercury levels—had children with the highest IQ's.

7. **Studies published in 2005 in the *American Journal of Preventive Medicine* found that even eating small amounts of fish each week can result in a 17 percent lower risk of heart disease, a 12 percent lower risk of stroke, and (when eaten by pregnant women) a modest increase in children's IQ.** The Omega-3 fats found in fish can also protect against Alzheimer's disease, arthritis, breast and prostate cancer, and many other conditions.

8. **Researchers at Harvard University concluded that the health benefits of fish "greatly outweigh the risks," including those from trace amounts of mercury.** Their study was published in *JAMA* (the Journal of the American Medical Association) in October 2006.

9. **Over forty years of scientific research has established that selenium, a plentiful nutrient in fish, can effectively neutralize the toxicity of trace amounts of mercury in seafood.** According to the U.S. Department of Agriculture, 16 of the 25 best sources of dietary selenium are ocean fish.

10. **There's solid scientific evidence that the amount of mercury in fish has remained the same (or even *decreased*) during the past century.** Researchers from Princeton University, Duke University, and the Los Angeles County Natural History Museum have all compared specimens of ocean fish preserved between 25 and 120 years ago with current samples of the same species. In these studies, mercury levels in the fish stayed the same or declined.

mercuryfacts.org

*MercuryFacts.org is a project of the nonprofit Center for Consumer Freedom. Write us at info@ConsumerFreedom.com or call 202.463.7112 to request an interview.*

# The Challenge:

Looking for gold in California streams and rivers is a recreational activity for thousands of state residents. Many gold enthusiasts simply pan gravels and sediments. More serious recreational miners may have small sluice boxes or suction dredges to recover gold bearing sediments. As these miners remove sediments, sands, and gravel from streams and former mine sites to separate out the gold, they are also removing mercury.

This mercury is the remnant of millions of pounds of pure mercury that was added to sluice boxes used by historic mining operations between 1850 and 1890. Mercury is a toxic, persistent, and bioaccumulative pollutant that affects the nervous system and has long been known to be toxic to humans, fish, and wildlife. Mercury in streams can bioaccumulate in fish and make them unfit for human consumption.

**The Solution:**
Taking mercury out of streams benefits the environment. Efforts to collect mercury from recreational gold miners in the past however, have been stymied due to perceived regulatory barriers. Disposal of mercury is normally subject to all regulations applicable to hazardous waste.

In 2000, EPA and California's Division of Toxic Substance Control worked in concert with other State and local agencies to find the regulatory flexibility needed to collect mercury in a simple and effective manner. These groups agreed to test two different mechanisms for collecting mercury during the summer of 2000. One approach was to add mercury to the list of materials that are collected at regularly scheduled or periodic household hazardous waste collection events sponsored by local county agencies.

Another mercury collection approach was to set up collection stations in areas where mercury is being found by recreational miners. One possibility would be to advertise a fixed location where people could bring mercury on a specific date and time. Another was to create a mercury "milk run" where state, local, or federal agency staff would come to locations specified by individuals or organizations such as suction dredging clubs, and pick up mercury that had been collected.

**The Results:**
In August and September, 2000 the first mercury "milk runs" collected 230 pounds of mercury. Not only was mercury received from recreational gold miners, but others such as retired dentists, also participated by turning in mercury that was in their possession. A Nevada County household waste collection event held in September 2000 collected about 10 pounds of mercury. The total amount of mercury collected was equivalent to the mercury load in 47 years worth of wastewater discharge from the city of Sacramento's sewage treatment plant or the mercury in a million mercury thermometers. This successful pilot program demonstrates how recreational gold miners and government agencies can work together to protect the environment. In the summer of 2001, State agencies planned to extend the program to six counties and include collection of mercury at summer mining fairs.

**Contact:**

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# Get The Lead Out!

by Alan Trees

When I first thought of doing an article on dredging for lead, I was concerned that it would look bad for anglers, and cast a dark light on fishermen. I have been a salmon and steelhead angler for over 50 years, and a gold dredger for over 40 years. I do not want to tarnish the sport of fishing. Most environmental groups and the Environmental Protection Agency (EPA) have long been trying to end any activity that sportsmen enjoy in the outdoors, whether it is fishing, hunting, gem collecting, wood cutting, camping, and the list goes on.

I know this article can be a double-edged sword, but as far as I can see, gold dredging is the only activity happening in our rivers and streams that addresses the deep cleaning and recovery of toxic metals. The EPA has classified lead as a toxin.

It was only a few years ago the EPA and the Idaho Department of Environmental Quality (IDEQ) contacted me to help create a mercury recovery program grant. They mentioned to me on the phone, as well as in an e-mail, that “Idaho gold dredgers were the only ones on the front lines cleaning up toxic metals from streams and rivers.” Keep in mind that in the same department in the same building, other officials are trying to shut down gold dredging in Idaho. It seems in the EPA and IDEQ, the left hand does not know what the right hand is doing. This controversy is best addressed in another article, so for now, I will stay on task to show positive aspects and address how to cleanup lost lead in the bottoms of our rivers and streams.”

I want to cover four things—where to start your search, how to identify the best locations to find lead in our waterways, the best techniques and the proper equipment, and how to market the lead you recover.

There are many locations here in Idaho, as well as a number of other western states, that have tons of lead lost in the streams and riverbeds. I am sure that in 42 years of gold diving I have recovered at least 3,000 pounds of lead. During my gold dredging, I have not only recovered and removed lead, but hundreds of pounds of mercury, old car parts, soft drink and beer cans, boat parts, glass, sunglasses, miles of fishing line and tons of other modern-day “artifacts.”

Let’s start out with a few guidelines that I incorporate when starting my lead recovery dredging.

First, locate a river that has been well used and is known for many years of fishing. I try to dredge where the water is most accessible. By working in these areas, you will be finding larger quantities of lead because the fisherman are concentrated in these areas. If you just start out dredging in a river without considering the access issue, you will probably find lead but it will be in smaller quantities and much more scattered.

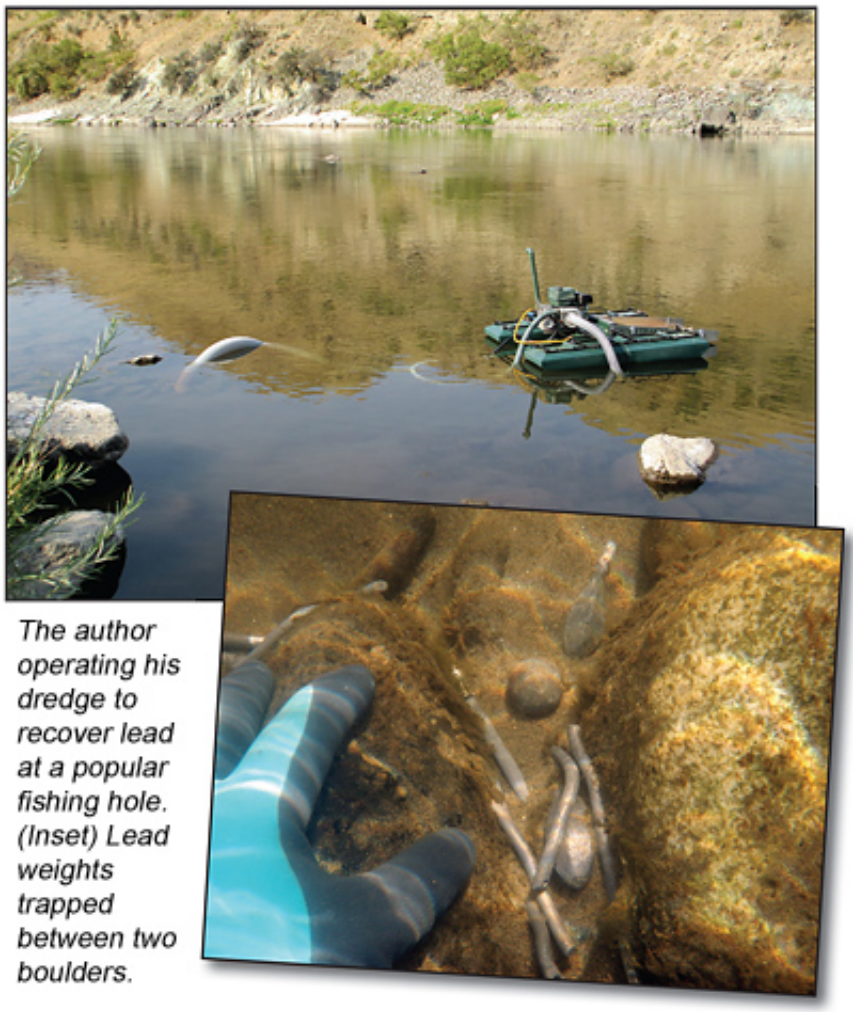
My favorite lead rivers are usually where many anglers fish for salmon or steelhead. This type of fishing requires the use of larger lead weights to get down to the best fishing. The average angler loses about 3-4 pounds of lead a year in larger bodies of water. If you are not familiar with the area, ask a fisherman where most anglers fish, or better yet, stop by during the salmon or steelhead season and take note of where the groups are fishing.

I like to wait until the salmon or steelhead season is over before dredging. This way, I won’t interfere with others and usually the water is lower and warmer by then. I like to draw the least amount of attention as possible because most fishermen do not understand gold dredgers or the positive aspects of this activity.

Second, knowing where to look while underwater is very important in order to find the larger concentrations of lead. Any obstacles you see, such as tree limbs, submerged logs, old fencing, large boulders, or anything protruding from the river bottom, needs investigating. The first day underwater, I found an old section of square wire fencing that had washed downriver during flooding. It had 30 or 40 fishing lures of all kinds tangled in it. Some were red and blue spinners, orange spoons, and all kinds of secret weapons to outsmart the most weary salmon or steelhead. It looked like a bait shop wall and nearly every one of them had a large piece of lead hanging down from them.

I started dredging on the opposite side of the obstacle facing away from the shoreline. I’ve found that lead is usually tangled or lost on the far side of the obstacles. When the angler casts his line over these areas, line and lead often become entangled with the obstacle and will shear or break off. On my first dive, I began searching the river bottom to first pick up the easier lead that was recently lost. Oftentimes, the most recent season’s lead weights are still visible and are protruding from the sand or stony bottoms.

Many times, I find very large accumulations of lead that have been building up for many years. In one such spot behind a large boulder, I recovered three-quarters of a 5-gallon bucket of lead in a two-foot area. This is not uncommon on most river bottoms. One of my favorite ways to spot hidden lead weights is to follow the line or strings. It seems that fishing line does not disintegrate for several years. Short pieces of string poking from the rocky and sandy bottoms can easily be followed to the lead weights. Not only do they lead you to the weights on the ends, but they lead you to a treasure chest of many pounds of lost lead that are hidden from sight.



*The author operating his dredge to recover lead at a popular fishing hole. (Inset) Lead weights trapped between two boulders.*

It is a good idea to wear gloves and to beware of sharp hooks lost in the sandy bottoms. In my first two days of diving, I did not get stuck by a single hook. By being careful and going slow, you should be okay, too.

When determining the right equipment for the job, there are several factors to consider: Am I in a gold-bearing river? Is the dredging season open? Is the location sensitive to dredging due to spawning areas? How deep is the water? Some rivers that I dive in are not gold-bearing rivers, and lead recovery would be my only interest. In this case, I would use my standard dredge, but slightly modify the riffles. Most riffle systems are a bit lower than I like, so instead of being perhaps three inches tall, I change the riffles to 1-1/8 inch tall with about a 70 degree “lean forward” angle. Because most dredge manufacturers are trying to design the right riffle to keep larger stones from clogging the sluice box, the riffles are fairly short and the angle is closer to a 50 degree “lean forward” angle. With this type of standard riffle, lead can get to rolling and be flushed out.

I use a water blaster on my dredge to clear away sand, but the garden hose type that we usually use for gold dredging does not work well for lead. It takes at least a one-inch hose with lots of volume to blast the sand away. If the season is open for gold dredging and gold is present, the basic dredge will do well. The main objective is to recover gold, and lead will be a secondary consideration. Due to the fact that the larger gold is deep in the overburden and the lead seems to be closer to the surface, your operation will be slowed down some just to recover lead.

When diving for lead in more sensitive areas, such as spawning beds or boat landings, I don’t use my standard gold dredge. In these areas, I disassemble my dredge and remove my sluice box as well as my pump housing and impeller. This way the pump seal is not damaged from lack of water and the air compressor drive pulley is free to operate the air compressor. You may have to place a large rock or bucket of rocks on the pontoons to balance them out so it will sit level in the water. All you are interested in is the hookah diving system because the lead recovery will now be hands-on. Hand fanning, or any simple technique you are familiar with, will work fine. When lead diving in these areas, I use a 5-gallon bucket with the bottom cut out and a one-half-inch screen fitted into the bottom. I move this along with me as I pick the pieces of lead from the stream bed.

The depth of the water is important for obvious reasons. If the water is no more than 12-18 inches, then just a snorkel and mask will do fine. I am more comfortable using my hookah system. I feel I am more productive and less worried about breathing in a wave or splash of water with a snorkel.

There is another benefit of lead recovery besides the value of cleaning up the waterways from toxic metals. That would be your recovered lead! Lead is expensive to buy and easy to market or sell. When shopping at my local bait shop, I spend about $7.00 for a 1-pound roll of 1/4-inch lead. I average about 600 pounds of lead in a 3-hour dive in a well fished Idaho river. Most fishermen would gladly pay $2.50/pound for used lead. This is one-third the price of finished lead. They can melt down and re-cast the lead to the size and shape that meets their needs. If you do the math, it equates to about $1,500 of heavy metal value per three-hour dive. This is good wages, and you are doing a service to the environment by removing this toxic metal. I feel that gold divers are working hand-in-hand with the sport of fishing to minimize the effects of toxic metals.

Here is a situation that happened to me awhile back that every gold diver and angler alike can appreciate. I was fishing one morning for salmon from my boat in a fairly fast portion of the river. I was using my wife’s special favorite recipe of salmon eggs with tuna in a bait ball to outsmart a wily Chinook salmon. All of a sudden my pole bent over and I was nearly yanked from the boat as I struggled to regain my balance, plant my feet, and get ready to drag in this monster. It went

upstream, then caught a fast current, and down the river it went. I wrestled desperately to regain control. I could visualize in my mind that this was going to be an Idaho State record salmon—at least 40 pounds or more! All the bait shops and fishing stores—even Cabela's would have my picture plastered all over the front door.

*The author with lead recovered during six hours of dredging in a popular salmon fishing area. The total was 1,250 pounds of lead!*

After considering that I had only 18-pound test line, I knew I had to baby it. The slightest wrong move could end it all. I was attempting to keep my line tight, and I reached down with one hand and slowly retrieved the tape measure so I could be ready for the big event. It seemed like an hour long struggle, but I am sure the whole ordeal only lasted 7 or 8 minutes. Then, as I wrestled the giant creature closer to the boat, the monster seemed to be tiring. I readied my net in my left hand. SWOOP! I somehow managed to hoist the monster over the side and into the boat. I could not believe my eyes! It was a monster, alright! It was a 235-15 Michelin tire! This goes to show you, you never know what you will find lurking in the depths of our rivers and streams.

When you see a gold dredger, thank him for his efforts in cleaning up our streams and rivers. Thank a gold dredger for the removal of trash from our rivers and streams. Thank a gold dredger for creating a better environment for our fisheries. Thank a gold dredger because he or she is the only one on the front lines, ridding the streams and rivers of toxic metals and waste. Can you think of any other program currently in place, by the EPA or any other environmental agency, that you can thank for their equal effort to clean and preserve our fisheries?

# Incidental Fallback

"Incidental Fallback" represents a net withdrawal, not an addition of material. Incidental Fallback cannot be a discharge within the meaning of the Clean Water Acts (CWA) as the CWA only permits and regulates additions. All gold mining suction dredges are designed to withdraw heavy metal (based on their specific gravity) from gravels and soils, it cannot be said that suction dredges add anything within the meaning of the CWA. It is simple math, the difference between addition and subtraction. Those activities that add can require a 401, 402, or 404 permit, those that subtract do not require a permit at all. That is the intent of Congress. The EPA and the Army Corp has for the past 30 years tried to redefine "Incidental Fallback" under a regulated and permitted "redeposit" category, but the courts have found this agency practice invalid on numerous occasions and instructed the EPA and Army Corp to remove their offending regulatory expansion.

To illustrate this point originally in *Nat'l Mining Ass'n v. U.S. Army Corps of Eng'rs,* 145 F.3d 1399, 1404 (D.C.Cir.1998). The court explained that, *"[b]ecause incidental fallback represents a net withdrawal, not an addition, of material**, it cannot be a discharge**" and questioned "how there can be an addition of dredged material when there is no addition of material."* Emphasis added.
And
"*This understanding of "discharge" excludes the small-volume incidental discharge that accompanies excavation and landclearing activities. Senator Muskie explained that "the bill tries to free from the threat of regulation those kinds of manmade activities which are sufficiently de minimis as to merit general attention at the State and local level and little or no attention at the State and local level and little or no attention at the national level." Senate Report on S. 1952, 95th Cong., reprinted in 1977 Legis.Hist. at 645. Senator Domenici stated that "we never intended under section 404 that the Corps of Engineers be involved in the daily lives of our farmers, realtors, people involved in forestry, anyone that is moving a little bit of earth anywhere in this country that might have an impact on navigable streams." Senate Debate, id. at 924.*

This holding stands today and is reflected from the *National Association of Homebuilders v. Corps* decision (D.D.C. 2007) invalidating the January 17, 2001, amendments to the Clean Water Act Section 404 regulatory definition of "discharge of dredged material" (referred to as the "Tulloch II" rule). The U.S. Army Corps of Engineers (Corps) and the Environmental Protection Agency (EPA) have promulgated a joint final rule to amend this definition by conforming the Corps' and EPA's regulations to the language of the court's opinion by deleting language from the regulation that was invalidated.

The State, as mandated by the CWA and funded by federal law, cannot carry out an objective when it conflicts or is inconsistent with express Congressional intent, exemptions, and purpose. See *CA Coastal Commission v. Granite Rock 480 U.S. 572*. State law is preempted if Congress has evidenced intent to occupy entirely given field (as is the case here) or, where Congress has not entirely displaced state regulation, if state law actually conflicts with federal law. If the State thinks otherwise, the clear legislative history of Congress demonstrates that the state law is federally pre-empted on this matter of "Incidental Fallback"as illustrated previously by Senator Muskie

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# Ruling on Motions for Summary Adjudication on Issue of Federal Preemption filed.

NEW FILE

## SUCTION DREDGE MSA RULING

**Included Actions**

- ***Kimble, et al. v. Harris, et al.***, Case No. CIVDS1012922, San Bernardino County, filed September 15, 2010 ("*Kimble*");
- *Karuk Tribe, et al, v. Calif. Dept. of Fish & Game,*[] *et al.*, Case No. RG12623796, Alameda County, filed April 2, 2012 ("*Karuk II*");
- ***Public Lands for the People, et al. v. State of California, et al.***, Case No. CIVDS1203849, San Bernardino County, filed April 12, 2012 ("*PLP*");
- ***The New 49'ers, Inc., et al. v. Calif. Dept. of Fish & Game, et al.***, Case No. SCCVCV1200482, Siskiyou County, filed April 13, 2012 ("*New 49'ers*");
- *Walker v. Kamala Harris, et al.*, Case No. 34-2013-80001439, Sacramento County, filed March 14, 2013 ("*Walker*"); and
- *Foley v. California Dept. of Fish and Wildlife, et al.* Case No. SCCVCV13-00804, Siskiyou County, filed July 1, 2013 ("*Foley*").

**Motions: Motions for Summary Adjudication on Issue of Federal Preemption:**

**(1) Plaintiff Kimble, et al. motion for summary adjudication on its 1st Cause of Action**

**(2) Plaintiff PLP, et al. motion for summary adjudication on its 4th Cause of Action**

**(3) Plaintiff New 49'ers, et al. motion for summary adjudication on its 2nd Cause of Action**

**(4) Defendant CDFW motion for summary adjudication re Kimble Second Amended Complaint (SAC), 1st Cause of Action**

**(5) Defendant CDFW motion for summary adjudication re PLP First Amended Complaint (FAC), 4th Cause of Action**

**(6) Defendant CDFW motion for summary adjudication re New 49'ers FAC, 2nd Cause of Action**

***Request for Judicial Notice***

**a.** In each of the three of CDFW's motions for summary adjudication, CDFW requests judicial notice under Evid. Code § 452(c) (official legislative acts) of the following:

Exhibits A, B, C, and E , various statutes or bills before Congress; Exhibits D, F, G, H, I, J, and M, excerpts from the Congressional Globe or Congressional Record; and Exhibits K and L, congressional committee reports or excerpts from such reports. CDFW argues that all of these documents are relevant to the sole issue presented in this motion - preemption - because the "critical question" in every preemption analysis is congressional intent. (*Louisiana Public Service Com. v. F.C.C.* (1986) 476 U.S. 355, 369.) The Court **Grants judicial notice of CDFW's Ex. A – M.**

**b.** In opposition to CDFW's motion, New 49'ers request judicial notice under Evid. Code § 452(c) (official executive acts) of: Exhibit 1, a Federal Register Notice issued by the Forest Service on June 6, 2005, "Clarification as to When a Notice of Intent to Operate and/or Plan of Operation is Needed for Locatable Mineral Operations on National Forest System Lands," 70 Fed. Reg. 32,713 (June 6, 2005); Exhibit 2 , a high-level administrative appeal from an adverse decision by the Tahoe National Forest Supervisor to the Deputy Regional Forester; and Exhibit 3, an excerpt of a Forest Service Schedule of Proposed Action (SOPA) in the Plumas National Forest.

In opposition to CDFW's motions, Kimble and PLP also request judicial notice under Evid. Code § 452(c) of New 49'ers Ex. 1 and Ex. 3. The Court **Grants judicial notice of Plaintiffs Kimble, PLP and New 49'ers Ex. 1-3.**

**c.** In each of the Kimble and PLP motions for summary adjudication, Kimble and PLP request judicial notice under Evid. Code 452(c) (official executive acts) of : Exhibit A, an E-mail from Mark Stopher, Environmental Project Manager, CDFW, Subject: Suction dredge status July 26, 2011, sent July 26, 2011, 3:49 PM; Exhibit B, April 1, 2013, CDFW Report to the Legislature Regarding Instream Suction Dredge Mining Under The Fish and Game Code (April 1, 2013) ("Report to Legislature"); and Exhibit C, Cal. Code Regs, tit. 14, §228 and §228.5 Suction Dredging. The Court **Grants judicial notice of Kimble and PLP Ex. A-C.**

**d.** *For the first time in reply*, Kimble and PLP make a second request for judicial notice under Evid. Code § 452(c) (official executive acts) of: Exhibit 1, United States Department of the Interior, Bureau of Land Management, Colorado, Minerals/Mining Frequently Asked Questions; Exhibit 2, United States Department of the Interior, Office of the Solicitor Memorandum, Dated November 14, 2005 To: Secretary, Director, Bureau of Land Management From: Solicitor Subject: Legal Requirements for Determining Mining Claim Validity Before Approving a Mining Plan of Operations Concurrence by Secretary of Interior, Gale S. Norton November 17, 2005; and Exhibit 3, State of California, Office of Administrative Law, Notice of Approval of Regulatory Action dated April 27, 2012.

Consideration of evidence offered for the first time in reply or evidence not referenced in the moving party's separate statement rests with the sound discretion of the trial court, as explained by the court in *San Diego Watercrafts v. Wells Fargo Bank* (2002) 102 Cal. App. 4th 308, 315-316. Here, the 2nd RJN of Kimble and PLP is not evidence in support of any particular undisputed fact, but rather, part of Plaintiffs' legal

argument that federal mining claims are presumed valid, i.e., that the Mining Law does not require determination of claim validity before allowing exploration or mineral development. The Court **Grants judicial notice of Kimble and PLP Ex. 1-3.**

**e.** The New 49'ers motion for summary adjudication does not include any request for judicial notice.

**f.** In opposition to the Kimble, PLP and New 49'er motions for summary adjudication, the Karuk Tribe and Coalition request judicial notice under Evid. Code § 452(c) (official executive and legislative acts) of: Ex. A - Legislative Counsel's Digest, California 2009 Legislative Service, 2009 Portion of 2009-2010 Regular Session; 2009 Cal. Legis. Srv. Ch. 62, §§ 1, 2(S.B. 670) (West), dated August 6, 2009 (enactment of Fish and Game §5653.1); Ex. B - Legislative Counsel's Digest, California 20l l Legislative Service, 2011 Portion of 2011-2012 Regular Session; 2011 Cal. Legis. Serv. Ch. 133, §6 (A.B. 120) (West), dated July 26, 2011 (2011 amendment of Fish and Game §5653.1); Ex. C - Bill Analysis, AB 120, (Budget Committee), dated June 8, 2011 (2011 amendment of Fish and Game §5653.1); Ex. E - Chapter 4.2, Water Quality and Toxicology, "Draft" Subsequent Environmental Impact Report from the California Department of Fish and Wildlife (previously named Department of Fish and Game), dated February 2011; Ex. F - California Department of Fish and Wildlife Report to the Legislature Regarding Instream Suction Dredge Mining Under the Fish and Game Code, Department of Fish and Wildlife, Charlton Bonham, Director, April 1, 2013;
Ex. G - Mercury Contamination from Historic Gold Mining in California, Fact Sheet FS-061-00, United States Geological Survey, Department of the Interior, Charles N. Alpers and Michael P. Hunerlach, dated May 2000; Ex. H - Chapter 43, Biological Resources,

"Draft" Subsequent Environmental Impact Report from the California Department of Fish and Wildlife (previously named Department of Fish and Game), dated February 2011; Ex. I - Suction Dredge Permitting Program, Final Subsequent Environmental Impact Report, California Department of Fish and Game, March 2012; and Ex. J - Findings of Fact of the California Department of Fish and Game, Suction Dredge Permitting Program Final SEIR, pursuant to CEQA, dated March 16, 2012. The Court **Grants judicial notice of Karuk Tribe Ex. A-C and E- J.**

***Suction Dredge Mining in California***

In general, CDFW regulates suction dredging and the use of any related equipment in California pursuant to F & G Code § 5653 specifically. Under that authority since 1995, the use of any vacuum or suction dredge equipment by any person in any river, stream or lake in California is prohibited, unless authorized under a permit issued by CDFW (F & G Code, § 5653 (a).)

F & G Code § 5653 states in its entirety:

> (a) The use of any vacuum or suction dredge equipment by any person in any river, stream, or lake of this state is prohibited, except as authorized under a permit issued to that person by the department in compliance with the regulations adopted pursuant to Section 5653.9. Before any person uses any vacuum or suction dredge equipment in any river, stream, or lake of this state, that person shall submit an application for a permit for a vacuum or suction dredge to the department, specifying the type and size of equipment to be used and other information as the department may require.
> (b) Under the regulations adopted pursuant to Section 5653.9, the department shall designate waters or areas wherein vacuum or suction dredges may be used pursuant to a permit, waters or areas closed to those dredges, the maximum size of those dredges that may be used, and the time of year when those dredges may be used. If the department determines, pursuant to the regulations adopted pursuant to Section 5653.9, that the operation will not be deleterious to fish, it shall issue a permit to the applicant. If any person operates any equipment other than that authorized by the permit or conducts the operation in any waters or

area or at any time that is not authorized by the permit, or if any person conducts the operation without securing the permit, that person is guilty of a misdemeanor.
(c) The department shall issue a permit upon the payment, in the case of a resident, of a base fee of twenty-five dollars ($25), as adjusted under Section 713, when an onsite investigation of the project size is not deemed necessary by the department, and a base fee of one hundred thirty dollars ($130), as adjusted under Section 713, when the department deems that an onsite investigation is necessary. In the case of a nonresident, the base fee shall be one hundred dollars ($100), as adjusted under Section 713, when an onsite investigation is not deemed necessary, and a base fee of two hundred twenty dollars ($220), as adjusted under Section 713, when an onsite investigation is deemed necessary.
(d) It is unlawful to possess a vacuum or suction dredge in areas, or in or within 100 yards of waters, that are closed to the use of vacuum or suction dredges.

[Added Stats 1986 ch 1368 § 23. Amended Stats 1988 ch 1037 § 1; Stats 1994 ch 775 § 1 (AB 1688); Stats 2006 ch 538 § 185 (SB 1852), effective January 1, 2007.]

Pursuant to SB 670 (effective 8/6/09), AB 120 (effective 7/26/11) and SB 1018 (effective 6/27/12), F & G Code § 5653.1, a conditional proscription against vacuum and suction dredging activities was enacted.

Suction dredge mining entails the use of a vacuum or suction system to remove and return material at the bottom of a river, stream, or lake for the extraction of minerals, primarily gold. (*People v. Osborn* (2004) 116 Cal.App.4th 764, 768; 14 Cal. Code Regs. (CCR), § 228(a).) "In suction dredge mining, the gravel within the active stream channel is suctioned from the bottom of the stream and processed over a sluice on a floating platform. A gasoline powered motor and pump are mounted on the floating platform for powering the suction apparatus and for driving the air pump which supplies air to the persons working underwater. The size of dredges used in California ranges from 2-inches to up to 10-inches or more." (*Karuk Tribe of Cal. v. U.S. Forest Service*

(N.D. Cal. 2005) 379 F.Supp.2d 1071, 1080, fn. 5, citations, quotation marks, and brackets omitted, rev'd on other grounds (9th Cir. 2012) 681 F.3d 1006.)

As set forth above under F & G Code 5653.1, *suction dredge mining throughout the State is prohibited* ***until*** *the Director of the CDFW certifies that (1) the Department has completed environmental review of its suction dredge regulations pursuant to the California Environmental Quality Act (CEQA); (2) CDFW promulgates new regulations, as necessary, based on that environmental review; (3) the new regulations are operative; (4) the new regulations "fully mitigate all identified significant environmental effects"; and (5) a "fee structure is in place that will fully cover all costs to the Department" related to administration of its suction dredge permit program. F & G Code, §5653.1(b). The Legislature found this moratorium necessary because "suction or vacuum dredge mining results in various adverse environmental impacts to protected fish species, the water quality of this state, and the health of the people of this state." (Stats. 2009, ch. 62, § 2.)*

On March 16, 2012, the CDFW completed the required environmental review and adopted updated regulations, effective April 27, 2013. But it has not certified completion of all five items required by § 5653.1(b), and ***the moratorium remains in effect.***

On April 1, 2013, CDFW pursuant to F and G Code § 5653.1(c) submitted its required report to the Legislature "on statutory changes or authorizations that, in the determination of the department, are necessary to develop the suction dredge regulations required by paragraph (2) of subdivision (b), including, but not limited to, recommendations relating to the mitigation of all identified significant environmental impacts and a fee structure that will fully cover all program costs."

***Federal Preemption in General***

Federal law can preempt state law in four ways: express, field, conflict and obstacle. (See generally *Viva! Intern. Voice for Animals v. Adidas Promotional Retail Ops., Inc.* (2007) 41 Cal.4th 929, 935-936; *California Federal Sav. & Loan Assn. v. Guerra* (1987) 479 U.S. 272, 280-281). (1) Congress can "pre-empt state law by so stating in express terms." (*Guerra, supra*, 479 U.S. at p. 280.) (2) In so- called field preemption, "congressional intent to pre-empt state law in a particular area may be inferred where the scheme of federal regulation is sufficiently comprehensive to make reasonable the inference that Congress left no room for supplementary state regulation." (*Id.* at pp. 280-281, citation and quotation omitted) Finally, federal law may conflict with state law either (3) "because compliance with both federal and state regulations is a physical impossibility" (*id.* at p. 281), or (4) if it "stands as an obstacle to the accomplishment and execution of the full purposes and objectives of Congress." (*Ibid.*)

"Courts are reluctant to infer preemption, and it is the burden of the party claiming that Congress intended to preempt state law to prove it." *Viva!, supra*, 41 Cal.4th at p. 936.

The Supreme Court has set forth several rules regarding preemption. First, "in all pre-emption cases, and particularly in those in which Congress has legislated ... in a field which the States have traditionally occupied, [courts must] start with the assumption that the historic police powers of the States were not to be superseded by the Federal Act unless that was the clear and manifest purpose of Congress." *Wyeth v. Levine* (2009) 555 U.S. 555, 565; see also *Bronco Wine Co. v. Jolly* (2004) 33 Cal.4th

943, 957. Second, if two readings of a statute are plausible, courts "have a duty to accept the reading that disfavors pre-emption." *Bates v. Dow Agrosciences LLC* (2005) 544 U.S. 431, 449. Finally, a general federal purpose to encourage a particular activity does not, on its own, preempt state laws that do the opposite. See *Commonwealth Edison Co. v. Montana* (1981) 453 U.S. 609, 633-34. Instead, "it is necessary to look beyond general expressions of 'national policy' to specific federal statutes with which the state law is claimed to conflict." (*Id.*, at p. 634.)

**People v. Rinehart**

Subsequent to argument in the instant case, the case of People v. Rinehart, (2014) 230 Cal. App. 4th 419, was decided. Defendant Brandon Rinehart was charged with a violation of F & G. C. § 5653(a), in that he used vacuum and suction dredge equipment in a river, stream, or lake without a permit, and with a violation of F. & G. C. § 5653 (d), in that he possessed a vacuum and suction dredge within an area closed to the use of that equipment and within 100 yards of waters closed to the use of that equipment. The trial court rejected defendant's affirmative defense that § 5653 was unenforceable against him because the statute, as applied, was preempted by federal law, and it disallowed evidence relevant to the issue. The trial court then found defendant guilty of both offenses.

The Third Appellate District Court of Appeal reversed the judgment and remanded the cause. The court noted that F. & G. C. § 5653***, requiring a permit from the state before persons may conduct suction dredge mining operations does not, standing alone, contravene federal law.*** However, the court could not determine on the record before it that, as a matter of law, the criminal provisions of § 5653, read in

light of the provisions of F. & G. C. § 5653.1, are rendered unenforceable because the California statutes have rendered the exercise of rights granted by the federal mining laws commercially impracticable, *given that the trial court had disallowed evidence relevant to the issue.* The matter thus had to be returned to the trial court for further proceedings on the issue of preemption, admitting whatever evidence, and hearing whatever argument, the trial court, in its discretion, deemed relevant and then ruling accordingly. Specifically, the trial court had to address at least whether § 5653.1, as currently applied, operated as a practical matter to prohibit the issuance of permits required by § 5653; and if so, whether that de facto ban on suction dredge mining permits had rendered commercially impracticable the exercise of defendant's mining rights granted to him by the federal government.

The *Rinehart* court addressed the fundamental principles of federal preemption as follows:

> The property clause of the United States Constitution "provides that 'Congress shall have Power to dispose of and make all needful Rules and Regulations respecting the Territory or other Property belonging to the United States.' U.S. Const., Art. IV, § 3, cl. 2." (*Kleppe v. New Mexico* (1976) 426 U.S. 529, 535 [].) The United States Supreme Court has "repeatedly observed that '[the] power over the public land thus entrusted to Congress is without limitations.' " (*Id.* at p. 539 [], quoting *U.S. v. San Francisco* (1940) 310 U.S. 16, 29 [].)
>
> Even so, " 'the State is free to enforce its criminal and civil laws' on federal land so long as those laws do not conflict with federal law. [Citation.] The Property Clause itself does not automatically conflict with all state regulation of federal land. Rather, ... '[a]bsent consent or cession a State undoubtedly retains jurisdiction over federal lands within its territory, but Congress equally surely retains the power to enact legislation respecting those lands pursuant to the Property Clause. And when Congress so acts, the federal legislation necessarily overrides conflicting state laws under the Supremacy Clause.' [Citation.]" (*Granite Rock, supra*, 480 U.S. at pp. 580–581 [], italics added, quoting *Kleppe v. New Mexico, supra*, 426 U.S. at p. 543.) Put differently, "[T]he Property Clause gives Congress plenary

power over ... federal land ... ; however, even within the sphere of the Property Clause, state law is pre-empted only when it conflicts with the operation or objectives of federal law ... [citation]." (*Granite Rock*, at p. 593 [].)

"[S]tate law can be pre-empted in either of two general ways. If Congress evidences an intent to occupy a given field, any state law falling within that field is pre-empted. [Citations.] If Congress has not entirely displaced state regulation over the matter in question, state law is still pre-empted to the extent it actually conflicts with federal law, that is, when it is impossible to comply with both state and federal law [citation] or where the state law stands as an obstacle to the accomplishment of the full purposes and objectives of Congress, [citation]." (*Silkwood v. Kerr-McGee Corp.* (1984) 464 U.S. 238, 248 []; see *Viva!, supra*, 41 Cal.4th at pp. 935–936.) (*Rinehart, supra*, 230 Cal.App.4th at pp. 430-431.)

The *Rinehart* court went on to describe the applicable federal mining law as follows:

The federal government's policy relating to mining and minerals is set forth at title 30 United States Code section 22: "Except as otherwise provided, all valuable mineral deposits in lands belonging to the United States, both surveyed and unsurveyed, shall be free and open to exploration and purchase, and the lands in which they are found to occupation and purchase, by citizens of the United States ... under regulations prescribed by law, and according to the local customs or rules of miners in the several mining districts, so far as the same are applicable and not inconsistent with the laws of the United States."

We deal here mainly with the General Mining Act of 1872.
"Under the Mining Act of 1872, 17 Stat. 91, as amended, 30 U.S.C. §22 *et seq.*, a private citizen may enter federal lands to explore for mineral deposits. If a person locates a valuable mineral deposit on federal land, and perfects the claim by properly staking it and complying with other statutory requirements, the claimant 'shall have the exclusive right of possession and enjoyment of all the surface included within the lines of their locations,' [citation], although the United States retains title to the land. The holder of a perfected mining claim may secure a patent to the land by complying with the requirements of the Mining Act and regulations promulgated thereunder [citation] and, upon issuance of the patent, legal title to the land passes to the patent holder." (*Granite Rock, supra*, 480 U.S. at pp. 575–576 [].)

The United States Supreme Court has recognized that the intent of Congress in passing the mining laws "was to reward and encourage the

> discovery of minerals that are valuable in an economic sense." (*United States v. Coleman* (1968) 390 U.S. 599, 602 [].)
>
> Constitutionally speaking, under most circumstances, the states are free to enact environmental statutes and regulations binding on those holding unpatented mining claims on federal lands so long as those statutes and regulations do not rise to the level of impermissible state land use regulations. (See *Granite Rock, supra*, 480 U.S. 572 [].) "The line between environmental regulation and land use planning will not always be bright; for example, one may hypothesize a state environmental regulation so severe that a particular land use would become commercially impracticable. However, the core activity described by each phrase is undoubtedly different. Land use planning in essence chooses particular uses for the land; environmental regulation, at its core, does not mandate particular uses of the land but requires only that, however the land is used, damage to the environment is kept within prescribed limits." (Id. at p. 587 [].)
> (*Rinehart, supra*, 230 Cal.App.4th at pp. 431-432.)

The *Rinehart* court then noted that "[i]n 1961, the State of California enacted section 5653 directing California's Department of Fish and Wildlife (formerly known as the Department of Fish and Game) (Department) to issue permits if it determined the particular vacuum or suction dredge mining operation "will not be deleterious to fish." (Stats. 1961, ch. 1816, § 1, p. 3864.) Suction dredging is the use of a suction system to remove and return materials from the bottom of a stream, river or lake for the extraction of minerals. (Cal. Code Regs., tit. 14, § 228.) In 1988, amendments to the statute made it a misdemeanor to possess a vacuum or suction dredge in or within 100 yards of waters closed to the activity. (Stats. 1988, ch. 1037, § 1, p. 3371.)" (*Rinehart, supra*, 230 Cal.App.4th at p. 432.)

Ultimately, the legislature prohibited issuing any new permits under section 5653, and imposed a statewide moratorium on instream suction dredge mining. The current F. & G. C. § 5653.1 allows for the statutory moratorium to end upon the Department's certification that the following five conditions had been satisfied:

"(1) The [D]epartment has completed the environmental review of its existing [(1994)] suction dredge mining regulations . ...

"(2) The [D]epartment has transmitted for filing with the Secretary of State ... a certified copy of new regulations adopted, as necessary, pursuant to ... the Government Code.

"(3) The new regulations described in paragraph (2) are operative.

"(4) ***The new regulations described in paragraph (2) fully mitigate all identified significant environmental impacts.***

"(5) A fee structure is in place that will fully cover all costs to the [D]epartment related to the administration of the program." (Former § 5653.1, subd. (b); see § 5653.1, as amended by Stats. 2012, ch. 39, § 7, eff. June 27, 2012.)
(*Rinehart, supra*, 230 Cal.App.4th at pp. 432-433.)

In *Rinehart*, Defendant argued that because of a lack of funding, the Department is unable for financial reasons to fulfill the conditions set forth in section 5653.1, which results in a continuing, if not permanent, moratorium on suction dredge mining permits, which stands as an obstacle to congressional intent. In response to the argument that such permits may be issued again at some point in the future, Defendant responded that to accept that argument would be to allow any moratorium to stand on the promise that it would be lifted in the future. Defendant also argued that, where the government has authorized a specific use of federal lands, a state may not prohibit that use, either temporarily or permanently, in an attempt to substitute its judgment for that of Congress. (*Rinehart, supra*, 230 Cal.App.4th at p. 433.)

The *Rinehart* court thus framed its analysis as whether sections 5653 and 5653.1, as presently applied, stand as obstacles to the accomplishment of the full purposes and objectives of Congress in passing the federal mining laws. (*Rinehart, supra*.) The court acknowledged that section 5653 requiring a permit from the state

before persons may conduct suction dredge mining operations does not, standing alone, contravene federal law, citing *Granite Rock, supra*, 480 U.S. 572 , which established that the requirement of a state permit to conduct certain activities on federal land is not categorically prohibited. (*Rinehart, supra.*)

Addressing the conditions attending the permit, the court stated:

> The question here is whether the requirements of section 5653.1, which requirements, defendant argues, cannot at the present time be met by the state, in fact operate to prohibit the issuance of a permit under section 5653. That is, according to defendant, there is at the current time a de facto ban on suction dredge mining in California imposed by the state through the operation of sections 5653 and 5653.1. Moreover, according to defendant, there is no economically feasible way to extract valuable mineral deposits at the site of his claim. Put simply, according to defendant, this combination of circumstances has the practical effect of the state taking away from him what the federal government has granted. Therefore, he argues, the state statutes are unenforceable because their operation, as to defendant, is preempted by federal law.
> (*Rinehart, supra*, 230 Cal.App.4th at p.434.)

The *Rinehart* court specifically found the opinion of the United States Court of Appeals for the Eighth Circuit in *South Dakota Mining Assn. Inc. v. Lawrence County* (8th Cir. 1998) 155 F.3d 1005 (*South Dakota Mining*) nearly directly on point:

> In *South Dakota Mining*, the voters of Lawrence County, South Dakota, enacted an ordinance prohibiting the issuance of new or amended permits for surface metal mining in what was known as the Spearfish Canyon area. Plaintiffs in the action to permanently enjoin enforcement of the ordinance included mining companies that held federally patented and unpatented mining claims in the area and that had conducted surface mining operations consistent with federal law within Lawrence County for the 15 years before the ordinance was enacted. (*South Dakota Mining, supra*, 155 F.3d at p. 1007.)
>
> The record in the district court showed that surface metal mining was the only mining method that had been used to mine gold and silver deposits in the area for the previous 20 years. The record also showed that surface metal mining was the only mining method that could extract gold and silver within the Spearfish Canyon area even though, in other parts of South Dakota, underground and other types of gold and silver mining were

prevalent. Surface metal mining in the Spearfish Canyon area was the only mining method available, as a practical matter, because the gold and silver deposits in that area were located, geologically, at the earth's surface. The record showed that the mining companies had invested substantial time and money to explore the area for mineral deposits and to develop mining plans that conformed to federal, state, and local permitting laws. (*South Dakota Mining, supra*, 155 F.3d at pp. 1007–1008.)

The district court permanently enjoined enforcement of the ordinance holding that the General Mining Act of 1872 preempted the ordinance. (*South Dakota Mining, supra*, 155 F.3d at p. 1008.)

The Eighth Circuit Court of Appeals affirmed the district court's order. The court first found that the purposes and objectives of Congress in passing the General Mining Act of 1872 included "the encouragement of exploration for and mining of valuable minerals located on federal lands, providing federal regulation of mining to protect the physical environment while allowing the efficient and economical extraction and use of minerals, and allowing state and local regulation of mining so long as such regulation is consistent with federal mining law." (*South Dakota Mining, supra*, 155 F.3d at p. 1010.)

The court then found that "[t]he Lawrence County ordinance is a per se ban on all new or amended permits for surface metal mining within the area. Because the record shows that surface metal mining is the only practical way any of the plaintiffs can actually mine the valuable mineral deposits located on federal land in the area, the ordinance's effect is a de facto ban on mining in the area. ...

"The ordinance's de facto ban on mining on federal land acts as a clear obstacle to the accomplishment of the Congressional purposes and objectives embodied in the Mining Act. Congress has encouraged exploration and mining of valuable mineral deposits located on federal land and has granted certain rights to those who discover such minerals. Federal law also encourages the economical extraction and use of these minerals. The Lawrence County ordinance completely frustrates the accomplishment of these federally encouraged activities. A local government cannot prohibit a lawful use of the sovereign's land that the superior sovereign itself permits and encourages. To do so offends both the Property Clause and the Supremacy Clause of the federal Constitution. The ordinance is prohibitory, not regulatory, in its fundamental character." (*South Dakota Mining, supra*, 155 F.3d at p. 1011.)
(Rinehart, supra, 230 Cal.App.4th at pp. 434-435.)

The Rinehart court distinguished its case from *South Dakota Mining* in that sections 5653 and 5653.1, read together or alone, do not *expressly* prohibit the issuance of suction dredge mining permits. Nevertheless, the Rinehart court determined that has no bearing on the result because while the F. & G.C. sections here "do not expressly ban suction dredge mining, they do require a state permit for such mining and, however, as currently applied, California law as embodied in the words and application of section 5653.1 acts to prevent the issuance of such permits." (*Rinehart, supra*, 230 Cal.App.4th at pp. 435-436.) In the case at hand, t*here is no particular argument from any party, that permits will not and cannot, be issued in the near or far future for years if ever. This is fundamentally unfair and clearly operates as a de facto ban.*

In any event, as argued by Rinehart, "in practical operation, sections 5653 and 5653.1, have, since 2009, banned suction dredge mining in California" and "there is no commercially viable way to discover and extract the gold or other minerals lying within his mining claims other than suction dredge mining, [so] the effect of the statutory scheme is to deprive him of rights granted to him under federal law." (*Rinehart, supra*, 230 Cal.App.4th at p. 436.)

The *Rinehart* court then stated:

> Put differently, and in the language of the hypothetical used by the court in *Granite Rock*, if sections 5653 and 5653.1 are environmental regulations that are "so severe that a particular land use [(in this case mining)] ... become[s] commercially impracticable" (*Granite Rock, supra*, 480 U.S. at p. 587), then they have become de facto land use planning measures that frustrate rights granted by the federal mining laws and, thus, have become obstacles to the realization of Congress's intent in enacting those laws. If that is the case, as defendant alleges, the Fish and Game Code provisions at issue here are unenforceable as preempted by federal mining law.

(*Rinehart, supra*, 230 Cal.App.4th at p. 436.)

Nonetheless, the *Rinehart* court, while acknowledging that defendant had made a colorable argument to that end, could not determine on the record before it that, as a matter of law, the criminal provisions of section 5653, read in light of the provisions of section 5653.1, were rendered unenforceable because the California statutes have rendered the exercise of rights granted by the federal mining laws "commercially impracticable." (*Granite Rock, supra*, 480 U.S. at p. 587.) (*Rinehart, supra*, 230 Cal.App.4th at p. 436.) In contrast, the record made by the miners in the instant case is sufficient.

Therefore, the *Rinehart* court returned the matter to the trial court for further proceedings on the issue of preemption, admitting whatever evidence, and hearing whatever argument, the trial court, in its discretion, deems relevant and then ruling accordingly. "Specifically, the trial court must address at least these two questions: (1) Does section 5653.1, as currently applied, operate as a practical matter to prohibit the issuance of permits required by section 5653; and (2) if so, has this de facto ban on suction dredge mining permits rendered commercially impracticable the exercise of defendant's mining rights granted to him by the federal government?" (*Rinehart, supra.*) The Court here, answers yes to both questions.

**Kimble MSA on it's 1st COA and PLP MSA on it's 4th COA**

**Kimble** argues that most suction dredge mining in California occurs on Federal lands where a miner has validly located and filed a Federal mining claim pursuant to Federal mining law. This creates, for the miner, an enforceable property right under Federal law to extract all minerals from his mining claim. Suction dredge mining is the only

economical and environmentally sound method for extracting minerals from California's rivers and streams. But F & G Code § 5653.1, since 2009, along with the CDFW new regulations in 2012, **prohibits** Federal prospectors and miners, who hold Federal mining claims and mineral estates, from engaging in suction dredge mining on Federal lands. Accordingly, Kimble contends they are entitled to summary adjudication of the federal preemption cause of action as a matter of law since the California statute and regulations impermissibly conflict with the 1872 General Mining Law, as amended, 30 U.S.C. §§ 22-54, and the 1976 Federal Land Policy Management Act, 43 U.S.C. §§ 1701 et seq. which provide that all valuable mineral deposits in lands belonging to the United States shall be "free and open" to mineral development.

Kimble argues that CDFW has admitted that its § 5653.1 constitutes a *complete prohibition* on suction dredge mining because the mandated new regulations have not and cannot fully mitigate all identified significant environmental impacts pursuant to F & G Code § 5653.1(b)(4) [1] and therefore constitutes a physical impossibility to comply with both State and Federal law, citing among other cases, *California Coastal Commission v. Granite Rock Co.* (1987) 480 U.S. 572, 581 ("*Granite Rock*"). Kimble argues:

[1] Based on the 2012 FSEIR determinations of project-specific significant and unavoidable effects under CEQA in the areas of water quality and toxicology, biological resources, cultural resources, and noise, and significant and unavoidable cumulative effects under CEQA re: wildlife species and their habitats, water turbidity/TSS discharges and mercury resuspension and discharge, the CDFW's new (2012) regulations cannot "fully mitigate all identified significant environmental effects". (http://www.dfg.ca.gov/suctiondredge/ ). See, CDFW Findings of Fact for Suction Dredge Permitting Program, March 16, 2012. (Karuk Tribe RJN, Ex. J.)

"The general rule is that "where the state law stands as obstacle to the accomplishment the full purposes and objectives of Congress," it is preempted. [*Granite Rock, supra,*] 480 U.S. 575, 592, ...; see also *Perez v. Campbell*, 402 U.S. 637 (1971) ("any state legislation which frustrates the full effectiveness of Federal law is rendered invalid by the Supremacy Clause" regardless of the underlying purpose of its enactors). The "all-pervading purpose of the mining laws is to further the speedy and orderly development of the mineral resources of our country," *United States v. Nogueira*, 403 F.2d 816, 823 (9th Cir. 1968); see also 30 U.S.C. § 21a(1) ("The continuing policy of the Federal Government in the national interest to foster and encourage private enterprise in...the development of the economically sound and stable domestic mining, minerals, metal and mineral reclamation industries").

"To further these vital public policies the 1872 Mining Act declares:

> "...all valuable mineral deposits in lands belonging to the United States, both surveyed and unsurveyed, shall be free and open to exploration and purchase, and the lands in which they are found to occupation and purchase, by citizens of the United States..." 30 U.S.C. § 22.

PLP makes essentially the same arguments.

**Ruling**

On their motions for summary adjudication, the Court finds there is no triable issue of material fact on the issue of Federal Preemption and that as a matter of law and in actual fact, that the State's extraordinary scheme of requiring permits and then refusing to issue them whether and/or being unable to issue permits for years, stands "as an obstacle to the accomplishment of the full purposes and objectives of Congress" under *Granite Rock and a de facto ban.*

**Material facts-1-5 (Kimble) Material Facts 1-6 (PLP)**

**Evidence - Declarations of Goldberg, Hobbs, Keene, Tyler, Maksymyk.**

**New 49'ers MSA on it's 2nd COA**

In the second causes of action of the ***New 49'ers* FAC**, Plaintiffs allege that through the 1872 Mining Law, as amended and related statutes, Congress created federal property rights in mining claims in furtherance of general federal policy to foster mineral development on federal lands. Also Congress possesses plenary power over federal property under the Property Clause (U.S. Const. Art. IV, § 3.) (FAC, ¶62.) The New 49'ers allege that the CDFW Actions (F & G Code 5653.1 and regulations thereunder), individually and/or in any combination thereof, are void as against the U.S. Constitution on the ground of the Supremacy Clause (U.S. Constitution, Article VI, Clause 2), insofar as they interfere with the federal purpose of fostering mineral development on federal property, and stand as an obstacle to the accomplishment and execution of the purposes and objectives of Congress." (FAC, ¶63.)

The **New 49'ers** argue they are entitled to summary adjudication of their second cause of action for federal preemption of F & G Code § 5653.1 and portions of the regulations set forth at 14 Cal. Code of Regs. §§ 228 et seq., which operate to forbid Plaintiffs from mining their claims. *The New 49'ers acknowledge that the State of California has lawful power to enact reasonable environmental regulations* that do not materially interfere with mining operations (*Granite Rock*), however, the New 49'ers argue that the State cannot lawfully require permits and then refuse to issue them, forbid mining entirely in certain areas, or require miners to participate in a lottery to obtain a very limited number of permits.

Specifically, the New 49'ers contend the challenged statutory and regulatory restrictions on suction dredge mining are preempted by federal law based on its arguments regarding the nature of rights in mining claims under Federal law and regulations and the doctrine of federal preemption, generally, and in the mining context. The arguments of the New 49'ers are similar to those of PLP and Kimble.

**Ruling**

On its motions for summary adjudication, the Court finds there is no triable issue of material fact on the issue of Federal Preemption and that as a matter of law and in actual fact, that the State's extraordinary scheme of requiring permits and then refusing to issue them whether and/or being unable to issue permits for years, stands "as an obstacle to the accomplishment of the full purposes and objectives of Congress" under *Granite Rock and a de facto ban.*

**Material Facts- 1-6**

**Evidence – Buchal declaration**

**CDW MSA against Kimble, PLP, and New 49'ers**

**The** CDW motions for summary adjudication as to Kimble, PLP, and New 49'ers is denied for reasons discussed above.

**Prevailing parties to prepare notice and order.**

No. 11-460

IN THE

# Supreme Court of the United States

LOS ANGELES COUNTY FLOOD CONTROL DISTRICT,

*Petitioner*,

v.

NATURAL RESOURCES DEFENSE COUNCIL, INC. and SANTA MONICA BAYKEEPER,

*Respondents.*

**On Writs of Certiorari to the United States Court of Appeals for the Ninth Circuit**

## BRIEF FOR AMICI CURIAE LAW PROFESSORS ON THE "ADDITION OF A POLLUTANT" QUESTION IN SUPPORT OF RESPONDENTS

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## INTEREST OF *AMICI CURIAE* [1]

*Amici* are professors of environmental and administrative law. *Amici* have a long-standing interest in the proper interpretation of environmental statutes, such as the Clean Water Act ("CWA"). In particular, *Amici* have an interest in ensuring the correct interpretation of the CWA's jurisdictional provisions.

Collectively, *Amici* have spent decades interpreting and teaching both the CWA and the statutory interpretation principles at issue in this case. The purpose of this submission is to urge this Court not to address the jurisdictional question whether there is an "addition of a pollutant," as that phrase is used in Section 502(12), 33 U.S.C. § 1362(12), in situations in which pollutants are discharged—or, in the parlance of several courts, "redeposited"—from one point to another within a particular water body. *Amici* urge this for two reasons. First, we agree with Respondents and the United States that this issue simply is not present in this case. And second, we believe that this is an open issue, and one that is both complicated and exceedingly significant.

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[1] In accordance with S. Ct. Rule 37.3(a), all parties have consented to the filing of this brief. Petitioner and Respondents have done so by filing consent letters directly with the Clerk. Pursuant to S. Ct. Rule 37.6, Counsel for *Amici* states that no counsel for a party authored this brief in whole or in part and no person or entity other than *Amici* or their counsel made a monetary contribution to the preparation or submission of this brief.

Most importantly, *Amici* believe that this Court should not resolve this complex jurisdictional issue in the absence of appropriate briefing. Proper analysis of the question at hand requires careful consideration of the intersection between four key statutory provisions under the CWA: Section 301(a), 33 U.S.C. § 1311(a) (establishing the basic jurisdictional dynamics of the CWA), Section 502(12), 33 U.S.C. § 1362(12) (defining "discharge of a pollutant," the key jurisdictional phrase in Section 301(a)), Section 402, 33 U.S.C. § 1342 (establishing the National Pollutant Discharge Elimination System ("NPDES") program), and Section 404, 33 U.S.C. § 1344 (establishing the "dredge and fill" permit program). The issues are further complicated by the fact that the U.S. Environmental Protection Agency ("EPA") has taken divergent positions under the two permit programs.

Neither the parties nor the United States has adequately briefed these issues. If the Court definitively rules on this question in the absence of such briefing, its ruling could have unforeseen consequences that transcend the NPDES program.

A further description of *Amici* is set forth in the Appendix to this brief.

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## SUMMARY OF THE ARGUMENT

Respondents in this case brought an action under the citizen suit provision of the CWA, 33 U.S.C. § 1365, based on the simple notion that Petitioner was violating the conditions of its NPDES permit. Now, relying on an unfortunate paragraph in the

court of appeals' opinion, Petitioner seeks to reframe the case as one involving the jurisdictional question whether it has added pollutants to the navigable waters from a point source. This reformulation simply does not fit.

Most fundamentally, Petitioner *concedes* that its stormwater system adds pollutants to the relevant waterways and is properly "subject to the Clean Water Act's proscriptions." Pet. Br. 44. This concession shifts the focus from whether the statute applies to whether its requirements are being met. And under the NPDES program, those requirements are set forth *in the permit*. 33 U.S.C. § 1342(a)(1), (b)(1). This principle has three important corollaries. First, both EPA and citizens are empowered to enforce any and all NPDES permit requirements. *See* 33 U.S.C. §§ 1319(b), (d), 1365 (a)(1)(A), (f)(6). Second, a permittee cannot collaterally attack its permit requirements in an enforcement proceeding. 33 U.S.C. § 1369(b)(2). And third, subject to narrow exceptions, compliance with a permit is deemed to be compliance with the CWA. 33 U.S.C. § 1342(k).

In line with these principles, this case involves nothing more than an issue of permit interpretation. The Court should reject Petitioner's effort to recast the case as one involving jurisdictional dynamics. Because this case involves permit enforcement, *South Florida Water Management District v. Miccosukee Tribe of Indians*, 541 U.S. 95 (2004) ("*Miccosukee*"), is simply inapposite.

If, however, this Court deems the jurisdictional questions to be relevant, it should first recognize that

the relevant portion of the *Miccosukee* opinion constitutes dictum, not a holding. In that case, this Court determined that the Tribe had conceded there would be no jurisdiction if the two channels of water were considered to be two parts of the same water body. 541 U.S. at 109–10. Thus, this Court's treatment of the relevant legal principle was based on the parties' "agreed-upon law." *Id.* at 104. As such, this Court had no occasion to consider the many complexities involved in resolving this issue.

Beyond that, there are sound jurisprudential reasons why this Court should not consider the "addition" question here. As discussed in Section III, below, this issue is a statutory minefield, involving both the NPDES and "dredge and fill" permit programs. This issue also poses difficult questions involving the intersection of this Court's opinions in *Clark v. Martinez*, 543 U.S. 371 (2005) (holding that courts cannot give the same word or phrase in a statutory provision different meanings in different contexts), and *Chevron, U.S.A., Inc. v. Natural Resources Defense Council*, 467 U.S. 837 (1984) (holding that courts should defer to reasonable agency interpretations of ambiguous statutory language, so long as the agency has spoken with the force of law). This is because EPA, with the acquiescence of the U.S. Army Corps of Engineers ("the Corps") in the Section 404 context, has taken contradictory positions regarding whether a pollutant must come from the "outside world" in order for a discharge of a pollutant to constitute an "addition" within the meaning of Section 502(12). While EPA historically has not chosen to regulate these

movements of pollutants under the NPDES program, *see*, *e.g.*, *Nat'l Wildlife Fed'n v. Gorsuch*, 693 F.2d 156 (D.C. Cir. 1982), both EPA and the Corps have long asserted jurisdiction over redeposits of dredged spoil within the same water body. *See*, *e.g.*, 40 C.F.R. § 232.2(1) (EPA regulation); 33 C.F.R. § 323.2(d)(1) (Corps regulation). Indeed, that latter scenario is the very lifeblood of the "dredge" component of Section 404's "dredge and fill" permit program; the vast majority of "dredged material" cases involve that very fact pattern.

Given these complexities, it is clear that neither Petitioner nor Respondents (nor the United States) has adequately briefed the "addition" question in this case. If the Court deems the jurisdictional issue to be relevant, it should at the very least require further briefing addressing these concerns.

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## ARGUMENT

### I. RESPONDENTS MAY ENFORCE THE RELEVANT PERMIT CONDITIONS; PETITIONER MAY NOT COLLATERALLY ATTACK THOSE PERMIT REQUIREMENTS

As the court of appeals noted, "'[t]he plain language of CWA § 505 authorizes citizens to enforce *all* permit conditions.'" Pet. App. 34 (quoting *Nw. Envtl. Advocates v. City of Portland*, 56 F.3d 979, 986 (9th Cir. 1995) (emphasis in original)). Section 505 does this by authorizing citizens to bring suit against persons alleged to be in violation of any "effluent

standard or limitation under this chapter." 33 U.S.C. § 1365(a)(1)(A) (2006). The phrase "effluent standard or limitation" is defined in Section 505(f), and includes "a permit or condition thereof issued under section [402] of this title. . . ." 33 U.S.C. § 1365(f)(6) (2006). Because Section 402 establishes the NPDES permit program, the language of Section 505(f)(6) provides that citizens may enforce NPDES permit conditions in suits under Section 505.

This plain meaning must control absent a clearly expressed legislative intention to the contrary. *Consumer Prod. Safety Comm'n v. GTE Sylvania, Inc.*, 447 U.S. 102, 108 (1980). In this case the legislative history underscores the plain meaning of Section 505(f)(6). Most significantly, the Senate Report accompanying the passage of the CWA in 1972 explained in unmistakable terms that "in addition to violations of section 301(a) [permit-less discharges], citizens are granted authority to bring enforcement actions for the violation of . . . *any* condition of *any* permit issued under section 402." Federal Water Pollution Control Amendments of 1972, S. REP. NO. 92-414 (1972) *reprinted in* 1972 U.S.C.C.A.N. 3668, 3747 (emphasis added).

Related to this, both the CWA and California law bar any collateral attack on Petitioner's permit. Section 509(b)(2) of the CWA makes this explicit with regard to EPA-issued permits. 33 U.S.C. § 1369(b)(2) (2006) (precluding review "in any civil or criminal proceeding for enforcement" of any "[a]ction of the Administrator with respect to which review could have been obtained under" Section 509(b)(1), which

includes "issuing or denying" any NPDES permit). As authorized under Section 304(i), 33 U.S.C. § 1314(i) (2006), EPA extended this dynamic to State-issued permits through its "duty to comply" regulation, which on its face applies to State programs. 40 C.F.R. § 122.41(a) (2012) ("Any permit noncompliance constitutes a violation of the Clean Water Act."). As recognized by the D.C. Circuit, this is a permissible interpretation of the CWA. *Gen. Motors Corp. v. EPA*, 168 F.3d 1377, 1381–83 (D.C. Cir. 1999) ("Certainly the EPA, acting in accordance with [an earlier case], the division of authority in the Act between state and federal permitting agencies, and the Senate Committee's expectation that enforcement proceedings would be straightforward and speedy, could reasonably interpret the Act to remit to a state forum any attack upon the validity of a state permit.").

As Respondents point out, California law follows suit. Resp. Br. 44 (citing Cal. Water Code §§ 13320, 13330). Indeed, Petitioner and its co-permittees filed a series of unsuccessful challenges to its permit in state court. *Id.* at 44–46. Seen in this light, both California law and comity concerns preclude any collateral attack regarding the appropriateness of the permit's terms.

## II. *MICCOSUKEE* DOES NOT RESOLVE THE QUESTION UPON WHICH THE COURT GRANTED CERTIORARI

Respondents, Petitioner, and the United States summarily agree on the answer to the question presented. That is, they agree that the "transfer of

water through a concrete channel within a single river does not constitute a discharge of pollutants from a point source under the Clean Water Act." Resp. Br. 1; Pet. Br. 27; U.S. Br. 19. However, this position rests on the incorrect assumption that the relevant analysis in *Miccosukee* constituted a holding in that case. It did not. The Court's treatment of the meaning of "discharge" in *Miccosukee* was based on the parties' "agreed-upon law." 541 U.S. at 104, 109–12. As such, it did not establish binding precedent and the meaning of "discharge" thus remains an issue unsettled by this Court. *See Central Va. Cmty. College v. Katz,* 546 U.S. 356, 363 (2006) (treating an assumption about the law in a prior case as dictum and refusing to apply that assumption when "[c]areful study and reflection have convinced [the Court] . . . that that assumption was erroneous").

In *Miccosukee*, this Court characterized the "discharge" issue as involving the application of "agreed-upon law to disputed facts." *Miccosukee*, 541 U.S. at 104; *see also id.* at 109 (noting that the Tribe "does not dispute" that if the canal and reservoir at issue factually constitute the same water body then "pumping water from one into the other cannot constitute an 'addition' of pollutants"). Because the only controversy regarding this issue was factual, the legal question was not decided by the Court and the Court's acquiescence in the parties' legal position reflected an untested assumption about the law that is not binding. *See Katz,* 546 U.S. at 363 (noting that this Court is not bound to follow its own "dicta in a prior case in which the point now at issue was not fully debated")*; see also Cohens v. State of Virginia*, 19 U.S. 264, 399 (1821) ("It is a maxim not to be

disregarded, that general expressions, in every opinion, are to be taken in connection with the case in which those expressions are used. If they go beyond the case, they may be respected, but ought not to control the judgment in a subsequent suit when the very point is presented for decision.")[2]

Further, this Court should hesitate to adopt law that has not been subjected to adversarial dispute. *See United States v. Crawley*, 837 F.2d 291, 293 (7th Cir. 1988) (noting that where an issue is "not presented as an issue," it has not been "refined by the fires of adversary presentation" and there is reason to believe it is not a "fully measured judicial pronouncement . . . and indeed that it may not have been part of the decision that resolved the case or controversy on which the court's jurisdiction depended"); *see also* Pierre N. Leval, *Judging Under the Constitution: Dicta About Dicta*, 81 N.Y.U. L. Rev.

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[2] This Court's decision in *S.D. Warren Co. v. Maine Bd. of Envtl. Prot.*, 547 U.S. 370 (2006), also does not resolve the meaning of "addition" here. First, the Court expressly distinguished the issue in that case from Section 402 because it involved the meaning of "discharge" under Section 401, 33 U.S.C. §1341 (2006). *Id.* at 381 ([T]he understanding that something must be added in order to implicate § 402 does not explain what suffices for a discharge under § 401."). Second, while the Court mentioned the "addition" discussion in *Miccosukee, see id.* at 381 n.6, and noted that the Court "accepted the shared view of the parties," *id*. at 381, its discussion of *Miccosukee* did not transform it into binding precedent on this question. *See United States v. Dixon*, 509 U.S. 688, 706 (1993) ("Quoting . . . suspect dictum multiple times . . . cannot convert it into case law."); *Metro. Stevedore Co. v. Rambo*, 515 U.S. 291, 300 (1995) ("Breath spent repeating dicta does not infuse it with life.").

1249, 1261–62 (2006) ("Our readiness to trust a court's rulings of law depends on the assumption that the adverse parties will each vigorously assert the best defense of its positions."). In *Miccosukee*, the question regarding whether moving pollutants within a water body can constitute an "addition" was not presented as an issue requiring the Court's resolution because both lower courts "rested their holdings on the predicate determination that the canal and reservoir are two distinct water bodies." 541 U.S. at 99. Acceptance of the parties' untested view of the law on an issue seemingly immaterial to the litigation would undermine an important purpose of the adversarial process, which is to safeguard accuracy.[3]

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[3] Courts have applied the same analysis in the analogous context of preclusion law, and for similar reasons. In the collateral estoppel context, for example, the general presumption is that stipulations of law are not preclusive. This presumption holds unless it is clear the parties intended a preclusive effect. *See e.g.*, *Pelham Hall Co. v. Hassett*, 147 F.2d 63, 67 (1st Cir. 1945) (noting parties should not be precluded "from raising a relevant point of law unless it appears beyond doubt that the precise point was actually contested and decided (not merely assumed) in the prior litigation"); *Anderson, Clayton & Co. v. United States*, 562 F.2d 972, 992 (5th Cir. 1977) (When a party "concedes or stipulates the issue upon which the court bases its judgment, the issue is not conclusively determined for purposes of collateral estoppel unless it is clear that the parties so intended."); RESTATEMENT (SECOND) OF JUDGMENTS § 27 cmt. e (1982) (An issue is not actually litigated "if it is the subject of a stipulation between the parties" although it may be binding "if the parties have manifested an intention to that effect."). The purpose of this limitation on stipulation is that the legal question, when conceded, has lost the safeguard of the

Moreover, even if the relevant pronouncement in *Miccosukee* were not technically dictum, this Court should be reluctant to conclude that it established binding law because the issue was not fully reviewed. *See Cohens,* 19 U.S. at 399 (declining to give weight to the Court's treatment of an issue that was not "investigated with care, and considered in its full extent"). This Court has exhibited a healthy skepticism about being bound by legal assertions that have not been the subject of plenary review. *Washington v. Confederated Bands & Tribes of Yakima Indian Nation*, 439 U.S. 463, 478 (1979) (refusing to resolve an issue on *stare decisis* grounds that had not received plenary review, and recognizing a lesser degree of precedential value for summary dismissals than for "opinion[s] of th[e] Court after briefing and oral argument on the merits").

*Miccosukee* presents a perfect example of why courts should be reluctant to treat untested stipulations of "agreed-upon law" as establishing holdings. As will be seen in Section III, below, the question whether redeposits of pollutants within waterways should be deemed "addition[s]" within the meaning of Section 502(12) of the CWA involves complex statutory dynamics, conflicting agency interpretations, and many years of sometimes contradictory case law. Neither the parties nor the

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adversarial process. *Anderson,* 562 F.2d at 992 n. 32 ("Adversary litigation is an important safeguard in the judicial process."). This Court should not adhere to the point of law in *Miccosukee* because it was not actually contested and decided.

United States briefed any of the underlying tensions in *Miccosukee*; nor have they done so here. This Court should be reluctant to conclude that it has established binding precedent on a complex subject absent the safeguards of an adversarial process. As Justice Kennedy once put it, these issues are "best reserved for a later case" because "neither the Court of Appeals in deciding the case nor the parties in their briefing before this Court devoted specific attention to the subject." *Friends of the Earth, Inc. v. Laidlaw Envtl. Serv. (TOC), Inc.*, 528 U.S. 167, 197 (2000) (Kennedy, J., concurring) (referring to Art. II and separation of powers issues implicated in CWA case).

## III. THE JURISDICTIONAL ISSUES RELATING TO DISCHARGES WITHIN WATERS OF THE UNITED STATES ARE COMPLICATED AND HAVE IMPLICATIONS WELL BEYOND THIS CASE

Section 301(a) is the fundamental jurisdictional provision of the CWA. It provides that, except as in conformance with specified sections of the Act, "the discharge of any pollutant by any person shall be unlawful." 33 U.S.C. § 1311(a) (2006). Two of the referenced exceptions to this prohibition are Sections 402 and 404. Section 402(a) allows EPA to issue permits "for the discharge of any pollutant. . . notwithstanding section [301(a)]," 33 U.S.C. § 1342(a) (2006), and Section 404(a) allows the Corps to issue permits "for the discharge of dredged or fill material." 33 U.S.C. § 1344(a) (2006); *see also Rapanos v. United States*, 547 U.S. 715, 723 (2006) (plurality) (recognizing that Sections 402 and 404 are "exceptions" to the Section 301 prohibition on the

discharge of any pollutant and that EPA and the Corps have the authority to issues permits under the respective provisions). The key point here is that Section 301(a) underlies both of these programs. If there is no "discharge of [a] pollutant," there is no jurisdiction for purposes of either Section 402 or Section 404.

Section 502(12) defines the phrase "discharge of a pollutant" to require four elements: (1) an addition; (2) of a pollutant; (3) to the navigable waters; (4) from a point source. 33 U.S.C. § 1362(12) (2006). The term "addition" is the only one of the four jurisdictional elements from the definition of "discharge of a pollutant" that is not further defined in Section 502. As this Court recognized in *Miccosukee*, the key jurisdictional question in this context is whether there is an "addition" of a pollutant when pollutants from within a water body are discharged at a different point within the same water. With no one disputing the issue in the case, and without further analysis, this Court merely quoted an earlier bit of dicta from the Second Circuit: "[I]f one takes a ladle of soup from a pot, lifts it above the pot, and pours it back into the pot, one has not 'added' soup or anything else to the pot." *Miccosukee*, 541 U.S. at 109–10 (quoting *Catskill Mountains Chapter of Trout Unlimited, Inc. v. New York*, 273 F.3d 481, 492 (2d Cir. 2001) ("*Catskill*")). While the *Catskill* analogy has some surface appeal, it ignores the structure of the CWA.

Though the federal government has properly and consistently regulated redeposits under Section 404 of the Act, the government's approach to similar "addition" questions under Section 402 of the Act has

not been consistent. The meaning of addition is an open question and should remain so here because of its complex jurisdictional implications under the Act.

### A. Redeposits Are Properly Regulated Under Section 404

As a starting place, it is important to establish that redeposits of material within the same water body are properly regulated under CWA Section 404. The plain language of the Act, its legislative history, the agencies' interpreting regulations, and all cases that have addressed this issue support the conclusion that a pollutant need not come from outside of the water body to constitute an "addition" in the context of Section 404 discharges.

Section 404(a) specifically contemplates that the Corps will issue permits for discharges of dredged material. 33 U.S.C. § 1344(a). Section 502(6) underscores this dynamic, defining the term "pollutant" to expressly include "dredged spoil." 33 U.S.C. § 1362(6). As many courts have noted, the fact that Sections 301(a) and 404(a), taken together, govern discharges of dredged material strongly suggests that regulated pollutants need not come from outside of the waters of the United States. In the oft-quoted language of the Fifth Circuit, "'dredged' material is by definition material that comes from the water itself. A requirement that all pollutants must come from outside sources would effectively remove the dredge-and-fill provision from the statute." *Avoyelles Sportsmen's League, Inc. v. Marsh*, 715 F.2d 897, 924 n.43 (5th Cir. 1983); *see also infra* n. 4.

The legislative history fully supports this reading. Senator Ellender, who sponsored the floor

amendment that first gave the Corps permitting authority over discharges of dredged material, noted that these discharges involve "moving spoil material from *one place in the waterway to another, without the interjection of new pollutants*." 117 CONG. REC. 38853 (Nov. 2, 1971) (statement of Sen. Ellender) (emphasis added); *see also id.* ("The disposal of dredged material does not involve the introduction of new pollutants; it merely moves the material from one location to another.").

Further, Congress acted in reliance on this understanding in adopting the 1977 Amendments to the CWA. Those Amendments created conditional exemptions for specified types of discharges involving dredged material, but provided that the exemption would be unavailable if the impacts on the waters of the United States would be significant. 33 U.S.C. § 1344(f). Tellingly, these conditional exemptions include several that involve the relocation of dredged material within the same water body, such as discharges associated with "plowing" and "the maintenance of drainage ditches." 33 U.S.C. § 1344(f)(1)(A), (C).

EPA and the Corps have rules bearing out these dynamics. First, both agencies define the term "dredged material" to mean "material that is excavated or dredged from waters of the United States." 40 C.F.R. § 232.2 (2012) (EPA regulation); 33 C.F.R. § 323.2(c) (2012) (Corps regulation). Their rules also indicate that the phrase "discharge of dredged material" includes not only "[t]he addition of dredged material to a . . . discharge site located in waters of the United States," but also "[a]ny addition, including redeposit other than incidental fallback, of dredged

material . . . into waters of the United States . . . . " 40 C.F.R. § 232.2 (EPA regulation); 33 C.F.R. § 323.2(d)(1) (Corps regulation).

In line with the above, it is unsurprising that there is extensive judicial authority supporting the application of Section 404 in situations where the discharge of dredged spoil occurs within the same aquatic system from which it was dredged; in fact, the courts of appeals have been virtually unanimous on this score.[4] The only case that is even slightly

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[4] *See United States v. Brace*, 41 F.3d 117, 121 (3d Cir. 1994) (farmer violated the CWA when he "cleared, mulched, churned, leveled, and drained the formerly wooded and vegetated site" and "paid for excavation in the site and the burying of plastic tubing or drainage tile" without a 404 permit); *United States v. Deaton*, 209 F.3d 331, 335–36 (4th Cir. 2000) ("What is important is that once that material was excavated from the wetland, its redeposit in that same wetland *added* a pollutant where none had been before.") (emphasis in original); *Avoyelles Sportsmen's League, Inc. v. Marsh*, 715 F.2d 897, 923–25 (5th Cir. 1983) (noting, *inter alia*, that "[t]he word 'addition', as used in the definition of the term 'discharge,' may reasonably be construed to include 'redeposit'"); *United States v. Cundiff*, 555 F.3d 200, 213 (6th Cir. 2009) (following *Deaton* and *Avoyelles* on facts substantially identical to those in *Deaton*); *Greenfield Mills, Inc. v. Macklin*, 361 F.3d 934, 947–49 (7th Cir. 2004) (following *Avoyelles* in expressly rejecting the argument that it mattered that the dredged materials were being redeposited into the same aquatic system); *Green Acres Enterprises, Inc. v. U.S.*, 418 F.3d 852, 856 (8th Cir. 2005) (requiring a 404 permit for excavation and dredging activities conducted in connection with levee maintenance that involved more than incidental fallback); *Borden Ranch P'ship v. U.S. Army Corps of Eng'rs*, 261 F.3d 810, 815 (9th Cir. 2001), *aff'd*, 537 U.S. 99 (2002) (affirmance by an equally-divided Court) ("deep ripping" in wetlands "can constitute a discharge of a pollutant"); *United*

discordant is the D.C. Circuit's decision in *National Mining Association v. U.S. Army Corps of Engineers*, 145 F.3d 1399 (D.C. Cir. 1998), in which the court determined that the Corps overstepped its statutory authority by defining "discharge of dredged material" to include incidental fallback. Even there, however, the court went on to indicate its general agreement with *Avoyelles* and its progeny. It started by quoting the above-mentioned language from *Avoyelles* regarding the concern about reading the dredge-and-fill provisions out of the law. *Id.* at 1405. It continued in the following terms:

> But we do not hold that the Corps may not legally regulate some forms of redeposit under its § 404 permitting authority. We hold only that by asserting jurisdiction over "*any* redeposit," including incidental fallback, the [rule] outruns the Corps's statutory authority. Since the Act sets out no bright line between incidental fallback on the one hand and regulable redeposits on the other, a reasoned attempt by the agencies to draw such a line would merit considerable deference.

*Id.*[5]

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*States v. Hubenka*, 438 F.3d 1026, 1035 (10th Cir. 2006) ("[U]se of a bulldozer to move river bottom materials in order to construct . . . dikes unquestionably falls within the scope of [Corps' regulations].").

[5] What is equally telling is the overall percentage of dredged-material cases in which the dredged spoil is redeposited into the same aquatic system from which it is taken. In our research, we identified 97 reported decisions under Section 404 involving

### B. The "Addition" Question is Complicated Under Section 402 and Should Not Be Decided in this Case

The clarity of the above approach to the "addition" question vanishes when one considers the same issues in the context of Section 402. As evidenced by this case, even Respondents are disinclined to argue that CWA jurisdiction should be triggered by the mere channelization of polluted water through a culvert or the like. Moreover, EPA has long taken the position that dams do not require NPDES permits, even where their operation results in the discharge of pollutants, such as sediments, that would otherwise be collected in the water behind the dam. And this position has been met with some judicial approval. *See Nat'l Wildlife Fed'n v. Gorsuch*, 693 F.2d 156, 175 (D.C. Cir. 1982) (concluding that "addition" is ambiguous and deferring to EPA's interpretation that water passing through a dam was not an "addition" because the

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dredged material, as distinct from fill material. In only two of those decisions did it appear that the dredged material either had been or was to be placed or discharged into a wholly separate body of water. *See Save Our Sound Fisheries Ass'n v. Callaway*, 387 F. Supp. 292, 294–95 (D.R.I. 1974) (regarding dredged spoil generated from "the removal of three shoals from the Providence River" that was proposed to be discharged at the Brenton Reef site off the coast of Rhode Island); *S. La. Envt'l Council, Inc. v. Sand*, 629 F.2d 1005, 1010 (5th Cir. 1980) (upholding a Section 404 permit authorizing the discharge of dredged spoil from Bayou Black and Bayou Boeuf into Avoca Island Lake, near Morgan City, Louisiana "to facilitate the movement of offshore drilling rigs and related marine equipment").

dam did not "physically introduce[] a pollutant from the outside world"); *Nat'l Wildlife Fed'n v. Consumers Power Co.*, 862 F.2d 580, 584 (6th Cir. 1988) (relying on *Gorsuch* to conclude that the deposit of water containing "entrained fish" generated by a turbine was not an "addition" because the fish did not come from the outside world). More recently, EPA promulgated its "Water Transfers Rule," which exempts from the NPDES program conveyances of water, even when they contain pollutants, from one water course to another, so long as the transferred water is not subjected to intervening industrial, municipal, or commercial use. 40 C.F.R. § 122.3(i) (2012); 73 Fed. Reg. 33697 (June 13, 2008).

The statutory waters are further muddied when one considers whether it is permissible for either the agencies or the courts to take this bifurcated approach, analyzing the "addition" question quite differently in the Section 402 and 404 contexts. In *Clark v. Martinez*, 543 U.S. 371 (2005), this Court held that courts cannot interpret a word or phrase in a particular statutory provision differently in different factual contexts. This is because "[t]o give these same words a different meaning for each category [of aliens] would be to invent a statute rather than to interpret one." *Id.* at 378; *see also Unites States v. Santos*, 553 U.S. 507, 522–23 (2008) (plurality opinion) ("*Clark v. Martinez* held that the meaning of words in a statute cannot change with the statute's application. To hold otherwise would render every statute a chameleon and would establish within our jurisprudence . . . the dangerous principle that judges can give the very same statutory text

different meanings in different cases.") (internal quotations omitted).

It is an open question whether *Clark v. Martinez* applies in the context of deference to agency interpretations under *Chevron*. It is well established that courts afford considerable deference to reasonable agency interpretations of ambiguous statutory provisions. *See, e.g., Chevron,* 467 U.S. at 843–44 (When the agency charged with implementing a statute interprets an ambiguous provision, "a court may not substitute its own construction of a statutory provision for a reasonable interpretation made by the administrator of an agency."). And this Court has recognized that EPA can interpret the same ambiguous term or phrase differently when it is used in separate or cross-referenced provisions with different policy dynamics. *See, e.g.*, *Envtl. Def. v. Duke Energy Corp.*, 549 U.S. 561, 576 (2007) (concluding that incorporating a definition of the same term from one Clean Air Act program in another program did not "eliminat[e] the customary agency discretion to resolve questions about a statutory definition by looking to the surroundings of the defined term"). But here the relevant phrase, "addition of [a] pollutant," is used only once, in the definition of "discharge of a pollutant" in Section 502(12). 33 U.S.C. § 1362(12). That same definition establishes the jurisdictional requirements for *both* the Section 402 and 404 permitting programs. Thus it remains to be seen how much discretion is afforded to an agency in its

interpretation of the same term in the same provision. [6]

Should this question be properly presented to this Court, the interpretation of "addition," as used in

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[6] Few lower courts have addressed whether *Clark v. Martinez* limits the discretion that agencies would otherwise possess under *Chevron,* let alone under the Court's more recent decision in *Duke Energy*. At least one circuit has indicated that even under *Clark v. Martinez*, an agency may interpret the same statutory provision to have different meanings in different contexts so long as the agency explains its reason for doing so and its interpretation is reasonable. *Dongbu Steel Co., Ltd. v. United States*, 635 F.3d 1363, 1370–72 (Fed. Cir. 2011) (rejecting the argument that *Clark v. Martinez* means the agency's inconsistent interpretation of a term is deemed unreasonable and noting that the agency's interpretation will be upheld so long as it "provide[s] an explanation for why the statutory language supports its inconsistent interpretation"). There is a circuit split regarding whether the Attorney General's interpretation of the provision at issue in *Clark v. Martinez* should be afforded *Chevron* deference. *Compare Hernandez-Carrera v. Carlson*, 547 F.3d 1237, 1242 (10th Cir. 2008) ("[A] subsequent, reasonable agency interpretation of an ambiguous statute . . . is due deference notwithstanding the Supreme Court's earlier contrary interpretation of the statute.") *with Tran v. Mukasey,* 515 F.3d 478, 481 (5th Cir. 2008) (concluding that the interpretation was not entitled to deference because the agency was bound by the Court's interpretation in *Clark v. Martinez*). *Tran*, however, failed to account for the discretion afforded to agencies under the *Brand X* doctrine. *See Nat'l Cable & Telecomm. Ass'n v. Brand X Internet Serv.,* 545 U.S. 967, 982 (2005) ("A court's prior judicial construction of a statute trumps an agency construction otherwise entitled to *Chevron* deference only if the prior court decision holds that its construction follows from the unambiguous terms of the statute and thus leaves no room for agency discretion.").

Section 502(12), would require consideration of the statutory and practical differences between Sections 402 and 404 and whether the term can have a different meaning as it relates to each permitting program. As discussed above, it is clear that Congress intended to regulate redeposits under Section 404; the inclusion of "dredged spoil" in the definition of statutory "pollutant[s]" demonstrates as much. What is less clear is whether or how pollutants within one water body are to be regulated under Section 402.

To be sure, if properly faced with the question, this Court would have several pathways to harmonize these issues. Initially, it could—and should—start with the plain language establishing that redeposits of dredged material fall within the scope of the Act. One conclusion, perhaps the soundest that could follow, is that the clear congressional intent under Section 404 combined with the shared definitional terms between the two programs indicates that pollutants need not come from the outside world to be "added" under *either* provision. Beyond that, the Court could conclude that given the different dynamics at play under Section 402, a different definition is appropriate. It would then need to recognize that these different interpretations of "addition" are acceptable in the two contexts, notwithstanding *Clark v. Martinez*. This Court's decisions in *Duke Energy* and the deference afforded to agencies under *Chevron* would likely support such a result.

Importantly, however, these dynamics and options demonstrate that this is an open question, and one that should not be decided in passing.

## CONCLUSION

For the foregoing reasons, the Court should decline to address the "addition of a pollutant" issue.

Respectfully submitted,

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November 5, 2012

## DESCRIPTION OF *AMICI CURIAE*

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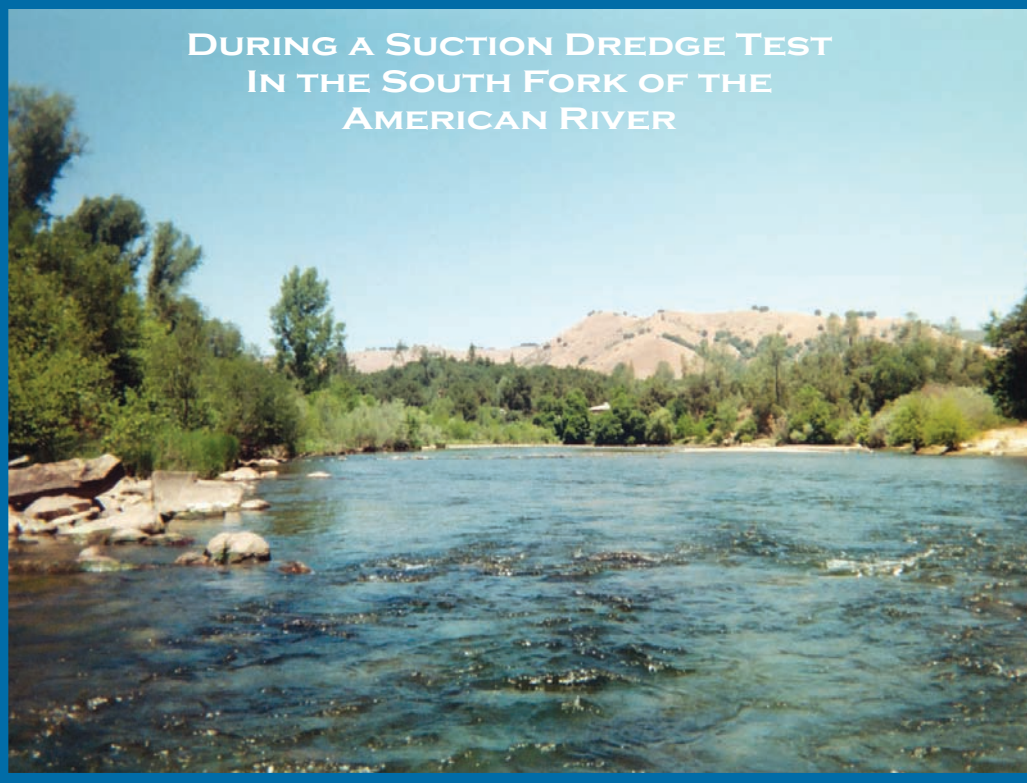
STAFF REPORT

# MERCURY

## LOSSES AND RECOVERY

During a Suction Dredge Test
In the South Fork of the
American River



CALIFORNIA
Water Boards
STATE WATER RESOURCES CONTROL BOARD
REGIONAL WATER QUALITY CONTROL BOARDS

MAY 2005

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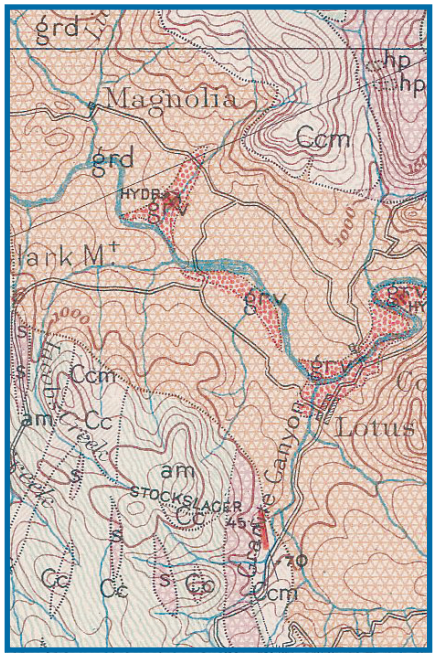


*FIGURE 1: Historical Map of Coloma, California by Waldemar Lindren (1894), United States Geological Survey Folio#3 - Placerville, California, Economic Geology - northwest (Courtesy of: Craig Couch)*

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# ABOUT A KILOGRAM OF MERCURY

## Introduction

Mercury has been used widely since the dawn of recorded history for gold mining. During California's gold rush, gold miners used about 6 million kilograms or 6.6 thousand tons of mercury (Churchill, 2000) to recover over 3.6 thousand tons of gold (Bulletin 193). **The weight of mercury used is roughly equal to the total weight of a 9-mile long line of 2,750, full sized pickup trucks (note: the pick up truck line equaling gold recovered would only be 5 miles long). The miners lost about half of the mercury to the environment.**

Using historical records,Churchill (2000) estimated that total mercury losses ranged between 2.3 million and 2.6 million kilograms for placer and lode mining in the Sierra Nevada Geomorphic Province. Consequently, elemental mercury from the gold rush is still found, sometimes in amounts that constitute a local hotspot (i.e., a location where visible elemental mercury is found) in Sierra Nevada watersheds where gold mining occurred. In March 2003, a recreational gold miner reported a mercury hotspot in the South Fork of the American River near Coloma, to State Water Resources Control Board staff. It was the first time a recreational gold miner had revealed a hotspot locations to agency staff. Coloma is California's historic "Gold Discovery" site as James W. Marshall's discovery there in January 1848 initiated the 1849 gold rush. Steve Franklin, the recreational gold miner who reported the hotspot, claimed to have recovered about a kilogram of mercury while gold mining from the hotspot during January and February 2003.

**Finding a hotspot near Coloma raised questions about its potential threat to human health, effects on local fish, and threat to water quality. Moreover, its discovery presented an opportunity to test the notion that recreational gold miners effectively clean up mercury hotspots while suction dredging for gold.** There is no record of any attempts by state or federal agencies to clean up a mercury hotspot in a California river. But State and federal agencies have discussed whether encouraging or even providing support for recreational gold miners to clean up hotspots is viable and wise. The pros are that there is a potentially large, volunteer workforce. The cons are that oversight would be difficult and, up to now, no data supported the notion that suction dredges could recover mercury efficiently.

*FIGURE 2: Steve Franklin and SWRCB staff sampled the hotspot on July 8, 2003, and recovered about 125 grams of mercury in about three hours from the river using simple suction recovery tools. Mercury was visible as droplets ranging from one to ten millimeters on bedrock in the river channel. (Photo by: Rick Humphreys, DWQ)*

Recreational gold dredging on public and private lands is a moderately popular activity in California. The Department of Fish and Game (DFG) issues several thousand permits annually to recreational gold dredgers. Along with gold, recreational dredgers recover iron (nails bolts, etc.), lead (fishing weights, buckshot, and spent bullets) and mercury (elemental mercury, mercury/gold amalgam, and mercury stained gold). Over the past several years, United States Forest Service (USFS), Bureau of Land Management (BLM) and State agency staff have discussed setting up a mercury recovery program for recreational dredgers. Incentives (e.g., cash for mercury, free dredging permits, new areas opened for dredging) were proposed in exchange for mercury turned in by recreational dredgers. Offering such incentives was and remains controversial for a variety of reasons and a mercury recovery program was not started. **Moreover, an important drawback was that the efficiency of a standard suction dredge at recovering mercury was unknown.** Consequently, no one knew if mercury would be lost along with waste sediment from a suction dredge. Clearly, a mercury recovery program that dispersed elemental mercury back into a stream in substantial amounts would be unacceptable. The hotspot presented an opportunity to determine the mercury recovery efficiency of a suction dredge.

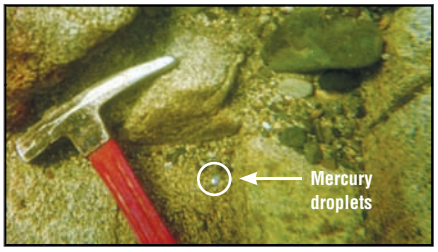


*FIGURE 3: Under water photograph showing river sediment, bedrock, and mercury droplets. (Photo by: Rick Humphreys, DWQ)*

Studying the hotspot may also reveal bedrock characteristics and sediment transport conditions that cause hotspots, and the effects that concentrated mercury has on local fish. This report documents the results of a suction dredge test that was completed in September 2003 by State Water Board, USFS, and DFG staff.

## Hotspot Setting

The hotspot is located mid-channel in the South Fork of the American River, a few miles downstream from the Marshall Gold Discovery State Park at Coloma. Surface placers and in-river gravel accounted for most gold produced from the area during the gold rush and in-river dredging recovered more gold during the 1930s and 1940s (Bulletin 193). These historic mining operations are the likely mercury source.

The hotspot is located on the downstream side of a low bedrock hump that extends across the river channel perpendicular to its flow. Because the hotspot remains underwater under all observed flow conditions, State Water Board skin divers recorded how the mercury occurred on bedrock and in river sediment visually. The bedrock hump is shaped like a low-pitched roof. River sediment forms wedge-shaped deposits on the up and downstream sides of the hump. Easily visible, small (e.g., 1mm) mercury droplets permeate the sediment at the thin upstream edge of the downstream wedge (see fig.2). Hand "fanning" stirs up fine-grained sediment, which is carried away by the river current. Elemental mercury, however, remains on bedrock, and continued fanning causes small mercury droplets to fall into bedrock depressions and fractures. When mercury droplets touch, they fuse into much **large** droplets (up to 25 millimeters). **Hand fanning the upstream sediment wedge also exposes elemental mercury in bedrock depressions and fractures** but in much smaller amounts than on the downstream side.

*FIGURE 4: "The hotspot is located mid-channel in the South Fork of the American River, a few miles downstream from the Marshall Gold Discovery State Park at Coloma." (Photo by: Rick Humphreys, DWQ)*

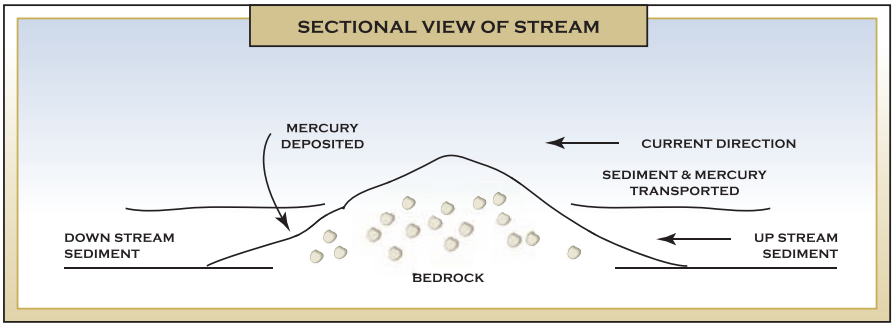


*FIGURE 5: Cross-sectional view of stream graphic showing where mercury deposits on bedrock.*

River flow at the hotspot is uncontrolled during winter and spring runoff but controlled for hydroelectric and recreational rafting purposes for the rest of the year. During controlled flow periods, flows typically range from 200 to 1,200 cubic feet per second (cfs) daily. High runoff coincides with winter storms, and these flows have ranged to 80,000 cfs as recently as 1997. Post dredge test inspections show that during low flow periods (200 cfs), sediment does not travel over the bedrock hump. But post dredge test inspections also showed that mercury had re-deposited on bedrock that had been dredged clean. Higher controlled flows may be moving sediment and mercury over the hump but attempts to observe sediment movement directly at higher flows proved too dangerous.

Mercury may concentrate at the hotspot because after it is carried over the bedrock hump during high flows, it encounters a low flow velocity zone on the downstream side of the bedrock hump. The river current on the downstream side lacks the power to move mercury anymore so it drops out on bedrock on the downstream side. If this scenario is correct, **periodic mercury recovery from this location might be practical.** A mercury removal system's design would depend on the site's the physical characteristics which are unknown. A detailed evaluation of mercury and sediment transport and flow velocity at the hotspot surface would be necessary if periodic mercury removal from this site is considered.

## Suction Dredge Test

The USFS volunteered their mineral evaluation team, based in Redding (Rich Teixeiria, Jim DeMaagd, and Tera Curren), to perform the test. According to Rich Teixeiria, the team's dredge is a Keene Engineering floating 4 inch dredge powered by a Honda 5.5 horsepower engine. It is similar to those used by recreational dredgers to recover gold (see fig.3). A single sluice box used carpet and riffles but no "miners" moss (i.e., woven nylon fabric placed between the riffles and carpet for enhanced gold recovery).

The team performed the dredge efficiency test on Sept.15, 2003. The 63.5kg sediment sample used in the test had been collected by State Water Board staff from the hotspot and characterized for grain size and mercury content. State Water Board staff analyzed the sample's grain size at the Cal Trans Laboratory in Sacramento. The sample classifies as a "clean gravel with sand" under Unified Soil Classification System. Visual inspection of size fractions showed that almost all the liquid mercury rested in the fraction that passed a 30-mesh sieve (0.6mm). The mercury content of this fraction served as a surrogate for the mercury content of the entire sample. Chris Foe of the Central Valley Regional Water Quality Control Board had two 30-mesh passing fractions of the sample analyzed for mercury by ALS Chemex Laboratory in Reno, NV. Two suspended sediment samples of the bulk sample (i.e., samples of sediment that settled out of water used for sieving after an hour) were sent to ALS Chemex Laboratory for mercury analysis. A second set of samples from archived material was sent to Frontier Geosciences in Seattle, WA after reliability problems were discovered with analyses performed on standards by ALS Chemex. During the test, the USFS team captured sediment lost off the sluice in a catch basin for later analysis. Small mercury droplets and fine, barely discernable droplets (i.e., floured mercury) were characteristic of these samples. After the test, 30 mesh and finer dredge concentrates and "waste" sediment were sent to ALS Chemex Laboratory. ALS Chemex Laboratory used an analytical method that could not quantify the high mercury concentration in the mercury-rich samples. So a second set of samples was sent to Frontier Geosciences for analyses.

The team (USFS and State Water Board staff) dredged the hotspot the next day on Sept. 16, 2003, and DFG staff recorded the test on video.

*FIGURE 6. Dredging the hotspot. (Photo by: Rick Humphreys, DWQ)*

> THE MERCURY CONTENT OF THIS FRACTION SERVED AS A SURROGATE FOR THE MERCURY CONTENT OF THE ENTIRE SAMPLE.

## Results - Laboratory Data

ALS Chemex reported that the mercury content of the samples received exceeded the upper detection limit of the analysis used and did not reanalyze the samples. As a result, the Frontier Geosciences analyses were used for this report. The bulk sample mercury concentration was 1,170ppm; the mercury concentration of the sediment captured by the dredge was 1,550ppm, and the mercury concentration of the sediment lost by the dredge was 240ppm. The suspended sediment sample mercury concentration was 298ppm. Note that these mercury concentrations are quite high. **Mercury concentrations of the waste and suspended sediment are over an order of magnitude higher than the minimum concentration necessary for classification as a California hazardous waste (20mg/kg).** The suspended sediment's high mercury content is problematic because after resuspension by dredging, it can be carried long distances by stream current.

## Results - Suction Dredge Efficiency

It is necessary to know how elemental mercury, which is a dense liquid, behaves physically when evaluating the laboratory results. During dredging, large mercury droplets were broken up into small droplets by turbulence. The phenomenon is called "flouring" and it is described as a major cause of mercury loss by historic hydraulic gold mining operations. Confounding matters is mercury's ability to form large droplets from small droplets. This causes mercury enrichment of sediment captured on the sluice because small mercury droplets that are caught in the low velocity area behind the sluice riffles fuse into large droplets just as they do on the downstream side of the bedrock hump. Sluice sediment samples had large and small mercury droplets. Such samples are subject to analytical bias from either a single large mercury droplet, or the absence of any mercury droplets.

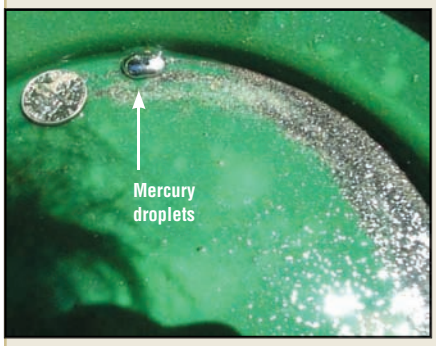


*FIGURE 7: Mercury panned from a small creek below the Sailor Flat Hydraulic Mine, Nevada County. (Photo by: Rick Humphreys, DWQ)*

Bias probably is affecting the analytical results for the efficiency test. The mercury concentration for the captured sediment is 32 percent higher than that of the parent sample, and that may be because the captured sediment sample analyzed had one or two large mercury droplets. However, in absolute terms, the mercury concentration of both samples agrees fairly well. Mercury concentrations in sediment lost by the dredge was averaged (30-mesh and finer and suspended sediment). The mercury concentration of the lost sediment fractions is about 2 percent that of the test sediment's mercury concentration. Thus, the dredge removed about 98 percent of the mercury from the test sample based on concentration. Unfortunately, a mass balance of sediment captured and lost, as part of the test was not performed because we did not have an accurate total mass for the lost fraction.

The test showed that a typical suction dredge set up to recover gold recovered about 98 percent of the mercury in the high-mercury, test sediment sample. However, the loss was in sediment that had high mercury content and is easily transported away by the river.

## Results - In-River Test

The team dredged about four yards or about 5,900 kilograms (6.5 tons) of sediment from the hotspot. Team members used special care to find and dredge large liquid mercury droplets as well as mercury-laden sediment from the site. During clean up after the test, team members noted large mercury droplets captured on the sluice. From the 30-mesh passing fraction, SWRCB staff separated about 0.5kg liquid mercury (see fig. 4). The remaining 2.2kg of sediment retained a substantial amount of liquid mercury as small (e.g., 1mm) and fine droplets of floured mercury, which floated on water used to immerse the sediment. Separating residual mercury from the sediment by physical means proved impossible. The mercury content of a 1.1kg sample was determined directly heating the sample and recovering the mercury vapor (i.e., retorting). The retorted sample contained 20gm of mercury or 1.8 percent. The dredge concentrate contained 540gm of mercury (liquid mercury + retorted mercury/ 1.1kg x 2), which accounted for about 20 percent of the sample mass (540gm mercury/2.7kg sieved sample x 100). Note that the mercury concentration of captured sediment from the in river test is about ten times higher than that reported for the efficiency test. The difference likely reflects the success of the dredge team in finding and dredging up mercury droplets during the in river test.

*FIGURE 8: Jim DeMaagd and Rich Teixeiria setting up the dredge. (Photo by: Rick Humphreys, DWQ)*

# CONCLUSIONS

## Conclusions and Recommendations

1. A suction dredge set up to recover gold recovered liquid mercury from the mercury hotspot. The dredge recovered about 98 percent of the mercury in a test sediment sample enriched in mercury. Mercury concentrations in the fine and suspended sediment lost from the dredge were more than ten times higher than that needed to classify it as a hazardous waste.

2. Lost sediment with high mercury levels is, in effect, mercury recycled to the environment. Floured mercury in fine sediment and mercury attached to clay particles in suspended sediment may be carried by the river to environments where mercury methylation occurs and where fish have high mercury concentrations. The consequences of having floured mercury added to biologically active areas where mercury methylation already occurs are currently unknown because the methylation potential of floured elemental mercury is unknown. But tests are underway at the DFG laboratory at Moss Landing to determine the methylation potential of floured mercury in sediment samples from this hotspot.

3. It is unacceptable to encourage suction dredgers to "clean up" in stream mercury hotspots because dredges release too much mercury in easily transportable forms. There may be other reasons to discourage suction dredging of mercury hotspots once the bioavailablity of floured mercury becomes known. It would be advisable for land management agencies to contact dredgers through their clubs and discourage them from trying to dredge liquid mercury from in-river hotspots on public lands. Removing mercury with hand-operated suction tubes, or better yet, reporting hotspot locations to land management agencies is a better strategy.

4. It might be possible to design a shore-based recovery system for the Coloma hotspot and recover mercury annually. Such a system would need to minimize mercury loss. Recovery equipment would need to be held in storage during nonuse and operated by trained staff. Proper permits (e.g., in stream alteration, and, mercury disposal or recycling) would be needed. Such a project is more complex and costly in time, money, and commitment than previously considered projects. Developing such a system might result in technical advances that could be applied to dredges used by gold dredgers.

5. The sediment transport parameters that cause mercury to concentrate should be characterized. Such a characterization at Coloma might be useful for predicting where other hotspots are located in the South Fork of the American River and other watersheds, and it would provide the data for a recovery project described above.

6. The hotspot's effect on fish and invertebrates in this segment of South Fork of the American River should be determined.

*FIGURE 9. Liquid mercury (about 0.5kg) separated from sediment captured by the dredge. (Photo by: Rick Humphreys, DWQ)*

*FIGURE 10: Under water diver searches for Mercury. (Photo by: Rick Humphreys, DWQ)*

...REMOVING MERCURY WITH HAND-OPERATED SUCTION TUBES, OR BETTER YET, REPORTING HOTSPOT LOCATIONS TO LAND MANAGEMENT AGENCIES IS A BETTER STRATEGY.

## References

*Contributions of Mercury to California's Environment from Mercury and Gold Mining Activities - Insights from the Historical Record.* Unpublished report, 18 pages. Ron Churchill, California Geological Survey, Division of Mines and Geology, 2000.

*Gold Districts of California, Bulletin 193, Sesquicentennial Edition*. William B. Clark, 1963 with some revisions through 1969. California Geological Survey, Division of Mines and Geology, 1998.

*Laboratory Classification of Soils, Unified Soils Classification System*, Geotechnical Branch, Division of Research and Laboratory Services, Bureau of Reclamation Engineering and Research Center Denver Colorado, 1986.



## Acknowledgements

Special thanks to Steve Franklin for reporting the hotspot and to Bill Center for granting us access to the area and use of his camp at Lotus. Thanks to Janine Clayton (USFS) for making the USFS minerals evaluation crew available. Thanks also to Chris Foe of the Central Valley Regional Water Quality Control Board and Mark Stephenson (DFG) for arranging laboratory analyses, and Chris Foe and Dr. Charles N. Alpers for their reviews. Finally, thanks to dredge crewmembers Rich Teixeiria, Tera Curren, Jim DeMaagd, Dominic Gregorio, and Janna Herren for making the project a success.

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# Planning for Statewide Mercury Program for Reservoirs meeting January 14, 2015

**Alex J. Horne**, Emeritus Professor

Ecological Engineering Group,

Department of Civil & Environmental Engineering,

University of California, Berkeley

&

Principal, Alex Horne Associates

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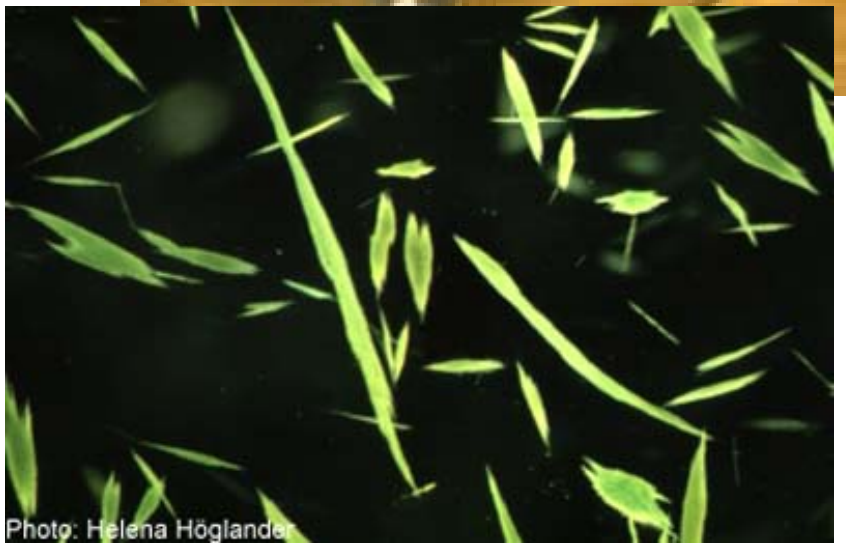
# Background

- 160 Californian reservoirs may be affected by proposed/possible new methyl-mercury regulations
- Reservoirs are relatively neglected in terms of using them as part of a treatment train for drinking water supply relative to treatment plants themselves
- Similarly, reservoir fish management is focused on fish biomass not contamination but this could change

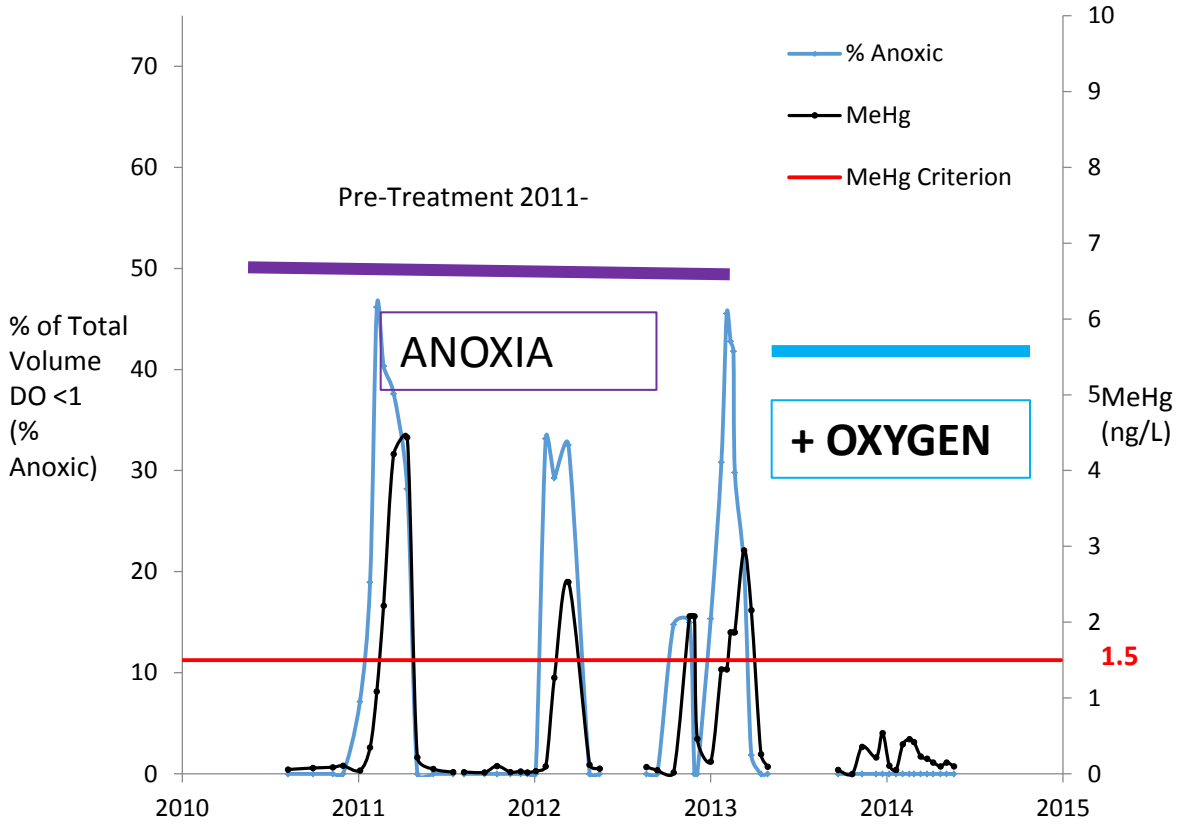

# Problems

- Methyl-mercury (MeHg) can accumulate in fish via the food web hundreds or thousand fold to reach dangerous levels for humans, animals & birds
- Algae are the main culprit in converting dissolved MeHg from water to cell matter (as with selenium, copper & some organic compounds)
- Mercury loading via the air & runoff is a major factor



# Methyl-mercury (MeHg)

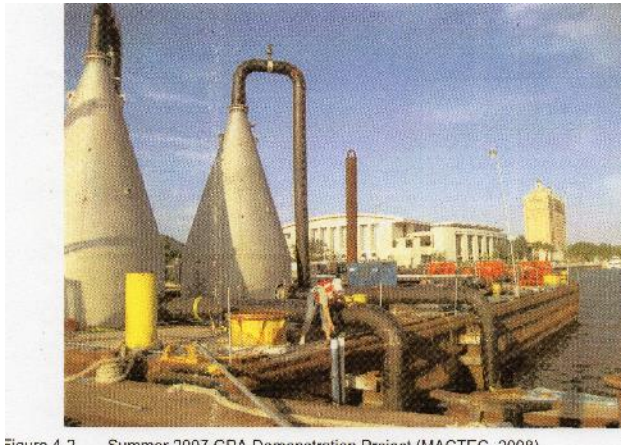
- Produced by bacteria, usually in sediments or close by & under **ANOXIC** (no oxygen) conditions
- *A review of > 100 publications finds almost no mention of anoxia being needed*
- Same bacteria also make hydrogen sulfide ($H_2S$) under anoxia
- **Getting ride of anoxia removes $H_2S$ so will it get ride of MeHg?**
- More or less true but not been put into practice very often



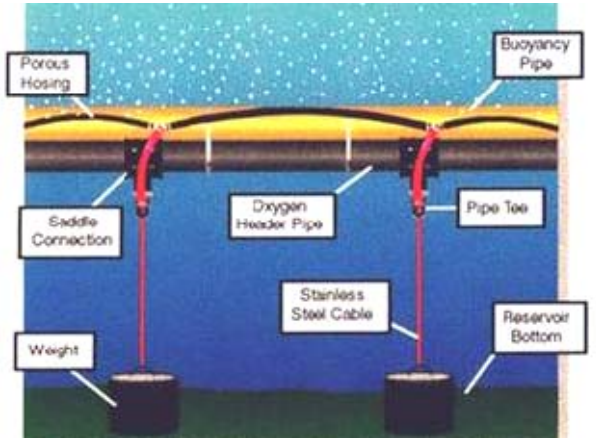

Preliminary fall 2014 data from Dave Drury, Santa Clara Valley WD for Calero Reservoir HOS oxygen treatment

# Hypolimnetic Oxygenation Systems HOS

- HOS covers a range of method that I like to use to get oxygen into the deep waters and sediments of:
- Lakes & reservoirs
- Rivers & streams
- Estuaries (Chesapeake Bay)
- Coastal dead zones (Mississippi River offshore)
- Some areas of the deep ocean (Black Sea or Baltic Sea)



ECO2 Speece cones in estuary

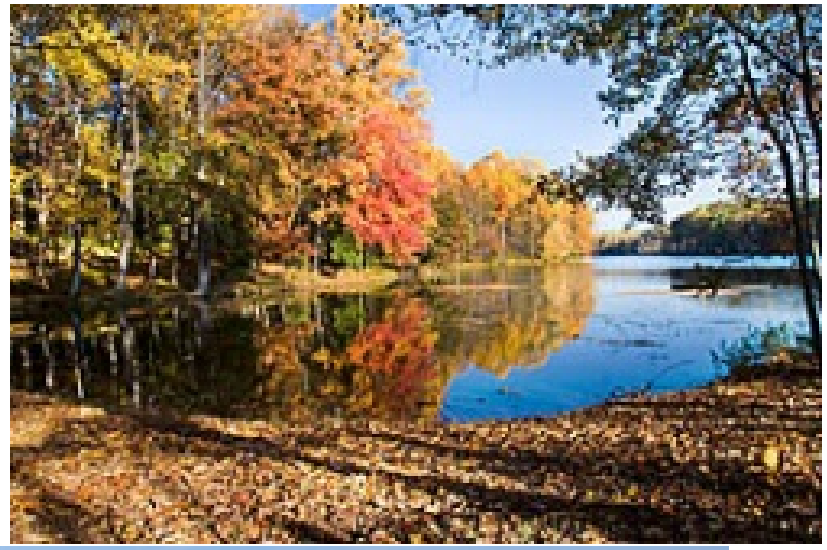



TVA-Mobley HOS diagram

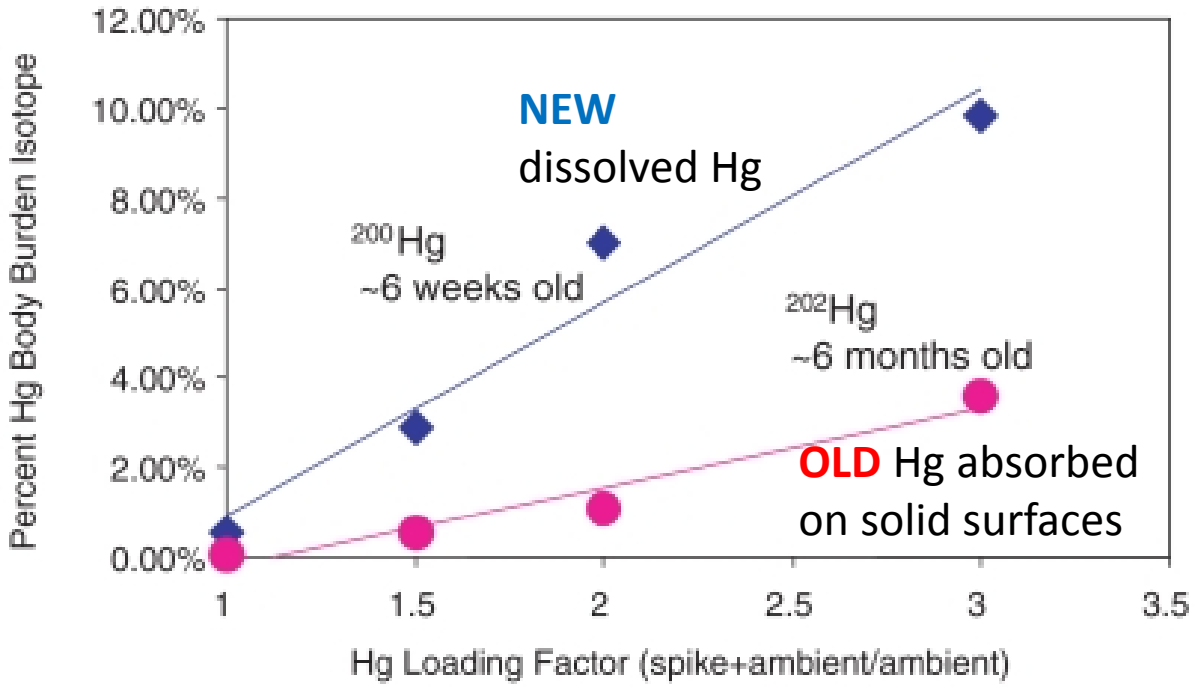
# Correlations: good study of 20 reservoirs in Maryland

Chesapeake Bay & Watershed Programs CBWP-MANTA-ASD-03-1

- **No one single factor** controlled MeHg in largemouth bass; most important were MeHg concentrations, $SO_4$ in water, & lake morphometry ($\hat{z}$ = A/V) = 44% of Σvariance
- **Shallow lakes** that stratify may be worst. Shallow + algae = eutrophication = benthic anoxia
- **Their Solutions**: add forest buffers & reduce acid rain (sulfates high). No use for us.



# Hg aging: Supply of New Mercury important:

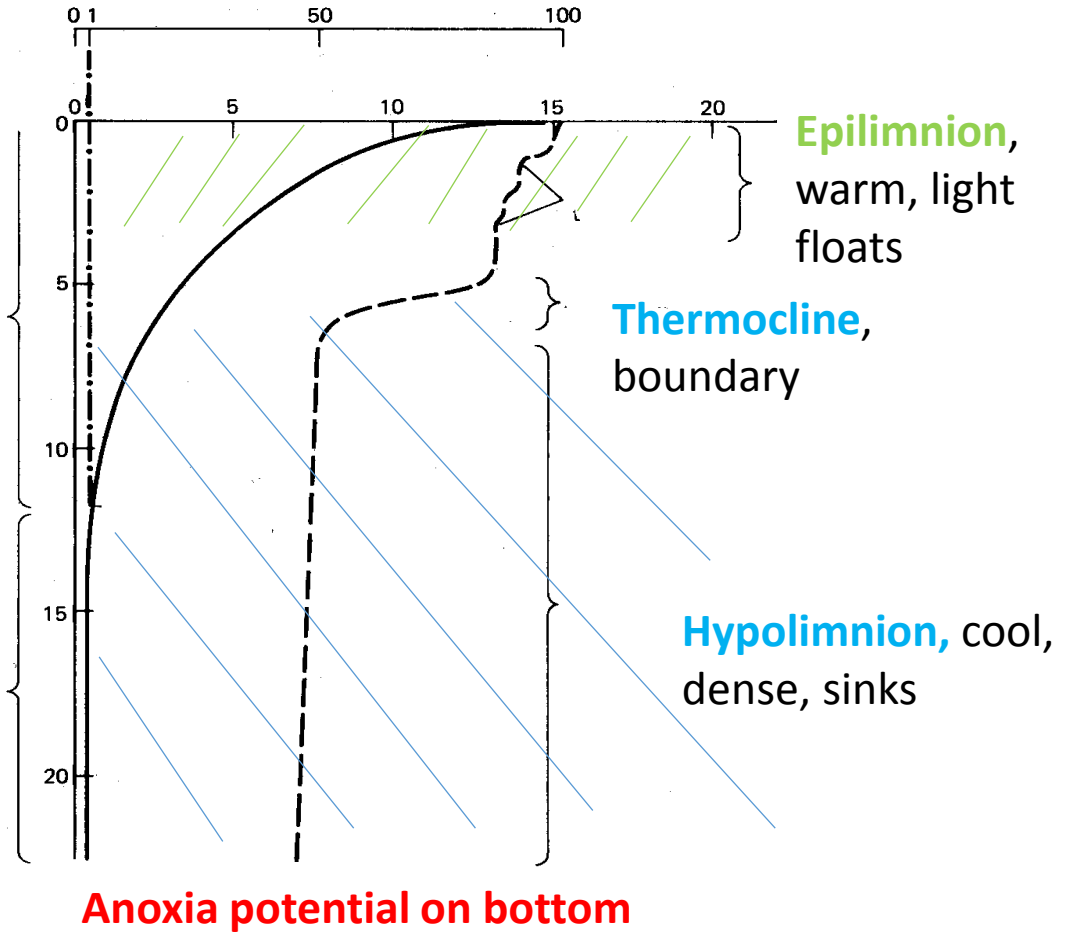



From D. P Krabbenhoft, USGS Wisconsin

- In Florida Everglades new Hg rather than old was most important for MeHg uptake to fish
- Old Hg absorbed onto solid surfaces, new is more dissolved
- So legacy MeHg in sediments may not be too important

# How do reservoirs get anoxic water?

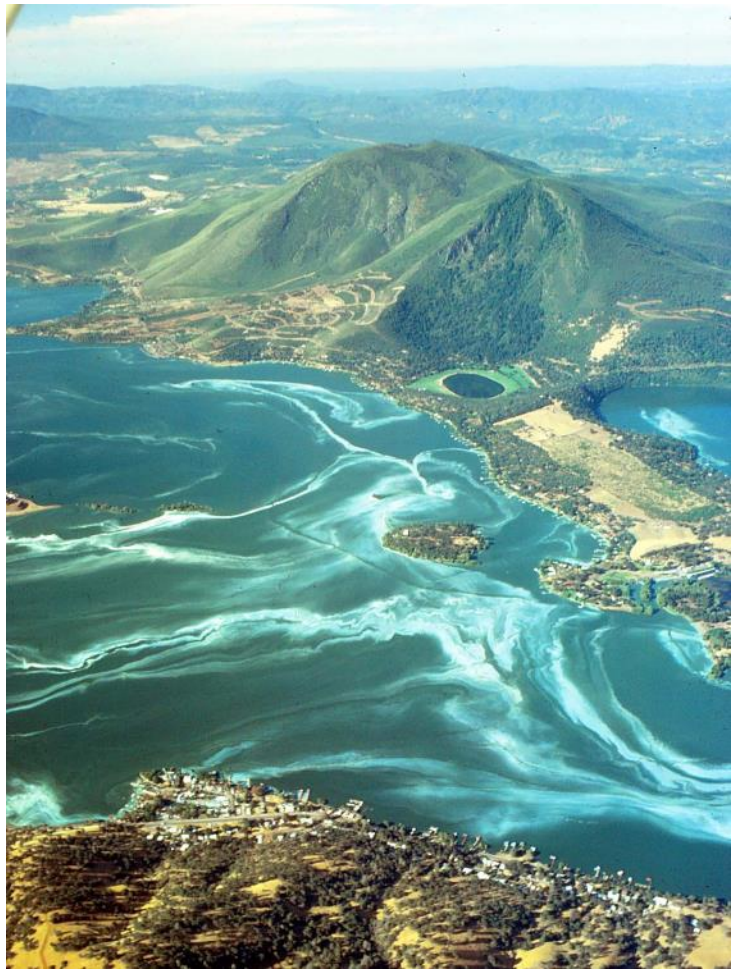
- In spring most waters > 10 feet deep stratify; warm, less dense water on top, cool denser water below.
- The stratification creates a deep block of water is isolated from new supplies of oxygen
- A fixed amount of oxygen in the deep water has to last all summer




From Horne & Goldman, Limnology textbook 1994

# More on anoxic bottom water due to algae

- Algae grow at surface where there is light, die, sink & rot in bottom water & sediments
- Rotting uses up oxygen so too much algae = anoxia
- Algae depend on nutrients
- Hard to reduce nutrients in watershed (unless unit process treatment wetlands used)
- **In-lake treatment** is the only option for most reservoirs

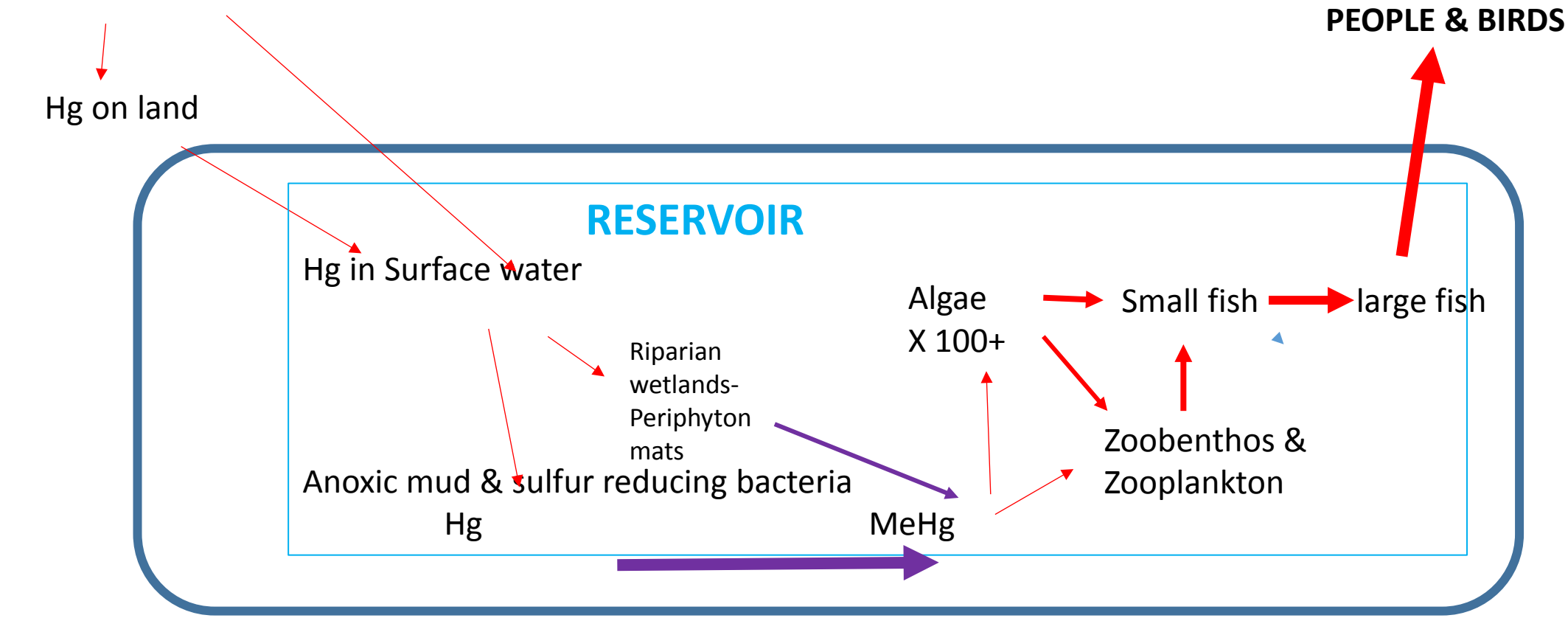


Algae bloom in Clear Lake, Lake Co. CA. ~ 1973
Photo Alex Horne

# Critical path for mercury methylation to humans & birds

TWO KEY STEPS

- **ANOXIC BACTERIAL METHYLATION** OF INORGANIC MERCURY
- **BIO-MAGNIFICATION** OF MeHg UP THE FOOD CHAIN



# Other possible methylation sites

- Low DO in algae mats –periphyton on rocks.  Would need to be thick and anoxic – at least at night
- Low DO possible in decaying layers of the metalimnion (thermocline). Could occur round lumps of decaying matter
- I am not very clear how this could happen very much in California reservoirs
- Riparian wetlands on reservoir edges

Photos: Alex Horne

# Questions

- Algae, zoobenthos, zooplankton & fish contain concentrated MeHg
- Uptake by algae is active uptake is from MeHg itself
- Some passive uptake by organic detritus with S-H bonds
- Will more algae & detritus reduce (biodilute) MeHg in reservoir? That is; some oligotrophic lakes fish have less MeHg in fish than green eutrophic ones (2 to 3 times dilution in zooplankton; Pickhard et al, PNAS 2002)
- Will this prevent HOS from working well?

# Bio-dilution decreases in MeHg vs HOS

**Increased algae = bio-dilution**

- Experimental data: with equal concentrations of aqueous Hg, an increase in algae could result in a decrease in Hg uptake—by zooplankton grazers
- Result: increasing algae reduced $CH_3Hg^+$ concentrations in zooplankton 2–3-fold
- Bloom dilution may provide mechanistic explanation for lower $CH_3Hg^+$ accumulation by zooplankton & fish in algal-rich relative to algal-poor systems.

**HOS = reduced MeHg in water**

- Moderate HOS will reduce MeHg by at least 7 times
- This will overwhelm the dilution effect 2:1
- Really effective HOS might reduce MeHg by up to 100 fold but needs to be tested.

# HOS – reservoir oxygenation

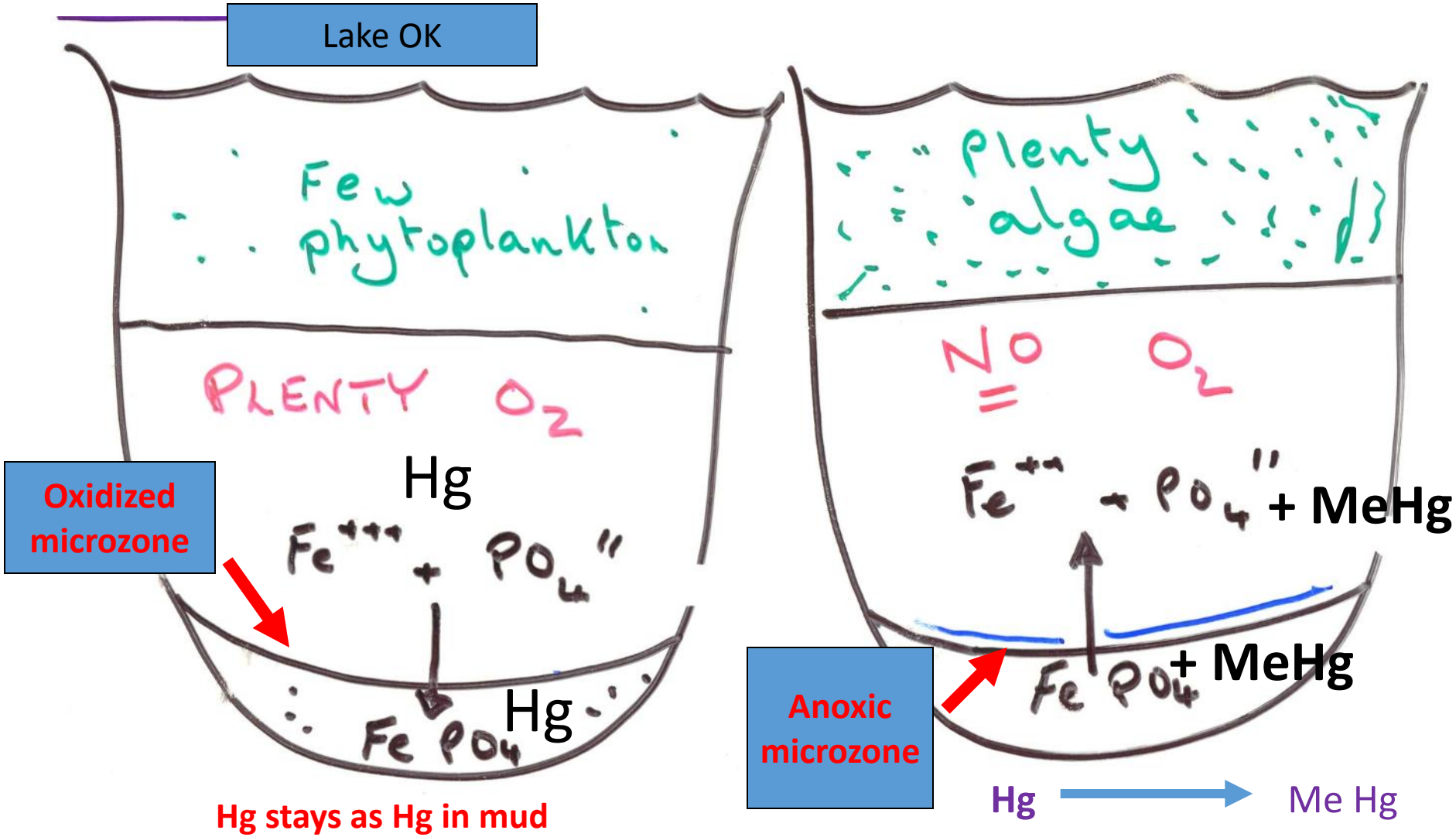
# ALL YOU NEED TO KNOW ABOUT OXYGEN

- **DISSOLVED OXYGEN (DO) IN WATER BECOMES DEPLETED MUCH MORE EASILY THAN GASEOUS OXYGEN IN AIR. LOW DO IN THE HYPOLIMNION IS POSSIBLY THE MOST IMPORTANT PROBLEM IN LAKES & RESERVOIRS**

- **DO CAN EASILY BE ADDED TO WATER ARTIFICIALLY AS COMPRESSED AIR OR PURE OXYGEN & IS THE MOST COMMON LAKE MANAGEMENT TECHNIQUE**

- **DO IS PRODUCED BY PHYTOPLANKTON PHOTOSYNTHESIS & USED BY THEM & ALL OTHER AQUATIC ORGANISMS, GIVING DAILY, SEASONAL & ANNUAL CYCLES OF DO.**

# Effect of oxygen on lakes

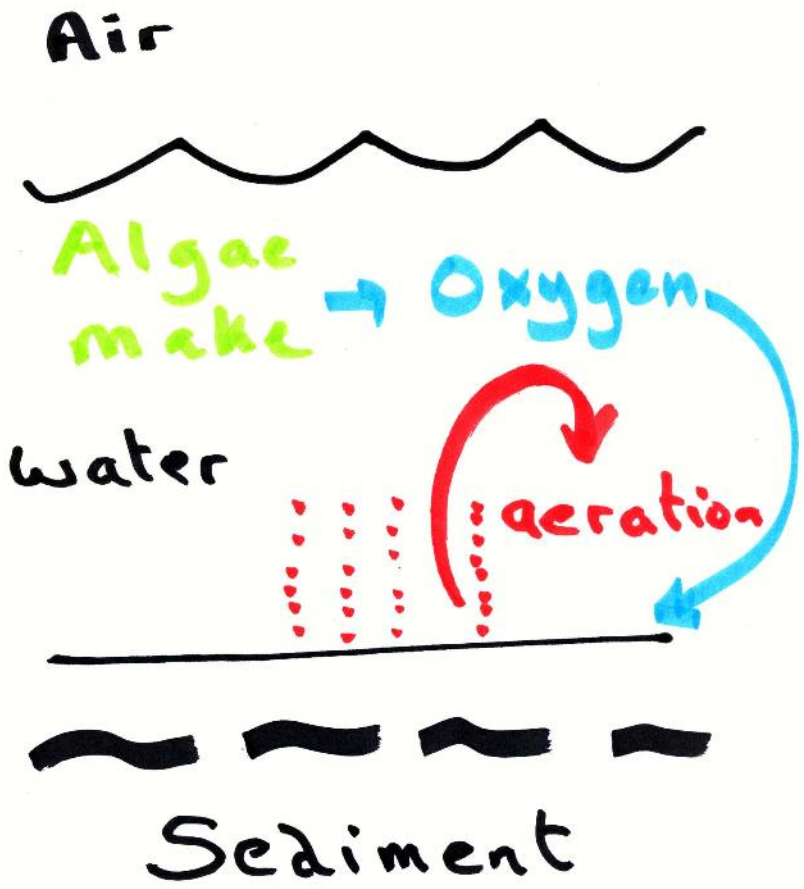


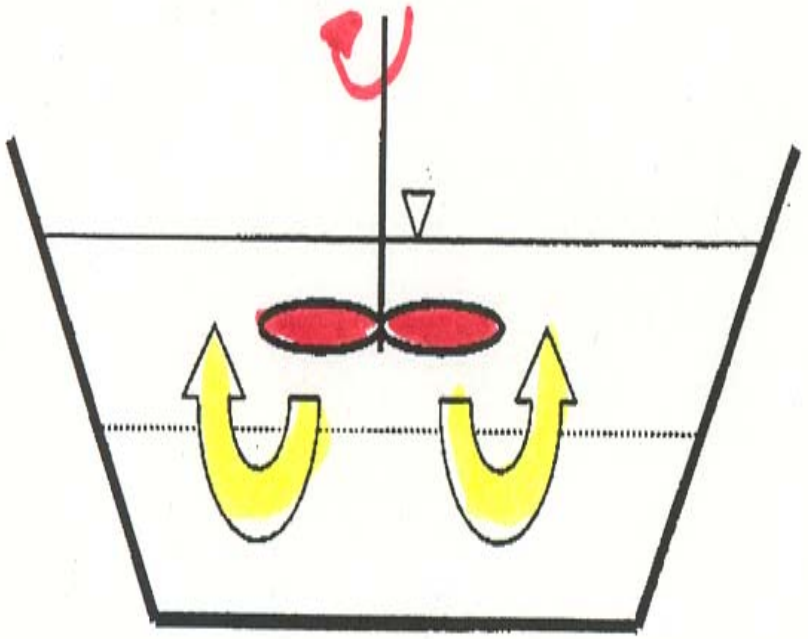
# What oxygenation/aeration/mixing choices are there?

- Destratification: mixes oxygen-rich surface water with anoxic deep water
- Compressed air lift pumps as unconstrained free bubbles or inside double tubes
- Pure oxygen as unconstrained free bubbles or inside a cone
    - Choice depends on reservoir & local folk

# Aeration: destratification with compressed air or propeller

## Oxygen comes from algal photosynthesis NOT from air

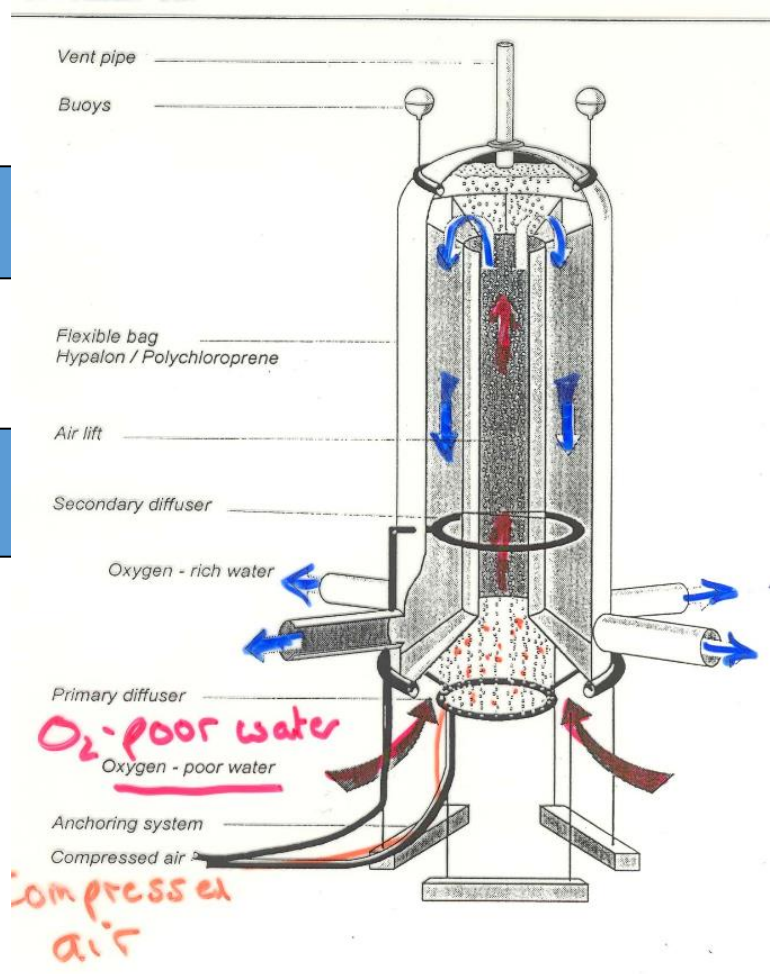


# Hypolimnetic Aeration: Partial air lift pump - Limnos unit

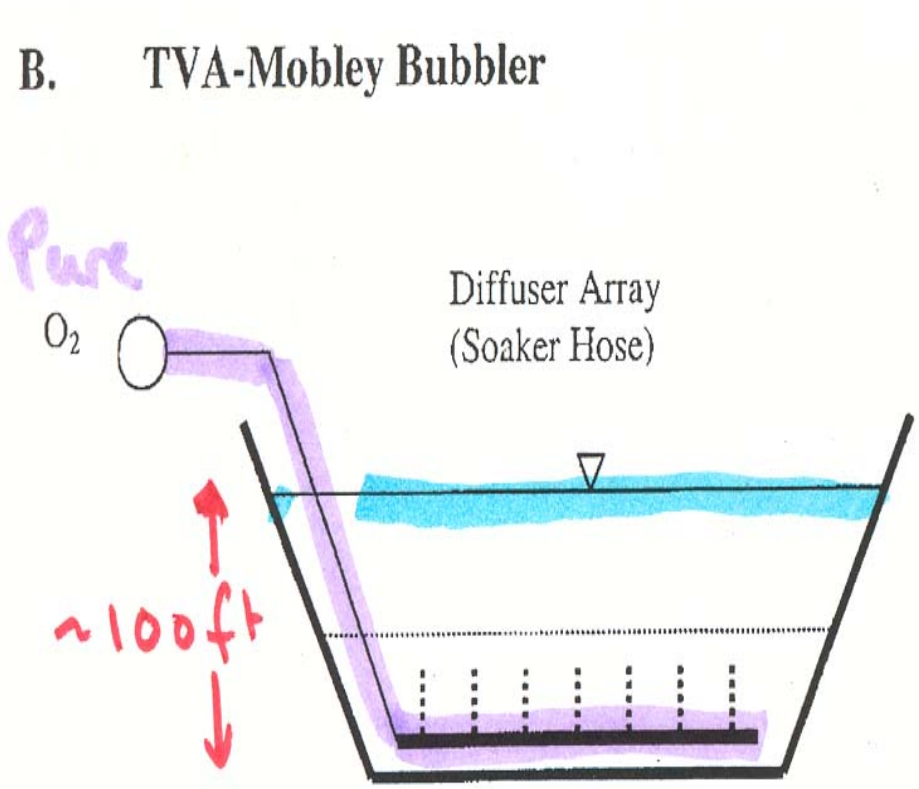
Epilimnion

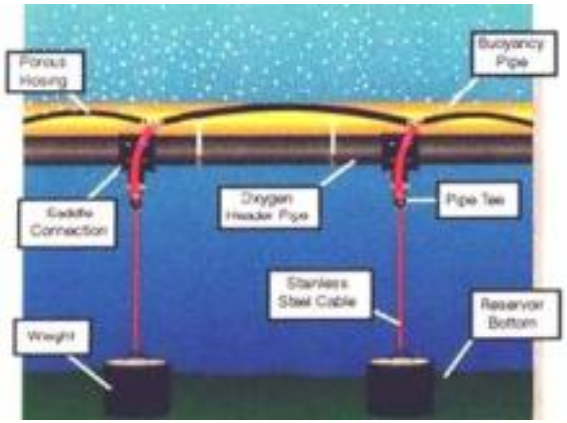
Hypolimnion

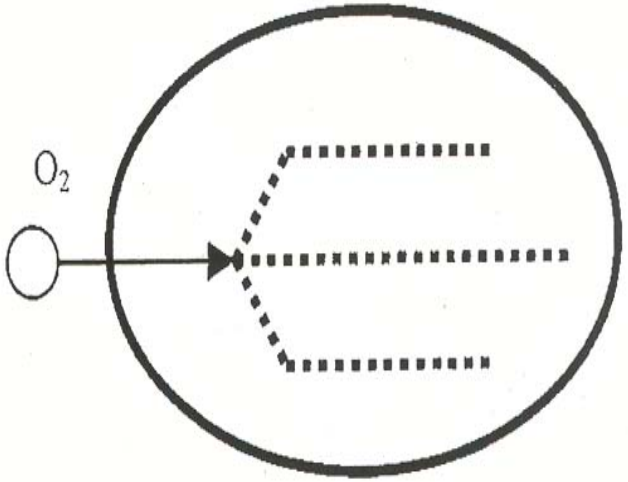



- Double tube
- Adds air, transfer efficiency 5-35% probably ~ 10%
- Oxygenated water can be passed out at any level including over sediments
- Outlet plume less dense than bottom water

# Unconstrained oxygen bubble plume



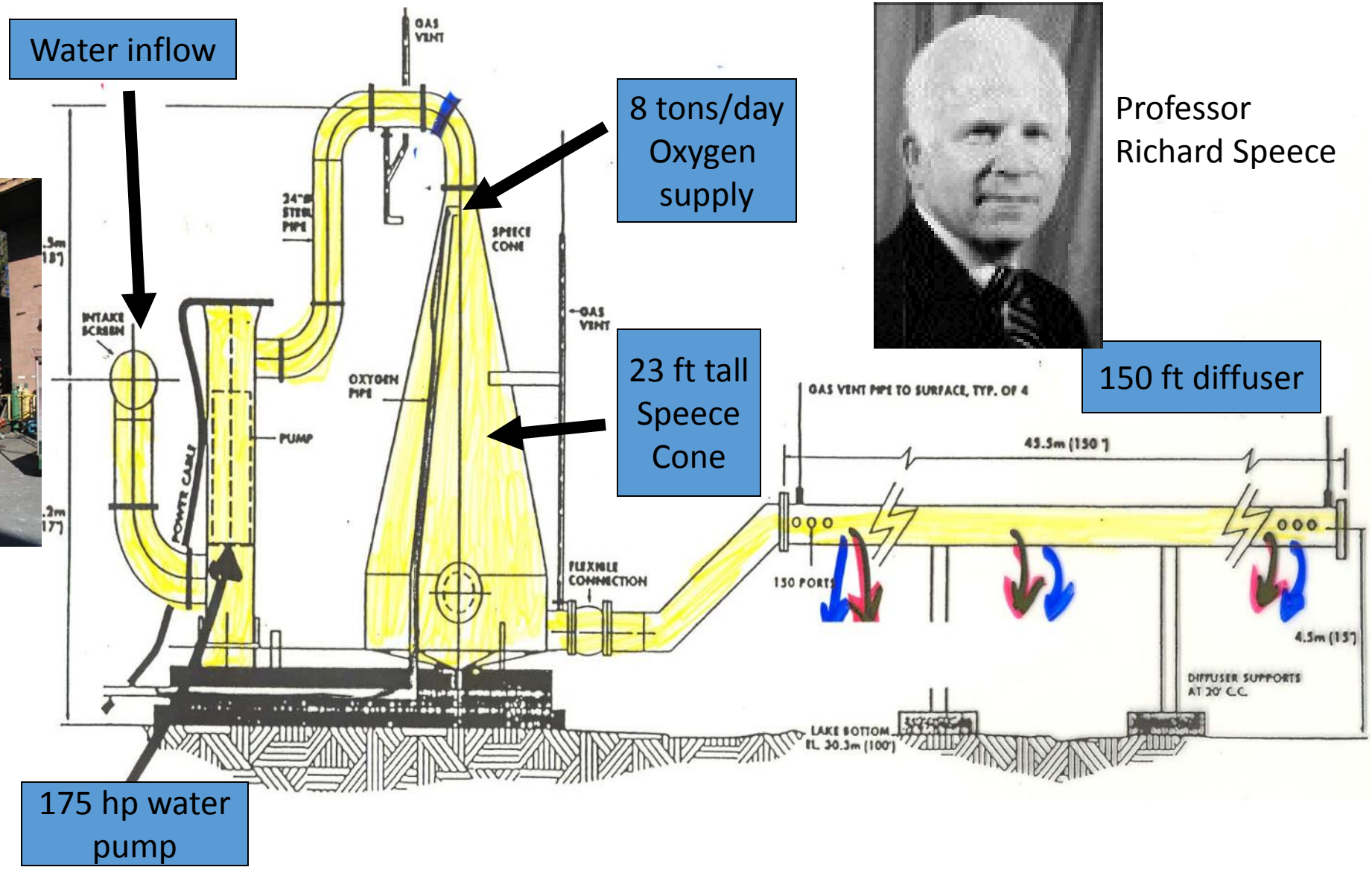




Mark Mobley

# Bubble-free plume: Speece Cone

Water inflow




8 tons/day Oxygen supply

23 ft tall Speece Cone

175 hp water pump

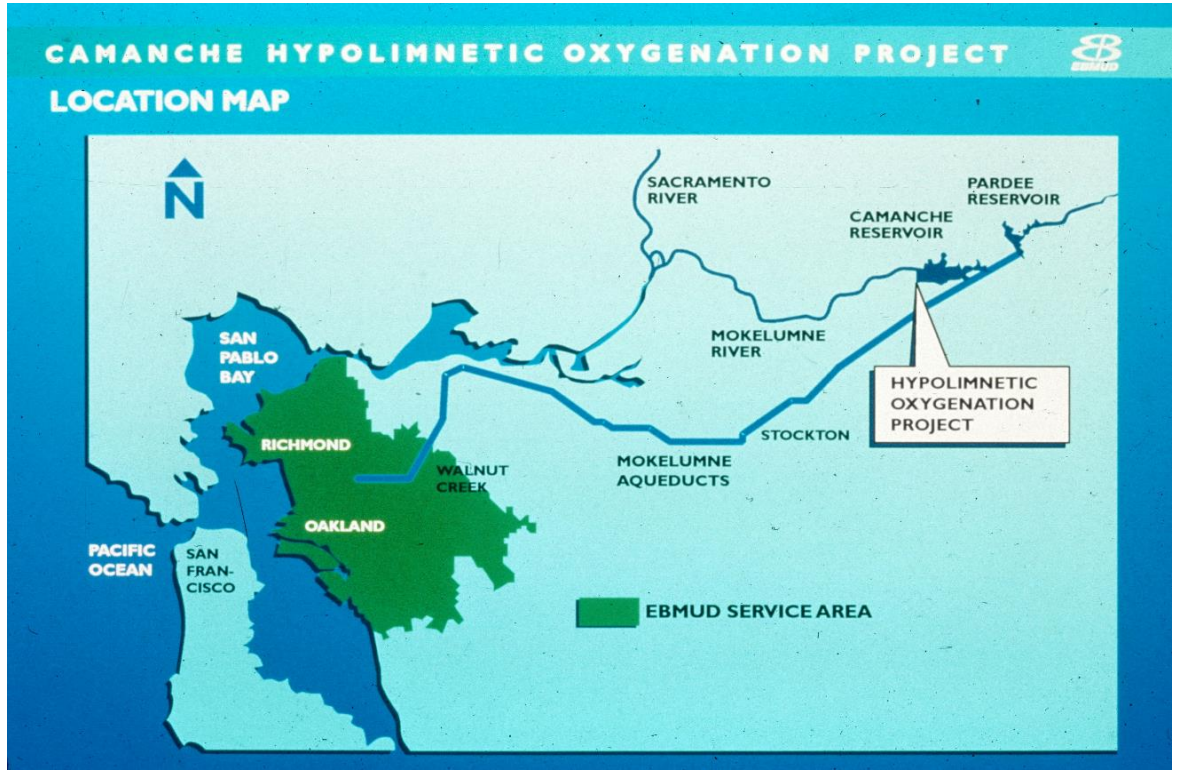


Professor Richard Speece

150 ft diffuser

# Camanche Reservoir
## EBMUD

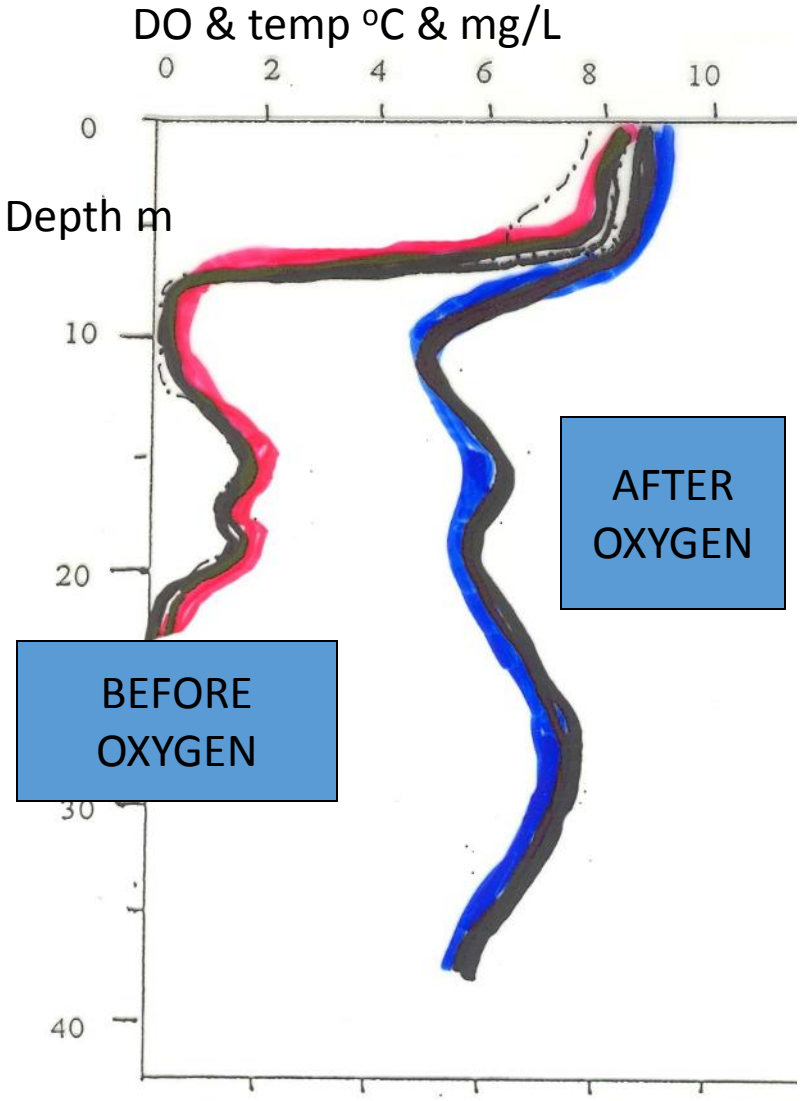
- In 1986-90 over 300,000 salmonids died in fish hatchery below Camanche & many more may have died in Mokelumne River
- Cause of death was ascribed by Prof. Horne to hydrogen sulfide
- Hydrogen sulfide is generated in anoxic muds
- So oxygenation of water above muds needed



# Camanche Reservoir Summary

- Oxygenation installed in 1992 **– no fish kills since**
- Can also now use hydropower at dam
- Natural Chinook spawning increased from 3,000 to 8,000+
- Capital cost $1.3 m ($1992)
- O&M $90,000/y (electricity for water pump + liquid oxygen supply)
- **Cost benefit ratio: ~ 1:30**

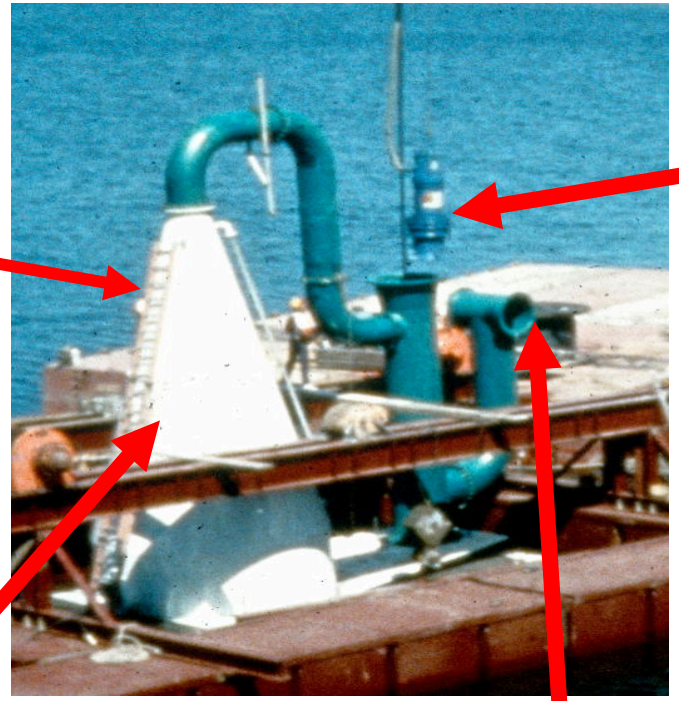
# Results of Camanche Reservoir HOS oxygenation




- Before hypolimnetic oxygenation DO below thermocline was absent
- After oxygenation DO minimum in hypolimnion was ~ 5 mg/L

# Speece Cone close up (8 tons/day)

Oxygen Supply line



Water pump being lowered into place


Speece Cone 23 ft high

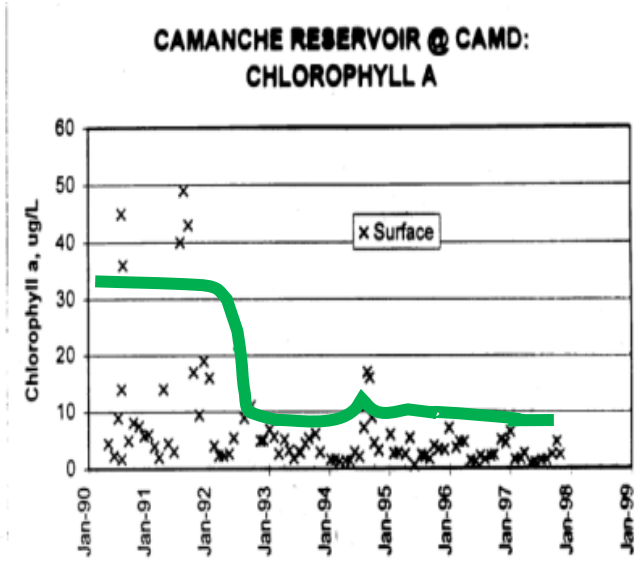
Deep Water inlet

- Evaporator + oxygen tank
- Camanche Reservoir EBMUD

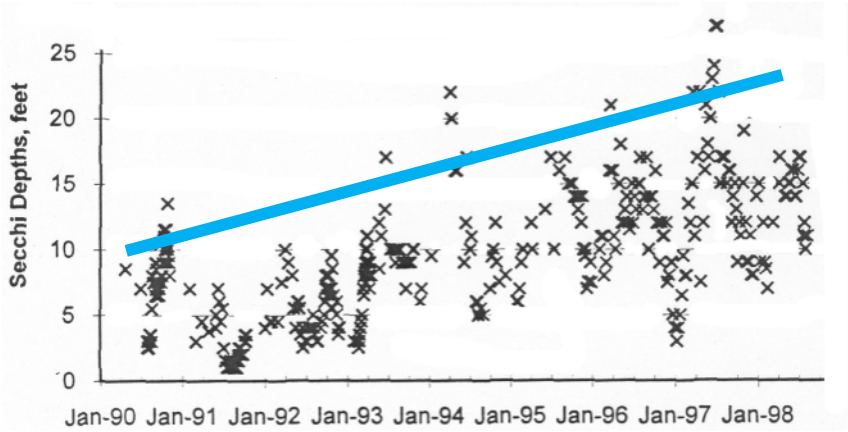
# What other benefits are there with HOS?

- HOS & similar methods have been used for decades for other water quality advantages for drinking & recreational lakes & reservoirs
- While reducing MeHg you could get a lot of other benefits – lower algae, pH, particulates, toxicity, taste & odor
- Cost/benefit ratio can be very favorable $$$ saved?

# HOS Oxygenation in Camanche Reservoir: first decade of eutrophication reversal

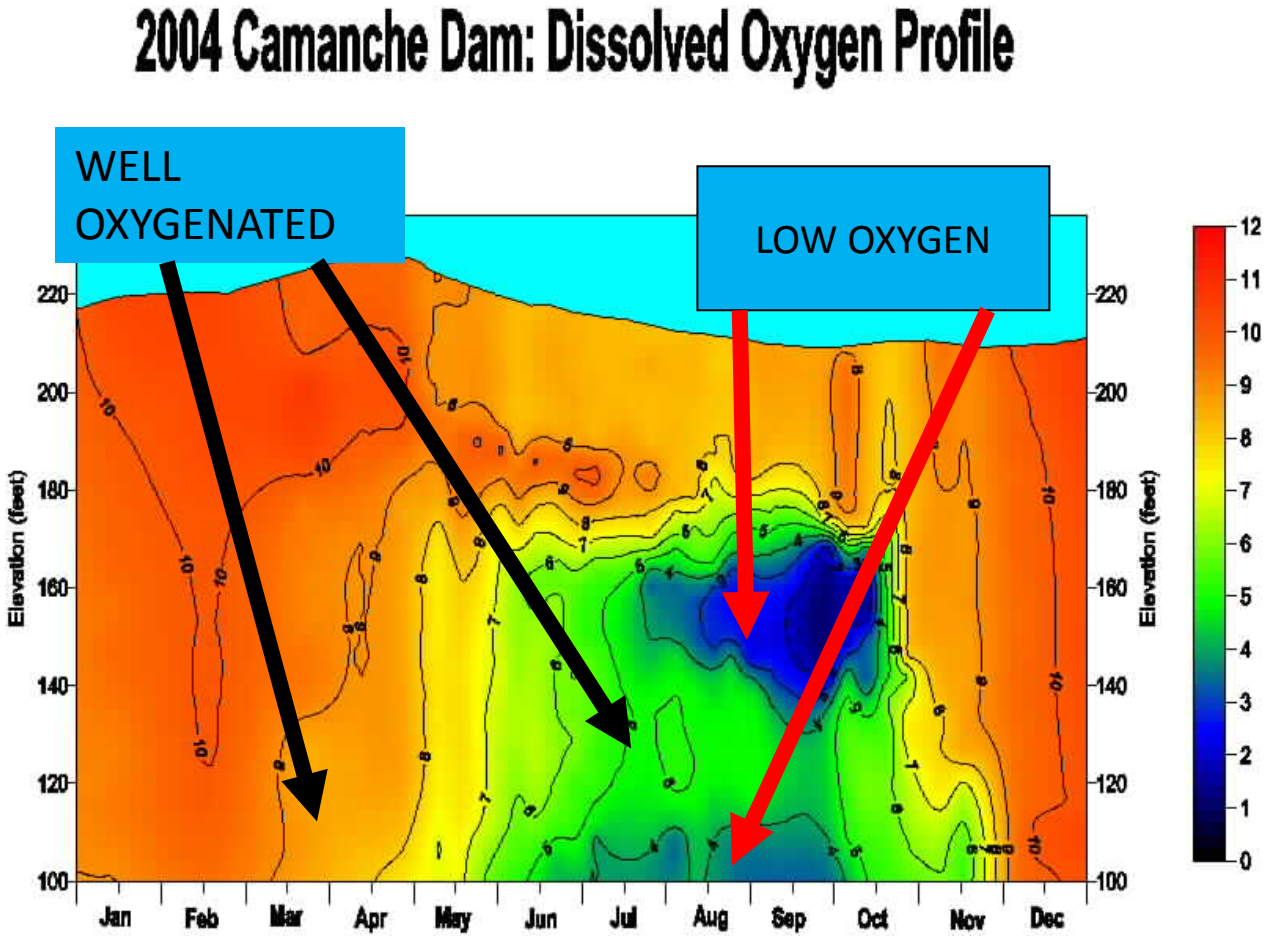



Secchi Depth



# Important similarities & differences between HOS for removal of $H_2S$ & MeHg

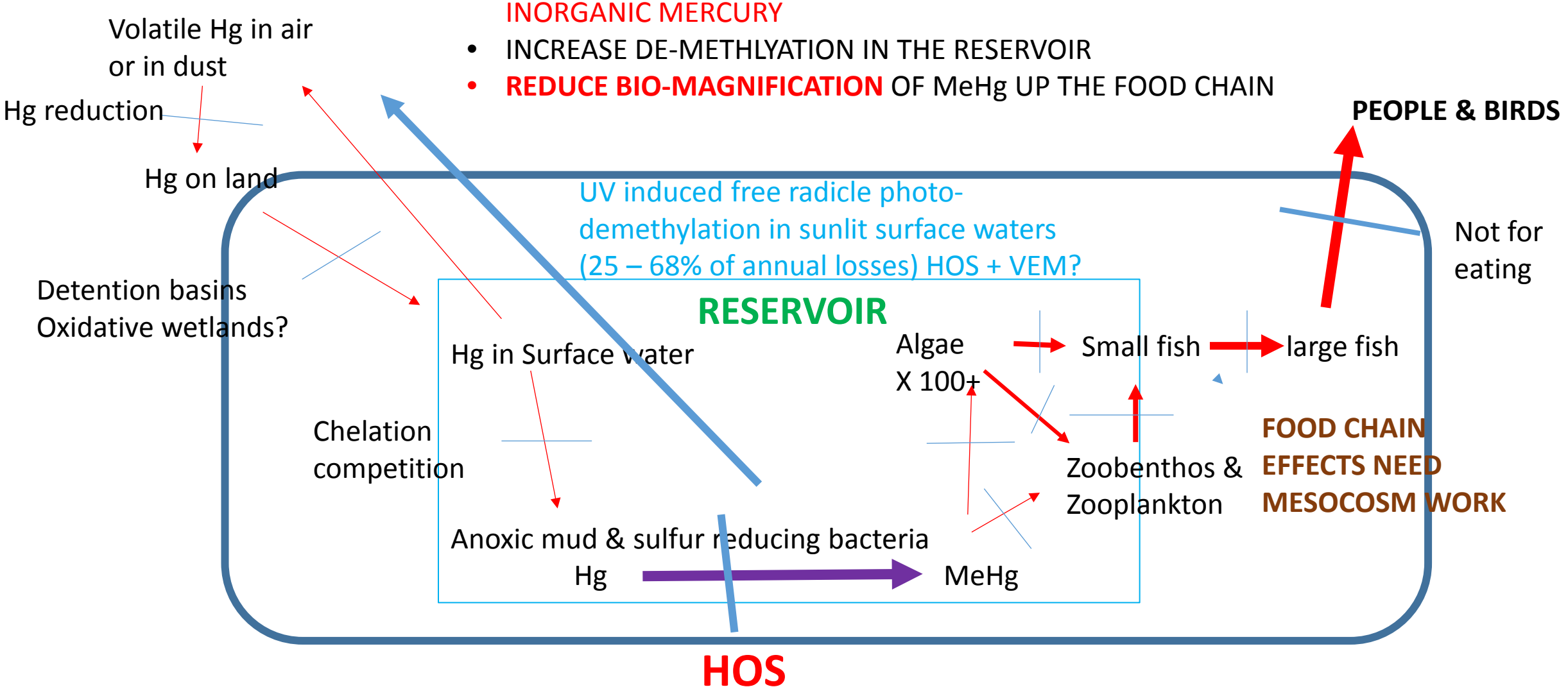
- Oxygen will easily & rapidly suppress production of $H_2S$ or MeHg
- Any $H_2S$ that is in the reservoir will be converted to harmless $SO_4$ or S within a day after mixing with oxygenated water
- Any MeHg in the reservoir will not easily or quickly be converted to Hg



# MeHg removal: Critical path for mercury de-methylation & other losses from reservoirs (to be completed)

TWO KEY STEPS

- **REDUCE OR ELIMINATE ANOXIC BACTERIAL METHYLATION** OF INORGANIC MERCURY
- INCREASE DE-METHLYATION IN THE RESERVOIR
- **REDUCE BIO-MAGNIFICATION** OF MeHg UP THE FOOD CHAIN



# More questions about MeHg

## CHELATION

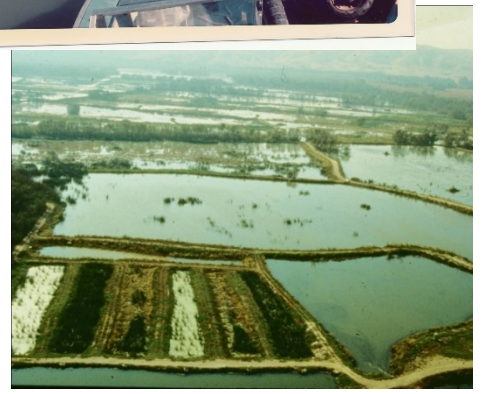
- Can we use methods used to decrease harmful effects of other metals for mercury? E.g. use of EDTA CHELATION for 6 heavy metals in the Los Angeles ACTA project.
- Method transports metal in an unavailable form to dilution & eventual breakup in the ocean
- Will it work for Hg & prevent methylation?

## FOOD WEB MeHg REDUCTION

- Seems to be some contradictions in results. Could be due to test details
- Need to sort these out with MESOCOSM experiments in reservoirs along with pilot project work (a la Orange County Water Districts wetlands projects)

# Cosms

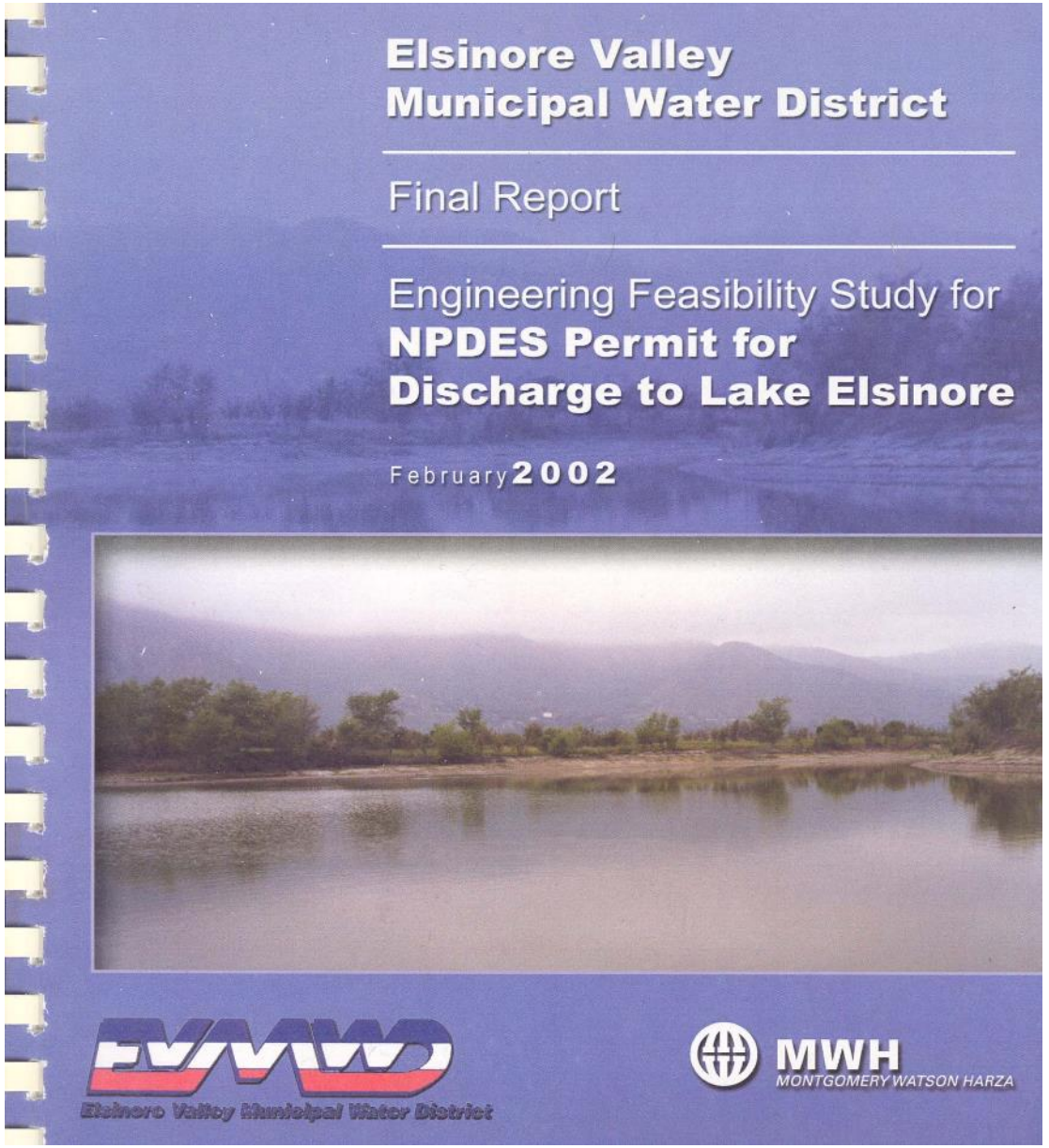
- Microcosms are test tubes or small flasks in laboratories (~ 1 - 100 liters) that can be replicated (triplicate samples) easily
- Mesocosms are lager containers (1-10 m3) that can be replicated with some logistic problems
- Macrocosms are large, hard to replicate (acres or land or hundreds of m3)



# THE 17 IN-LAKE & LAKE BED METHODS

- Physical controls: **Change** lake bathymetry, water/nutrient residence times, sediment chemistry, or light regime.  Harvest weeds, algae, trash & fish.
- Chemical controls: **Poison** the undesirables or **restrict** anoxia, light or nutrient recycling.
- Biological controls: **Eat** or **harvest** the undesirables
- Biomanipulation: **Change** the food web and trophic pyramid

# Lake Elsinore improvement



# The 17 methods in action!

**Table 5-2**
**Applicability Review of In-Lake Methods for Lake Elsinore**

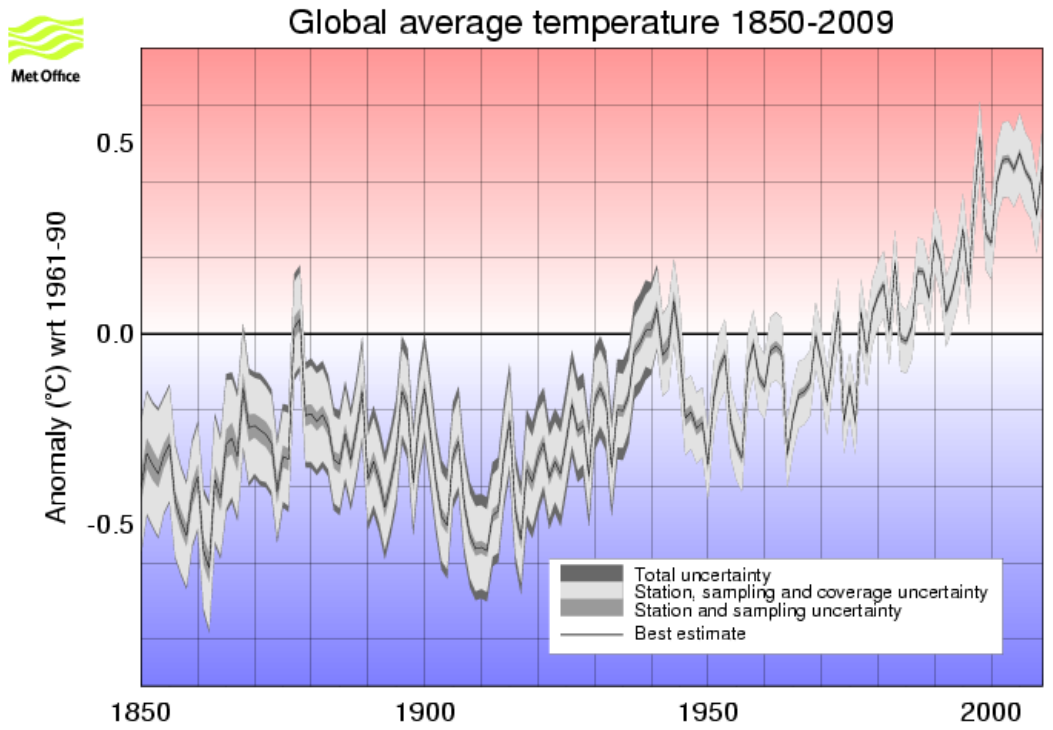
| No. | Method | Applicability for Lake Elsinore | Use |
|---|---|---|---|
| 1 | **Dredging** | Too costly for a large lake where over 20 feet must be removed | No |
| 2 | **Water level fluctuation** | Stable lake level will improve shoreline for humans and riparian & submerged vegetation. Addition of recycled water is feasible from local sources. Nutrients added can be offset with oxygenation, wetlands and at-plant treatment. Is needed for bio-manipulation. | Yes |
| 3 | **Destratification and lake mixing** | Possible method using air mixing to move oxygenated surface water to anoxic sediments. Will require most energy when problems are worse. Not shown to work in warm climates | No |
| 4 | **Macrophyte (water weed) harvesting** | No weeds at present, mechanical harvesting may be needed along with herbicides in enhanced lake for inshore swimming/boating areas. Combine with bio-manipulation | Maybe |
| 5 | **Wetland algae filters (off-line wetlands)** | Good method for direct removal of algae using redesigned 350 acres of current lakebed "wetlands". High pumping cost, successful in tests in Florida. Discontinue after bio-manipulation | Yes |
| 6 | **Algae (phytoplankton) harvesting** | Cost is high unless algae are harvested and sold as high priced health food or food dye. Possible use with Oregon firms. | No |
| 7 | **Selective withdrawal of hypolimnion water** | No spare water to lose, would require a large siphon from the deeper lake to outlet | No |
| 8 | **Dilution/flushing** | No spare or clean water available in large amounts | No |
| 9 | **Sediment sealing (fabric liners, barriers)** | Lake too large for these methods. Could be used for weed control alongside docks, swim areas | Limited |
| 10 | **Herbicides (for algae or macrophytes)** | Will be needed to shape the expected vegetation growth following bio-manipulation. Combine with harvesting? | Yes |
| 11 | **Oxygenation or aeration** | Main method to prevent fish kill, odors, internal nutrient loading. Oxygenation only feasible method in Elsinore due to huge pipe runs needed for aeration in very shallow water. Reduce after bio-manip. | Yes |
| 12 | **Shading (dyes)** | Lake too large, dye lasts only a few months. | No |
| 13 | **Sediment sealing (alum, Phosloc or calcium carbonate)** | Lake very large for these methods, high cost, reserve for limited use if aeration/oxygenation is not fully effective for $PO_4$. Not recommended for Elsinore; increases toxicity and pH. Recent experiments show alum can be replaced with oxygenation. | No |
| 14 | **Pathogens of algae or macrophytes** | Ineffective for blue-green algae due to resistance buildup. None known for macrophytes | No |
| 15 | **Fish grazers on algae or macrophytes** | Not applicable, lake needs more submerged macrophytes, not less | No |
| 16 | **Nutrient harvesting from fish or other biota** | Many small fish and large carp will be harvested as part of the bio-manipulation process. N and P removal expected to be small relative to inflows. Combine with bio-manipulation | Yes |
| 17 | **Bio-manipulation** | Main sustainable method to remove nuisance algae, tie up nutrients, reduce sediment re-suspension | Yes |

# Limnological metrics for anoxia

- MeHg bacteria are inhibited by even a whiff of dissolved oxygen (~ 0.1 mg/L)
- This is easy with many methods of aeration-oxygenation-mixing
- However, getting oxygen deeper into the mud depends on method used
- So DO > 2 mg/L in bottom water essential, really need > 5 mg/L

# Climate change & MeHg

- Assume an increase over next 50 years of 0.5-3.0 $^{o}$C (0.01 to 0.06$^{o}$C/y - current rate of increase is – 0.006$^{o}$C/y, NASA)
- Reservoirs will stratify earlier in year and de-stratify later = longer anoxia
- Hypolimnion deep water will be warmer = faster MgHg production



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***Chapter 7***

# MERCURY METHYLATION VERSUS DEMETHYLATION: MAIN PROCESSES INVOLVED

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## ABSTRACT

It is well known that mercury presents high toxicity, causing a great damage to the environment and living organisms; however, its properties depend on the mercury species present. Organomercury compounds, where methylmercury is included, cause more concern.

Since 60-70's, several methylation mechanisms are known. Generally, methylmercury can be formed naturally in the aquatic environment by two general pathways: chemical methylation (abiotic) and microbial (biotic) processes. At the same time, methylmercury can be also decomposed abiotically or by the action of several demethylating microbes, or demethylators, ranging from anaerobes to aerobes. Regarding the biotic methylmercury demethylation, two distinct vias - oxidative and reductive - might be used by those microorganisms, differing in the final products obtained. In relation to the reductive processes, two pathways might occur. The first one involves the mercury resistance operon (*mer*) whereas the second one involves sulfide ions; however, the former is considered to be the most common pathway. Regarding the *mer* operon, some bacteria only carry on a narrow-spectrum operon (*mer*A), being only able to reduce inorganic mercury (Hg(II)) to elemental mercury ($Hg^0$). On the other hand, others beyond this operon also carry on a broad-spectrum operon (*mer*B). These microorganisms are able to decompose methylmercury to $Hg^0$.

Taking into account all of these processes, in the present work the most referred methylation and demethylation mechanisms found in aquatic environments are discussed, as well as the environmental factors that influence them. Factors related with the inorganic mercury/methylmercury availability and those that affect directly the activity of methylators and demethylators are also referred. Generally, the relationships encountered are complex and sometimes significant shifts on the microbial communities may be

observed. These changes can alter the processes involving the mercury species, as well as the final products obtained.

In conclusion, the abiotic factors and the type of microorganisms that are present in the environment, including their genetic patrimony, influence significantly the presence and the type of the mercury species. Furthermore, there are environmental factors, such as redox conditions, sulfides and organic matter that also affect the mercury dynamic and the equilibrium existents.

# 1. INTRODUCTION

The amount of mercury in the environment is much higher than the global background level as a result of the anthropogenic activities during the 20$^{th}$ century (Eckley and Hintelmann, 2006). Mercury has been used in several industrial or agricultural applications for ages and mercury species are stable and persistent in the natural systems. In this sense, several harmful situations to the environment and human health have been associated to mercury and its compounds. Methylmercury poisoning in Minamata (Japan), the organic mercury poisoning in Iraq, the methylmercury exposure in the Amazon (Brasil) and the elemental mercury spill in Catamarca (Peru), are examples of real situations that involved mercury species (Gochfeld, 2003).

Mercury properties are well known and have been reported in numerous works (Weast, 1975; Andren and Nriagu, 1979; Nriagu, 1979; Filella *et al.*, 1995; Cutnell and Johnson, 1998; Jackson, 1998; Mukherjee *et al.*, 2004). Mercury can be found in three oxidation states: 0 (elemental), 1+ (mercurous) and 2+ (mercuric) (Andren and Nriagu, 1979; Jackson, 1998), such as in $Hg^0$, $Hg_2^{2+}$ and $Hg^{2+}$; however, the last form is the most common in aquatic environments. This mercury form has strong tendency to form extremely stable coordination complexes and organometallic compounds (Jackson, 1998). Several complexes might be formed between mercury and different ligands. These can be sulfur (Leermakers *et al.*, 1995; Lobinski, 1998; Ravichandran, 2004), namely thiol groups (Carty and Malone, 1979; IPCS, 1991) and sulfides (Jackson, 1998), nitrogen (e.g. R-$NH_2$), phosphorous or carbon (Jackson, 1998). In relation to oxygen ligands, mercury has low affinity to them (Carty and Malone, 1979).

Due to mercury affinity to ligands containing sulfur, low molecular weight thiols, i.e. sulfhydryl containing molecules such as cysteine, are emerging as important factors in the transport and distribution of mercury throughout the body (Rooney, 2007) due to the phenomenon of "Molecular Mimicry" (Bridges and Zalups, 2005), whereby the bonding of metal ions to nucleophilic groups on certain biomolecules results in the formation of organo-metal complexes that can behave or serve as a structural and/or functional homolog of other endogeneous biomolecules or of the molecule to which the metal ion has bonded. When observed with mercury, this phenomenon might cause significant injuries.

## 1.1. Organomercury Compounds

Organomercury compounds are the most toxic mercury species, not belonging to this group of compounds the mercury complexes formed with organic matter originally present in

the aquatic systems. The organomercury compounds can be divided into two groups: one in which mercury atom is linked to an organic radical (RHgX), and another group to that mercury is linked to two organic radicals ($R_2Hg$) (Benes and Havlík, 1979).

The compounds that belong to the first group are soluble in water, dissociating in the $R\text{-}Hg^+$ cation and $X^-$ anion, being the most common the $Cl^-$, $OH^-$, $NO_3^-$ and $SO_4^{2-}$ anions (Benes and Havlík, 1979). Depending on anion nature, the compounds obtained will have different properties. Poorly coordinating anions, such as $ClO_4^-$, $NO_3^-$, $PF_6^-$ and $BF_4^-$ anions confer an ionic character to $RHg^+X^-$ salt (Carty and Malone, 1979), and are correspondingly more hydrophilic (Jackson, 1998), while $Cl^-$, $Br^-$ and $I^-$ anions confer on a linear covalent character (C-Hg-X) (Carty and Malone, 1979), being these methylmercury halides among the more lipophilic methylmercury species (Jackson, 1998).

The second group includes compounds such as dimethylmercury and diphenylmercury (Benes and Havlík, 1979). These compounds are volatile, non polar and have low solubility in water (Benes and Havlík, 1979; Carty and Malone, 1979; Jackson, 1998), not being affected by air, and weak acids and bases (Andren and Nriagu, 1979). These properties might be due to their covalent bonds (Benes and Havlík, 1979; Carty and Malone, 1979; Jackson, 1998).

Both groups of organomercury compounds - RHgX and $R_2Hg$ - are broad-spectrum biocidal agents acting via diverse mechanisms in biological systems. Organomercurials are supposed to induce membrane associated oxidative stress in living organisms through different mechanisms, including the enhancement of the lipid peroxidation and intracellular generation of reactive oxygen species (ROS) (Milaeva, 2006).

Methylmercury is the most common organomercury compound found in aquatic environments. It is also one of the most hazardous mercury species, due to its high stability in combination with its lipid solubility, leading to a high ability to penetrate membranes in living organisms (Beijer and Jernelöv, 1979). Methylmercury is of particular public health concern due to its bioaccumulation and biomagnification within the aquatic food web (Wiener *et al.*, 2002; Orihel *et al.*, 2007; Coelho *et al.*, 2008). In terms of the biomagnification factor that corresponds to the concentration increase for each trophic transfer it is about two- to five-fold for various aquatic ecosystems and to all typical trophic levels, being an order of magnitude higher than the one for inorganic mercury (Meili, 1997).

Although most of mercury emitted to the environment is in inorganic form, nowadays it is well-known that inorganic mercury can be naturally methylated in the environmental ecosystems being it transformed into methylmercury. Since 60-70's, several methylation mechanisms are known. In the aquatic environment, methylmercury can be formed by two general pathways: chemical methylation (abiotic processes) and microbial metabolism (biotic processes) (Celo *et al.*, 2006). On the other hand, methylmercury can be decomposed abiotically, as for example, by light (Jackson, 1998), or biotically, by various free-living demethylating microorganisms (IPCS, 1990; Ebinghaus and Wilken, 1996). As both processes might occur simultaneously, methylmercury presence in the aquatic environments depends on the existing balance of methylation versus demethylation.

## 2. MERCURY METHYLATION/DEMETHYLATION PROCESSES

The knowledge of the efficiency of the different pathways of mercury methylation and demethylation is one of the key steps to predict methylmercury concentrations in the different environmental compartments and to estimate the mercury bioaccessibility to the organisms. However, the factors that influence the competing methylation and demethylation reactions are yet insufficiently understood and little to no attempt has been made to determine end products. The relative importance of each reaction and the resulting net effect will probably depend on the environmental conditions and biological factors with spatial and temporal variations (Hintelmann *et al.*, 2000).

In this sense, it is important to consider that the net amount of biologically available methylmercury is a function of the processes that regulate its formation, degradation and exchanges between compartments. So, methylation and demethylation are two important processes regulating the mercury cycle in natural environments (Rodríguez Martín-Doimeadios *et al.*, 2004; Monperrus *et al.*, 2007a) and they can be driven by both biotic and abiotic mechanisms.

The biogeochemical cycle of mercury has been extensively studied whereas the mechanism of natural mercury methylation in the environment is not still clear. If methylmercury production, for example, is the most significant process that is occurring in the aquatic environment, hazardous effects on living organisms may occur due to methylmercury presence and its related high toxicity. Microbial methylation (biotic processes) is widely accepted as the main conversion mechanism of inorganic mercury into methylmercury in natural environment (Barkay *et al.*, 2003; Eckley and Hintelmann, 2006; Monperrus *et al.*, 2007b; Raposo *et al.*, 2008). Nevertheless, the relative importance of mercury chemical methylation (abiotic processes) is ambiguous. Some authors emphasize that the abiotic pathway is possible in natural environments but it appears to play a minor role (Ullrich *et al.*, 2001; Benoit *et al.*, 2003; Gårdfeldt *et al.*, 2003; Eckley and Hintelmann, 2006; Dominique *et al.*, 2007; Monperrus *et al.*, 2007b), especially photochemical methylation (Dominique *et al.*, 2007). On the other hand, other authors suggest that the biotic processes can not account for all the methylmercury formed naturally (Celo *et al.*, 2006).

If demethylation of methylmercury is occurring in a significant extent, this is advantageous; however, in some situations the substrate of mercury methylation might be formed, inducing in this way the methylmercury formation. On the other hand, the demethylation process that occurs in the aquatic environments depends also on abiotic and biotic factors. Generally, the existent relationships are quite complex and variable. Nevertheless, in the following sections the most common mercury methylation and demethylation processes found on the environment, as well as the environmental factors that influence these, will be discussed.

### 2.1. Mercury Methylation Processes

#### *2.1.1. Chemical Methylation – Abiotic Processes*

In the case of the abiotic pathway, mercury methylation is possible only in the presence of a suitable methyl donor (Ullrich *et al.*, 2001; Celo *et al.*, 2006). Moreover, this process

may be photochemically induced. The latter reaction mechanism is likely little relevant, since the methyl radicals produced photochemically will be rapidly scavenged by oxygen (Gårdfeldt *et al.*, 2003). Potential methylating agents for abiotic methylmercury formation in natural environments include small organic molecules, such as methyliodide and dimethylsulfide (Celo *et al.*, 2006), and larger organic components of dissolved organic matter, such as fulvic and humic acids (Ullrich *et al.*, 2001; Celo *et al.*, 2006). Transmethylation reactions involving organometallic complexes like methylcobalamin, methyllead or methyltin compounds have also been considered as possible pathways for chemical mercury methylation. Transmethylation reactions can occur as a result of the transference of carbocationic $Me^+$, carbanionic $Me^-$ or radical $Me\cdot$, depending on the chemical properties of the metal component of the methylating agent (Celo *et al.*, 2006). Therefore, a large variety of chemical variables may influence the methylation process (Celo *et al.*, 2006).

Methyliodide (MeI) plays an important role in the biogeochemical cycle of mercury in the marine environment as being it an effective solubilizing agent for mercury sulfides (Minganti *et al.*, 2007). MeI is mainly produced in the marine environment by algae and plankton whereas its dispersion by human activity can be overlooked. So, this compound is present at relatively high concentrations in areas where biomass productivity is high. MeI has not the ability to directly methylate oxidized mercury ($Hg^{2+}$) if methyltin and methyllead species, acting as transferring agents for the methyl group from MeI to mercury, are not present in the medium (Minganti *et al.*, 2007). Hence, the reaction mechanism corresponds to the $Hg^{2+}$-assisted hydrolysis of MeI, resulting in quantitative formation of methanol (Celo, 2003; Celo and Scott, 2005) ($Hg^{2+} + 2\ MeI + 2\ H_2O \rightarrow HgI_2 + 2\ MeOH + 2\ H^+$). The methylation reaction for mercury requires reducing/anaerobic conditions as MeI only methylates reduced forms of metals. Therefore, the oxidative addition ($Hg^0 + MeI \rightarrow MeHgI$) is presumably the methylation mechanism evolved (Celo *et al.*, 2006). In the presence of 200 ng/L MeI, methylmercury formation could therefore be as high as 0.2 pg/L/year (Celo *et al.*, 2006).

Humic matter contains different kinds of functional groups and, besides the linkage of oxidized mercury to thiol groups, it has most likely an additional complexation to neighbouring carboxylic groups. Taking into account that organic acids with methyl groups in the $\alpha$-position show high methylation efficiency for mercury (Falter, 1999a,b), humic matter is the most promising environmental methylating agent as consequence of its high concentration in waters and sediments and of its association with the solubility and thus mobility of mercury in freshwaters and marine waters. Only three humic substances, namely 2,6-di-tert-butyl-4-methylphenol (BHT), *p*-xylene and mesitylene, have the ability to methylate inorganic mercury at pH 3.5 but only at temperatures exceeding 37 °C. At pH 7, only BHT produces methylmercury. In terms of fulvic acids, all of them are able to methylate inorganic mercury but the lower molecular weight compounds (M.W. 200) are the most active ones.

Methylcobalt (III) compounds like methylcobalamin are considered potential mercury methylators because their ability for the transference of a methyl group to free $Hg^{2+}$. Although some authors propose a reaction mechanism based on the enzymatic transference of methyl radicals from methylcobalamin to $Hg^{2+}$ via sulfate-reducing bacteria (Barkay *et al.*, 2003), others reivindicate that the reaction takes place in the absence of biological activity (Celo *et al.*, 2006; Chen *et al.*, 2007). In the latter case, inorganic mercury acts as an

electrophile to attack methylcobalamin with the subsequent transference of a methyl carbanion to the most oxidized mercury specie ($MeCo(dmg)_2H_2O + Hg^{2+} + H_2O \rightarrow Co(dmg)_2(H_2O)_2^+ + MeHg^+$; dmg = dimethylglyoximate). In spite of this, methylcobalamin readily methylates $Hg^{2+}$ in non-environmental matrices but it is unlikely in the aquatic environment because its low abundance.

The reaction products of methylcobalamin and $Hg^{2+}$ are methylmercury and dimethylmercury. The first specie to be formed is methylmercury, the first methylation rate being two times faster than the second one. Chen *et al.* (2007) also studied inorganic mercury methylation by methylcobalamin in aquatic systems and identified methylmercury as the reaction product. On the other hand, kinetic experiments showed that the methylation reaction is fast but the salinity and pH modify the electron density of the methyl donor and the electrophilicity of metal ion in the reaction system, which affects to methylmercury formation (Chen *et al.*, 2007). So, the reaction rate is 0.00612 and 0.000287 $min^{-1}$ for pH 5.0 and 1.5, respectively (Chen *et al.*, 2007). Celo *et al.* (2006) refer that the most favourable environmental conditions to mercury methylation by methylcobalamin are acidic pH, high ionic strength and low chloride concentration that are more usually present in fresh waters. Furthermore, they found that methylcobalamin is unlikely to methlylate in moderate or highly saline environments because it is apparently unreactive towards chloride complexes of $Hg^{2+}$ (Celo *et al.*, 2006). Nevertheless, there are controversies and other authors have also reported that the inorganic mercury methylation by methylcobalamin is possible even in highly saline solutions, which emphasizes its importance in aquatic environments (Chen *et al.*, 2007).

Organotin compounds, particularly methyltin species, are suitable methyl donors and their role in abiotic mercury methylation has been evidenced in the aquatic environment (Rosenkranz *et al.*, 1997). Furthermore, methyltin compounds have been frequently detected in all the environmental compartments of the aquatic system. The favourable conditions for the transmethylation reaction among methyltin compounds and $Hg^{2+}$ ($Me_nSn$ (IV) + Hg (II) $\rightarrow$ $Me_{n-1}Sn$(IV) + MeHg (II)) include alkaline pH and the presence of high amounts of chloride (Celo *et al.*, 2006). Therefore, the greater contribution of this methylation mechanism occurs in seawaters than in freshwaters. Furthermore, organotin compounds are efficient methylators of inorganic mercury only at pH values higher than 6. It can be due to methyltin cations (aqua complexes) are unreactive, while neutral methyltin hydroxide complexes are reactive. Within methyltin compounds, $MeSn(OH)_3$ is the most reactive specie and $Me_3SnOH$ is the least one to transfer methyl groups to $Hg^{2+}$. A transition state involving simultaneous methyl transfer from $Sn^{4+}$ to $Hg^{2+}$ and chloride transfer from $Hg^{2+}$ to $Sn^{4+}$ is suggested. Celo *et al.* (2006) estimate that the mercury methylation rate is 0.5 pg/L/day for typical environmental concentrations of monomethyltin (~ 1200 ng Sn/L) and $Hg^{2+}$ (~ 1 ng/L) under pH and temperature values appropriate for seawaters (8 and 20 ºC, respectively). Evidence that methyllead compounds may also methylate mercury exists (Ebinghaus and Wilken, 1996; Rosenkranz *et al.*, 1997), being methylmercury produced by transmethylation.

The artifactual formation of methylmercury when acetic acid is used as an analytical chemical for mercury speciation has been reported (Falter, 1999a,b). Gårdfeldt *et al.* (2003) investigated the mechanism and kinetics of the methylmercury formation from a solution containing $Hg^{2+}$ and acetic acid. The reaction occurs via mercury acetate complexes [$(Hg(CH_3COO)_n)^{2-n} \rightarrow CH_3Hg^+ + CO_2 + (n-1)((CH_3COO)_n)^-$; n = 1-4]. Since the dominant mercury complexes vary with pH, the reaction rate is dependent on this one. Although there

are controversies in the effect of sunlight or UV radiation on the net rate of methylmercury formation, it is not significantly enhanced by photolysis when methylmercury photodegradation is also considered. Gårdfeldt *et al.* (2003) estimate a maximum methylmercury formation rate of 6.5 pg/dm$^3$/h for $1.5\times10^{-4}$ M acetic acid and $10^{-10}$ M $Hg^{2+}$ at pH 3.6. The main parameter limiting the mercury methylation rate via this reaction mechanism is the presence of other ligands, which may compete with acetate for mercury complexation, e.g. chloride, oxalate and sulfide. Recently, the minimal predicted complexation of inorganic mercury by acetate suggests that the methylation is unlikely to account for the methylmercury found in rainwater, or that the mechanism of this reaction in the atmosphere differs from that previously reported (Conaway *et al.*, 2010).

### *2.1.2. Biotic Processes*

Several microorganisms are able to methylate mercury, such as some sulfate-reducing bacteria (Berman *et al.*, 1990; Rodríguez Martín-Doimeadios *et al.*, 2004; Sunderland *et al.*, 2006; Dias *et al.*, 2008; Duran *et al.*, 2008; Holloway *et al.*, 2009), iron-reducing bacteria (Holloway *et al.*, 2009), sulfide and sulfur-oxidizers (Rodríguez Martín-Doimeadios *et al.*, 2004), among others (Rodríguez Martín-Doimeadios *et al.*, 2004). Nevertheless, the first have been identified as the dominant methylators in the aquatic environments.

Some studies involving mercury methylators have been done in order to get insight on the pathway of carbon and on the nature of methyl donors used. Several pathways have been proposed but one of the most studied and well understood is the referred to the *Desulfovibrio desulfuricans* (Berman *et al.*, 1990; Choi *et al.*, 1994a). The most likely source of the methyl group seems to be the C-3 of serine. This compound is the principal methyl donor to tetrahydrofolate and is formed during the carbon flow from the pyruvate. The proposed pathway is represented briefly in Figure 1 and was adapted from the works performed by Berman *et al.* (1990) and Choi *et al.* (1994a).

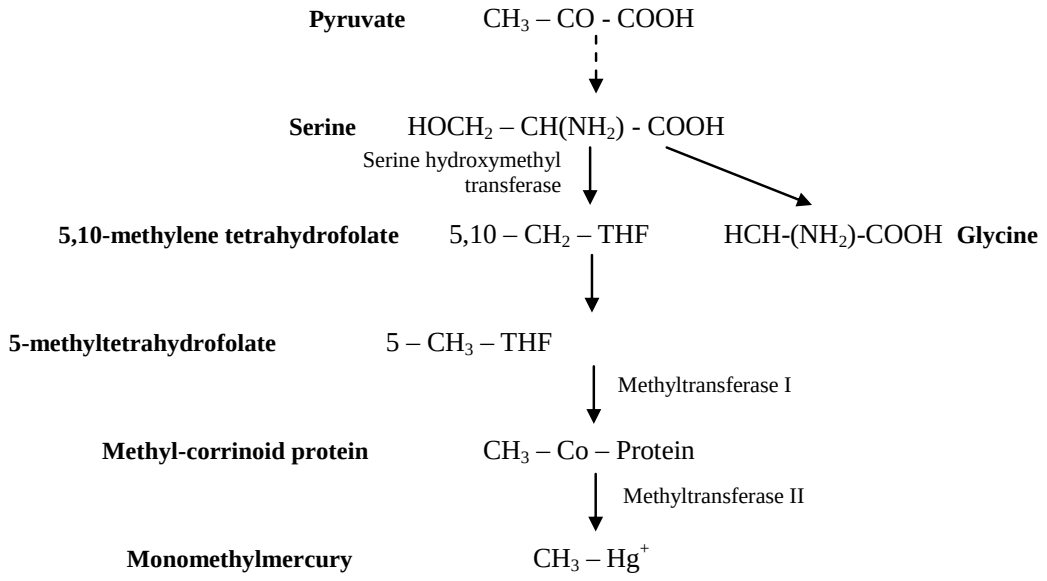


Figure 1. Proposed pathway for methylation of mercury in *Desulfovibrio desulfuricans*.

In more detail, when performing incubations with radiocarbon of C-1-labeled pyruvate, C-3-labeled pyruvate and C-3-labeled serine, the last specie was incorporated into methylmercury with 95% preservation of specific activity, a much higher percentage than those obtained with the other labeled substrates (11 and 21%, respectively) (Berman *et al.*, 1990). These results indicated that the methyl group is probably donated as C-3 of serine to tetrahydrofolate by the action of the enzyme serine hydroxymethyl transferase (Berman *et al.*, 1990; Choi *et al.*, 1994b). After that, tatrahydrofolate transfers the methyl group to cobalamin (vitamin $B_{12}$) or a closely related methyl carrier, such as a corrinoid protein, that finally transfers the methyl group to mercuric ions (Berman *et al.*, 1990; Choi and Bartha, 1993; Choi *et al.*, 1994a). Nevertheless, it has been reported that methylcobalamin is able to methylate spontaneously inorganic mercury (Choi and Bartha, 1993) as referred in the previous section. These observations raised the question of how mercury methylation process occurs *in vivo*. In fact the role of methylcobalamin in the methylation of inorganic mercury in organisms, as well as, its ability of methylating spontaneously mercuric ions, has been readily demonstrated. Therefore, it was necessary to verify if the principal source of methylmercury determined *in vivo* was due to spontaneous transmethylation or to an enzymatically catalyzed process. Choi *et al.* (1994a) proved clearly that inorganic mercury methylation occurring *in vivo* is an enzymatically catalyzed process, rather than a spontaneous transfer of methyl group from methylcobalamin, after observation of saturation kinetics and of the higher rate of inorganic mercury methylation (at pH 7.0) by cell extracts of *Desulfovibrio desulfuricans*, when compared with transmethylation by free methylcobalamin. Therefore, in these processes two methyltransferases seem to be involved (Choi *et al.*, 1994a). Latter on Choi *et al*. (1994b) have further proposed that methyl group may also originate from formate via the acetyl-CoA synthase pathway.

In biological systems, beyond methylcorrinoid derivatives (such as, methylcobalamin), there are two possible other microbial methylating agents: *S*-adenosylmethionine (SAM) and 5-methyltetrahydrofolate (5-MTHF). Nevertheless, Gadd (1993) refers that the major methylating agent involved in mercury methylation is the methylcobalamin.

Due to the important role of the enzymes mentioned before, sometimes it might be difficult to differentiate between biotic and abiotic methylation because it has been suggested that the formation of abiotic methylmercury may result from dead communities of bacteria that can continue to methylate mercury by releasing enzymes (Eckley and Hintelmann, 2006). Thus, these enzymes seem to have potential to promote extracellular methylation (Eckley and Hintelmann, 2006).

Regarding culture conditions, these also play an important role on methylmercury synthesis. It has been reported, for example, that *Desulfovibrio desulfuricans* produced more methylmercury under fermentative than under sulfate-reducing conditions (Choi and Bartha, 1993).

*i. Environmental Factors that Affect Mercury Biotic Methylation*

As biotic methylmercury production is related to microorganism's activity, it will depend on several factors. It must always be remembered that the factors that affect mercury methylation can be separated into those that affect the activity of mercury methylating bacteria and those that affect the bioavailability of mercury to the methylating organisms. The relative importance of these factors is generally difficult to assess.

In relation to the first type of factors, Holloway *et al.* (2009), for example, observed that the soil type influenced more the soil microbial communities than season, when they studied the spatial and seasonal variations in mercury methylation in soils collected in a historic mercury mining area in Yolo County (California). Soil moisture is another factor that might have an important role. When it increases, water-saturated micropores in soils also increase, being induced the reduced environment required by both sulfate- and iron-reducing bacteria (Holloway *et al.*, 2009) that might promote mercury methylation. Owing to this, strong correlations between soil moisture and methylmercury concentrations are sometimes encountered (Holloway *et al.*, 2009). Nutrients are also required by microorganisms and when there is a shift in these compounds concentrations, variations in soil microbial communities are driven (Holloway *et al.*, 2009). Nutrients might be also the reason why higher methylation rates are generally determined in sediments than in water column. This might be due to the highest nutrient and carbon contents of sediments (Eckley and Hintelmann, 2006), leading to more prolific bacteria populations, including mercury methylators. Sediment temperature also affects the activity of the microbial community present and so mercury methylation might vary seasonally (Raposo *et al.*, 2008). Methylation is generally increased with temperature, as stated for sediments collected in Lavaca Bay (Texas) (Bloom *et al.*, 1999), Gulf of Trieste (Hines *et al.*, 2006), Hudson River (Heyes *et al.*, 2006) and New York/New Jersey Harbor (Hammerschmidt *et al.*, 2008); however, methylation might be quite active in Winter (Hines *et al.*, 2006).

Since sulfate-reducing bacteria appear to be the primary mercury methylators in sediments and they are able to reduce sulfate to sulfide (Eckley and Hintelmann, 2006), it is common to find out similar depth distributions for mercury methylation activity and sulfide (Hines *et al.*, 2006). Also, an increase in methylmercury concentrations are generally associated with increasing sulfate concentrations (Muresan *et al.*, 2007; Holloway *et al.*, 2009). Nevertheless, both the availability of sulfate and the presence of high quality carbon (electron donor) in organic matter are the two major variables affecting sulfate-reducing bacteria populations and activities (Heyes *et al.*, 2006) and thus mercury methylation (Mitchell *et al.*, 2008).

As already mentioned, other factors, such as, sulfide and organic matter, affect the bioavailability of mercury to the methylating organisms. The substrate for mercury methylation in sediments, for example, is the inorganic mercury present in pore waters, whose bioavailability for methylating bacteria tends to decrease as sulfide concentrations increase, since dissolved mercury tends to form non-neutral complexes with sulfur (Benoit *et al.*, 1999; Hines *et al.*, 2006; Lambertsson and Nilsson, 2006). At low sulfide concentrations, neutral HgS complexes in pore waters tend to dominate (Lambertsson and Nilsson, 2006). These species can diffuse through bacterial membranes and can be methylated to methylmercury (Hines *et al.*, 2006). On contrary, as dissolved sulfide concentrations increase, these species are replaced by non-neutral complexes, which are not able to pass through the bacterial membranes. Several studies (Heyes *et al.*, 2006; Lambertsson and Nilsson, 2006; Muresan *et al.*, 2007; Hammerschmidt *et al.*, 2008) propose the existence of these processes in natural environments, after considering the methylmercury formation rates and the mercury and sulfide pore waters concentrations; however, some exceptions have been reported. Sunderland *et al.* (2006), for example, verified an increase in methylmercury percentage in high sulfide sediments containing high levels of dissolved organic carbon. This might result of the formation of bioavailable Hg(II) complexes that contains both sulfur and dissolved organic

carbon (Sunderland *et al.*, 2006). Taking into account the important role that sulfate-reducing bacteria, as well as, the sulfide content have on mercury methylation, the relationship between mercury methylation and sulfate/sulfide chemistry is complex (Eckley and Hintelmann, 2006). While sulfate controls microbial activity, sulfide controls mercury speciation.

Organic matter is another important factor that influences methylation because it acts as a terminal electron acceptor and as a carbon source to microorganisms; however, the relationship between dissolved organic matter and mercury methylation is more complex than this. On one hand, dissolved organic carbon has been shown to increase mercury methylation by stimulating microbial activity. On the other, mercury bioavailability might change. It has been suggested in some works that, for example, impoundments cause increases in methylmercury concentrations by creating organic-rich anoxic deposits conducive to mercury methylation (Hines *et al.*, 2000). In fact, the microbial community uses the organic carbon pool to build new cells and to include $CH_3$ that combines with inorganic mercury to form methylmercury. Add to this, high contents of organic matter in the sediment are a prerequisite for maintaining low redox potentials while supplies of "high-quality" organic matter, providing electron donors for the sulfate-reducing bacteria (Lambertsson and Nilsson, 2006). Nevertheless, organic matter also provides complexing agents for $Hg^{2+}$ (Ravichandran, 2004; Eckley and Hintelmann, 2006) and methylmercury (Cai *et al.*, 1999; Ravichandran, 2004), thus influencing both the total sediment and water column concentrations and the partitioning between solid and dissolved phases (Lambertsson and Nilsson, 2006; Sunderland *et al.*, 2006). When organic matter complexes the inorganic mercury, this specie becomes less biologically available for methylation because dissolved organic carbon molecules are generally too large to cross the cell membranes of the bacteria (Ravichandran, 2004). Besides, dissolved organic carbon-mediated reduction of inorganic mercury to the volatile $Hg^0$ species would also reduce the bioavailability of mercury for methylation and subsequent biological uptake (Ravichandran, 2004). Another fact to be considered is that the measurement of total organic matter content might have little relevance in terms of the concentration of organic substrate required really by mercury methylating organisms. These require some specific compounds, such as acetate, and not the total pool (Heyes *et al.*, 2006; Drott *et al.*, 2008b). Moreover, the type of the organic matter present is another important factor that influences mercury methylation because as hypothesized by Ravichandran (2004) when organic matter is largely labile and readily biodegradable, it may promote methylation by stimulating microbial growth and when the organic matter is relatively recalcitrant and consists of high molecular weight humic and fulvic acids, the abiotic methylation may be favored.

Organic matter also affects the redox potential of the sediments (Sunderland *et al.*, 2006). A high content of organic matter in the sediment promotes heterotrophic microbial activity, which consumes oxygen and lowers the redox potential close to the sediment surface (Lambertsson and Nilsson, 2006). On the other hand, the lower the organic matter in the sediment the deeper can oxygen and other competing electron acceptors (mainly manganese and iron) penetrate before being depleted by heterotrophic microbial activity (Lambertsson and Nilsson, 2006).

It must also be referred that not always a positive correlation between total mercury and methylmercury is observed in soils (Holloway *et al.*, 2009) and sediments (Heyes *et al.*, 2006; Lambertsson and Nilsson, 2006). Several hypotheses have been formulated in order to explain these results. One of the possibilities is the occurrence of microbial growth inhibition due to the high mercury concentrations, leading to the inhibition of the biotic mercury methylation

(Holloway *et al.*, 2009). The other is linked to geochemical factors as insufficient supply of new organic matter and inadequate redox conditions that also not favor the mercury methylation (Lambertsson and Nilsson, 2006). As already mentioned, for example, a high organic content in sediment maintains a low redox potential, which is a prerequisite for sulfate reduction (performed by sulfate-reducing bacteria) and concomitant mercury methylation (Lambertsson and Nilsson, 2006). In spite of this, in sediments with high organic content the redox potential required for sulfate reduction can be maintained closer to the sediment surface (Lambertsson and Nilsson, 2006). In sediments with lower organic contents, mercury methylation occurs deeper in the sediment (Lambertsson and Nilsson, 2006).

## 2.2. Mercury Demethylation Processes

### *2.2.1. Chemical Demethylation – Abiotic Processes*

The photolytic decomposition of methylmercury remains the only abiotic demethylation mechanism that is significant in surface waters exposed to sunlight (Sellers *et al.*, 1996; Gårdfeldt *et al.*, 2001; Chen *et al.*, 2003; Hammerschmidt and Fitzgerald, 2006; Monperrus *et al.*, 2007a). However, the overall impact on the aquatic Hg cycle is still unclear and the end products of the methylmercury degradation have not been clearly identified yet. Hammerschmidt and Fitzgerald (2006) demonstrate that the methylmercury decomposition in surface waters is an exclusively abiotic and sunlight-induced process. Monperrus *et al.* (2007b) estimate demethylation rates of methylmercury in coastal and marine waters (6.4–24.5 % $day^{-1}$) and suggest that an important part of the demethylation is mostly driven by sunlight because those rates decrease severely under dark conditions. Monperrus *et al.* (2007b) and Whalin *et al.* (2007) refer that, in coastal and marine surface waters, although methylmercury is mainly photochemically degraded, the demethylation yields observed under dark conditions may be attributed to microbial mediated pathways. Furthermore, higher demethylation potentials are predicted in marine surface waters in comparison with the water masses located deeper in the euphotic zone as the methylmercury degradation is inhibited under dark conditions. In sediments, the abiotic mechanism is also more conductive to the environmental methylmercury decomposition than the biotic one (Rodríguez Martín-Doimeadios *et al.*, 2004).

Hammerschmidt and Fitzgerald (2006) demonstrated that the rate of the methylmercury degradation is positively correlated with the intensity of photosynthetically active radiation (PAR) at a 0.75-6 m depth in the water column. Nevertheless, methylmercury can be degraded more rapidly at lower depths due to the additional influence of the ultraviolet (UV) light. In this sense, other authors suggested that the methylmercury photodecomposition is largely limited to the upper 0.5-1 m layer of surface waters, which is consistent with the penetration of the UV light in the water column (Krabbenhoft *et al.*, 2002). Moreover, Lehnherr and Vincent (2009) attribute the most important driver of the methylmercury photodecomposition to the UV radiation in freshwaters because wavelengths in the visible spectrum degrade methylmercury at a much slower rate than the former. However, they also recognize that the visible light plays an important role in deepening waters as it is attenuated much less rapidly than the UV radiation. Therefore, the modeling of the methylmercury photodecomposition requires the mechanistic knowledge of the role of the UV radiation

versus visible light, since wavelengths in both UV and visible regions of the solar spectrum are attenuated at very different rates in the water column of freshwaters.

It is important to take into account that photodecomposition rates are comparable among several lakes with widely varying water chemistry. It suggests that the kinetics of the methylmercury photodecomposition is not influenced by environmental factors apart from those affecting the light intensity and methylmercury concentration in natural surface waters (Sellers *et al.*, 1996; Hammerschmidt and Fitzgerald, 2006).

Since methylmercury cannot absorb sunlight wavelengths at all and thus the direct photodegradation cannot occur, the only possible mechanism is the indirect photolysis involving the photochemical formation of aqueous free radicals in sunlit natural waters. Chen *et al.* (2003) investigated the kinetics and mechanism of the methylmercury photodegradation mediated by hydroxyl radicals. They used the nitrate photolysis from 285 to 800 nm as the hydroxyl radical source. The products identified were $Hg^{2+}$, $Hg^0$, chloroform and formaldehyde, the main aqueous product being divalent mercury. The effects of chloride concentration and methylmercury speciation have also been investigated. The presence of chloride can lead to a higher methylmercury degradation rate that can be attributed to the chlorine radicals produced during the aqueous oxidation of chloride by hydroxyl radicals. The chlorine radicals formed may also attack the C–Hg bond and lead to an enhanced methylmercury degradation. Although the pH value does not significantly affect the degradation rate constant for reactions induced by hydroxyl radicals, a small decrease in the degradation rate is observed when the pH value increases from 5 to 8.5. It seems to be due to an increase in the relative concentration of methylmercury hydroxide, whose degradation rate is lower than that of methylmercury chloride. The two mechanisms proposed for the methylmercury degradation by hydroxyl radicals are both the dissociation of $CH_3$ group ($CH_3HgCl + {}^{\cdot}OH \rightarrow {}^{\bullet}CH_3 + HgOHCl$) and the dissociation of HgCl ($CH_3HgCl + {}^{\cdot}OH \rightarrow CH_3OH + {}^{\bullet}HgCl$) to form HgOHCl or other divalent mercury products. So, the Hg–C bond is attacked by the electronically excited hydroxyl radicals. Based on the typical concentration of hydroxyl radicals in natural waters, the methylmercury degradation rate was calculated. It ranges from 0.008 to 3.204 ng $L^{-1}$ $d^{-1}$ assuming a methylmercury concentration of 0.9 ng $L^{-1}$ in natural waters, except for seawaters due to their lower OH radical concentration. The methylmercury photodegradation mediated by hydroxyl radicals may be one of the most important pathways in sunlit surface waters.

Other possible mechanisms of indirect photodegradation could involve the singlet oxygen mediated pathway or the organic peroxy radical mediated pathway. However, no laboratory data is available to assess the importance of these reactions for the methylmercury decomposition.

As above mentioned, the methylmercury photodecomposition occurs via indirect photolysis and, therefore, it requires the presence of a photosensitizing species such as nitrate or dissolved organic matter (Chen *et al.*, 2003). Several studies have shown that this reaction is enhanced in the presence of organic compounds (Sellers *et al.*, 1996; Gårdfeldt *et al.*, 2001). Lehnherr and Vincent (2009) showed that the contribution of the UV radiation to the methylmercury degradation is greater in high dissolved organic matter waters (76 %) than in low ones (54 %) where the visible light acquires a similar role (46 %). Within UV radiation, the region A (320-400 nm) is a more important driver of the methylmercury photodecomposition than the region B (280-320 nm). On the other hand, the

photosensitization of dissolved organic matter by wavelengths in the PAR spectrum appears to be an important factor influencing the methylmercury photodecomposition in not very surface photic zones (Hammerschmidt and Fitzgerald, 2006). However, the demethylation mechanism of methylmercury by PAR (400-700 nm) still remains unknown.

The chemical methylmercury demethylation mediated by selenoamino acids via a bis(methylmercuric)selenide intermediate has been suggested, which is readily degraded to mercury selenide and dimethylmercury (Khan and Wang, 2010). The latter one is then decomposed further to methylmercury. This demethylation reaction can occur in vivo. In the aquatic environment, although there has been no report on the concentrations of selenoamino acids in natural waters, their sulfur counterparts have been reported in surface and sediment pore waters. Similarly, the sulfur-aided demethylation pathway gives mercury sulfide as ultimate reaction product.

### *2.2.2. Biotic Processes*

Microorganisms in contaminated environments have developed resistance to mercury and play a major role in natural decontamination. Mercury resistance occurs widely on Gram negative and Gram positive bacteria, in environmental (Chatziefthimiou *et al.*, 2007; Ramond *et al.*, 2008), clinical (Soge *et al.*, 2008) and industrial isolates. On contrary to mercury methylation that seems to be restricted to a subset of bacteria, mercury demethylation appears to be a process that is more widely spread. Research works on molecular biology shows that methylmercury degradation performed by microorganisms generally proceeds through two distinct vias (Hines *et al.*, 2006), oxidative and reductive, being the last one mainly linked to the mercury resistance (*mer*) operon. Both biotic pathways for methylmercury degradation are encountered in the environment and will be further discussed in the following sections.

*i. Reductive Methylmercury Degradation*

The reductive methylmercury degradation might occur through two pathways, one involving the *mer* operon and other that does not; however, the former process is the most studied and considered to be the most common.

When microorganisms use the reductive pathway via *mer* operon to perform methylmercury degradation, two stages are involved which are catalyzed by two enzymes. The *mer*-mediated methylmercury degradation pathway may be represented easily by:

$$\text{R-Hg} \xrightarrow[\text{Lyase}\ (\textit{mer}\text{B})]{} \text{Hg(II)} \xrightarrow[\text{Reductase}\ (\textit{mer}\text{A})]{} \text{Hg}^0$$

In general terms, the organomercurial lyase breaks the carbon-mercury bond in toxic substrates, such as methylmercury and phenylmercury, being released methane or benzene, respectively, and inorganic mercury (Hg(II)), which is subsequently reduced to $Hg^0$ by the action of the mercuric reductase.

Based on the organization of *mer* operon genes, two modes of mercury resistance are encountered in bacteria (Hines *et al.*, 2006): narrow-spectrum resistance and broad-spectrum

resistance. In the first, Hg(II) is reduced to the less toxic, inert and volatile elemental form ($Hg^0$), by the action of the mercuric reductase (MerA). On contrary, in broad-spectrum resistance, both organic and inorganic mercury will be remediated due to the presence of a *merB* gene that encodes an enzyme organomercurial lyase, beyond the presence of the mercuric reductase.

Genes encoded by the *mer* operon have been reported to be located on plasmids (Summers and Silver, 1978; Brown *et al.*, 1986; Griffin *et al.*, 1987; Radstrom *et al.*, 1994; Osborn *et al.*, 1997; Barkay *et al.*, 2003), chromosomes (Wang *et al.*, 1989; Inoue *et al.*, 1989, 1991), transposons (Kholodi *et al.*, 1993; Hobman *et al.*, 1994; Liebert *et al.*, 1997; Mindlin *et al.*, 2001; Ng *et al.*, 2009), as well as on integrons (Kholodii *et al.*, 1993; Liebert *et al.*, 1997). The mobile elements (plasmids, transposons and integrons) play an important role in the dissemination of mercuric resistance throughout microbial communities (horizontal transfer). Furthermore, these mobile elements may be occasionally combined with other resistance determinants, promoting the spreading of these plasmids with multiple resistance genes. It has been demonstrated that *mer* operon is associated to multidrug-resistance (Soge *et al.*, 2008, Silver and Phung, 2005, Ball *et al.*, 2007). Moreover, it has been verified that mercuric multiple resistant bacteria can effectively transfer the phenotype to potentially pathogenic species (Ball *et al.*, 2007). This proves clearly that it is of extreme importance to study the *mer* operon frequency in the microorganisms present in the environment, in order to obtain valuable data to be used in the prediction of the evolution of drug resistance.

In other hand *mer* operon has been used in mercury bioremediation studies, performed with the aim to decontaminate environments with high levels of mercury. In spite of this, *mer* operon has been used to modify microorganisms which become able to reduce inorganic mercury to elemental mercury (Deng *et al.*, 2008), and plants with the capacity of also degrade methylmercury (Bizily *et al.*, 2003); however, it is still necessary to perform more studies in order to increase the efficiency. Furthermore, the role of *mer* operon over heavy metals and xenobiotics detoxification has also been demonstrated (De and Ramaiah, 2007; De *et al.*, 2008).

*ii. Organization of the Mer Operon*

The *mer* operon(s), as well as the protein gene products found in a Gram negative bacteria are represented in Figure 2. Generally, when a bacteria is in the presence of methylmercury and inorganic mercury that are very toxic species to the cells, the bacteria tries to bring quickly these species to the cytoplasm where they will be converted enzymatically to the volatile and low-toxicity elemental form, $Hg^0$.

Several studies show that once at the cell surface, methylmercury passes through the cell's outer membrane via passive diffusion in its neutral/hydrophobic form(s) (Step 1) (Barkay *et al.*, 2003; Kritee *et al.*, 2009). Simmons-Willis *et al.* (2002), for example, suggests that methylmercury is transported as a complex with molecules containing thiol groups (i.e., cysteine or glutathione); however, more studies are needed in order to better understand the process involved. Nevertheless and considering the detailed work performed recently by Kritee *et al.* (2009), it seems that the uptake rate across the bacterial membrane (*V*) will always be lower than the rate of diffusion across the diffusion boundary layer (*J*) (Step 1'), remaining the cell uptake limited.

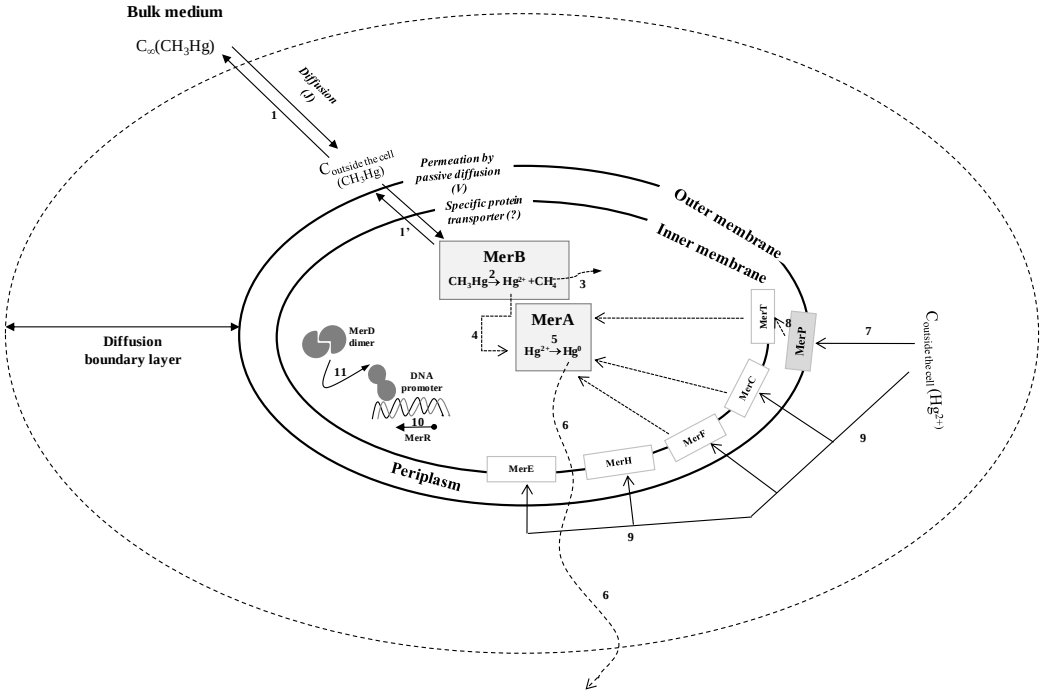


Figure 2. The products of the *mer* operon(s) of a Gram-negative bacteria.

The organomercury lyase (MerB), a small monomeric enzyme that cleaves the Hg-C covalent bond (Step 2) releasing Hg(II) (the substrate of mercuric reductase) (Step 3) and reduced organic compounds, such as methane from methyl mercury (Step 4) (Silver and Phung, 2005), is known as key enzyme in bacterial detoxification and bioremediation of organomercurials species.

The gene *mer*B has been found in the *mer* operon located on the plasmid of some bacteria, such as in *Escherichia coli*, *Pseudomonas* (almost 50%) and *Staphylococcus aureus* with "penicillinase" plasmids (Silver and Phung, 2005). This gene is also found on the chromosomes of methicillin-resistant *S. aureus* (MRSA) and of some *Bacillus* strains isolated from the environment. Frequently, Gram negative bacteria have two copies of *mer*B gene and *Bacillus* has three copies.

Several *mer*B sequences have been identified in a variety of microorganisms and most of the *mer*B genes are very homologous to each other; however, phylogenetic analysis revealed that MerB is an enzyme without known homologs in prokaryotic or eukaryotic proteins (Silver and Phung, 2005; Pitts and Summers, 2002). Furthermore, the MerB accepts a wide range of substrates. Chien *et al.* (2010) investigated the substrate specificities by resistant strains that have different *mer*B gene(s), and observed that *mer*B1 gene from *Bacillus Megaterium* MB1 conferred the highest volatilization ability to methylmercury chloride, ethylmercury choride and thimerosal, while *mer*B3 conferred the faster volatilization activity to *p*-chloromercuribenzoate; however, further work needs to be done in order to relate the MerB sequences with the wide range of organomercurial substrates known. In the presence of phenylmercury that is other organomercurial extremely toxic to the cells, this enzyme is capable to reduce the phenyl moiety to benzene (Silver and Phung, 2005). The enzymatic

reaction of organomercurial lyase has been studied and explained tentatively by Narita *et al.* (2003), Begley and Ealick (2004) and Silver and Phung (2005), indicating the occurrence of a proton attack on the Hg-C bond. Until now the catalytic mechanism of MerB has been controversial. Recently, Li *et al.* (2010) when studying the degradation mechanism of methylmercury by the tris(2-mercapto-1-*tert*-butylimidazolyl)hydroborate ([$Tm^{t\text{-}Bu}$]) ligand system, which shows a coordination environment that resembles closely the active site of the organomercurial lyase MerB, as well as Lafrance-Vanasse *et al.* (2009) after performing crystal structure studies on MerB in its free and mercury-bond forms, verified that two conserved cysteines, namely Cys-96 and Cys-159 are essential for enzymatic activity, playing a role in substrate binding, carbon-mercury bond cleavage, and controlled product (ionic mercury) release. Moreover, these authors also observed that an aspartic acid (Asp-99) in the active site plays a crucial role in the proton transfer step required for the cleavage of the carbon-mercury bond, acting as a proton mediator. The result of this bond cleavage is the release of methane and the retention of the ionic mercury in the active site. The way that methylmercury is bound to MerB is still under discussion. Nevertheless, the ionic mercury formed seems to be transferred directly to the reductase MerA (Step 3) for conversion to the less toxic elemental mercury (Lafrance-Vanasse *et al.*, 2009). In this manner, MerB and MerA keep the toxic organomercurial and ionic mercury species bound at all times and thus minimize their damaging interactions with other cellular proteins (Lafrance-Vanasse *et al.*, 2009). Indeed, Benison *et al.* (2004) proposed that the carboxyl-terminal cysteines of MerA are involved in removing the mercuric ion directly from MerB. Hg(II) is reduced in MerA to $Hg^0$ by electron transfer from FAD cofactor (Step 5) (Silver and Phung, 2005) and released (Step 6).

Mercuric reductase also reduce Hg(II) from cell outside, which is delivered into cytoplasm by mercuric transporters. A recently developed bacterial two-hybrid protein system showed that N-terminal region of MerA interacts with the cytoplasmatic face of mercuric transporter MerT (Schué *et al.*, 2007); however, in relation to mercuric reductases, diversity among different microorganisms is observed and lies in the N-terminal of the enzyme. MerA protein may have this N-terminal domain once or twice or lack it as for example in *Streptomyces*. The N-terminal domain of mercuric reductase can be proteolytically removed in vivo, and it appears to have little effect on overall rates of mercury reduction, suggesting that mercuric ions can be transferred in a different way (Schué *et al.*, 2008). In more detail, the resistance mechanism against Hg(II) ions (narrow-spectrum resistance) is completed by a mercury transport system. This may be formed by, for example, one (MerC), two (MerT and MerP), or three (MerT, MerP and MerC) proteins, which deliver mercury ions inside the cell cytoplasm where they are reduced to $Hg^0$ (Velasco *et al.*, 1999). Other membrane transport proteins have been reported, such as MerF, MerE and MerH. These transport systems hinder the toxic mercury to be free, not being able to cause damage into the cell. Studies performed in *E.coli* TG2 showed that the highest rate of cellular mercury volatilisation was observed in bacteria that expressed both MerP and MerT (Wilson *et al.*, 2000). MerP seems to make the system more efficient, as it acts not only as a periplasmic mercury binding protein (Step 7), but also as a metallochaperone, delivering Hg(II) to the membrane-anchored protein MerT (Step 8); however, MerP is not essential for Hg(II) transport (Wilson *et al.*, 2000; Nascimento and Chartone-Souza, 2003). Simplified schemes of some of these mercury transporters are represented in Figure 3.

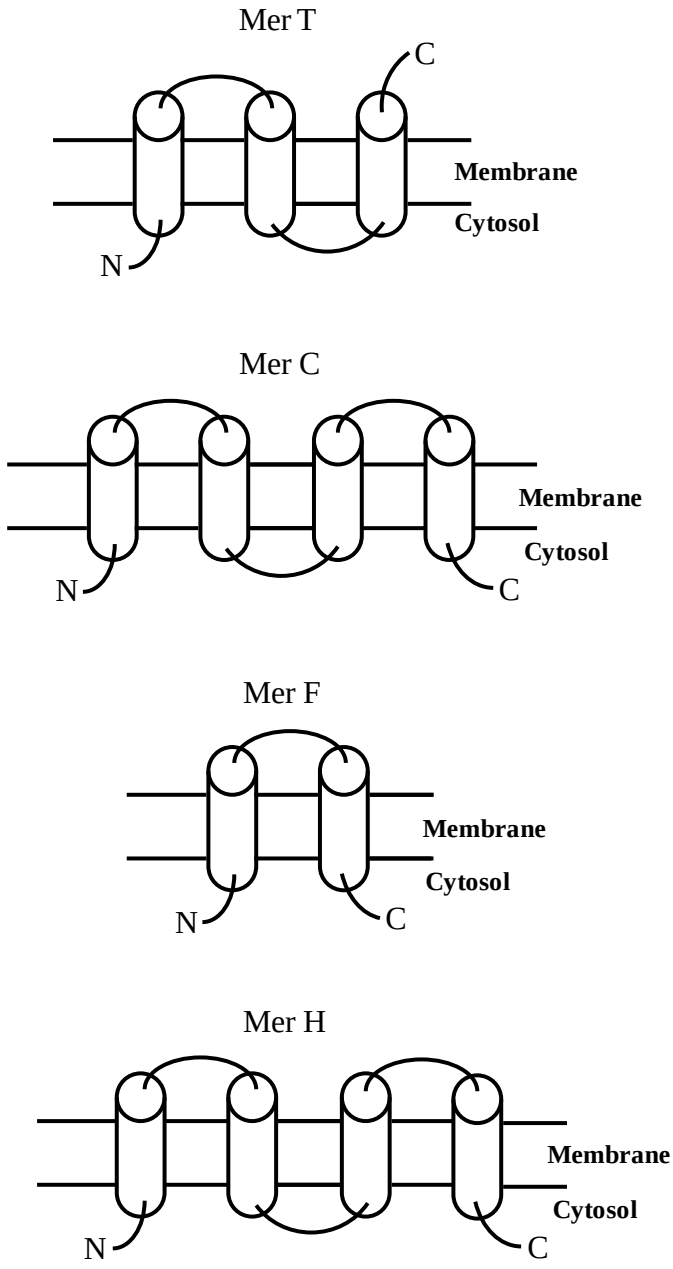


Figure 3. Simplified schemes of the MerT, MerC, MerF and MerH mercury transporters.

MerT has three trans-membrane alfa domains. The first cysteine pair, located in the first *trans*-membrane helix, receives mercury from the periplasmatic MerP. A second cysteine pair, located in a cytoplasmic loop connecting the second and the third *trans*-membrane helices, is important for optimal mercury transport, but not for the interaction with mercuric reductase (Schué *et al.*, 2008).

In relation to other membrane transport proteins, MerC and MerH cross the membrane four times, unlike MerF and MerE that just cross twice. Nevertheless, it is supposed that these

proteins function in a similar way. For acquiring further data over the structure of these proteins we recommend the reading of the works of Wilson *et al.* (2000) and Silver and Phung (2005). MerP does not make any difference to the rate of volatilization in the presence of MerC or MerF (Wilson *et al.*, 2000), indicating that neither of these transport proteins interacts with MerP in vivo (Step 9). It is interesting to observe that among all of the Hg(II) transporters only MerT presents the C-terminal in the periplasm and the N-terminal in cytosol, while all the others present the both terminals in cytoplasm, suggesting no interaction domain with MerP. Moreover, differences have been observed among all these Hg(II) transporters, suggesting that they can have different specificity to different mercury species and/or different $K_m$'s (Wilson *et al.*, 2000; Kyono *et al.*, 2009; Schué *et al.*, 2009); however, further studies must be done in order to clarify the role of these Hg(II) transporters.

The expression of the *mer* operon is regulated by MerR that bind to the upstream operator DNA regions (Step 10). The product of *mer*R is a 144-amino acid MerR protein that represses the transcription of *mer* operon in the absence of Hg(II) and induces it in the presence of Hg(II). MerR also represses its own synthesis whether or not Hg(II) is present (Heltzel *et al.*, 1990). The MerR protein has a distinctive protein fold consisting of a DNA-binding helix-turn-helix motif, followed by another helix-turn-helix motif that communicates between the metal binding and DNA binding domains. Half of the C-terminal of this small protein is a 35-residue leucine-zipper helix that forms the dimer interface as an anti-parallel coiled coil giving the protein an overall shape like a twisted staple (Song *et al.*, 2007). In Gram-negative bacteria the regulatory *mer*R gene is separated from the others genes by the operator-promoter region and is transcribed in the other direction. In Gram-positive bacteria *mer*R is transcribed in the same direction of the other genes of the operon. MerR is an unusual repressor that never leaves its operator that lies in a region of dyad symmetry located between the region consensus -35 and -10, a RNA polymerase recognition site. Moreover, in the absence of Hg(II) it captures an RNA polymerase in an inactive but stable preinitiation complex (Lee *et al.*, 1993). Studies of conformational dynamics of MerR after Hg(II) binding revealed allosteric conformational change from the metal-binding site to the two DNA binding domains leading to distortion of the operator and freeing the pre-bond RNA polymerase to begin transcription of the structural genes (Guo *et al.*, 2010).

In some *mer* operons, a second regulator gene, *mer*D, is present and possibly is an antagonist of *mer*R necessary to turn off expression by binding to the same promoter-operator region to which the Mer R protein bind (Mukhopadhyay *et al.*, 1991) (Step 11).

Regarding the induction of the *mer* operon, Hg(II) and phenylmercury acetate are able to do that (Nucifora *et al.*, 1989); however, this has not been unequivocally demonstrated for methylmercury. Schaefer *et al.* (2004) after performing a very interesting work on the role of the MerB in controlling methylmercury accumulation in mercury-contaminated natural waters, verified that Hg(II) induces quantitatively the expression of both *mer*A and *mer*B. In this work, the bacterium *Pseudomonas stutzeri* OX was used. This bacterium is resistant to inorganic and organic mercury as it carries two discrete *mer* operons, a narrow-spectrum *Tn*501-like operon and a broad spectrum *Tn*5053-like operon. Another interesting point stated by these authors was that at inducing Hg(II) concentrations higher than 2 μM, *mer*A transcript abundance continued to increase while *mer*B transcripts leveled off. One possible explanation for these results is differences in transcription kinetics between *mer*B and *mer*A. Moreover, *mer*A transcripts were consistently 7- to 15-fold more abundant than *mer*B transcripts at a given inducing Hg(II) concentration, which may be expected as this bacteria strain contains

two copies of *mer*A and a single copy of *mer*B. Similar results were achieved by Kritee *et al.* (2009) when studying the strain *Escherichia coli* JM 109 that carries the broad spectrum *mer* operon of the soil denitrifying bacterium previously referred, *Pseudomonas stutzeri* OX. These authors refer that MerB has lower turnover rates (lower $k_{cat}$ – 0.7 to 20 $min^{-1}$ – as compared to MerA – 400 to 800 $min^{-1}$ - i.e. it is less efficient in causing product formation per unit time and therefore the rate of Hg(II) reduction per cell is much higher than the rate of methylmercury degradation). Moreover, there is no evidence until now to suggest active involvement of radical pairs or paramagnetic species in non-photochemical biological reactions involving mercury, either in MerB catalysis and MerA reduction mechanism (Kritee *et al.*, 2009).

Recently, Kritee *et al.* (2009) verified that when performing experiments with a microorganism able to perform the degradation of methylmercury by the action of the *mer* operon, cell density may also have a significant role in the methylmercury degradation rates. These authors observed that at low cell densities, methylmercury seems to be bioavailable for diffusion and uptake into the cells, being the activity of the MerB the rate limiting. As the cell density increases, the bioavailability of methylmercury might be decreased due to sorption of this compound to the bacterial cell surfaces, decreasing methylmercury availability in the cytoplasm, originating lower rates of methylmercury degradation when compared to those obtained with lower cell densities.

Another reductive degradation pathway, a non-*mer*-mediated detoxification, has been proposed. Baldi *et al.* (1993) reported that the sulfate reducing bacteria *Desulfovibrio desulfuricans* might use an alternative anaerobic, non-*mer*-mediated degradation pathway, where methylmercury reacted with microbially produced sulfide to form an unstable dimethylmercury sulfide $(MeHg)_2S$ intermediate, which decomposes to dimethylmercury ($Me_2Hg$) and mercury sulfide (HgS). Dimethylmercury is then degraded to methylmercury and methane. Thus, the production of methane from methylmercury is common to both of the reductive demethylation pathways. Furthermore, as this non-*mer*-mediated degradation pathway implies the reaction of methylmercury with sulfide, it is expected to be most prevalent in sulfide-rich sediments. Such process has been suggested to occur in the environment. In fact, an increase on methylmercury degradation rate has been observed when pore-water sulfide concentrations have also increased (Marvin-Dipasquale *et al.*, 2000).

*iii. Oxidative Methylmercury Demethylation*

Oxidative demethylation is another demethylation pathway of monomethylmercury found in the environment (Oremland *et al.*, 1991; Oremland *et al.*, 1995; Hines *et al.*, 2006) that has been observed under aerobic and anaerobic conditions (Oremland *et al.*, 1991; Marvin-Dipasquale and Oremland, 1998; Hines *et al.*, 2000; Marvin-Dipasquale *et al.*, 2000; Hines *et al.*, 2006). Several kinds of microorganisms have been proposed to be involved in the process; however, the most common are sulfate reducers (Oremland *et al.*, 1991; Marvin-Dipasquale and Oremland, 1998; Marvin-Dipasquale *et al.*, 2000) and methanogens (Oremland *et al.*, 1991; Marvin-Dipasquale and Oremland, 1998; Marvin-Dipasquale *et al.*, 2000).

It seems that methylmercury is demethylated in part by biochemical pathways used for the metabolism of one-carbon compounds, such as methanol (Oremland *et al.*, 1991; Marvin-Dipasquale *et al.*, 2000), methylamines and methyl sulfides, as the addition of this kind of compounds has originated a substantial inhibition in methylmercury demethylation

(Oremland *et al.*, 1991). In oxidative demethylation, methylmercury is converted primarily to $CO_2$ and inorganic mercury, on contrary to the reductive degradation pathway of *mer*-detoxification, characterized by the nearly exclusive production of methane; however, it has been suggested that different microbial groups are capable of oxidative demethylation but with different stoichiometric end-product $CO_2/CH_4$ ratios and/or at different rates (Oremland *et al.*, 1995; Marvin-Dipasquale *et al.*, 2000). For methanogenic bacteria, for example, is expected the production of both $CO_2$ and $CH_4$ during oxidative demethylation, since these are the products of the $C_1$ metabolism by methanogens (Oremland *et al.*, 1995). Moreover, some of the carbon dioxide formed by the demethylators can be fixed into acetate pools by acetogenic bacteria (Oremland *et al.*, 1991).

The following reactions for the oxidative demethylation pathways used by sulfate reducers (Eq. 1) and methanogens (Eq. 2) have been proposed (Marvin-Dipasquale and Oremland, 1998), respectively:

$$SO_4^{2-} + CH_3Hg^+ + 3\ H^+ \rightarrow H_2S + CO_2 + Hg^{2+} + 2\ H_2O \text{ (Eq. 1)}$$

$$4\ CH_3Hg^+ + 2\ H_2O + 4\ H^+ \rightarrow 3\ CH_4 + CO_2 + 4\ Hg^{2+} + 4\ H_2 \text{ (Eq. 2)}$$

Considering Eq. 2, the oxidative metabolism of methylmercury during methanogenis will yield methane and carbon dioxide at a ratio of 3:1 (Oremland *et al.*, 1995), while $CO_2$ will be the only product formed under conditions of sulfate reduction or of respiration of other anaerobic electron acceptors (Oremland *et al.*, 1995). Nevertheless, the knowledge about oxidative demethylation is limited and it is not certain in what form and by what mechanism methylmercury is taken up (Drott *et al.*, 2008a). Further studies on pore water speciation of methylmercury must be performed.

*iv. Environmental Factors that Affect Mercury Biotic Demethylation*

Regarding the microbial communities found in environment, they seem to be quite well adapted to mercury toxicity. Schaefer *et al.* (2004) during the study of microbial adaptation to mercury in two natural waters collected in New Jersey, one water highly mercury contaminated and other much less contaminated, observed that the microbial community found in the most contaminated site was well adapted to mercury toxicity as indicated by the enrichment of Hg(II)-resistant bacteria ($2\text{-}4 \times 10^3$ Hg(II) resistant CFU/ml versus < 80 Hg(II) resistant CFU/ml determined in the less contaminated site), as well as by the presence and expression of *mer*A genes in the microbial biomass.

Some environmental factors seem to play an important role in controlling the magnitude and the pathway of methylmercury degradation (Marvin-Dipasquale and Oremland, 1998; Marvin-Dipasquale *et al.*, 2000) as they influence the microorganism's activity. As expected, the microorganism's activity, for example, increases with temperature. In several studies it has been stated that demethylation of methylmercury in sediments generally increases in summer (Hines *et al.*, 2006).

In relation to nitrate, for example, Marvin-Dipasquale and Oremland (1998) when performing incubations of anaerobic sediments with [$^{14}$C]MeHg observed that nitrate addition did not stimulate methylmercury degradation, indicating that nitrate-respiring bacteria were not directly involved in this degradation process; however, an increase in $^{14}CO_2/^{14}CH_4$ ratio in

some of the sediments was observed, suggesting nitrate inhibition on methanogenesis and possibly on the activity of sulfate reducers (Marvin-Dipasquale and Oremland, 1998). On contrary, addition of sulfate both increased total methylmercury degradation and increased $^{14}CO_2/^{14}CH_4$ ratios to values > 1 at all sites sampled. Similar results were reported by Marvin-Dipasquale *et al.* (2000). Thus, $CO_2$ production from methylmercury degradation seems to be enhanced under sulfate-reducing conditions and suggests that sulfate reducing bacteria oxidize the methyl group of methylmercury entirely to $CO_2$ (Eq. 2), in a similar way that they oxidize acetate (Eq. 3) (Marvin-Dipasquale and Oremland, 1998):

$$SO_4^{2-} + CH_3COO^- + 3\ H^+ \rightarrow H_2S + 2\ CO_2 + 2\ H_2O \quad \text{(Eq. 3)}$$

On contrary, phosphate seems not to have any effect on methylmercury degradation (Marvin-Dipasquale and Oremland, 1998).

Organic matter might play also an important role on the methylmercury degradation, in the same way as mentioned before in mercury methylation. On one hand, dissolved organic carbon might increase methylmercury demethylation by stimulating the demethylators activity. On the other, methylmercury-organic complex formation may be occurring, thereby decreasing methylmercury availability to bacteria (Marvin-Dipasquale *et al.*, 2000).

Methylmercury concentration can also influence the type of bacterial community present. In some sediments an increase of $^{14}CO_2/^{14}CH_4$ ratios with methylmercury concentration has been observed (Marvin-Dipasquale and Oremland, 1998). This fact may reflect a shift from methanogen-dominated demethylation at the low concentrations to sulfate reducers-dominated demethylation at higher concentrations (Marvin-Dipasquale and Oremland, 1998); however, Marvin-Dipasquale and Oremland (1998) stated that above ~800 ng of methylmercury /(g of dry sediment), the $^{14}CO_2/^{14}CH_4$ ratio remained constant, suggesting that the individual contributions of both groups to total methylmercury degradation did not varied.

In relation to sediment depth, some shifts on demethylation processes might also occur due probably again to changes in the bacterial community present. Hines *et al.* (2000), for example, when studying methylmercury demethylation in sediments in the Gulf of Trieste observed that methylmercury was mainly demethylated oxidatively with carbon dioxide as the primary carbon end product, indicative of the action of sulfate reducers, following Eq. 1; however, the percentage of carbon recovered as methane increased with depth, probably due to the enhancement in methanogens activity (Eq. 2).

Regarding the two distinct vias, oxidative and reductive, involved in the methylmercury degradation, the recent use of isotopic labelled methylmercury in determinations of demethylation rates allowed to find out the total mercury concentration is an important parameter that promotes shifts on these processes. In severely mercury contaminated environments, with total mercury concentrations in sediments around 22 to 106 nmol/g, the reductive methylmercury degradation via the *mer* operon seems to dominate, while in less contaminated environments (total mercury concentrations determined in sediments of 0.01-63 nmol/g) the oxidative demethylation is the main process (Marvin-Dipasquale *et al.*, 2000). Similar results were also reported by Schaefer *et al.* (2004) after determining the $^{14}C$-MeHg demethylation in two natural waters collected in New Jersey. The MerB-mediated reductive demethylation was the dominant process in the most contaminated sample whereas oxidative demethylation process was predominant in the less contaminated water (Schaefer *et al.*, 2004). All of these results indicate that reductive demethylation pathway seems to be

triggered when Hg and/or methylmercury contents surpass a determined value; however, it still remains uncertain which threshold of Hg and/or methylmercury concentrations is required for reductive *mer*-mediated demethylation to dominate.

Sediment redox potential and sometimes its conjunction with mercury concentration are important parameters that also control the mercury demethylation processes (Marvin-Dipasquale *et al.*, 2000; Schaefer *et al.*, 2002; Rodríguez Martín-Doimeadios *et al.*, 2004). The reductive pathway seems to dominate in aerobic incubations or under anaerobic incubations of highly contaminated sediments (Marvin-Dipasquale *et al.*, 2000; Schaefer *et al.*, 2002). Similar results were reported by Hines *et al.* (2006) for Gulf of Trieste sediments. These authors verified that demethylation was restricted to the oxidative pathway as evidenced by the production of $CO_2$, being only observed one exception in winter. In this occasion, the sediment tended to harbour a deeper oxidizing region at the surface, since oxygen consumption was slow, becoming more oxidizing and inducing the occurrence of the reductive demethylation pathway. So that, in the surficial sediments of Gulf of Trieste an increased contribution of reductive demethylation in winter was observed when oxidizing conditions penetrated further into the sediment (Hines *et al.*, 2006).

Another important aspect that must be referred is the inverse relationship that sometimes is observed between the proportion of total mercury present as methylmercury and the concentration of total mercury, known as "mercury accumulation paradox" (Schaefer *et al.*, 2004). This phenomenon might be due to the higher number of mercury resistant bacteria found in these mercury contaminated environments, to the existence and expression of *mer* genes in those organisms and to the occurrence of MerB-mediated reductive demethylation. All these factors will lead to a decrease in methylmercury concentration, even high total mercury concentrations are found. Nevertheless, alternative explanations for these results exist, such as enhanced methylation in less contaminated sites due, for example, to the water acidification that can stimulates Hg(II) transport into bacterial cells. This will lead to an increase on methylation rates and so higher methylmercury concentrations may be found in less mercury contaminated environments.

Moreover, the reductive and oxidative methylmercury degradation processes should be regard with great care. The effect of Mer-B mediated methylmercury degradation and the immediate reduction of Hg(II) to $Hg^0$ by MerA, corresponds to a net loss of mercury from the system via the volatilization of $Hg^0$ (Schaefer *et al.*, 2004). On the other hand, the end product of the oxidative demethylation is thought to be Hg(II) that is the substrate for methylation. In spite of this, methylmercury might be produced and its concentration increases, resulting in higher toxicity.

In order to evaluate the relative importance of biotic processes versus photochemical degradation of methylmercury in a given ecosystem, mercury isotope studies seem to be very promising. The detailed study performed recently by Kritee *et al.* (2009) provided evidence that the evaluation of mass dependent and independent fractionations, MDF and MIF, respectively, will allow to differentiate between microbial and abiotic mercury transformation pathways (Kritee *et al.*, 2009). For example, the extent of MDF evaluated by the ratio of $\alpha_{202}/\alpha_{198}$, will be different if microbial or abiotic mercury transformation pathways are occurring, being equal to 1.0004 and 1.0016, respectively (Kritee *et al.*, 2009). In relation to MIF, if microbial mercury transformations are occurring, MIF will not be observed (Kritee *et al.*, 2009). On contrary, if photochemical transformations exist, MIF will occur.

## 3. CONCLUSIONS

The methylation/demethylation processes that occur in the aquatic environments establish a methylmercury pool continually available for bioaccumulation. This is of great concern as methylmercury is one of the most toxic mercury specie. Both processes - mercury methylation and methylmercury demethylation - can involve abiotic or biological processes, the last one involving the action of microorganisms. Generally, the biological processes are more significant; however, sometimes the abiotic processes might have also an important role. In fact, the relative importance of mercury abiotic methylation is controversial. Some authors emphasize that the abiotic pathway appears to play a minor role in natural environments whereas others suggest that the biotic processes can not account for all the methylmercury formed naturally.

In relation to the biological processes, methylmercury demethylation appears to be a process that is more widely spread across the microbial genera in comparison to mercury methylation. Sulfate-reducing bacteria are considered to be the most important methylators present in the aquatic ecosystems. Moreover, these bacteria are able to donor a methyl group by the C-3 of serine (which is a compound formed during the carbon flow from pyruvate) or by formate via the acetyl-CoA synthase pathway.

The photolytic decomposition of methylmercury remains the only abiotic demethylation mechanism that is significant in surface waters exposed to sunlight. However, the overall impact on the aquatic mercury cycle is still unclear and the end products of the methylmercury degradation have not been clearly identified yet. In sediments, the abiotic mechanism is also more conductive to the environmental methylmercury decomposition than the biotic one.

Regarding the biotic methylmercury demethylation, two distinct vias - oxidative and reductive - might be used by microorganisms. The former is mainly conducted by sulfate reducers and methanogens. In this process, methylmercury is primarily converted to $CO_2$ and inorganic mercury; however, sometimes methane is also formed. The reductive process might occur through two pathways, one involving the *mer* operon and other does not. Nevertheless, the process involving the *mer* operon is the most studied and it is considered the most common pathway. In this process, two enzymes participate - the MerB (organomercurial lyase) and the MerA (mercuric reductase) - and so methylmercury will be converted to elemental mercury. In spite of this, the mentioned process represents a net loss of mercury ($Hg^0$) from the system, while the oxidative process might be only a source of substrate to mercury methylation due to the formation of Hg(II). Thus, if oxidative demethylation is not associated with a subsequent Hg(II) reduction, this has major implications in natural systems where oxidative demethylation dominates. In fact, this mercury can be remethylated to methylmercury if conditions are appropriate. Oxidative demethylation is presumed to result in Hg(II) as an end product, so no net elimination of Hg(II) takes place in this process, in contrast to the reductive demethylation.

The *mer* operon shows that the genetic patrimony of the microbial community is very important and affects significantly the methylmercury presence in the aquatic environments. In fact, there are bacteria that only transcribe MerA (narrow-spectrum resistance) and so they are only able to reduce Hg(II) to $Hg^0$, but there are others that have MerB and MerA (broad-spectrum resistance) and the latter are able to decompose methylmercury to $Hg^0$. Both

microbial mercury resistances might assume nowadays an important role, for example in the remediation of mercury contaminated environments. Therefore, the identification of mercury resistant strains is essential to the development of technological mercury bioremediation strategies.

Moreover, *mer* operon is present in various taxonomic groups and microbiocenoses, and *mer* genes are localized essentially in DNA mobile elements, promoting the horizontal transfer. It has been demonstrated that the *mer* operon is associated with multidrug-resistance and so the frequency study of this operon in the microorganisms present in the environment will give valuable data to be used in the prediction of the drug resistance evolution.

In terms of the action way of the *mer* operon, more research work is needed in order to better understand how the mercuric ion transporters work and the interaction between all *mer* products, as well as the role of different mercury species in induction of *mer* operon and its own regulation.

In this work, it was also stated that the mercury methylation and methylmercury degradation via abiotic and biotic pathways are affected by several environmental factors that influence inorganic mercury/methylmercury availability, as well as the activity of methylators/demethylators. These relationships are often very complex. Furthermore, these environmental factors might also change the microbial communities present, leading to shifts, for example in the biotic processes involved in methylmercury demethylation. As discussed in the present work, total mercury and the redox potential are factors likely to induce these changes. So, for example, the reductive demethylation pathway seems to dominate in both anoxic severely mercury contaminated and aerobic environments. Nevertheless, further works are needed to be performed in order to get more knowledge on these aspects.

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# Concurrent photolytic degradation of aqueous methylmercury and dissolved organic matter

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## HIGHLIGHTS

- MeHg photodegradation rates were similar in natural waters over a wide range of DOM.
- MeHg concentration was related to labile DOM but percent loss was related to humic DOM.
- Optical measurements of DOM could aid in monitoring in situ MeHg photodegradation.
- Physical characteristics of wetland systems control MeHg concentrations.

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## ABSTRACT

Monomethyl mercury (MeHg) is a potent neurotoxin that threatens ecosystem viability and human health. In aquatic systems, the photolytic degradation of MeHg (photodemethylation) is an important component of the MeHg cycle. Dissolved organic matter (DOM) is also affected by exposure to solar radiation (light exposure) leading to changes in DOM composition that can affect its role in overall mercury (Hg) cycling. This study investigated changes in MeHg concentration, DOM concentration, and the optical signature of DOM caused by light exposure in a controlled field-based experiment using water samples collected from wetlands and rice fields. Filtered water from all sites showed a marked loss in MeHg concentration after light exposure. The rate of photodemethylation was $7.5 \times 10^{-3}$ $m^2$ $mol^{-1}$ (s.d. $3.5 \times 10^{-3}$) across all sites despite marked differences in DOM concentration and composition. Light exposure also caused changes in the optical signature of the DOM despite there being no change in DOM concentration, indicating specific structures within the DOM were affected by light exposure at different rates. MeHg concentrations were related to optical signatures of labile DOM whereas the percent loss of MeHg was related to optical signatures of less labile, humic DOM. Relationships between the loss of MeHg and specific areas of the DOM optical signature indicated that aromatic and quinoid structures within the DOM were the likely contributors to MeHg degradation, perhaps within the sphere of the Hg-DOM bond. Because MeHg photodegradation rates are relatively constant across freshwater habitats with natural Hg–DOM ratios, physical characteristics such as shading and hydrologic residence time largely determine the relative importance of photolytic processes on the MeHg budget in these mixed vegetated and open-water systems.

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## 1. Introduction

Mercury (Hg) contamination in wetland environments poses significant risks to humans and wildlife because wetland processes convert Hg to monomethyl mercury (MeHg), the form that is more readily concentrated in aquatic food webs (Mergler et al., 2007; Selin, 2009). In fish and wildlife, Hg accumulation has been associated with neurological and behavioral abnormalities, low reproductive success, and direct toxicity (Crump and Trudeau, 2009; Mitro et al., 2008; Wiener et al., 2003). These concerns have led to the listing of Hg as an important pollutant across the world and prompting United Nations Environment Programme (UNEP) international negotiations to address the Hg problem (http://www.chem.unep.ch/mercury/default.htm).

Wetlands are locations of MeHg production and subsequent transport to aquatic systems because they possess the optimal conditions for Hg methylation (Gilmour et al., 1992; St. Louis et al., 1996). Shallow flooded systems of all kinds, including rice agriculture

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and managed wetlands, also possess the optimal conditions for Hg methylation because of their repeated wet–dry cycles and available substrates for microbial activity (Hall et al., 2009; Windham-Myers et al., 2009). In California, shallow flooded habitats have been identified as responsible for a majority of in situ MeHg production in the Sacramento–San Joaquin Delta (Wood et al., 2010a,b). Rice agriculture constitutes a large proportion of the managed flooded lands in California, greater than the acreage of natural and managed non-agricultural wetlands throughout the state (Hill et al., 2006). Globally, rice production also contributes to a significant proportion of the wetland acreage in the lower Mississippi River watershed and much of southern and southeast Asia (USGS, 2000).

Because Hg is a global pollutant and locations where methylation occurs are widespread, it is important to understand the pathways for MeHg removal within aquatic systems to better protect human and ecosystem health (Sellers et al., 1996; Wiener et al., 2003). Photolytic degradation of MeHg, also referred to as photodemethylation, is an important component of the MeHg cycle (Hammerschmidt et al., 2006; Lehnherr et al., 2012; Li et al., 2010; Sellers et al., 2001). In coastal waters where chloride complexes predominate, OH radicals may play the primary role in MeHg degradation (Hammerschmidt and Fitzgerald, 2010). In freshwater systems, MeHg is more strongly associated with reduced organic functional groups within dissolved organic matter (DOM) that will increase photodegradation rates compared to coastal or ocean waters (Black et al., 2012; Zhang and Hsu-Kim, 2010). Other recent studies used isotopic methods to quantify the contribution of photodemethylation to the MeHg cycle in a number of systems (Bergquist and Blum, 2007; Kritee et al., 2012), but questions remain about the effects that DOM has on Hg-isotope fractionation (Zheng and Hintelmann, 2009, 2010). Despite recent studies focused on mechanisms, photodemethylation remains a poorly defined process in the natural environment.

DOM plays a complex role in both Hg cycling and photolytic reactions in aquatic systems. DOM strongly binds with the reactive inorganic form $Hg^{II}$ (Han et al., 2006; Lamborg et al., 2003; Ravichandran, 2004) and MeHg (Hintelmann et al., 1997; Khwaja et al., 2010; Qian et al., 2002). Because of this DOM plays a role in the cycling and bioavailability of both $Hg^{II}$ (Bergamaschi et al., 2012; Brigham et al., 2009; Choe et al., 2003; Dittman et al., 2010; Gorski et al., 2008; Gerbig et al., 2011; Graham et al., 2012; Schuster et al., 2011) and MeHg (Bergamaschi et al., 2011; Choe and Gill, 2003; Pickhardt and Fisher, 2007; Tsui and Finlay, 2011). DOM also binds with other radical-forming constituents that may participate in photochemical processes (Gu et al., 2011; Gao and Zepp, 1998). Specific components within DOM can release labile organic compounds and nutrients when exposed to light (Dalzell et al., 2009; Engelhaupt et al., 2003; Mopper and Kieber, 2000; Moran and Zepp, 1997). Additionally, photolytic reactions within the DOM can physically alter DOM structure by breaking large macromolecules into smaller components that are more available for bacterial utilization (Cory and McKnight, 2005; Blough and DelVecchio, 2002; Mostafa et al., 2007; Spencer et al., 2009). Alternatively, DOM that is dominated by fresh, low molecular weight structures can lead to the formation of larger DOM molecules and particles during light exposure, further complicating the effects of light exposure on DOM dynamics in natural systems (Stepanauskas et al., 2005).

Recent studies have reported contradictory lines of evidence regarding the role of DOM in photodemethylation. Zhang and Hsu-Kim (2010) implicated DOM binding in promoting photodemethylation. In contrast, Li et al. (2010) suggested that spatial trends in MeHg concentrations in waters from the Florida Everglades may have been related to DOM effectively shading the MeHg from solar radiation, thus maintaining higher MeHg concentrations where DOM was high. Meanwhile, Black et al. (2012) observed only a minor effect of DOM on demethylation rates.

Despite the recent contributions of these studies to our understanding of DOM effects on demethylation, none of the work was performed on unadulterated samples. Previous laboratory-based experiments used model compounds (i.e. glutathione (GSH)), commercially available isolates (Suwanee River Humic Acid (SRHA), International Humic Substances Society, St Paul, MN) and other forms of altered or synthesized DOM. The use of commercial standards, concentrated DOM, or isotope-labeled MeHg is useful in mechanistic studies but can significantly alter the DOM and its reactivity thus limiting the extrapolation of these studies to natural systems (Shubina et al., 2010). For instance, GSH is a good model compound for testing the effects of reduced sulfur groups in organic molecules in a well-constrained manner necessary for mechanistic studies, but it lacks the complexity of interactions within the structure of natural DOM to justify extrapolation to natural systems without corroborative field evidence. Isolates derived from natural DOM, like SRHA, are preferable to model compounds when making inferences about natural systems, but commercial isolates are also limited because they are known to have different properties than natural DOM due to the loss of important structural components in the isolation process (Shubina et al., 2010). Because DOM structure and reactivity is so complex, and dependent on conditions (ie. pH, ionic strength and DOM concentration), studies using natural water samples are necessary to bridge the gap between these valuable mechanistic lab studies and what occurs in natural systems.

Characterizing DOM sources and transformations in natural systems is important for improved understanding of biogeochemical processes, but such information is typically difficult or expensive to obtain. There are many ways to measure DOM properties, but most approaches require solid material, which requires large quantities of water and the isolation process typically alters the DOM and includes only a fraction of the bulk pool. One non-destructive method for the characterization of natural DOM that has received recent attention is optical characterization. The use of absorbance and fluorescence spectroscopy uses the inherent optical properties of DOM structures to infer the presence and relative distribution of organic structure and functional groups within the DOM. Furthermore, optical properties can be measured in situ at time-scales relevant to natural processes (Romera-Castillo et al., 2011). Recently, optical proxies have been used successfully to determine temporal variability in THg and MeHg concentration in dynamic hydrologic settings (Bergamaschi et al., 2011, 2012; Dittman et al., 2009).

In this study, we investigated changes in MeHg concentration and the relationship to changes in DOM and inherent optical properties of surface waters collected from rice fields and exposed to solar radiation in a controlled, field-based, bottle experiment. Our objective was to test whether in situ proxies for dynamic biogeochemical settings could be identified that would provide a way to observe MeHg dynamics in the field at timescales relevant to production and degradation processes. This information would be useful for improving our understanding of Hg cycling in natural systems and aid in making informed management decisions that minimize MeHg exposure both within these systems and in downstream habitats.

## 2. Methods

### 2.1. Field procedures

Water samples were collected from five field outlets within the Yolo Bypass Wildlife Area on the morning of July 30, 2008 (Table S1; Fig. S1A). Two were collected at the outlets of domestic (white) rice fields (R20, R66), two at the outlets of wild rice fields (W31, W64), and one was collected from a permanently flooded open-water wetland (PW5). All samples were collected early in the morning to minimize light exposure prior to the experiment. From each field location approximately 10 L of filtered surface water was collected in a polycarbonate carboy by pumping water through an acid-cleaned 0.45 μm filter cartridge using a peristaltic pump (Fig. S1C). The samples were filtered to

minimize biological activity within the samples during the experiment. The peristaltic pump was equipped with acid-cleaned C-flex pump head tubing and FEP Teflon® tubing on both the inlet and outlet. Ultra clean handling protocols were followed throughout equipment cleaning, sample collection, experimental manipulation, and analysis (Choe and Gill, 2003; Choe et al., 2003; Gill and Fitzgerald, 1985).

After rigorous mixing, each field sample was split into eleven 500 mL fluorinated ethylene propylene (FEP) Teflon® bottles (Fig. S1C). Six clear bottles were used for the "light" treatment and five opaque bottles were used for the "dark" treatment. The dark bottles were used as a control for possible changes not related to light exposure. For each wetland site, all sample bottles were placed in a 13 mm polypropylene mesh net and floated together on the surface of an open water pond to mimic the maximum potential natural exposure to ambient light at the water surface (Fig. S1D). Five time points (t0, t1, t2, t3, and t4) were sampled over a two-day period, representing a cumulative photon flux of 0, 20, 30, 50 and 80 mol $m^{-2}$ of photosynthetically active radiation (PAR), respectively. Two bottles, one dark and one light, were not deployed, and these served as the time zero (t0) samples. At each successive time period, two bottles (one clear and one dark) were removed from each wetland site. Also, at each time point, one additional clear bottle was pulled from one of the wetlands to serve as a field replicate. Once collected, subsamples for DOM concentration and optical properties were poured from the clear bottles into an amber glass bottle and stored on ice until analysis. The remaining sample in each Teflon bottle was immediately preserved by acidification with high purity hydrochloric acid to 0.5% acid (v/v) and kept in the dark at room temperature until MeHg analysis. Whereas MeHg was analyzed at all time-points for both the clear and dark bottles, DOM subsamples were collected from only the dark bottles at the end of the experiment (t4) to serve as an experimental control.

Field measurements of ultraviolet (UV-A plus UV-B) and photosynthetically active radiation (PAR) were made continuously using a quantum sensor with nanologger (Apogee Instruments, Inc.) during the experiments to relate light exposure to MeHg and DOM degradation. The light sensor was located approximately 4 km from the location used for deployment of bottle incubations. Measurements are reported in moles of photons in the PAR wavelengths striking a square meter of water surface every second (mol $m^{-2}$ $s^{-1}$). These were multiplied by the number of seconds for each PAR integration interval, giving an estimate of total light exposure, or cumulative PAR photon flux, in mol per square meter (mol $m^{-2}$). Although radiative energy in the UV wavelengths is primarily responsible for photolytic degradation of MeHg (Black et al., 2012; Lehnherr and St. Louis, 2009), the measurements of PAR correlated well with UV-A and UV-B energy measured at the site and with total radiation measurements at the nearby California Irrigation Management Information System (CIMIS) meteorological station in Davis, CA (http://wwwcimis.water.ca.gov/cimis/frontStationDetailInfo.do?stationId=6&src=info). Thus, PAR represents a surrogate for the relative amount of total light exposure in the experiment rather than assigning the mechanism to a single wavelength or wavelength range. Furthermore, the clear FEP Teflon® bottles used in this study are known to inhibit some of the radiative energy; however, they have been widely used in photodegradation studies because their high optical transparency requires only a small correction to obtain absolute degradation rates (Byington, 2007; Lehnherr and St. Louis, 2009). Caveats aside, the comparisons contained within this study were made across equivalent methodologies and exposure conditions and represent a general, yet meaningful, response of MeHg and DOM to light exposure in natural surface water environments.

### 2.2. Laboratory procedures

#### 2.2.1. Monomethyl mercury analyses

Methylmercury analyses were performed at the Pacific Northwest National Laboratory Marine Sciences Laboratory, Sequim, WA. Concentrations were determined using distillation and aqueous phase ethylation followed by GC separation, pyrolysis and detection via cold vapor atomic fluorescence spectrometry (Bloom, 1989; Horvat et al., 1993). The accuracy and precision of the measurements were within 5% as indicated by an internal standard, laboratory replicates, and laboratory matrix spikes. The method detection limit for MeHg determinations was 0.012 ng $L^{-1}$ based on three times the standard deviation (s.d.) of 7 replicate measurements of a low MeHg content aqueous sample. Absolute differences in 8 field replicate bottles within the experiment averaged 0.002 ng $L^{-1}$ (s.d. 0.033 ng $L^{-1}$) which corresponded to a relative percent difference (RPD) of less than 3% for each site's field replicate except the lowest concentration site where the RPD was 15%.

#### 2.2.2. Dissolved organic matter concentration

Measurements of DOM concentration and optical properties were performed at the U.S. Geological Survey (USGS) carbon research laboratory in Sacramento, CA on a carbon basis as DOC in mg C $L^{-1}$ within 48 h of collection by high-temperature catalytic combustion using a Shimadzu TOC-$V_{CNS}$ total organic carbon analyzer according to a modified version of method EPA 415.3 (U.S. Environmental Protection Agency, 2005). The accuracy and precision of the measurements were within 5% as indicated by an internal standard (caffeine), laboratory replicates, and matrix spikes. The long-term method detection limit for DOM concentration was 0.30 mg C $L^{-1}$ based on three times the standard deviation of a low concentration standard measured over the annual cycle.

#### 2.2.3. Optical characterization of DOM

Optical measurements of DOM are related to the light sensitive (chromophoric) portions of the DOM pool that absorb or fluoresce radiation in the ultraviolet and visible spectra. Spectral absorbance (A) was measured at 1 nm increments between 200 and 750 nm in a 0.01 m quartz cuvette using a CARY-300 spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). Samples with $A_{254}$ greater than 3 absorbance units (AU) were diluted and reanalyzed to ensure linearity of response in the wavelengths of interest. Absorbance values for wavelengths greater than 500 nm were below the level of reliable detection for a 1 cm cuvette so were removed from the analysis. Specific (carbon-normalized) absorbances for all wavelengths were calculated for concentration-independent comparisons of spectral shape across sites. The specific absorption at 254 nm has been related to the aromatic content of the DOM (Weishaar et al., 2003). Concentration-independent spectral slopes were also calculated for several wavelength ranges ($S_{275–295}$, $S_{290–350}$, $S_{350–450}$ and $S_{412–676}$, respectively) using a non-linear least-squares curve fitting technique for each specified spectral range (Boss and Zaneveld, 2003; Del Vecchio and Blough, 2002). Slope ratios were also calculated for the wavelength ranges within the ultraviolet spectrum (UV $S_R$: $S_{275–295}/S_{290–350}$) and the ultraviolet to visible spectra (UV–vis $S_R$: $S_{275–295}/S_{412–676}$). Spectral slopes and slope ratios were calculated using MatLab R2008a (MathWorks, Natick, Massachusetts, USA) to infer DOM composition with higher slope values indicating a low molecular weight or "fresh" microbial or algal contribution to DOM and slope ratios further indicating the relative molecular size (Helms et al., 2008). The accuracy and precision of the measurements were within 5% as indicated by an internal standard (potassium dichromate) measured monthly, a laboratory reference material (Lipton® unsweetened iced tea, 1% by volume) measured daily, and laboratory replicates measured approximately every 10 samples. The long-term method detection limits vary by wavelength, ranging from 0.001 AU at 650 nm to 0.008 AU at 250 nm based on three times the standard deviation of field method blanks over the annual cycle.

Fluorescence was measured using a SPEX Fluoromax-4 spectrofluorometer equipped with a 150 W Xenon lamp (Horiba Jobin Yvon, NJ,

USA). Fluorescence intensity was measured at excitation wavelengths (ex) of 200 nm to 440 nm at 10 nm increments and emission wavelengths (em) of 300 nm to 600 nm at 5 nm increments on room temperature samples (25 °C) in a 0.01 m quartz cell. Instrument corrections were applied and results were water Raman-normalized. Concentration-related inner filter effects were corrected using wavelength and slit-width dependent corrections as described by Gu and Kenny (2009). Fluorescence results are shown as contour plots of fluorescence intensities in Raman-normalized fluorescence units (RFU) across the excitation and emission spectra creating a 3 dimensional excitation–emission matrix (EEM) for each sample (Cory et al., 2011). Individual diagnostic peaks within the EEMs spectra (Table 1) were identified according to previous efforts (Coble, 1996, 1990, 1998; Stedmon et al., 2003) with the addition of two peaks: 1) "FDOM" at excitation 370 and emission 460 (ex370em460) corresponding to field instrumentation (Bergamaschi et al., 2011; Downing et al., 2009) and 2) a previously unidentified area "peak Z" (ex420em460) that represented a baseline for humic-like fluorescence. The accuracy and precision of the measurements were within 5% as indicated by an internal standard (quinine sulfate) measured quarterly, a laboratory reference material (Lipton® unsweetened iced tea, 1% by volume) measured daily, and laboratory replicates measured approximately every 10 samples. The long-term method detection limits vary by excitation-emission pairs, ranging from 0.001 RFU throughout much of the EEM spectra to 0.354 RFU in the region of the peak B based on three times the standard deviation of field method blanks over the annual cycle. Fluorescence spectra were also analyzed using parallel factor analysis, or PARAFAC, to identify the important EEM pairs in the EEM spectra across all samples (Stedmon et al., 2003).

Changes in DOM composition were evaluated using published derivations of fluorescence properties (Table 1). For comparison of fluorescence EEM shape across sites with differing concentrations, carbon-normalized EEM plots were also calculated by dividing the fluorescence intensities by DOM concentration across the entire EEM spectra, hereafter referred to as C-normalized fluorescence. Three published fluorescent DOM compositional indicators were also calculated. Fluorescence index (FI) was calculated as the ratio of emissions 470 nm and 520 nm at excitation 370 nm for corrected spectra according to Cory et al. (2010) as an indicator of relative microbial versus terrestrial contributions to the chromophoric DOM pool. Humic Index (HIX) was calculated as an indicator of source, diagenesis and sorptive capacity (Ohno, 2002; Ohno et al., 2008). The freshness index (β:α), an indicator of the contribution of recently produced DOM, was measured as the ratio of emission intensity at 380 nm divided by the maximum emission intensity between 420 and 435 nm at excitation 310 nm with higher values representing a higher proportion of fresh DOM (Parlanti et al., 2000; Wilson and Xenopoulos, 2009). Finally, the optical ratio of fluorescence (ex370 em460) to absorbance at 370 nm, known as the relative fluorescence efficiency (RFE), was calculated as an indicator of the relative amount of algal and non-algal DOM (Downing et al., 2009).

### 2.3. Data analyses

Graphical and basic statistical analyses were performed in both Excel 2003 and Sigmaplot® (version 11, Systat Software, Inc., San Jose, Calif.). Analyses of variance (ANOVAs) were performed on the calculated MeHg degradation rates for each time point of the experiment using a one-way ANOVA test followed by the Holm–Sidak multiple pairwise comparison method to determine significance of differences between sites. To examine which part of the absorbance and fluorescence spectra were most strongly related to MeHg concentration and percent MeHg loss, we calculated the correlation coefficients between MeHg concentration and percent loss and each individual absorbance wavelength and fluorescence wavelength pair over the course of the light exposure incubation. This graphical approach has been used to identify regions within optical spectra related to reactivity of DOM and the production of disinfection byproducts (Kraus et al., 2010). Correlation coefficients (R) were calculated using the Pearson Product Moment function in Excel and confirmed with Sigmaplot® (v.11).

Exploratory data analyses were performed using The Unscrambler® X version 10.1 (CAMO Software, Oslo, NORWAY) on both concentration and percent change data. Principle component analyses (PCA) were performed using Non-linear Iterative Partial Least Squares of mean-centered, untransformed and data with cross validation. Because PCA allowed the simultaneous inclusion of both DOC and optical variables, all optical measurements in the PCA were carbon-normalized to focus on the relative difference in spectral shape rather than having redundant concentration effects.

## 3. Results and discussion

### 3.1. Initial conditions

The samples collected in this study had a wide range of MeHg and DOM concentrations, and DOM character (Table 1). Although DOM concentrations were higher than previous studies in lakes and wetland-derived waters, the $SUVA_{254}$ values were lower and absorption slopes ($S$) were higher than the DOM in those studies reflecting a smaller, relatively low aromatic content of the DOM within this study (cf. Helms et al., 2008; Weishaar et al., 2003). In general, water from two fields had similar absorbance signatures (domestic rice field R20 and wild rice field W31), and the three other fields had unique absorbance spectra (R66, W64, and PW5) providing four distinct absorbance signatures for the experiment (Table 1). Site W64 had the lowest $SUVA_{254}$ (1.24 versus 2.4 to 2.5 L $mg^{-1}$ $cm^{-1}$) and highest $S$ and $S_R$ values. The unique absorbance in W64 was likely related to high algal production observed during this study (data not shown). Site PW5 also had elevated $S$ and $S_R$ values relative to W31, R20 and R66 but not as elevated as W64. Site R66 had generally lower $S$ values than W31 and R20 but similar $S_R$ values which may indicate a similar source of DOM as R20 and W31 but different molecular size.

Initial (t0) carbon-normalized EEM spectra were relatively similar across sites and were dominated by humic-like fluorescence in the regions referred to as peak A and peak C which are common components in DOM spectra (Table 1; Stedmon et al., 2003). Although less pronounced and more variable, there were fluorescence signatures in the EEMs regions known as peaks N, T and B in some of the samples (Coble et al., 1998; Stedmon et al., 2003). Peak N has been associated with algal productivity (Coble et al., 1998) whereas peaks T and B have been associated with protein-like structures and lignin degradation products (Baker and Spencer, 2004; Hernes et al., 2009; Stedmon et al., 2003). One sample (W64) had generally lower carbon-normalized fluorescence intensities across the EEM spectra matrix compared to the other sites. Mentioned earlier, this site had the most algal production which may have contributed large amounts of non-chromophoric DOM thus decreasing the relative fluorescence across the spectra.

The humic index (HIX) and fluorescence index (FI) values were in the ranges 0.80 to 0.90 and 1.47 to 1.59, respectively. These values indicate DOM was largely terrestrial in origin with minor differences in the contribution of microbial DOM (McKnight et al., 2001; Ohno, 2002). All samples had similar HIX values around 0.9 except W64 (0.8); whereas, FI values were similar across all samples except PW5 (Table 1). The sample from PW5 was uniquely high in relative fluorescence efficiency. This may indicate a larger microbial contribution to the DOM signature for the PW5 site. In general, there were three fields with similar DOM optical signatures (R20, R66, and W31) and two other fields with unique DOM signatures (W64 and PW5) indicating three distinct fluorescence starting conditions (Table 1).

**Table 1**
Comparison of methylmercury and diagnostic dissolved organic matter measurements between sites for initial conditions (t0) prior to the light exposure incubation experiment. The sites are identified by their field number in the wetland complex preceded by the type of field management where "R" represents domesticated (white) rice, "W" represents wild rice, and "PW" represents permanent wetland pond (see Table S1).

| 1a. Concentration-based measurements | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Measurement | Name (units) | Property/purpose | Reference | R20 | R66 | W31 | W64 | PW5 |
| Dissolved (<0.45 μm filter-passing) monomethyl mercury | f-MeHg (ngHg $L^{-1}$) | Concentrations used to calculate photodemethylation rates | Horvat et al., 1993 | 1.50 | 0.50 | 0.70 | 3.75 | 0.18 |
| Dissolved organic carbon | DOC (mg C $L^{-1}$) | Dissolved organic matter concentration on a carbon-basis | U.S. EPA method 415.3 | 13.8 | 8.5 | 16.8 | 36.3 | 10.2 |
| Ratio of MeHg to DOC | MeHg/DOC (ng Hg mg $C^{-1}$) | Related to binding site strength and availability | Haitzer et al. (2002) | 0.11 | 0.06 | 0.04 | 0.10 | 0.02 |
| Total dissolved nitrogen concentration | TDN (mg $L^{-1}$) | Total dissolved nitrogen concentration | Merriam et al. (1996) | 1.34 | 0.81 | 1.75 | 5.92 | 0.93 |
| Absorbance intensity at 350 nm | $A_{350}$ (AU $cm^{-1}$) | General absorbance of DOM, related to general carbon bonding | Baker and Spencer (2004) | 0.07 | 0.05 | 0.09 | 0.11 | 0.04 |
| Absorbance intensity at 440 nm | $A_{440}$ (AU $cm^{-1}$) | General absorbance of DOM, related to algal activity in some cases | Hulatt et al. (2009); Zhao et al. (2009) | 0.01 | 0.01 | 0.02 | 0.03 | 0.01 |
| *1b. Absorbance-based DOM character measurements* | | | | | | | | |
| Specific ultraviolet absorbance at 245 nm | $SUVA_{254}$ (L $mg^{-1}$ $m^{-1}$) | Relative aromatic content of DOM | Weishaar et al. (2003) | 2.41 | 2.58 | 2.41 | 1.50 | 2.22 |
| Spectral slope between 275 and 295 nm | $S_{275–295}$ | Relative molecular weight/size of DOM | Helms et al. (2008) | 0.0179 | 0.0160 | 0.0179 | 0.0196 | 0.0198 |
| Spectral slope between 290 and 350 nm | $S_{290–350}$ | DOM composition | Blough and DelVecchio (2002) | 0.0175 | 0.0156 | 0.0173 | 0.0176 | 0.0188 |
| Spectral slope between 350 and 400 nm | $S_{350–400}$ | Relative molecular weight/size of DOM | Helms et al. (2008) | 0.0179 | 0.0160 | 0.0182 | 0.0140 | 0.0178 |
| Spectral slope between 412 and 676 nm | $S_{412–676}$ | DOM composition, photobleaching | Twardowski et al. (2004) | 0.0164 | 0.0156 | 0.0150 | 0.0116 | 0.0171 |
| Ratio of spectral slopes in the ultraviolet spectrum | UV $S_R$ | Relative molecular weight/size of DOM | Helms et al. (2008) | 0.98 | 0.97 | 0.95 | 1.26 | 1.06 |
| Ratio of spectral slope in the ultraviolet range to the spectral slope in the visible range | UV–vis $S_R$ | DOM source, degree of photolytic alteration | This study | 1.09 | 1.03 | 1.20 | 1.68 | 1.16 |
| *1c. Fluorescence-based DOM character measurements (carbon-normalized, value/DOC × 100)* | | | | | | | | |
| Carbon-normalized fluorescence intensity at ex260em450 | peak A (RFU, c-norm) | Relative amount of "humic-like" DOM | Coble, 1996 (1990); Stedmon et al. (2003) | 16.1 | 18.7 | 17.9 | 9.8 | 17.7 |
| Carbon-normalized fluorescence intensity at ex270em305 | peak B (RFU, c-norm) | Relative amount of "protein-like" DOM | Coble (1996, 1990); Stedmon et al. (2003) | 1.6 | 3.2 | 1.7 | 2.9 | 3.0 |
| Carbon-normalized fluorescence intensity at ex340em440 | peak C (RFU, c-norm) | Relative amount of "humic-like" DOM | Coble (1996, 1990); Stedmon et al. (2003) | 8.0 | 10.2 | 8.9 | 4.5 | 8.1 |
| Carbon-normalized fluorescence intensity at ex390em510 | peak D (RFU, c-norm) | Relative amount of soil "fulvic-like" DOM | Coble (1996, 1990); Stedmon et al. (2003) | 3.4 | 4.3 | 3.9 | 2.0 | 3.5 |
| Carbon-normalized fluorescence intensity at ex300em390 | peak M (RFU, c-norm) | Relative amount of "marine-like" DOM | Coble (1996, 1990); Stedmon et al. (2003) | 7.9 | 9.6 | 8.8 | 5.0 | 8.0 |
| Carbon-normalized fluorescence intensity at ex280em370 | peak N (RFU, c-norm) | Relative amount of algal derived DOM | Coble et al. (1998) | 6.0 | 7.5 | 6.9 | 5.0 | 6.6 |
| Carbon-normalized fluorescence intensity at ex270em340 | peak T (RFU, c-norm) | Relative amount of "protein-like" DOM | Coble (1996, 1990); Stedmon et al. (2003) | 3.3 | 5.0 | 3.8 | 4.2 | 4.1 |
| Carbon-normalized fluorescence intensity at ex370em460 | FDOM (RFU, c-norm) | Relative amount of "quinoid-like" humic DOM, in situ cdom fluorescence probe window | Downing et al. (2009) | 5.9 | 7.6 | 6.8 | 3.5 | 6.8 |
| Carbon-normalized fluorescence intensity at ex420em460 | peak Z (RFU, c-norm) | Baseline DOM fluorescence | This study | 1.5 | 2.0 | 1.8 | 0.9 | 1.5 |
| Humification index | HIX | Relative measurement of sorption capacity; C:O and C:N, carboxyl content | Ohno (2002) | 0.90 | 0.87 | 0.89 | 0.81 | 0.87 |
| Fluorescence index ex370em520/ex370em480 | FI | Relative contribution of terrestrial and microbial sources to the DOM pool | Cory et al. (2010) | 1.47 | 1.50 | 1.49 | 1.48 | 1.59 |
| Freshness index | β:α | Relative contribution of fresh DOM to recalcitrant DOM | Parlanti et al. (2000); Wilson and Xenopoulos (2009) | 0.75 | 0.76 | 0.78 | 0.86 | 0.81 |
| Relative fluorescence efficiency (FDOM/A370) | RFE (RFU/AU) | Microbial (non-algal) to algal ratio | Downing et al. (2009) | 15.8 | 15.4 | 17.4 | 13.2 | 22.1 |

### 3.2. Photolytic degradation of MeHg

Methylmercury concentrations in clear bottles decreased with increasing exposure to solar radiation at rates independent of initial concentration. Both MeHg loss in the clear bottles at each time point relative to the initial concentration ($[MeHg]_t/[MeHg]_0$), and the MeHg loss in clear bottles relative to the dark bottles at the same time point ($[MeHg]_{t,clear}/[MeHg]_{t,dark}$), showed similar trends (Fig. 1). Linear and exponential regressions provided strong fits of the data, although the regressions for $[MeHg]_t/[MeHg]_0$ were stronger than $[MeHg]_{t,clear}/[MeHg]_{t,dark}$ ($r^2 = 0.87$ and 0.88 versus $r^2 = 0.51$ and 0.52, respectively). The regression equation slopes represent the loss rate of MeHg as a rate constant ($k_{pd}$) dependent on cumulative PAR exposure and initial MeHg concentration. The linear

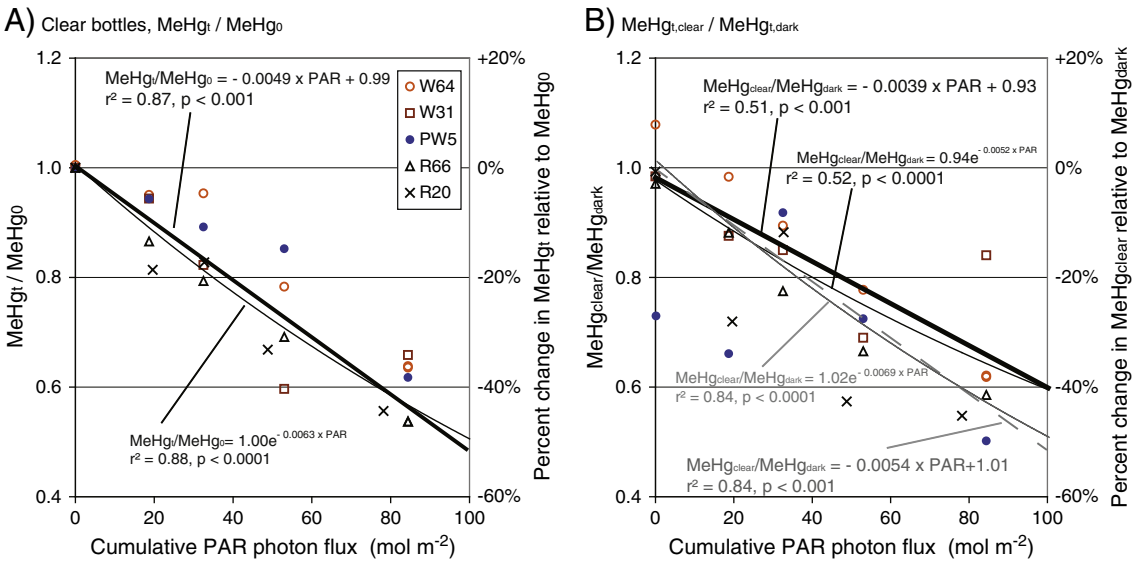


**Fig. 1.** Changes in MeHg concentration as a result of light exposure. Figure A shows the change in MeHg concentration plotted against cumulative photosynthetic available radiation (PAR) for clear bottles at each time point relative to the initial (t0) sample from that location. Figure B shows the ratio of MeHg concentration in clear bottles to their paired dark bottles at each time point in the experiment. Linear and exponential regressions are shown on both plots. The dashed line in Figure B shows the linear regression when four outliers were removed from the analysis.

regression slopes suggest a $k_{pd}$ of approximately 0.005 $m^2$ $mol^{-1}$ (Fig. 1); however, it is important to note that the $k_{pd}$ for MeHg was calculated over a time period of light exposure in which the loss appeared to be linear (cumulative PAR of 80 mol $m^{-2}$). Because other work that measured loss over a greater PAR exposure (180 to 320 mol $m^{-2}$) used the rate loss equation (Eq. (1)), and the exponential regression fits for the PAR exposure in this study were slightly better than the linear fits, we chose to report $k_{pd}$ using the same first-order rate loss equation similar to others so that we could better extrapolate the results of this study to systems with greater PAR exposure conditions (Lehnherr and St. Louis, 2009; Li et al., 2010).

$$\ln[\text{MeHg}]t = \ln[\text{MeHg}]0 - (\text{kpd} \times \text{Cumulative PAR photon flux}). \quad (1)$$

Using Eq. (1), we calculated the rate constants ($k_{pd}$) for each time point in the experiment to determine if any changes in the rate occurred throughout the light exposure period. Rate constants for the clear bottles ranged from 0.0043 to 0.0081 $m^2$ $mol^{-1}$, whereas the rate constants for the dark bottles ranged from −0.0017 to 0.0016 $m^2$ $mol^{-1}$ (Table S2). All $k_{pd}$ for clear bottles were significantly greater than their respective dark bottle pair ($p < 0.05$; Holm–Sidak post-hoc test). When dark bottle rates were subtracted from clear bottles to isolate changes due to the effects of light exposure alone, there were no differences between $k_{pd}$ across sites ($p < 0.05$; Holm–Sidak post-hoc test). The median $k_{pd}$ for the "clear-dark bottle treatment" was 0.0063 $m^2$ $mol^{-1}$ (s.d. = 0.0030), which is identical to the $k_{pd}$ from the exponential regression for $[MeHg]_t/[MeHg]_0$ (0.0063 $m^2$ $mol^{-1}$). When correcting for Teflon interference, the median rate constant ($k_{pd}$) for all clear bottles was 0.0075 $m^2$ $mol^{-1}$ in this study, well within the range of 0.006 to 0.015 $m^2$ $mol^{-1}$ reported by Black et al. (2012) for nearby wetland-derived water mixtures and other natural and experimental waters across North America (Table 2).

The degradation rate of MeHg did not differ between samples despite differences in DOM concentration and character. The differences in $k_{pd}$ across the DOM range were even less than those reported in experimental water mixtures prepared from nearby wetlands (Table 2). This result is consistent with the predominance of strong binding

**Table 2**
Summary of photodemethylation rates reported for sites across North America.

| $k_{pd} \times 10^{-3}$ | $k_{pd} \times 10^{-3}$ corrected[2] | Location | Water source | MeHg source | MeHg (ng $L^{-1}$) | DOM (mgC $L^{-1}$) | Reference |
|---|---|---|---|---|---|---|---|
| 4–10 | 5.2–13 | ELA, Ontario, Canada | Lake | Native | 1.1 | 17 | Sellers et al. (1996)[1] |
| 2–4 | 2.6–5.2 | ELA, Ontario, Canada | Lake | $CH_3HgCl$ spike | 4.5–6 | 17 | Sellers et al. (1996)[1] |
| 3.82 | 4.5 | ELA, Ontario, Canada | Lake | $CH_3^{199}Hg$ spike | 1.2 | 12.8 | Lehnherr and St. Louis (2009) |
| 3.93 | 4.5 | ELA, Ontario, Canada | Lake | Native | 0.8 | 12.8 | Lehnherr and St. Louis (2009) |
| 8 | 10 | Marcell, Minnesota, USA | Lake | $CH_3HgCl$ spike | 8.3 | 11 | Hines and Brezonik (2004)[1] |
| 10.87 | 13.67 | Everglades, Florida, USA | Freshwater wetland | $CH_3^{201}Hg$ spike | 0.6 | 6–22 | Li et al., 2010 |
| – | – | Laboratory experiments | Commercial isolate, model compounds | $CH_3HgCl$ spike | >100 | 0–2 | Zhang and Hsu-Kim (2010) |
| 3.0 | 3.8 | Alaska, USA | Lake | $CH_3HgCl$ spike | 1.2–4.2 | 0.4–10 | Hammerschmidt and Fitzgerald (2010)[1] |
| – | 9.9 +/− 2.0 | San Francisco, CA, USA | Coastal wetland | $CH_3HgCl$ spike | 0.02–1.25 | 1.5–11.3 | Black et al. (2012) |
| – | 3.2 +/− 1.0 | San Francisco, CA, USA | Coastal ocean | $CH_3HgCl$ spike | 0.02–1.25 | 1.5–11.3 | Black et al. (2012) |
| 6.3 +/− 3.0 | 7.5 +/− 3.5 | Sacramento, CA, USA | Freshwater wetland, rice field | Native | 0.2–3.8 | 8.5–36.3 | This study |

[1] $k_{pd}$ are estimated from calculations using data from tables or charts and/or local daily PAR during the study.
[2] Corrections range from 1.2 to 1.35 based on type of Teflon bottle used.

between Hg and DOM at reduced sulfur groups at environmentally relevant concentrations of Hg (<1 μg Hg mg $C^{-1}$; Haizer et al., 2002; Ravichandran, 2004). Although DOM alters MeHg photosensitivity because of the effects that the thiol binding between DOM and MeHg has on the Hg–C bond in the MeHg molecule (Zhang and Hsu-Kim, 2010), Hg and MeHg binding occurs at a small fraction of the DOM sites where thiol complexes occur thus minimizing any effect DOM composition may have on MeHg photolytic degradation. The exception to this scenario is the rare condition where aqueous Hg concentrations are elevated to the degree that the strong thiol binding site capacity of the DOM is exceeded (Haitzer et al., 2002; Zhang and Hsu-Kim, 2010), or the less rare condition where the actinic flux is significantly reduced in the water column by DOM absorbance effectively shading the MeHg from radiation in deeper water strata (Li et al., 2010). Neither condition was met under the conditions of this study.

### 3.3. Photolytic degradation of DOM

#### 3.3.1. Effect of light exposure on DOM concentration

The concentration of DOM measured on a carbon basis did not change significantly with light exposure. Concentrations changed by less than 7% in all clear bottles over the entire exposure period, and percent loss did not differ significantly from dark control bottles ($t = -1.53$, $p = 0.165$, $df = 8$). Bulk concentrations of DOM rarely change significantly over short periods of light exposure because the photosensitive DOM portion usually comprises a small fraction (1 to 5%) of the total DOM pool. However, changes in DOM composition (structural alterations) may occur within the bulk DOM pool without affecting bulk DOM concentrations (Cory et al., 2011; Spencer et al., 2007).

#### 3.3.2. Effect of light exposure on DOM absorbance

Although bulk DOM concentrations did not change significantly as a result of light exposure, changes in the absorbance spectra indicated changes in the light sensitive (chromophoric) structures within the DOM (Fig. S2; Table S3). In general, light exposure caused a decrease in the total amount of light absorbed across the full absorbance spectrum with maximum losses occurring in the wavelengths between 330 nm and 450 nm (Fig. S2B–D). Many DOM structures absorb light in this region including aromatic compounds, lignin degradation products, pigments, and other organic structures derived from the decay of terrestrial and emergent wetland plants (Del Vecchio and Blough, 2004; Hernes et al., 2009; Minor et al., 2007) and algal sources (Hulatt et al., 2009; Zhang et al., 2009).

Although DOM from all sites showed loss of absorbance between 330 and 450 nm, there was a difference in loss patterns between management types. DOM from the white rice fields (R20 and R66) showed similar patterns in the loss of absorbance with maximum losses occurring at all wavelengths of >350 nm (Fig. S3A, D). In contrast, DOM from the wild rice fields (W31 and W64) showed a similar maximum percent loss in absorbance near 350 nm but the loss diminished between 350 and 450 nm (Fig. S3B, C). The DOM from field W64 showed unique changes at wavelengths of >350 nm as the light exposure experiment continued. The relative standard deviation in absorbance for field W64 showed a strong inflection point for relative changes in absorbance at 440 nm (Fig. S2B), an area of DOM absorbance known to be related to algal activity. Field W64 possessed higher chlorophyll (data not shown) and may have had a wider distribution of algae and a more complex assemblage of pigments which could explain the unique absorbance spectral response to light exposure. The sample from field W64 may have even contained nanoplanktonic particles that were small enough to have passed through the filter and continued to be active throughout the experiment. Evidence of this possibility resides in the fact that the large increase in W64 absorbance at wavelengths over 400 nm was exaggerated by the spectral correction for scattering in which the absorbance in the range of 700 to 750 nm is used to calculate spectral slopes and the degree of increase was not observed in the raw spectra (Table 1). Alternatively, the higher contribution of DOM from algae or plankton in the sample from field W64 may have led to condensation reactions that caused the formation of colloids or large DOM structures that absorb in the visible wavelengths (Kieber et al., 1997; Stepanauskas et al., 2005).

Changes in the spectral slopes of the absorbance curve caused by light exposure indicated structural changes in the DOM related to molecular size and origin. The ultraviolet slope ratio ($S_{275-290}/S_{350-400}$) increased with light exposure by as much as 15%, suggesting that photolytic degradation decreased the average molecular size of the DOM in most cases (Helms et al., 2008). The large structures proposed to have formed in W64 sample were not captured in the absorption slope data because the slopes calculated in this study did not cover the range of absorbance caused by these structures (Downing et al., 2009). Future research efforts should consider extending the slope calculations into longer wavelengths to capture possible condensation products.

#### 3.3.3. Effect of light exposure on DOM fluorescence

Carbon-normalized fluorescence intensities decreased across the EEMs spectra following light exposure (Fig. 2). By comparing carbon-normalized EEMs spectra in the clear bottles prior to light exposure (t0) with the spectra after the full period of light exposure (t4) the areas where the greatest change in the spectra occurred is apparent (Fig. 2, Table S3). A comparison between the EEMs spectra of the clear bottles and dark bottles at t4 showed a similar trend whereas a comparison between the t0 samples and dark bottles at t4 showed little change, indicating that the changes were caused by photolytic processes (Fig. S4). The greatest percent loss in fluorescence intensity occurred in the region centered near excitation 370 and emission 400 nm (ex370 em400) for all samples (Fig. 2; Fig. S5). This EEMs region has not received much attention regarding studies linking structural information with fluorescence spectra, but the region falls near the edges of the regions typically associated with terrestrially derived humic acids (ex370, em420–480; Coble, 1996; Cory et al., 2011; Stedmon et al., 2003) and fulvic-like DOM derived from green algae (ex320–340, em400–450; Nguyen et al., 2005). More directly, this area falls at the lower edge of the region measured by in situ sensors (ex370 em400–460) in studies linking fluorescence and MeHg (Bergamaschi et al., 2011). In addition, there was a measurable increase in the region of peak B (ex280 em305) following light exposure in some cases. This region is typically associated with "protein-like" fluorescence (Baker and Spencer, 2004; Stedmon et al., 2003), but it also encompasses other refractory structures such as tannic phenols which complicates interpretation of structural relationships in this area of the EEMs (Hernes et al., 2009; Mostafa et al., 2007).

The fluorescence ratios used to identify DOM composition in previous studies changed little as a result of light exposure, at least for the period of exposure in this study (Table S3). The humic index (HIX) decreased with increasing light exposure for all samples but only by a small fraction. The fluorescence index (FI), used to assess the relative amount of terrestrial and microbial DOM in the bulk pool (Cory et al., 2010; McKnight et al., 2001), changed less than 2% with light exposure. The freshness index (β:α) changed even less with light exposure. Only the lesser-known calculated indicator known as the relative fluorescence efficiency (RFE), which is calculated from both absorbance and fluorescence data, changed markedly with light exposure in this study.

The lack of significant change in the fluorescence indicators suggests that, while these indicators may be sensitive to differences in DOM source and microbial alteration, they are minimally affected by changes due to abiotic processes, like solar radiation. That is not to

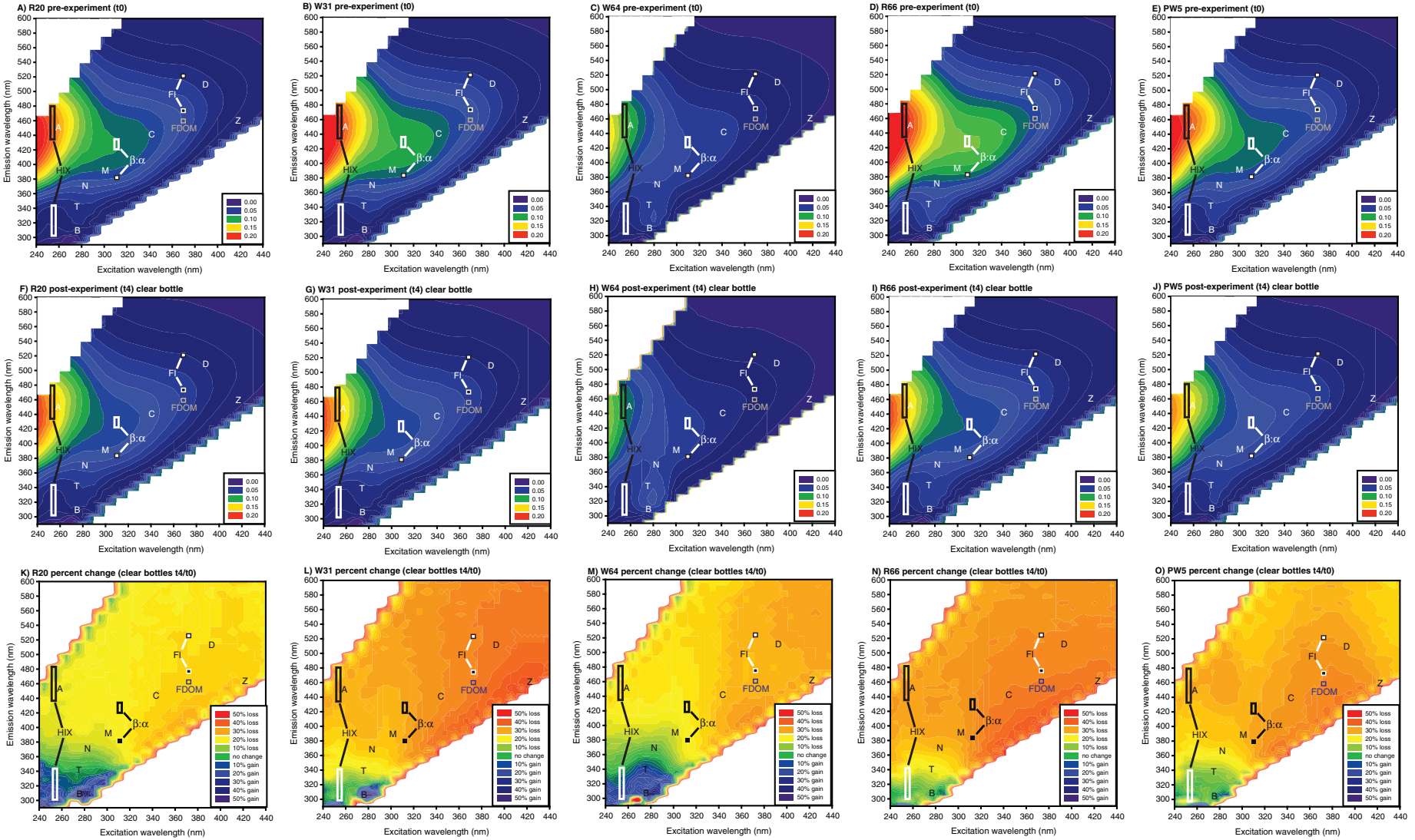


**Fig. 2.** Changes in carbon-normalized, excitation-emission matrix (EEM) fluorescence intensity spectra as a result of light exposure. Figures A through E show EEMs for clear bottles prior to light exposure (t0). Figures F through J show EEMs for clear bottles after the full period of light exposure (t4). Figures K through O show the percent change in fluorescence intensity across the EEMs in the clear bottles relative to t0 ($I/I_0$). In figures A though J, yellow to orange shades indicate higher fluorescence intensities. In figures K through T, orange and yellow shades indicate a decrease in fluorescence, blue shades indicate an increase in fluorescence, and green shades indicate minimal changes. Named peaks (Coble, 1996; Stedmon et al., 2003) and areas used for calculating indicators (Cory et al., 2010; Ohno, 2002) are labeled.

say the fluorescent DOM is not affected in these regions but that the regions are affected similarly such that the ratios do not change. For instance, the areas of the EEMs used to calculate the FI decreased to a similar degree during light exposure which resulted in little change in the FI ratio (Table S3; Fig. 2). This was true for the β:α ratio as well (Table S3). The lack of change in both FI and β:α reaffirms their use as indicators of microbial DOM and provides evidence that there was minimal microbial activity during incubation within this study. It is likely that the HIX did not change more because samples that are already predominantly humic are insensitive to losses in the non-humic region. Ratios of fluorescence to absorbance are better at discriminating between photodegradation and microbial processing of DOM than fluorescence or absorbance alone which may explain the changes in RFE seen in this study (Romera-Castillo et al., 2011).

It is worth noting that photolytic degradation led to a more uniform fluorescence signature across sites (Fig. 3, Fig. S6). The trend toward uniformity following light exposure may represent a base refractory pool of chromophoric DOM across the sites in this study. This was seen more clearly by comparing the relative standard deviation (RSD) in C-normalized EEMs across the sites prior to and after light exposure (Fig. 3). The difference in the EEMs across sites was high for bottles not exposed to light (t0, dark bottle t4) and showed specific areas of greater variance in the peak A and peak C regions (Fig. 3A) whereas the same samples exposed to light (clear bottle at t4) were more similar in EEMs spectra across sites (Fig. 3B). The difference between the relative standard deviation (RSD) for samples that were exposed to light (clear bottles t4) and those that were not exposed to light (t0, dark bottle t4) indicated that the greatest relative changes in carbon-normalized fluorescence between sites occurred in a band of wavelengths in the "Type IV" region of the EEMs including a marked decrease around peak N, an area previously attributed to DOM of phytoplankton origin and an increase in the area between peak B and peak T attributed to proteins containing ring structures and phenolic degradation products (Chen et al., 2003; Coble et al., 1998; Stedmon et al., 2003).

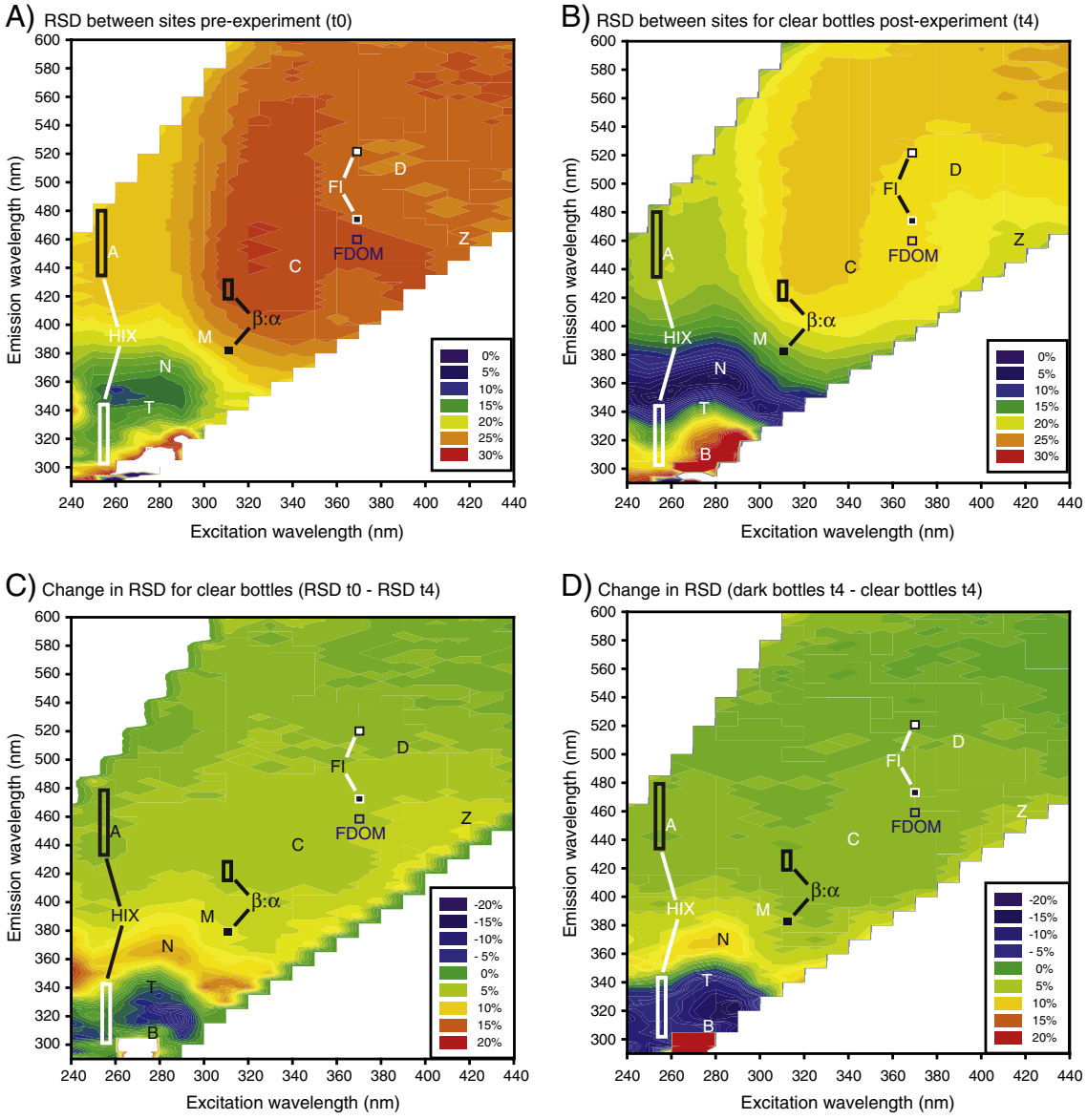


**Fig. 3.** Differences in fluorescence signatures between sites measured as the relative standard deviation (RSD) of carbon-normalized fluorescence intensities across the fluorescence spectra across all sites. Figure A shows the RSD across all sites prior to light exposure (t0). Figure B shows the RSD across all sites after light exposure in the clear bottles (t4). Figure C shows the difference between RSD at t0 and t4 in the clear bottles. Figure D shows the difference between the dark and clear bottles at t4. Figures C and D highlight the areas of the fluorescence signature that changed most across sites as a result of light exposure. The area of the spectra below the "B" peak has a large RSD because of large analytical error and not changes in measureable fluorescence. Absolute standard deviations and the RSD plots for the dark bottles are shown in Fig. S6.

Parallel factor analysis (PARAFAC) verified the identification of the areas of the EEMs where differences occurred. For this study, a four component PARAFAC model captured a majority of the variance in the EEMs spectra across sites and light exposure (Fig. S7A–D). Component 1 was in the area of peak A (ex < 280 em > 440) with a secondary component in the area of peak D (Stedmon et al., 2003). Component 2 was in the area of peak M and peak N (Coble, 1996; Coble et al., 1998). Component 3 was distinctly similar to the tryptophan-like peak T of bacterial or algal origin (Cory and McKnight, 2005; Stedmon et al., 2003). Component 4 was also distinctly in the humic region of peak C (Coble, 1996; Stedmon et al., 2003). In general, the components were similar to a subset of those identified by C-normalized EEMs intensity and difference plots (Fig. 2). Within the PARAFAC model, components 1 and 2 changed little over the time series of light exposure (Fig. S7E). In contrast, component 3 (protein-like) increased while component 4 (humic-like) decreased with increasing light exposure (Fig. S7E). This analysis corroborates the previous analysis that suggested photolytic degradation of DOM was focused in aromatic regions of the DOM. The use of the PARAFAC model in this analysis does not identify the total loss of fluorescence but rather is related to the relative changes in the EEMs spectra across sites and exposure. The small dataset for which PARAFAC could be performed would not allow for discrimination of EEMs differences between sites and light exposure independently, limiting our ability to elucidate between changes due to photodegradation within sites and differences in DOM between sites.

### 3.4. MeHg–DOM relationships

Although there was not an apparent relationship between DOM concentration and demethylation rate, the role of DOM in photolytic processes is complex and may still play a major role in MeHg degradation by mediating radical formation or transferring its energy to the Hg–C bond (Black et al., 2012; Blough, 2001). More information about DOM and its degradation may provide insights to the possible photodemethylation pathways and help narrow whether production of singlet oxygen ($^1O_2$) or hydroxyl radicals (OH•) in bulk water dominate demethylation (Hammerschmidt and Fitzgerald, 2010; Zhang and Hsu-Kim, 2010) or internal production of radicals within the sphere of influence of aromatic DOM (Latch and McNeil, 2006) further contribute to the complex role DOM plays in demethylation (Black et al., 2012).

The relationship between MeHg concentration and individual absorbance intensities was strongly positive across the entire range of wavelengths from 200 to 500 nm ($R > 0.85$, $p < 0.001$), with the correlation coefficient reaching its maximum ($R = 0.93$) between 420 and 440 nm (Fig. S8). The strong relationship across the entire absorbance spectrum indicates that the DOM concentration is the primary driver of differences between sites with DOM properties related to specific wavelengths contributing to the minor increase in R between 420 and 440 nm. Although small, the increase in correlation coefficient between 420 and 440 nm may indicate a specific loss of absorbance from photodegradation of residual pigments related to phytoplankton death or macroalgal exudation (Blough, 2001; Hulatt et al., 2009) or may merely be related to the unique behavior of W64 samples noted earlier (Fig. S3).

Because percent loss in absorbance at each wavelength was independent of DOM concentration, we also evaluated the relationship between percent MeHg loss and percent absorbance loss for each wavelength to identify possible wavelengths directly related to MeHg loss. Linear regressions between percent loss of MeHg and absorbance across the absorbance spectra showed maximum correlation coefficients ($R = 0.87$ to $0.88$; $p < 0.001$) occurring throughout the range 280 to 350 nm and dropping off markedly above 400 nm (Fig. S8). The loss of absorbance in the range of 280 to 320 nm has been observed previously and attributed to photo-production of low molecular weight carbonyl compounds from direct photolytic cleavage of the C–C bonds in humic substances by UV-B energy (Kieber et al., 1990), suggesting a linkage between MeHg degradation and the photodegradation of these DOM structures.

Similar to absorbance, fluorescence data showed specific regions of the EEMs spectra strongly related to MeHg. Relationships between MeHg and fluorescence intensities were strongest in protein-like region of peak T and peak N associated with algal origin (Fig. 4A), whereas the region of peak C and FDOM were areas of the weakest correlation coefficients. The higher correlation between MeHg and fluorescence in peak T and peak N suggests that the higher MeHg concentrations are related to sites with more labile DOM and higher ecosystem productivity in general. More labile forms of DOM are known to stimulate the production of MeHg (Windham-Myers et al., 2009), at least until the point at which biodilution occurs (Karimi et al., 2007; Pickhardt et al., 2005). In contrast, the loss of MeHg was more strongly related to the loss of the humic or fulvic portion of the DOM across a wide area of the EEMs spectra (Fig. 4B). The highest correlation coefficients were observed to be in the longer wavelengths in the area of peak C and the FDOM region, an area attributed to quinoid humic structures (Cook et al., 2008). These humic regions are the same structures believed to be photodegraded to form OH radicals (Blough, 2001) and carbonyl compounds (Kieber et al., 1990) as noted above.

The relationships observed between MeHg and specific portions of the DOM in the previous analyses were verified using principal component analysis (PCA). The use of PCA also allowed for the comparison of the relative influence of the absorbance and fluorescence properties listed in Table 1 with respect to MeHg concentration (Fig. S9) and percent change in MeHg with light exposure (Fig. S10). The concentration-based analysis confirmed that primary differences were between sites (PC1) with a secondary difference occurring within sites (PC2) separated along the light exposure time series (Fig. S9A). Bulk DOM concentration (as DOC) dominated PC1 whereas differences in humic fluorescence peaks appeared to dominate PC2 (Fig. S9B). In contrast to the previous analyses, the indicators related to microbial or algal DOM (i.e. peak B, peak T, $A_{440}$) did not factor into the correlation loadings for the concentration-based analysis, suggesting that these attributes did not contribute to variation in the data (Fig. S9C). The use of PCA for the percent change over light exposure was not influenced by bulk DOM concentration (Fig. S10). In this analysis, data clustered by light exposure across sites with the dark bottles falling in the lower right quadrant and clear bottles with greatest light exposure clustering in the upper left quadrant (Fig. S10A). The primary components (PC1 and PC2) in this analysis (Fig. S10B) consisted of a far more complex mixture of fluorescent and absorbance properties than the concentration-based analysis (Fig. S9B). The correlation loading indicated a close positive relationship between percent change in MeHg with the percent change in absorbance in the UV range and humic regions of the EEM spectra and a negative relationship with spectral slopes and slope ratios illustrating the complex relationship between MeHg and DOM (Fig. S10C).

## 4. Conclusions

The photodegradation rate measured in this study ($7.5 +/- 3.5$ $m^2$ $mol^{-1}$) was similar to previously reported rates for freshwater and estuarine systems. Despite a wide range in both DOM concentration and character, the photodegradation rate was a function of light exposure. High DOM concentrations were proposed to be responsible for relatively low MeHg degradation rates in some systems because the DOM attenuated the light, essentially shading MeHg within the water column (Li et al., 2010). In contrast, Black et al. (2012) observed only a small effect of DOM concentration on MeHg photodemethylation rates despite high attenuation of light in the water column, suggesting a complex role of DOM in demethylation.

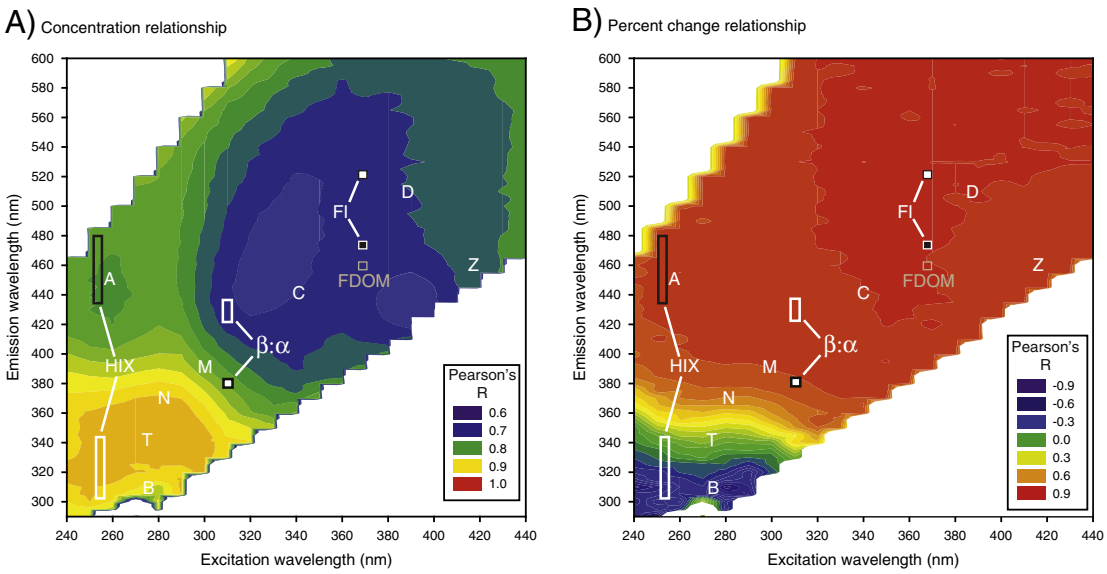


**Fig. 4.** Contour plots showing Pearson Product Moment correlation coefficients (Pearson's R) for each excitation–emission pair across the EEMs spectra. Figure A shows the correlation coefficients between MeHg concentration and fluorescence intensities. Figure B shows the correlation coefficients between the percent loss of MeHg and percent loss of fluorescence intensities.

The results in this study are more in line with those of Black et al. (2012), in that there was no observable effect of DOM concentration on photodemethylation rates over a large DOM concentration range (8 to 34 mg C $L^{-1}$) and a large ratio of MeHg to DOM (0.02 to 0.11 ng Hg mg $C^{-1}$). It is possible that this trend would change in more highly contaminated areas where higher ratios of MeHg (and Hg) to DOM affect binding conditions (Haizer et al., 2002; Zhang and Hsu-Kim, 2010).

The data shown here support the conclusion that DOM affects photodemethylation in complex ways that are not reflected in a simple relationship between DOM concentration and demethylation rates (Black et al., 2012). Results suggest photolytic reactions occur that affect specific regions across the absorbance and fluorescence spectra that represent different DOM components. Changes in the regions of the DOM optical spectra that were principally affected by light exposure are known to produce OH radicals (Blough, 2001), singlet oxygen ($^{\bullet}O_2$) (Latch and McNeil, 2006), and DOM radicals (Brezonik, 1994). Although this correspondence could occur without a mechanistic relationship, the relationships observed between the loss of specific fluorescent DOM and MeHg in this study are provocative. The strong relationships between the percent loss of MeHg and percent loss of chromophoric DOM containing aromatic and/or quinoid humic structures suggest light exposure alters DOM moieties directly bound with MeHg, similar to what has been reported for the photoreduction of $Hg^{II}$ (Gu et al., 2011; O'Driscoll et al., 2006). These data, particularly the relationship between percent loss of FDOM and MeHg during photolytic degradation, support the notion that light-induced reactions not only occur in the DOM sphere but are potentially linked to the binding site itself (Hines and Brezonik, 2004; Latch and McNeil, 2006; Zhang and Hsu-Kim, 2010).

The results of this study provide valuable information towards the understanding of MeHg photodemethylation and the relationship to DOM photodegradation in shallow flooded environments including areas of rice cultivation. Despite the complexity in the concurrent photolytic degradation of MeHg and DOM, demethylation appears to be driven primarily by cumulative exposure to solar radiation, therefore differences in photolytic degradation between aquatic systems will be driven more by physical factors such as shading by vegetation and particles within the water column and hydrologic controls on residence time than by chemical drivers. Differences in MeHg concentration between open water and vegetated areas may be more the result of limits on photodegradation processes than differences in net MeHg production processes in the sediment (Marvin-DiPasquale et al., 2014–in this issue; Windham-Myers et al., 2014a–in this issue). Alternatively, the degradation of DOM to more labile forms may have an effect on MeHg production rates caused by the stimulation of microbial activity. Further research should focus on the relative importance of photodegradation as both a competitive and synergistic process in the net production of MeHg in vegetated and open water areas of wetlands, with optical measurements of DOM providing critical information about both processes.

Additionally, the results of this study provide valuable information about the relationships between photolytic degradation of both MeHg and DOM that may be used to develop tools to improve monitoring programs and aid the development of a regulatory framework for reducing MeHg exposure in aquatic systems. Optical measurements of DOM may provide a useful tool for understanding factors controlling MeHg photodemethylation in situ. Initial MeHg concentrations appear to be related to bulk DOM concentration and possibly indicators of DOM lability or general field productivity (T peak) whereas the loss of MeHg appears to be related more to the loss of specific humic structures within the DOM which fluoresce in the area of FDOM (Cory et al., 2010). Simple deduction would suggest that a ratio between optical indicators in these regions could provide the information necessary to identify MeHg concentration across both its production and loss, but no such diagnostic ratio was identified in this study. Further efforts should attempt to identify ratios within and between absorbance and fluorescence spectra that may capture the integrative effect of MeHg production and loss processes. Other measures of MeHg production such as iron and sulfur speciation or dissolved manganese concentration may help predict initial conditions that control initial MeHg–DOM relationships (Alpers et al., 2014–in this issue; Marvin-DiPasquale et al., 2014–in this issue).

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# Investigative Report of Scientific Misconduct and Conflict of Interest, U.S. Geological Survey

Date Posted to Web: December 12, 2014

This is a version of the report prepared for public release.

## SYNOPSIS

This office received allegations of scientific misconduct and conflict of interest associated with U.S. Geological Survey (USGS) Open File Report 2010-1325A, titled "The Effects of Sediment and Mercury Mobilization in the South Yuba River and Humbug Creek Confluence Area, Nevada County, California: Concentrations, Speciation, and Environmental Fate—Part 1: Field Characterization."

Our investigation did not disclose any evidence of scientific misconduct or conflict of interest by the scientist in the USGS study.

This investigation is closed with no further action by this office. The allegations have been reviewed by this office, including consultations with the USGS ethics officer and the USGS scientific integrity officer, and determined to be unsubstantiated.

## DETAILS OF INVESTIGATION

The U.S. Department of the Interior, Office of Inspector General, received allegations that a USGS research chemist deliberately omitted data while conducting a study and concluding that suction dredge mining could contribute to the increase of methylmercury levels in biota in California waterways. According to the complaint, the research chemist withheld available scientific data from his study, which the complainant alleged would have resulted in a different scientific conclusion. The complainant obtained this additional data via USGS Freedom of Information Act (FOIA) Request 2013-00085.

The complaint also alleged that the research chemist's membership in and support of the Sierra Fund's (TSF) activities presented a conflict of interest and created the appearance that the research chemist used his professional capacity to support a private organization. TSF is a nonprofit organization whose mission is to protect and restore the natural resources and communities of the Sierra Nevada region; one of TSF's primary goals is to stop suction dredging. According to documents in the complaint, the research chemist spoke at several conferences hosted by TSF and was a private donor to the organization.

Coordination with the USGS deputy ethics officer and deputy ethics counselor revealed that the research chemist's membership in TSF was authorized and complemented USGS interests. Private donations to such organizations by USGS employees are not regulated because they do not create a conflict of interest; an ethical question would only arise if an employee were receiving compensation from the organization. The deputy ethics officer's review of the research chemist's file showed that he is in compliance with ethical rules and responsibilities and there were no other complaints against him.

According to the USGS scientific integrity officer (SIO), the research chemist's work on Open File Report 2010-1325A (South Yuba River Study) presented no scientific integrity issues. The SIO explained that there is a growing trend for people to file scientific integrity complaints in an effort to change legislative decisions they do not like; the object is to undermine the scientific

basis for the decision in an effort to have the decision reversed or overturned by the courts. The SIO and the deputy ethics officer discussed the research chemist's activities during his tenure at USGS and concluded that the research chemist's record is "above the board" regarding ethics issues.

An interview of the complainant revealed two primary concerns: whether the research chemist purposefully omitted data from the study and whether his association with TSF biased his scientific work product. The complainant questioned the research chemist's choice to analyze only 1 year of mercury data when many years' worth of mercury data was available. An associate of the complainant consolidated the mercury data received via the USGS FOIA request and the data from the research chemist's study into one graph. According to the complainant, the graph portrays the variation and natural fluctuation in mercury levels in the South Yuba River watershed, which would have led to a different scientific conclusion had the research chemist incorporated the data into his analysis. In addition, the complainant believes the research chemist's association with TSF is inappropriate; the research chemist's attendance at TSF functions created the appearance of a conflict of interest.

The research chemist confirmed that USGS Open File Reports are fully peer reviewed, just like any USGS report would be. Each report is reviewed for quality control purposes by two colleagues, a supervisor, a water specialist, and a data specialist; projects are also reviewed at the proposal level before the study begins. The Bureau of Land Management (BLM) and the California Water Board (CWB) funded the South Yuba River Study to determine mercury characterization and speciation, to characterize mercury levels in biota, and to evaluate the viability of suction dredging as a means to remove mercury from the watershed. In the study, the research chemist conducted a dry run with a 3-inch-diameter suction dredge in a low-mercury-level area, and he found little mercury (as expected). He planned to run another test in 2008 with a larger diameter dredge at a hotspot (a location known to have high levels of mercury), but CWB objected because of concern the test would cause more damage to the environment. According to the research chemist, CWB did not want dredging to be the solution to the mercury problem; instead, CWB wanted to ban suction dredging, which it did in 2008.

The research chemist emphasized that USGS is strictly a science agency with no regulatory function. USGS is concerned only with collecting and providing data while other agencies decide policy. Because the research chemist was precluded from determining whether dredging mobilizes mercury through direct testing (i.e., testing with the large diameter dredge), the second part of the study instead focused on characterizing the sedimentation process in the laboratory. The team also conducted some biological monitoring of mercury levels found in invertebrates within the study sites. The research chemist claimed he did not expect to find conclusive results in the 1 or 2-day invertebrate testing because the methylmercury integration process takes weeks to months, but the team collected what little data it could anyway. Additionally, lab simulations of mercury mobilization using the collected sediment samples were designed to show how mercury would transform (i.e., become methylated and/or reactive) if it was transported and deposited downstream as it would as a result of suction dredging.

The research chemist received the FOIA response containing biological mercury data; BLM paid

for a biological mercury study from 1999 to 2004 with samples taken from over 220 sites. He stated that he did not hide the additional data, but simply did not incorporate it into the South Yuba River Study because the older samples originated from different locations under unknown conditions. He did not know whether the additional data would have changed the conclusions of the report. He admitted to speculating that dredging may impact mercury levels in biota based on the results of his study; however, he also emphasized in the conclusion section of the South Yuba River Study that more study is required to verify the relationship between suction dredging and mercury level increases in biota. He believed the state may have selectively used the data from the South Yuba River Study for its Environmental Impact Report (EIR), but claimed he cannot control how his report is used by other entities (this EIR contributed to the legislative ban on suction dredge mining in California waters).

The research chemist confirmed that he sits on an advisory board for TSF, as do members of many other Federal and State agencies. He described TSF as a non-profit advocacy group in Nevada City, CA, which has completed several projects in the Sierra Nevada region related to mining and the environment. He classified his relationship with TSF as purely professional, and stated he keeps his distance because the chief executive officer of TSF has become a "target" due to her strong anti-mining stance. The research chemist donated his time to TSF by reviewing reports to ensure TSF was citing USGS reports accurately. He also attended TSF meetings, with many other agencies in attendance, to discuss environmental issues associated with mining. He claimed that TSF is trying to change laws and raise money for anti-mining lobbying, but that USGS is not involved in regulation or advocacy and has no bias regarding mining.

## SUBJECT

Research chemist, USGS.

## DISPOSITION

This investigation is closed with no further action by this office. The allegations have been reviewed by this office, including consultations with the USGS ethics officer and the USGS SIO, and determined to be unsubstantiated.

# Mercury Measurements Over Time

South Yuba River Collection Sites

Data Provided by the USGS in response to FOIA 2013-00085

# Explanation

- Not all sites had consistent sampling of the same species
- For those sites where multiple species were sampled in the same year, the sample with the largest sample size was selected
- No manipulation or analysis of the data was performed, this is a simple plot of the MeHg measurements as provided by the USGS
- Data received from the FOIA is provided separately exactly as we received it from the USGS

# Conclusions

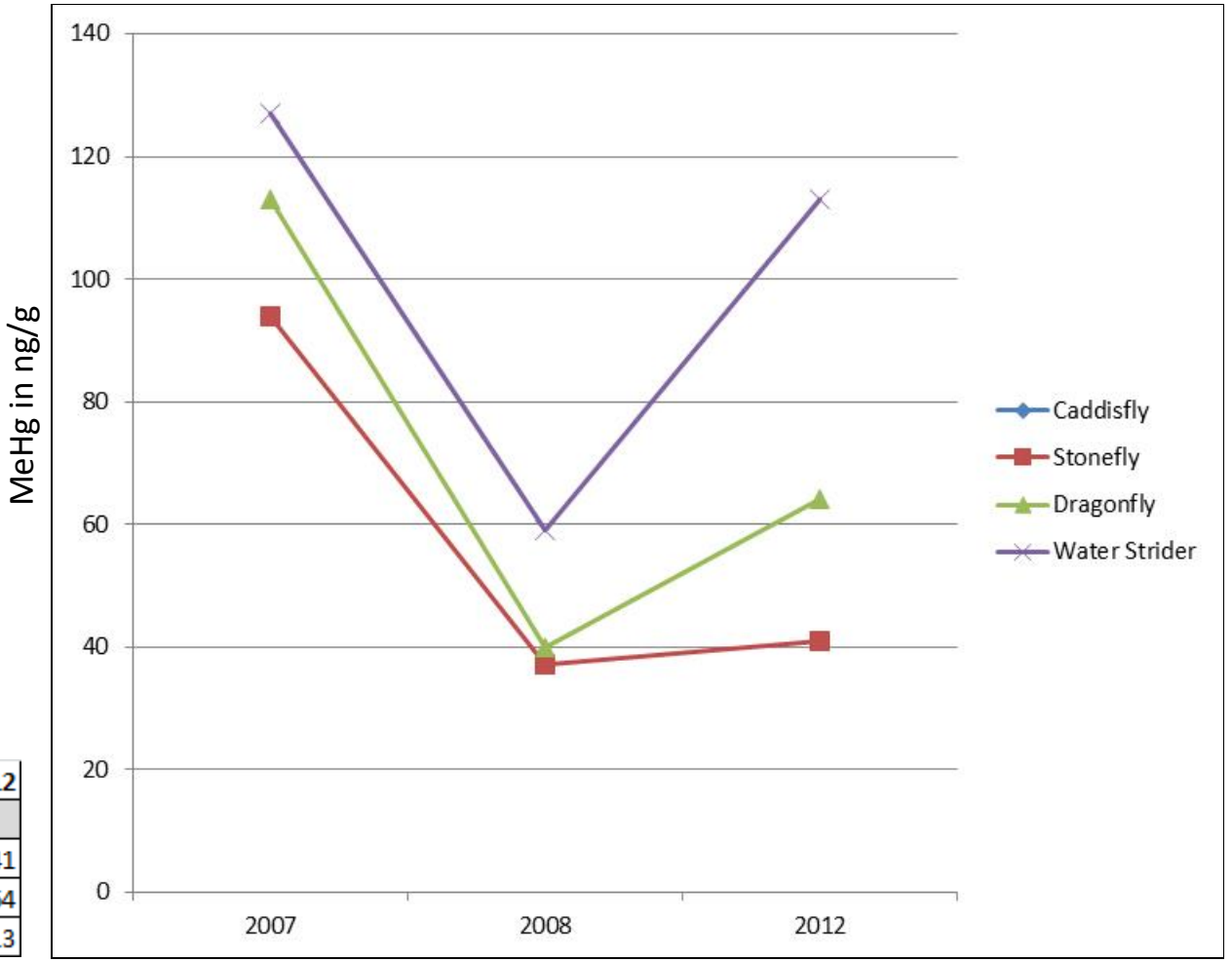
- There is natural variability in MeHg measurements year to year, month to month, and even with samples collected from the same site on different days
- Higher measurements in 2007 appear to be correlated to a 20% higher spring flood than in 2008. The corresponding decrease in 2008 appears to be related to the lack of a spring flood
- Other studies have confirmed there is a correlation (spike) in MeHg levels and the size and timing of the spring flood
  - Reference *Deer Creek Mercury Study* conducted by Sierra Fund scientist Dr. Carrie Monohan in 2006-2007
- No correlation can be drawn from the data that suction dredging, or the lack of suction dredging is in any way correlated to MeHg levels in biota

# Site HUM1

## Site Location:

| | |
|---|---|
| 39.20.18.6 | -120.55.55.2 |

Humbug Creek upstream from South Yuba River




Measurements of MeHG in ng/g

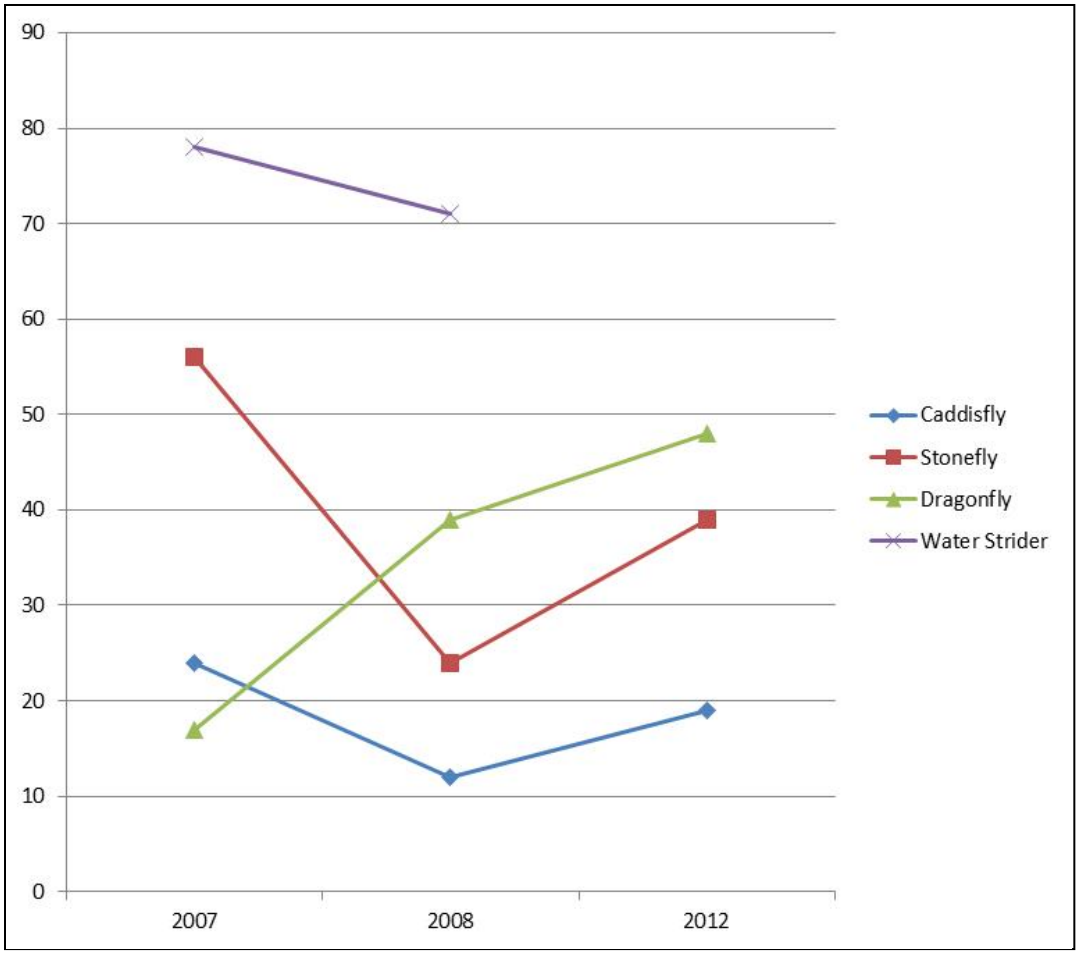
| | 2007 | 2008 | 2012 |
|---|---|---|---|
| Caddisfly | | | |
| Stonefly | 94 | 37 | 41 |
| Dragonfly | 113 | 40 | 64 |
| Water Strider | 127 | 59 | 113 |

# Site SYR-1

Site Location:

| | |
|---|---|
| 39.20.16.8 | -120.55.55.8 |

Junction of Humbug Creek and South Yuba River – Site of the dredge test, and soil sampling for the dredging SEIR in 2008




Measurements of MeHG in ng/g

| | 2007 | 2008 | 2012 |
|---|---|---|---|
| Caddisfly | 24 | 12 | 19 |
| Stonefly | 56 | 24 | 39 |
| Dragonfly | 17 | 39 | 48 |
| Water Strider | 226 | 78 | 71 |

# Site SYR-4

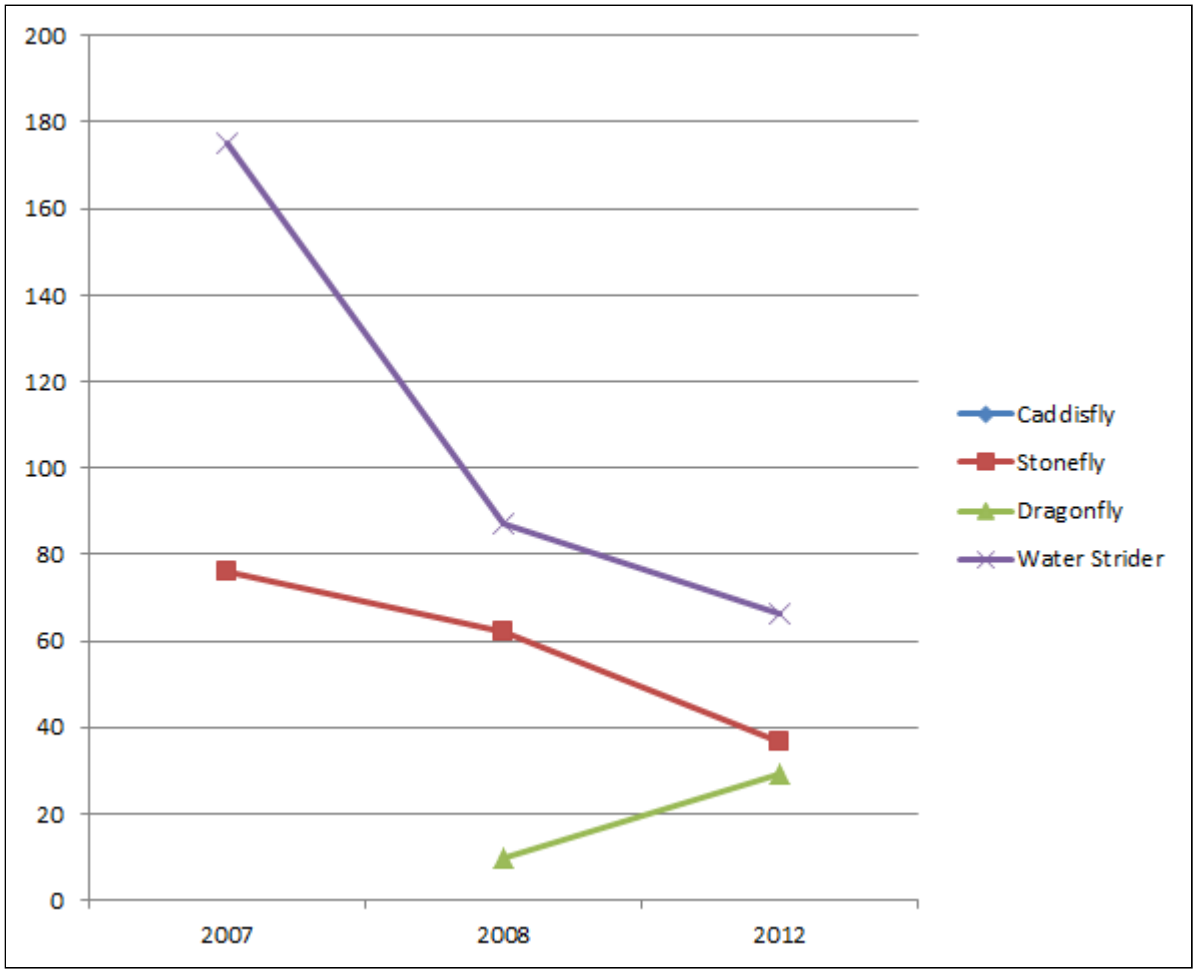
Site Location:

| | |
|---|---|
| 39.20.9.2 | -120.56.8.1 |

South Yuba River, downstream of the 2007 dredging pool

Measurements of MeHG in ng/g

| | 2007 | 2008 | 2012 |
|---|---|---|---|
| Caddisfly | | | |
| Stonefly | 76 | 62 | 36.8 |
| Dragonfly | | 10 | 29 |
| Water Strider | 175 | 87 | 66.3 |



# Site SYR-6

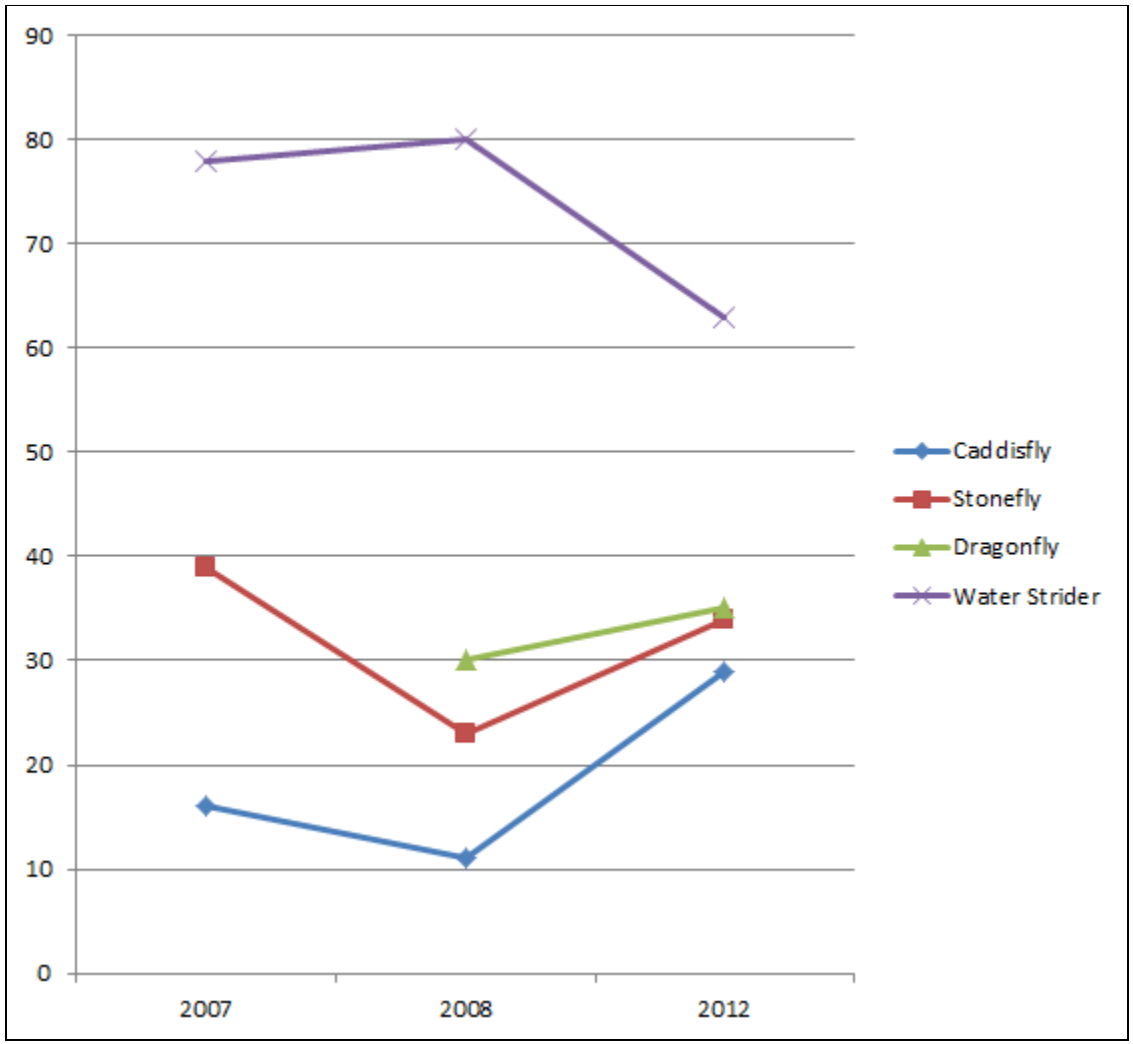
Site Location:

| | |
|---|---|
| 39.20.31.0 | -120.56.58.6 |

South Yuba River at North Canyon

Measurements of MeHG in ng/g

| SYR6 | 2007 | 2008 | 2012 |
|---|---|---|---|
| Caddisfly | 16 | 11 | 29 |
| Stonefly | 39 | 23 | 34 |
| Dragonfly | | 30 | 35 |
| Water Strider | 78 | 80 | 63 |

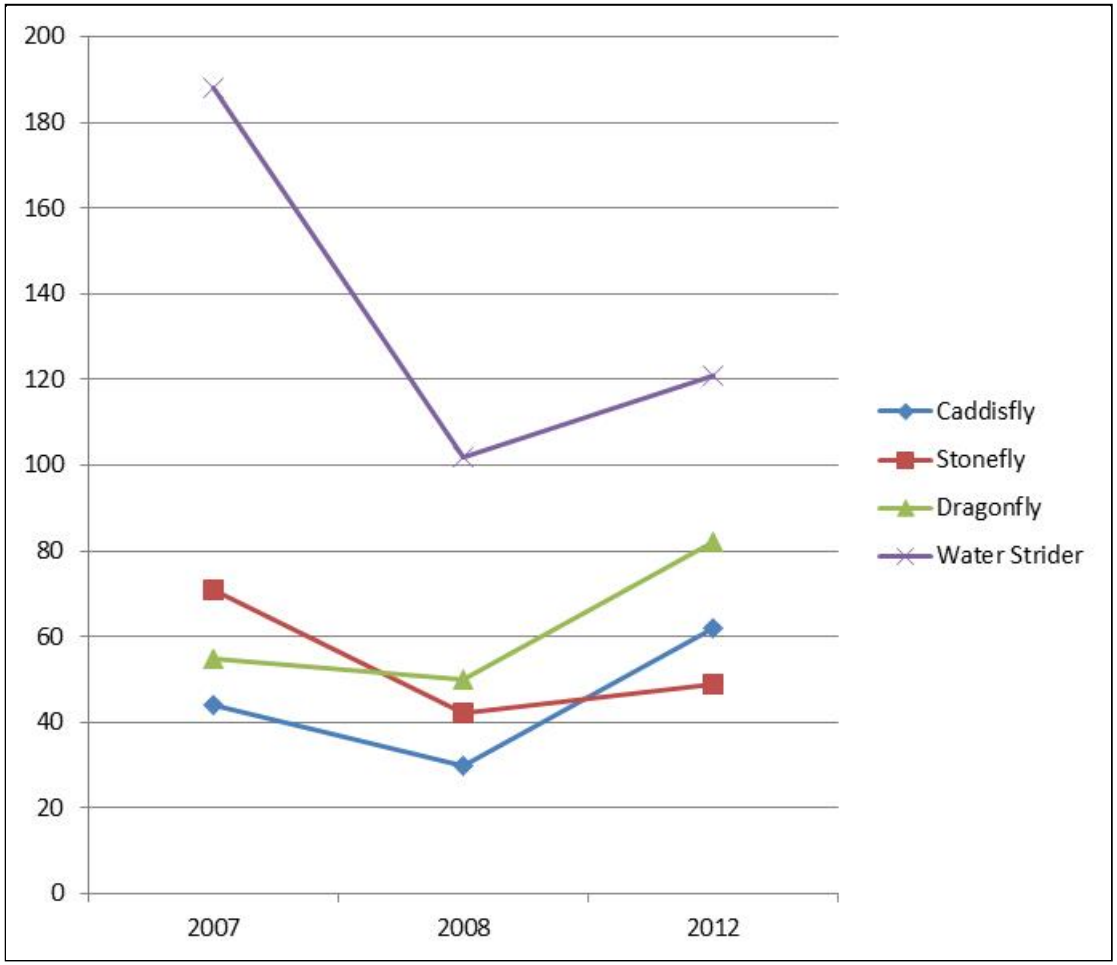


# Site SYR-7

Site Location:

39.19.48.2 -120.59..7.8

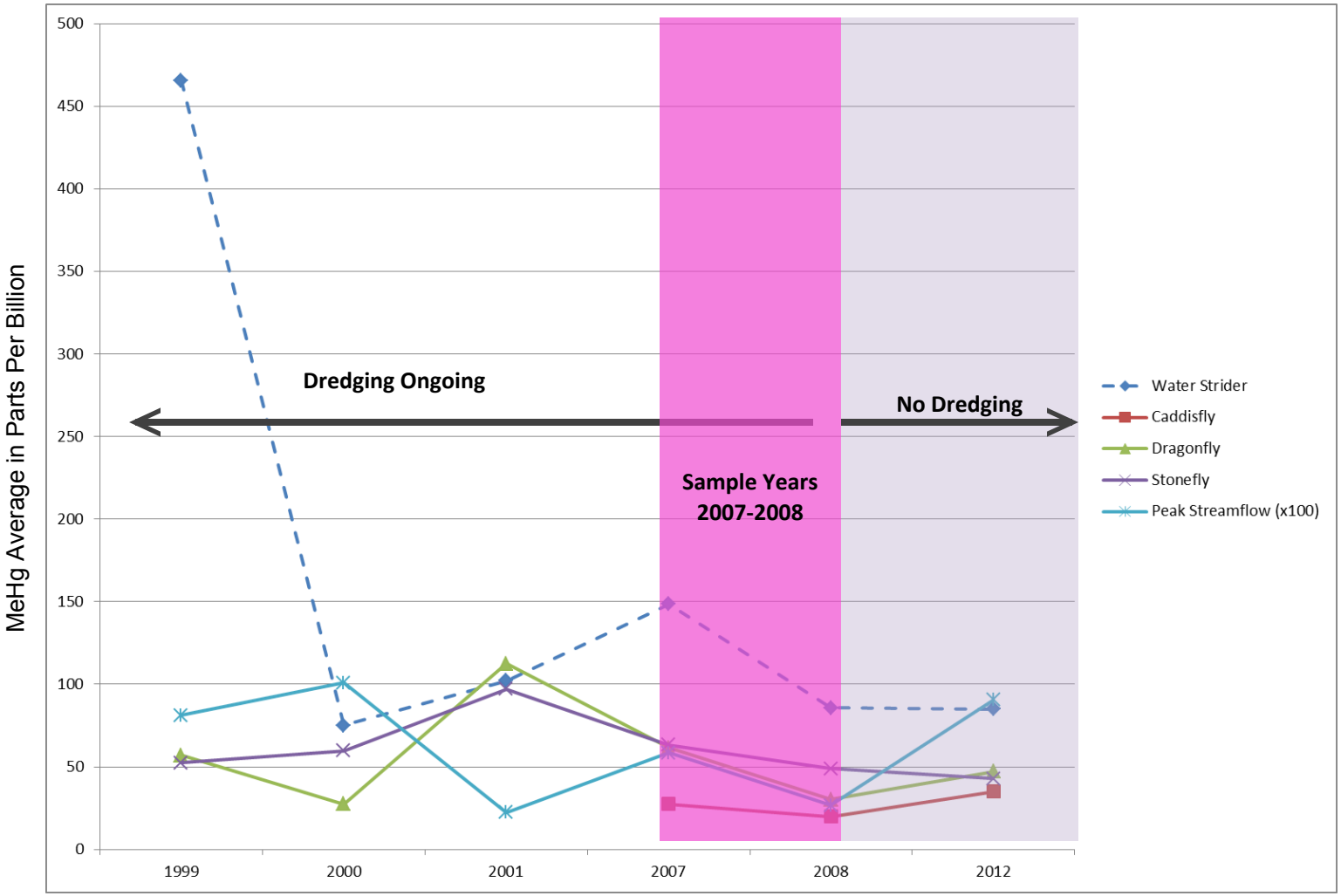
South Yuba River at Edwards Crossing




Measurements of MeHG in ng/g

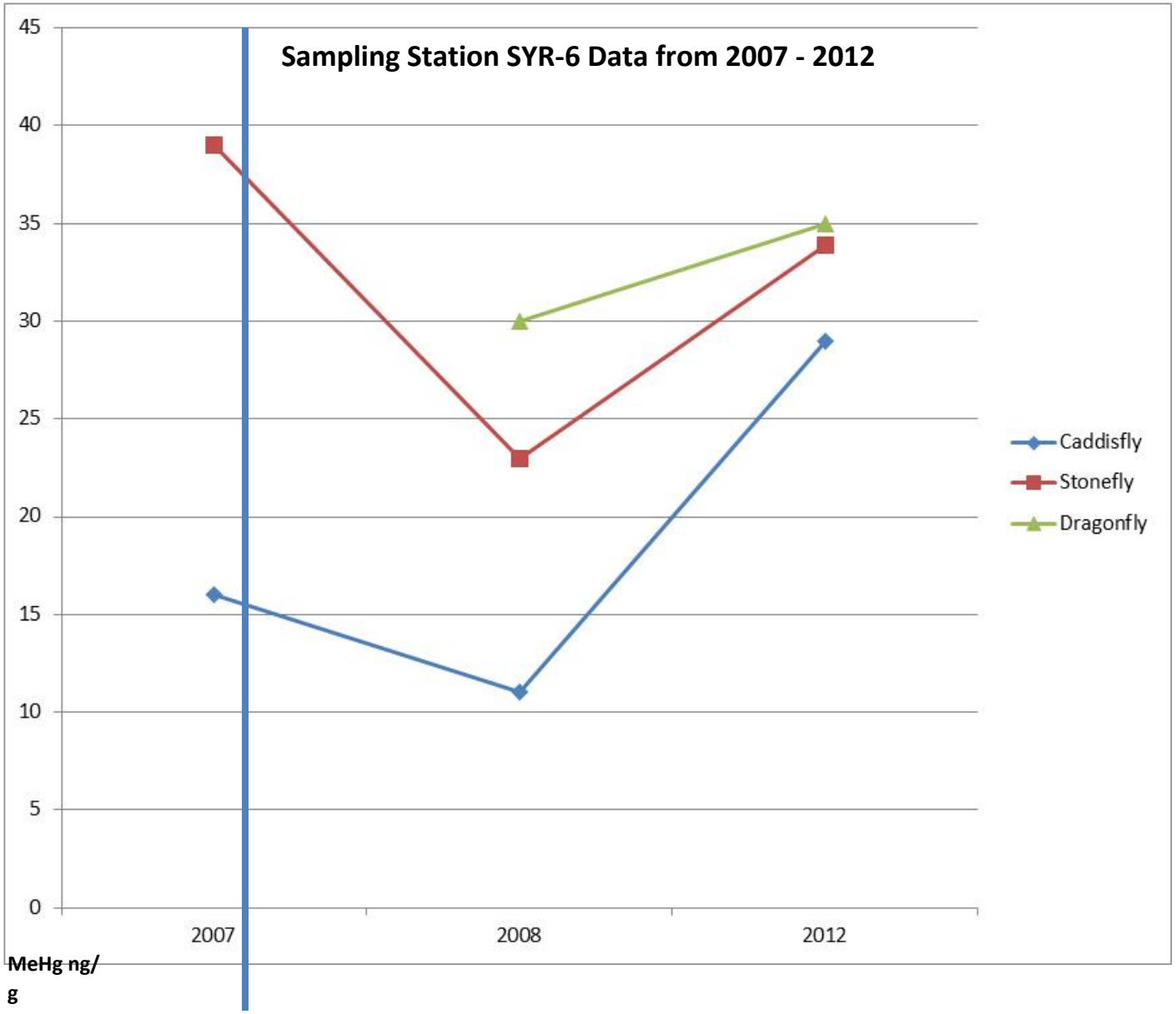
| SYR7 | 2007 | 2008 | 2012 |
|---|---|---|---|
| Caddisfly | 44 | 30 | 62 |
| Stonefly | 71 | 42 | 49 |
| Dragonfly | 55 | 50 | 82 |
| Water Strider | 188 | 102 | 121 |

# Mercury Measurements of Insects in S. Yuba River Over Time




Water Year

Note: This chart uses all of the USGS data from all relevant stations




Note: This chart uses only Sampling Station SYR -6 which just downstream from the dredge test site

MeHg Sampling Locations 1999-2012: Yellow Pins = 2012 sampling
Note: Dredge test sampling 2007/2008 and 2012 sampling are from identical locations

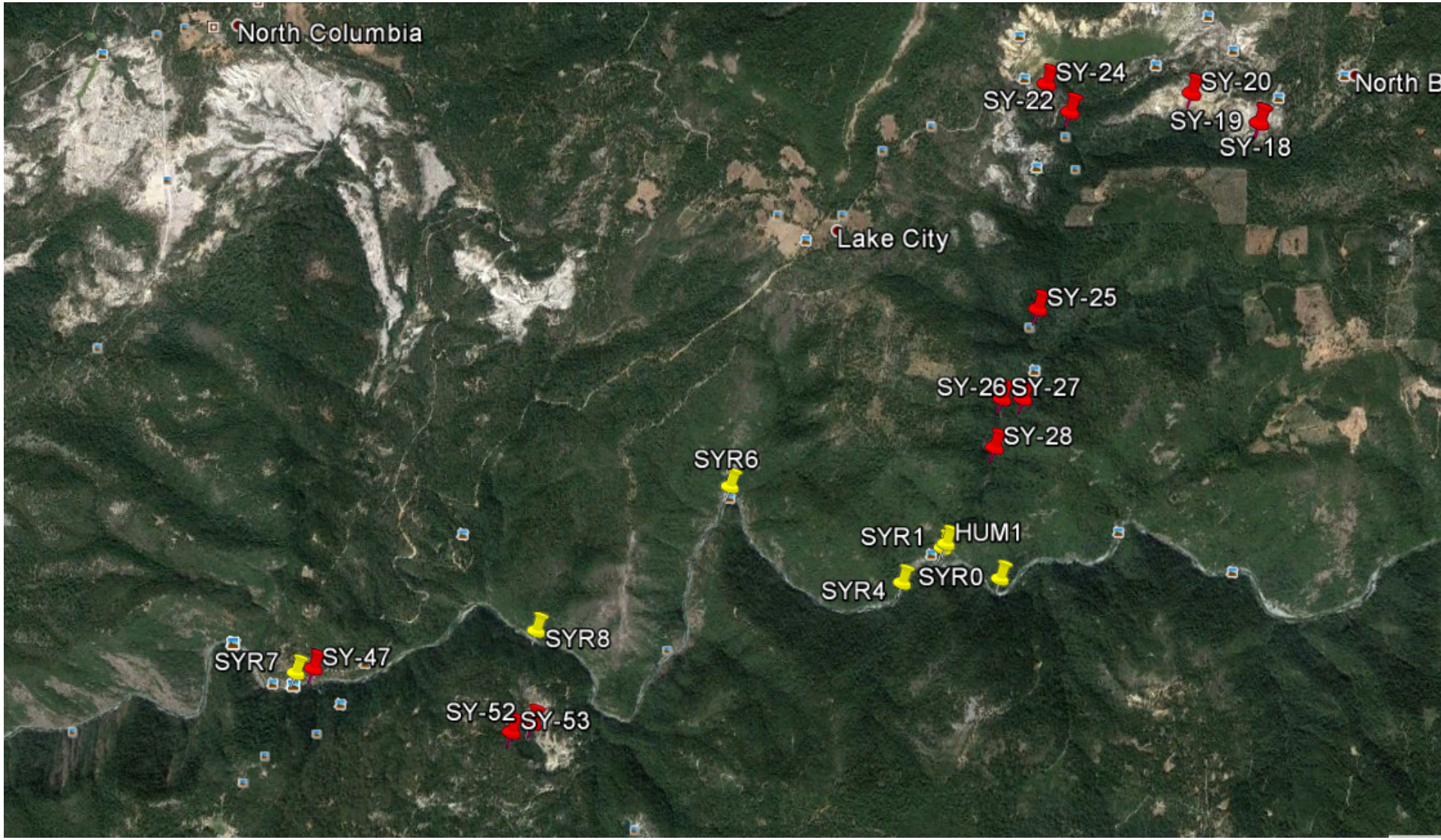


Table 1. Sampling sites for biota, South Yuba River and Humbug Creek, 2012

| | | | Planned | | Actual | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Site** | **Site Code** | **Distance upstream from SYR-7 (m)** | **Latitude** | **Longitude** | **Latitude** | **Longitude** | **Invert-ebrates** | **Fish** | **Invert-ebrates sampled in 2007, 2008** |
| South Yuba River 500 m upstream of Humbug Creek | SYR0 | 7,470 | 39°20'9.9" | 120°55'38.7" | 39°20'9.9" | 120°55'38.7" | Yes | Yes | |
| Humbug Creek near South Yuba River | HUM1 | 100 * | 39°20'18.6" | 120°55'55.2" | 39°20'18.6" | 120°55'55.2" | Yes | | Yes |
| South Yuba River just downstream of Humbug Creek confluence | SYR1 | 6,970 | 39°20'16.8" | 120°55'55.8" | 39°20'16.8" | 120°55'55.8" | Yes | | Yes |
| South Yuba River, bottom of pool downstream Dredge Site | SYR4 | 6,720 | 39°20'9.2" | 120°56'8.1" | 39°20'9.2" | 120°56'8.1" | Yes | | Yes |
| South Yuba River downstream North Canyon | SYR6 | 4,860 | 39°20'31.0" | 120°56'58.6" | 39°20'31.0" | 120°56'58.6" | Yes | | Yes |
| South Yuba River at Illinois Crossing | SYR8 | 1,860 | 39°20'1.7" | 120°58'2.8" | 39°19'48.2" | 120°59'7.8" | Yes | | |
| South Yuba River at Edwards Crossing | SYR7 | - | 39°19'48.2" | 120°59'7.8" | 39°19'58.4" | 120°57'55.7" | Yes | Yes | Yes |

* Distance upstream from SYR1

Table 2. Preliminary mercury data for invertebrates, South Yuba River and Humbug Creek, 2012

[g, gram; %, percent; ng, nanogram; µg, microgram; Hg, mercury; MeHg, monomethylmercury; ww, wet weight; u/s, upstream; d/s, downstream]

| Unique Sample Code | Site | Site Code | Latitude (WGS84) | Longitude (WGS84) | Collection Date | Collection Time | Process Date | Order | Family | Number of individual organisms | Total mass (g) | Avg. mass per organism (g) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HUM1-080612-001 | Humbug Ck. u/s of S. Yuba R. | HUM1 | 39°20'18.6" | 120°55'55.2" | 8/6/12 | 1:30 PM | 8/7/12 | Hemiptera | Gerridae | 25 | 1.62 | 0.065 |
| HUM1-080612-002 | Humbug Ck. u/s of S. Yuba R. | HUM1 | 39°20'18.6" | 120°55'55.2" | 8/6/12 | 1:30 PM | 8/7/12 | Plecoptera | Perlidae | 10 | 1.61 | 0.161 |
| HUM1-080612-003 | Humbug Ck. u/s of S. Yuba R. | HUM1 | 39°20'18.6" | 120°55'55.2" | 8/6/12 | 1:30 PM | 8/7/12 | Odonata | Gomphidae | 7 | 1.46 | 0.209 |
| SYR0-080612-001 | S. Yuba R. u/s of Humbug Ck. | SYR0 | 39°20'09.9" | 120°55'38.7" | 8/6/12 | 11:02 AM | 8/7/12 | Hemiptera | Gerridae | 23 | 1.29 | 0.056 |
| SYR0-080612-002 | S. Yuba R. u/s of Humbug Ck. | SYR0 | 39°20'09.9" | 120°55'38.7" | 8/6/12 | 11:02 AM | 8/7/12 | Hemiptera | Gerridae | 23 | 1.38 | 0.060 |
| SYR0-080612-003 | S. Yuba R. u/s of Humbug Ck. | SYR0 | 39°20'09.9" | 120°55'38.7" | 8/6/12 | 11:02 AM | 8/7/12 | Odonata | Gomphidae | 5 | 1.09 | 0.218 |
| SYR0-080612-004 | S. Yuba R. u/s of Humbug Ck. | SYR0 | 39°20'09.9" | 120°55'38.7" | 8/6/12 | 11:02 AM | 8/7/12 | Plecoptera | Perlidae | 10 | 1.60 | 0.160 |
| SYR0-080612-005 | S. Yuba R. u/s of Humbug Ck. | SYR0 | 39°20'09.9" | 120°55'38.7" | 8/6/12 | 11:02 AM | 8/7/12 | Plecoptera | Perlidae | 15 | 1.43 | 0.095 |
| SYR0-080612-006 | S. Yuba R. u/s of Humbug Ck. | SYR0 | 39°20'09.9" | 120°55'38.7" | 8/6/12 | 11:02 AM | 8/7/12 | Trichoptera | Hydropsychidae | 58 | 1.51 | 0.026 |
| SYR1-080612-001 | S. Yuba R. at Humbug Ck. | SYR1 | 39°20'16.8" | 120°55'55.8" | 8/6/12 | 2:10 PM | 8/8/12 | Trichoptera | Hydropsychidae | 57 | 1.37 | 0.024 |
| SYR1-080612-002 | S. Yuba R. at Humbug Ck. | SYR1 | 39°20'16.8" | 120°55'55.8" | 8/6/12 | 2:10 PM | 8/8/12 | Hemiptera | Gerridae | 22 | 1.09 | 0.050 |
| SYR1-080612-003 | S. Yuba R. at Humbug Ck. | SYR1 | 39°20'16.8" | 120°55'55.8" | 8/6/12 | 2:10 PM | 8/8/12 | Odonata | Gomphidae | 5 | 1.49 | 0.298 |
| SYR1-080612-004 | S. Yuba R. at Humbug Ck. | SYR1 | 39°20'16.8" | 120°55'55.8" | 8/6/12 | 2:10 PM | 8/8/12 | Plecoptera | Perlidae | 12 | 1.44 | 0.120 |
| SYR4-080612-001 | S. Yuba R. d/s of dredge pond | SYR4 | 39°20'9.2" | 120°56'8.1" | 8/6/12 | 4:00 PM | 8/8/12 | Odonata | Gomphidae | 7 | 1.79 | 0.256 |
| SYR4-080612-002 | S. Yuba R. d/s of dredge pond | SYR4 | 39°20'9.2" | 120°56'8.1" | 8/6/12 | 4:00 PM | 8/8/12 | Plecoptera | Perlidae | 6 | 1.00 | 0.167 |
| SYR4-080612-003 | S. Yuba R. d/s of dredge pond | SYR4 | 39°20'9.2" | 120°56'8.1" | 8/6/12 | 4:00 PM | 8/8/12 | Plecoptera | Perlidae | 7 | 0.80 | 0.114 |
| SYR4-080612-004 | S. Yuba R. d/s of dredge pond | SYR4 | 39°20'9.2" | 120°56'8.1" | 8/6/12 | 4:00 PM | 8/8/12 | Hemiptera | Gerridae | 28 | 1.49 | 0.053 |
| SYR6-080712-001 | S. Yuba R. at North Canyon | SYR6 | 39°20'31.0" | 120°56'58.6" | 8/7/12 | 11:00 AM | 8/8/12 | Hemiptera | Gerridae | 25 | 1.44 | 0.058 |
| SYR6-080712-002 | S. Yuba R. at North Canyon | SYR6 | 39°20'31.0" | 120°56'58.6" | 8/7/12 | 11:00 AM | 8/8/12 | Odonata | Gomphidae | 8 | 1.30 | 0.163 |
| SYR6-080712-003 | S. Yuba R. at North Canyon | SYR6 | 39°20'31.0" | 120°56'58.6" | 8/7/12 | 11:00 AM | 8/8/12 | Plecoptera | Perlidae | 9 | 1.37 | 0.152 |
| SYR6-080712-004 | S. Yuba R. at North Canyon | SYR6 | 39°20'31.0" | 120°56'58.6" | 8/7/12 | 11:00 AM | 8/8/12 | Plecoptera | Perlidae | 17 | 2.01 | 0.118 |
| SYR6-080712-005 | S. Yuba R. at North Canyon | SYR6 | 39°20'31.0" | 120°56'58.6" | 8/7/12 | 11:00 AM | 8/8/12 | Trichoptera | Hydropsychidae | 45 | 1.03 | 0.023 |
| SYR6-080712-006 | S. Yuba R. at North Canyon | SYR6 | 39°20'31.0" | 120°56'58.6" | 8/7/12 | 11:00 AM | 8/8/12 | Trichoptera | Hydropsychidae | 65 | 1.15 | 0.018 |
| SYR7-081712-001 | S.Yuba R. at Edwards Crossing | SYR7 | 39°19'48.2" | 120°59'7.8" | 8/17/12 | 11:00 AM | 8/17/12 | Odonata | Gomphidae | 3 | 1.04 | 0.347 |
| SYR7-081712-002 | S.Yuba R. at Edwards Crossing | SYR7 | 39°19'48.2" | 120°59'7.8" | 8/17/12 | 11:00 AM | 8/17/12 | Odonata | Gomphidae | 5 | 0.80 | 0.160 |
| SYR7-081712-003 | S.Yuba R. at Edwards Crossing | SYR7 | 39°19'48.2" | 120°59'7.8" | 8/17/12 | 11:00 AM | 8/17/12 | Plecoptera | Perlidae | 10 | 1.48 | 0.148 |
| SYR7-081712-004 | S.Yuba R. at Edwards Crossing | SYR7 | 39°19'48.2" | 120°59'7.8" | 8/17/12 | 11:00 AM | 8/17/12 | Plecoptera | Perlidae | 16 | 1.48 | 0.093 |
| SYR7-081712-005 | S.Yuba R. at Edwards Crossing | SYR7 | 39°19'48.2" | 120°59'7.8" | 8/17/12 | 11:00 AM | 8/17/12 | Trichoptera | Hydropsychidae | 40 | 0.77 | 0.019 |
| SYR7-081712-006 | S.Yuba R. at Edwards Crossing | SYR7 | 39°19'48.2" | 120°59'7.8" | 8/17/12 | 11:00 AM | 8/17/12 | Hemiptera | Gerridae | 30 | 1.64 | 0.055 |
| SYR7-081712-007 | S.Yuba R. at Edwards Crossing | SYR7 | 39°19'48.2" | 120°59'7.8" | 8/17/12 | 11:00 AM | 8/17/12 | Hemiptera | Gerridae | 30 | 1.60 | 0.053 |
| SYR8-082712-001 | S. Yuba R. at Illinois Crossing | SYR8 | 39°19'58.4" | 120°57'55.7" | 8/27/12 | 11:00 AM | 8/28/12 | Hemiptera | Gerridae | 30 | 1.57 | 0.052 |
| SYR8-082712-002 | S. Yuba R. at Illinois Crossing | SYR8 | 39°19'58.4" | 120°57'55.7" | 8/27/12 | 11:00 AM | 8/28/12 | Plecoptera | Perlidae | 10 | 1.77 | 0.177 |
| SYR8-082712-003 | S. Yuba R. at Illinois Crossing | SYR8 | 39°19'58.4" | 120°57'55.7" | 8/27/12 | 11:00 AM | 8/28/12 | Plecoptera | Perlidae | 14 | 1.77 | 0.126 |
| SYR8-082712-004 | S. Yuba R. at Illinois Crossing | SYR8 | 39°19'58.4" | 120°57'55.7" | 8/27/12 | 11:00 AM | 8/28/12 | Odonata | Gomphidae | 7 | 2.11 | 0.301 |
| SYR8-082712-005 | S. Yuba R. at Illinois Crossing | SYR8 | 39°19'58.4" | 120°57'55.7" | 8/27/12 | 11:00 AM | 8/28/12 | Trichoptera | Hydropsychidae | 80 | 1.43 | 0.018 |

Table 2. Preliminary mercury data for invertebrates, South Yuba River and Humbug Creek, 2012

[g, gram; %, percent; ng, nanogram; µg, microgram; Hg, mercury; MeHg, monomethylmercury; ww, wet weight; u/s, upstream; d/s, downstream]

| Unique Sample Code | Site | Site Code | % solids | % moisture | Hg (ng/g ww) | Hg (µg/g ww) | MeHg (ng/g ww) | MeHg (µg/g ww) | % MeHg |
|---|---|---|---|---|---|---|---|---|---|
| HUM1-080612-001 | Humbug Ck. u/s of S. Yuba R. | HUM1 | 34.3 | 65.7 | 118.0 | 0.118 | 113.0 | 0.113 | 95.76 |
| HUM1-080612-002 | Humbug Ck. u/s of S. Yuba R. | HUM1 | 21.3 | 78.7 | 61.6 | 0.062 | 41.3 | 0.041 | 67.05 |
| HUM1-080612-003 | Humbug Ck. u/s of S. Yuba R. | HUM1 | 21.8 | 78.2 | 87.6 | 0.088 | 64.1 | 0.064 | 73.17 |
| SYR0-080612-001 | S. Yuba R. u/s of Humbug Ck. | SYR0 | 31.4 | 68.6 | 85.0 | 0.085 | 81.2 | 0.081 | 95.53 |
| SYR0-080612-002 | S. Yuba R. u/s of Humbug Ck. | SYR0 | 32.4 | 67.6 | 84.1 | 0.084 | 62.2 | 0.062 | 73.96 |
| SYR0-080612-003 | S. Yuba R. u/s of Humbug Ck. | SYR0 | 21.7 | 78.3 | 43.0 | 0.043 | 40.0 | 0.040 | 93.02 |
| SYR0-080612-004 | S. Yuba R. u/s of Humbug Ck. | SYR0 | 18.6 | 81.4 | 54.5 | 0.055 | 39.9 | 0.040 | 73.21 |
| SYR0-080612-005 | S. Yuba R. u/s of Humbug Ck. | SYR0 | 20.3 | 79.7 | 45.7 | 0.046 | 41.7 | 0.042 | 91.25 |
| SYR0-080612-006 | S. Yuba R. u/s of Humbug Ck. | SYR0 | 19.2 | 80.8 | 51.4 | 0.051 | 32.7 | 0.033 | 63.62 |
| SYR1-080612-001 | S. Yuba R. at Humbug Ck. | SYR1 | 23.6 | 76.4 | 29.8 | 0.030 | 19.2 | 0.019 | 64.43 |
| SYR1-080612-002 | S. Yuba R. at Humbug Ck. | SYR1 | 30.4 | 69.6 | 133.0 | 0.133 | 71.2 | 0.071 | 53.53 |
| SYR1-080612-003 | S. Yuba R. at Humbug Ck. | SYR1 | 24.0 | 76.0 | 138.0 | 0.138 | 48.3 | 0.048 | 35.00 |
| SYR1-080612-004 | S. Yuba R. at Humbug Ck. | SYR1 | 20.8 | 79.2 | 59.6 | 0.060 | 39.4 | 0.039 | 66.11 |
| SYR4-080612-001 | S. Yuba R. d/s of dredge pond | SYR4 | 23.4 | 76.6 | 40.9 | 0.041 | 28.6 | 0.029 | 69.93 |
| SYR4-080612-002 | S. Yuba R. d/s of dredge pond | SYR4 | 26.7 | 73.3 | 63.3 | 0.063 | 40.8 | 0.041 | 64.45 |
| SYR4-080612-003 | S. Yuba R. d/s of dredge pond | SYR4 | 22.0 | 78.0 | 58.1 | 0.058 | 36.8 | 0.037 | 63.34 |
| SYR4-080612-004 | S. Yuba R. d/s of dredge pond | SYR4 | 30.6 | 69.4 | 78.2 | 0.078 | 66.3 | 0.066 | 84.78 |
| SYR6-080712-001 | S. Yuba R. at North Canyon | SYR6 | 33.0 | 67.0 | 80.7 | 0.081 | 63.0 | 0.063 | 78.07 |
| SYR6-080712-002 | S. Yuba R. at North Canyon | SYR6 | 20.5 | 79.5 | 61.3 | 0.061 | 36.0 | 0.036 | 58.73 |
| SYR6-080712-003 | S. Yuba R. at North Canyon | SYR6 | 24.3 | 75.7 | 75.7 | 0.076 | 35.7 | 0.036 | 47.16 |
| SYR6-080712-004 | S. Yuba R. at North Canyon | SYR6 | 23.7 | 76.3 | 50.4 | 0.050 | 33.9 | 0.034 | 67.26 |
| SYR6-080712-005 | S. Yuba R. at North Canyon | SYR6 | 29.9 | 70.1 | 51.9 | 0.052 | 31.2 | 0.031 | 60.12 |
| SYR6-080712-006 | S. Yuba R. at North Canyon | SYR6 | 26.1 | 73.9 | 41.3 | 0.041 | 28.8 | 0.029 | 69.73 |
| SYR7-081712-001 | S.Yuba R. at Edwards Crossing | SYR7 | 23.3 | 76.7 | 61.8 | 0.062 | 39.7 | 0.040 | 64.24 |
| SYR7-081712-002 | S.Yuba R. at Edwards Crossing | SYR7 | 27.5 | 72.5 | 115.0 | 0.115 | 82.0 | 0.082 | 71.30 |
| SYR7-081712-003 | S.Yuba R. at Edwards Crossing | SYR7 | 23.7 | 76.3 | 81.2 | 0.081 | 56.1 | 0.056 | 69.09 |
| SYR7-081712-004 | S.Yuba R. at Edwards Crossing | SYR7 | 24.9 | 75.1 | 80.8 | 0.081 | 49.4 | 0.049 | 61.14 |
| SYR7-081712-005 | S.Yuba R. at Edwards Crossing | SYR7 | 33.6 | 66.4 | 70.6 | 0.071 | 62.6 | 0.063 | 88.67 |
| SYR7-081712-006 | S.Yuba R. at Edwards Crossing | SYR7 | 34.5 | 65.5 | 190.0 | 0.190 | 121.0 | 0.121 | 63.68 |
| SYR7-081712-007 | S.Yuba R. at Edwards Crossing | SYR7 | 34.8 | 65.2 | 217.0 | 0.217 | 106.0 | 0.106 | 48.85 |
| SYR8-082712-001 | S. Yuba R. at Illinois Crossing | SYR8 | 33.8 | 66.2 | 136.0 | 0.136 | 81.0 | 0.081 | 59.56 |
| SYR8-082712-002 | S. Yuba R. at Illinois Crossing | SYR8 | 25.5 | 74.5 | 108.0 | 0.108 | 63.9 | 0.064 | 59.17 |
| SYR8-082712-003 | S. Yuba R. at Illinois Crossing | SYR8 | 23.0 | 77.0 | 60.9 | 0.061 | 47.3 | 0.047 | 77.67 |
| SYR8-082712-004 | S. Yuba R. at Illinois Crossing | SYR8 | 21.3 | 78.7 | 54.9 | 0.055 | 38.7 | 0.039 | 70.49 |
| SYR8-082712-005 | S. Yuba R. at Illinois Crossing | SYR8 | 29.1 | 70.9 | 50.1 | 0.050 | 36.0 | 0.036 | 71.86 |

Table 3. Preliminary mercury data for fish, South Yuba River and Humbug Creek, 2012
[TL, total length; SL, standard length; wt, weight; g, gram; Hg, mercury; %, percent; ww, wet weight; u/s, upstream]

**Site: SYR0 - South Yuba River u/s of Humbug Ck.**

**GPS Coordinates**
Latitude: 39°20'09.9"
Longitude: 120°55'38.7"

**Collection Date/Time:** 8/6/12 11:00 AM

| Sample ID | Species | TL (mm) | SL (mm) | Fish wt (g) | Sample wt (g) | Hg (ng/g ww) | % Solids | NOTES |
|---|---|---|---|---|---|---|---|---|
| SYR0-080612-001F | Rainbow Trout | - | 110 | 14.81 | 14.81 | 85.4 | 27.1 | |
| SYR0-080612-002F | Rainbow Trout | 122 | 106 | 12.71 | 12.71 | 142 | 29.3 | |
| SYR0-080612-003F | Rainbow Trout | 112 | 95 | 12.14 | 12.14 | 93.5 | 29 | |
| SYR0-080612-004F | Sacramento Sucker | 268 | 237 | 176.98 | 176.98 | 152 | 25.4 | |
| SYR0-080612-005F | Sacramento Sucker | 224 | 198 | 92.06 | 92.06 | 113 | 28.4 | |
| SYR0-080612-006F | Sacramento Sucker | 185 | 162 | 62.94 | 62.94 | 82.5 | 36.2 | |
| SYR0-080612-007F | Sacramento Sucker | 181 | 160 | 55.78 | 55.78 | 93.4 | 33.2 | |
| SYR0-080612-008F | Sacramento Sucker | 181 | 159 | 41.86 | 41.86 | 75.8 | 28 | |

Table 3 (continued)

**Site: SYR7 - South Yuba River at Edwards Crossing**

**GPS Coordinates**
Latitude: 39°19'48.2"
Longitude: 120°59'7.8"

**Collection Date/Time:** 8/17/12 9:00 AM

| Sample ID | Species | TL (mm) | SL (mm) | Fish wt (g) | Sample wt (g) | Hg (ng/g ww) | % Solids | NOTES |
|---|---|---|---|---|---|---|---|---|
| SYR7-081712-001LF | Rainbow Trout | 232 | 205 | 104.70 | 8.24 | 143 | 21.6 | |
| SYR7-081712-001RF | Rainbow Trout | 232 | 205 | 104.70 | 6.80 | 129 | 21.6 | |
| SYR7-081712-002LF | Rainbow Trout | 195 | 170 | 70.31 | 6.23 | 103 | 22.6 | |
| SYR7-081712-003LF | Rainbow Trout | 189 | 164 | 55.38 | 4.77 | 138 | 22.7 | |
| SYR7-081712-004LF | Rainbow Trout | 187 | 164 | 59.21 | 5.04 | 212 | 23.9 | |
| SYR7-081712-005LF | Rainbow Trout | 176 | 155 | 55.22 | 4.68 | 155 | 22.6 | |
| SYR7-081712-006LF | Rainbow Trout | 178 | 157 | 54.32 | 3.36 | 231 | 21.5 | |
| SYR7-081712-007LF | Rainbow Trout | 163 | 144 | 43.15 | 4.20 | 139 | 23.7 | |
| SYR7-081712-008LF | Rainbow Trout | 128 | 112 | 19.77 | 1.92 | 107 | 22 | |
| | | | | | | | | |
| SYR7-081712-009LF | Sacramento Sucker | 243 | 213 | 160.73 | 13.33 | 173 | 21.4 | |
| SYR7-081712-009RF | Sacramento Sucker | 243 | 213 | 160.73 | 8.07 | 152 | 20.1 | |
| SYR7-081712-010LF | Sacramento Sucker | 237 | 209 | 135.01 | 7.11 | 238 | 20.7 | |
| SYR7-081712-011LF | Sacramento Sucker | 229 | 200 | 148.00 | 10.20 | 170 | 21.5 | |
| SYR7-081712-012LF | Sacramento Sucker | 221 | 193 | 109.29 | 10.73 | 138 | 19.8 | |
| SYR7-081712-013LF | Sacramento Sucker | 186 | 164 | 72.33 | 6.92 | 172 | 20.2 | |
| SYR7-081712-014LF | Sacramento Sucker | 175 | 152 | 53.38 | 5.37 | 119 | 20 | |
| SYR7-081712-015LF | Sacramento Sucker | 169 | 149 | 54.13 | 4.57 | 132 | 19.6 | |
| SYR7-081712-016LF | Sacramento Sucker | 130 | 112 | 24.55 | 2.00 | 85.3 | 19.8 | |
| SYR7-081712-017LF | Sacramento Sucker | 135 | 117 | 26.39 | 2.75 | 110 | 19.6 | |
| SYR7-081712-018LF | Sacramento Sucker | 128 | 111 | 22.54 | 1.87 | 101 | 19.2 | |
| SYR7-081712-019LF | Sacramento Sucker | 131 | 113 | 24.51 | 2.43 | 81.6 | 19.9 | |
| SYR7-081712-020LF | Sacramento Sucker | 128 | 111 | 21.32 | 2.14 | 69.8 | 19.1 | |
| SYR7-081712-021LF | Sacramento Sucker | 129 | 114 | 21.86 | 1.95 | 72.4 | 18.6 | |
| SYR7-081712-022LF | Sacramento Sucker | 109 | 93 | 13.56 | 1.55 | 96.2 | 19.8 | |

Table 9. Concentrations of total mercury and methylmercury in individual composites of biological samples collected at Humbug Creek and the South Yuba River, California, in September 2007 and September 2008.

[g, gram; No., number of individual oranisms in composite; ng/g, nanogram per gram; ww, wet weight; THg, total mercury; MeHg, methylmercury; %, percent; nd, not determined]

| Unique Sample Code | Site identifier | Year | Order | Family | Age | No. | Total mass (g) | Ave. Mass (g) | % moisture | THg (ng/g ww) | MeHg (ng/g ww) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SYR6-091307-003 | SYR-6 | 2007 | Hemiptera | Gerridae | Adult | 25 | 1.44 | 0.057 | nd | 96 | 78 |
| SYR2-091307-003 | SYR-2 | 2007 | Hemiptera | Gerridae | Adult | 25 | 1.13 | 0.045 | nd | 94 | 85 |
| HUM1-091307-005 | HUM-1 | 2007 | Hemiptera | Gerridae | Adult | 25 | 1.35 | 0.054 | nd | 116 | 127 |
| SYR5-091307-003 | SYR-5 | 2007 | Hemiptera | Gerridae | Adult | 23 | 1.32 | 0.057 | nd | 148 | 145 |
| SYR3-091307-002 | SYR-3 | 2007 | Hemiptera | Gerridae | Adult | 23 | 1.11 | 0.048 | nd | 137 | 165 |
| SYR4-091307-003 | SYR-4 | 2007 | Hemiptera | Gerridae | Adult | 30 | 1.60 | 0.053 | nd | 174 | 175 |
| SYR7-091407-005 | SYR-7 | 2007 | Hemiptera | Gerridae | Adult | 25 | 1.29 | 0.052 | nd | 190 | 188 |
| SYR1-091307-003 | SYR-1 | 2007 | Hemiptera | Gerridae | Adult | 17 | 0.97 | 0.057 | nd | 188 | 226 |
| SYR3-091307-001 | SYR-3 | 2007 | Odonata | Libellulidae | Larva | 3 | 1.24 | 0.413 | nd | 20 | 23 |
| SYR2-091307-002 | SYR-2 | 2007 | Odonata | Libellulidae | Larva | 3 | 1.19 | 0.397 | nd | 51 | 55 |
| SYR7-091407-001 | SYR-7 | 2007 | Odonata | Gomphidae | Larva | 3 | 1.15 | 0.383 | nd | 56 | 55 |
| HUM1-091307-004 | HUM-1 | 2007 | Odonata | Gomphidae | Larva | 5 | 0.88 | 0.176 | nd | 82 | 113 |
| SYR6-091307-002 | SYR-6 | 2007 | Plecoptera | Perlidae | Larva | 12 | 1.66 | 0.138 | nd | 37 | 39 |
| SYR5-091307-002 | SYR-5 | 2007 | Plecoptera | Perlidae | Larva | 8 | 1.22 | 0.153 | nd | 53 | 44 |
| SYR1-091307-002 | SYR-1 | 2007 | Plecoptera | Perlidae | Larva | 8 | 1.12 | 0.14 | nd | 53 | 56 |
| SYR7-091407-003 | SYR-7 | 2007 | Plecoptera | Perlidae | Larva | 8 | 1.47 | 0.184 | nd | 90 | 71 |
| SYR4-091307-002 | SYR-4 | 2007 | Plecoptera | Perlidae | Larva | 9 | 1.84 | 0.204 | nd | 77 | 76 |
| HUM1-091307-002 | HUM-1 | 2007 | Plecoptera | Perlidae | Larva | 15 | 1.67 | 0.111 | nd | 86 | 94 |
| SYR6-091307-001 | SYR-6 | 2007 | Trichoptera | Hydropsychidae | Larva | 80 | 0.58 | 0.007 | nd | 24 | 16 |
| SYR1-091307-001 | SYR-1 | 2007 | Trichoptera | Hydropsychidae | Larva | 100 | 0.59 | 0.006 | nd | 46 | 24 |
| SYR5-091307-001 | SYR-5 | 2007 | Trichoptera | Hydropsychidae | Larva | 75 | 0.34 | 0.005 | nd | 26 | 24 |
| SYR4-091307-001 | SYR-4 | 2007 | Trichoptera | Hydropsychidae | Larva | 100 | 0.53 | 0.005 | nd | 35 | 30 |
| SYR7-091407-006 | SYR-7 | 2007 | Trichoptera | Hydropsychidae | Larva | 90 | 0.42 | 0.005 | nd | 61 | 44 |
| HUM1-091108-006 | HUM-1 | 2008 | Hemiptera | Gerridae | Adult | 25 | 1.62 | 0.065 | 64.27 | 63 | 59 |
| HUM1-091108-005 | HUM-1 | 2008 | Hemiptera | Gerridae | Adult | 25 | 1.54 | 0.062 | 64.86 | 73 | 61 |

Table 9. Concentrations of total mercury and methylmercury in individual composites of biological samples collected at Humbug Creek and the South Yuba River, California, in September 2007 and September 2008.

[g, gram; No., number of individual oranisms in composite; ng/g, nanogram per gram; ww, wet weight; THg, total mercury; MeHg, methylmercury; %, percent; nd, not determined]

| Unique Sample Code | Site identifier | Year | Order | Family | Age | No. | Total mass (g) | Ave. Mass (g) | % moisture | THg (ng/g ww) | MeHg (ng/g ww) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SYR1-091108-005 | SYR-1 | 2008 | Hemiptera | Gerridae | Adult | 22 | 1.11 | 0.050 | 75.57 | 80 | 78 |
| SYR6-091208-004 | SYR-6 | 2008 | Hemiptera | Gerridae | Adult | 23 | 1.21 | 0.053 | 61.81 | 115 | 80 |
| SYR4-091108-006 | SYR-4 | 2008 | Hemiptera | Gerridae | Adult | 24 | 1.31 | 0.055 | 78.19 | 81 | 87 |
| SYR7-091208-005 | SYR-7 | 2008 | Hemiptera | Gerridae | Adult | 27 | 1.37 | 0.051 | 71.92 | 116 | 102 |
| SYR1a-091108-004 | SYR-1a | 2008 | Hemiptera | Gerridae | Adult | 25 | 1.31 | 0.052 | 68.96 | 180 | 133 |
| SYR4-091108-005 | SYR-4 | 2008 | Odonata | Gomphidae | Larva | 8 | 0.99 | 0.124 | nd | 12 | 10 |
| SYR1a-091108-003 | SYR-1a | 2008 | Odonata | Gomphidae | Larva | 10 | 1.12 | 0.112 | 82.9 | 26 | 17 |
| SYR4-091108-003 | SYR-4 | 2008 | Odonata | Gomphidae | Larva | 4 | 1.49 | 0.373 | 85.77 | 26 | 22 |
| SYR1-091108-004 | SYR-1 | 2008 | Odonata | Gomphidae | Larva | 10 | 1.65 | 0.165 | 85.27 | 30 | 26 |
| SYR4-091108-004 | SYR-4 | 2008 | Odonata | Gomphidae | Larva | 4 | 1.54 | 0.385 | 82.61 | 29 | 26 |
| HUM1-091108-004 | HUM-1 | 2008 | Odonata | Gomphidae | Larva | 4 | 1.07 | 0.268 | 86.65 | 32 | 30 |
| SYR6-091208-002 | SYR-6 | 2008 | Odonata | Gomphidae | Larva | 4 | 1.39 | 0.348 | 76.16 | 42 | 30 |
| SYR7-091208-003 | SYR-7 | 2008 | Odonata | Gomphidae | Larva | 4 | 1.54 | 0.385 | 84.91 | 47 | 35 |
| SYR1-091108-003 | SYR-1 | 2008 | Odonata | Gomphidae | Larva | 4 | 1.63 | 0.408 | 78.19 | 48 | 38 |
| SYR1-091108-002 | SYR-1 | 2008 | Odonata | Gomphidae | Larva | 4 | 1.69 | 0.423 | 78.38 | 44 | 39 |
| HUM1-091108-003 | HUM-1 | 2008 | Odonata | Gomphidae | Larva | 4 | 1.05 | 0.263 | 81.58 | 46 | 40 |
| SYR7-091208-004 | SYR-7 | 2008 | Odonata | Gomphidae | Larva | 6 | 1.53 | 0.255 | 83.31 | 57 | 50 |
| SYR6-091208-001 | SYR-6 | 2008 | Plecoptera | Perlidae | Larva | 12 | 1.30 | 0.108 | 83.08 | 28 | 23 |
| SYR1-091108-001 | SYR-1 | 2008 | Plecoptera | Perlidae | Larva | 9 | 1.31 | 0.146 | 86.52 | 33 | 24 |
| HUM1-091108-002 | HUM-1 | 2008 | Plecoptera | Perlidae | Larva | 9 | 1.17 | 0.130 | 83.18 | 34 | 37 |
| SYR7-091208-002 | SYR-7 | 2008 | Plecoptera | Perlidae | Larva | 12 | 1.39 | 0.116 | 84.13 | 58 | 42 |
| SYR1a-091108-002 | SYR-1a | 2008 | Plecoptera | Perlidae | Larva | 9 | 1.31 | 0.146 | 86.28 | 59 | 51 |
| HUM1-091108-001 | HUM-1 | 2008 | Plecoptera | Perlidae | Larva | 6 | 1.60 | 0.267 | 83.50 | 57 | 52 |
| SYR4-091108-002 | SYR-4 | 2008 | Plecoptera | Perlidae | Larva | 9 | 1.06 | 0.118 | 83.51 | 67 | 62 |
| SYR1a-091108-001 | SYR-1a | 2008 | Plecoptera | Perlidae | Larva | 6 | 1.55 | 0.258 | 83.51 | 86 | 63 |
| SYR4-091108-001 | SYR-4 | 2008 | Plecoptera | Perlidae | Larva | 6 | 1.58 | 0.263 | 78.39 | 102 | 86 |
| SYR6-091208-003 | SYR-6 | 2008 | Trichoptera | Hydropsychidae | Larva | 150 | 0.77 | 0.005 | 89.69 | 18 | 11 |

Table 9. Concentrations of total mercury and methylmercury in individual composites of biological samples collected at Humbug Creek and the South Yuba River, California, in September 2007 and September 2008.

[g, gram; No., number of individual oranisms in composite; ng/g, nanogram per gram; ww, wet weight; THg, total mercury; MeHg, methylmercury; %, percent; nd, not determined]

| Unique Sample Code | Site identifier | Year | Order | Family | Age | No. | Total mass (g) | Ave. Mass (g) | % moisture | THg (ng/g ww) | MeHg (ng/g ww) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SYR1-091108-006 | SYR-1 | 2008 | Trichoptera | Hydropsychidae | Larva | 130 | 0.89 | 0.007 | 89.62 | 19 | 12 |
| SYR4-091108-007 | SYR-4 | 2008 | Trichoptera | Hydropsychidae | Larva | 70 | 0.60 | 0.009 | 88.91 | 26 | 20 |
| SYR1a-091108-005 | SYR-1a | 2008 | Trichoptera | Hydropsychidae | Larva | 100 | 0.70 | 0.007 | 82.94 | 41 | 27 |
| SYR7-091208-001 | SYR-7 | 2008 | Trichoptera | Hydropsychidae | Larva | 130 | 0.64 | 0.005 | 85.69 | 80 | 30 |

## Table AML-1. information on sample locations for biological samples, South Yuba River watershed, 1999-2002.

Station name abbreviations: AB, above; BL, below; C, Creek; CYN, Canyon; DR, Drain; MF, Middle Fork; MI, miles; NR, near; PK, Park; RD, Road; RES, Reservoir; S, south; SF, South Fork]

| Station map ID | Station name | Station number | Type | Map group | Latitude (NAD27) | Longitude (NAD27) | Latitude (NAD27) | Longitude (NAD27) | Biology collection dates |
|---|---|---|---|---|---|---|---|---|---|
| SY-4 | Omega drainage gulch at Scotchman Ck nr Washington | 392045120463301 | Stream | Alpha/Omega | 39.345732 | -120.776884 | 39° 20' 45" | -120°46' 37" | 9/2/1999 |
| SY-5 | Scotchman C ab Omega drainage gulch nr Washington | 392042120463401 | Stream | Alpha/Omega | 39.344899 | -120.777162 | 39° 20' 42" | -120°46' 38" | 9/2/1999 |
| SY-8 | Scotchman Ck at The Falls at Washington. | 392116120470101 | Stream | Alpha/Omega | 39.354343 | -120.784662 | 39° 21' 16" | -120° 47' 5" | 9/2/1999, 7/29/2002 |
| SY-10 | Scotchman Ck bl Falls nr Washington | 392119120470201 | Stream | Alpha/Omega | 39.355176 | -120.784940 | 39° 21' 19" | -120° 47' 6" | 7/29/2002 |
| SY-12 | Alpha Pit Marsh | 392003120470901 | Pond/Pit Lake/Wetland/Reservoir | Alpha/Omega | 39.334167 | -120.785833 | 39° 20' 3" | -120° 47' 9" | 7/29/2002 |
| SY-13 | Alpha Mine pit lake nr Washington | 392007120471001 | Pond/Pit Lake/Wetland/Reservoir | Alpha/Omega | 39.335176 | -120.787162 | 39° 20' 7" | -120°47' 14" | 8/23/2000, 10/18/2001, 7/29/2002 |
| SY-14 | Ancho Erie drainage at Tailings Dam nr Washington | 392512120453101 | Stream | Ancho Erie | 39.419899 | -120.759663 | 39° 25' 12" | -120°45' 35" | 7/31/2002 |
| SY-15 | Ancho Erie drainage at Poorman Ck nr Washington | 392512120453201 | Stream | Ancho Erie | 39.419899 | -120.759941 | 39° 25' 12" | -120°45' 36" | 7/31/2002 |
| SY-16 | Poorman Ck bl Ancho Erie Mine drainage nr Washington | 392513120453301 | Stream | Ancho Erie | 39.420176 | -120.760218 | 39° 25' 13" | -120°45' 37" | 7/31/2002 |
| SY-18 | Humbug Creek ab Le Du Mine | 392152120542601 | stream | Malakoff | 39.364444 | -120.907222 | 39° 21' 52" | -120°54' 26" | 5/15/2002 |
| SY-19 | Le Du Mine tunnel discharge nr N Bloomfield | 392151120542901 | Tunnel | Malakoff | 39.364062 | -120.909113 | 39° 21' 51" | -120°54' 33" | 5/15/2002 |
| SY-20 | Crystal Hill Mine pond nr N Bloomfield | 392159120544101 | Pond/Pit Lake/Wetland/Reservoir | Malakoff | 39.366284 | -120.912446 | 39° 21' 59" | -120°54' 45" | 8/20/1999, 9/15/1999 |
| SY-22 | Malakoff Diggings flooded shaft (red) nr N Bloomfield | 392155120551601 | Tunnel | Malakoff | 39.365173 | -120.922169 | 39° 21' 55" | -120°55' 20" | 9/3/1999, 9/15/1999 |
| SY-24 | Malakoff Diggings Pond nr N Bloomfield | 392201120552301 | Pond/Pit Lake/Wetland/Reservoir | Malakoff | 39.366839 | -120.924114 | 39° 22' 1" | -120°55' 27" | 11/12/1999, 12/1/1999 |
| SY-25 | Malakoff Diggings Lake City Tunnel discharge nr N Bloomfield | 392111120552501 | Tunnel | Malakoff | 39.352951 | -120.924669 | 39° 21' 11" | -120°55' 29" | 8/20/1999 |
| SY-26 | Humbug Ck ab Falls nr Nevada City | 392057120552901 | Stream | Malakoff | 39.349062 | -120.925780 | 39° 20' 57" | -120°55' 33" | 9/3/1999 |
| SY-27 | Malakoff Diggings N Bloomfield Tunnel discharge nr N Bloomfield | 392051120553501 | Tunnel | Malakoff | 39.347395 | -120.927447 | 39° 20' 51" | -120°55' 39" | 8/20/1999 |
| SY-28 | Humbug Ck bl Falls nr Nevada City | 392040120553701 | Stream | Malakoff | 39.344340 | -120.928002 | 39° 20' 40" | -120°55' 41" | 8/20/1999, 9/3/1999 |
| SY-29 | Relief Hill Deep Blue Tnnl Outflow Nr N Bloomfield | 392126120510201 | Tunnel | Relief Hill | 39.357119 | -120.851610 | 39° 21' 26" | -120° 51' 6" | 9/1/1999, 7/30/2002 |

## Table AML-1. information on sample locations for biological samples, South Yuba River watershed, 1999-2002.

Station name abbreviations: AB, above; BL, below; C, Creek; CYN, Canyon; DR, Drain; MF, Middle Fork; MI, miles; NR, near; PK, Park; RD, Road; RES, Reservoir; S, south; SF, South Fork]

| Station map ID | Station name | Station number | Type | Map group | Latitude (NAD27) | Longitude (NAD27) | Latitude (NAD27) | Longitude (NAD27) | Biology collection dates |
|---|---|---|---|---|---|---|---|---|---|
| SY-30 | Relief Hill Hydraulic Pit Mine drainage tunnel nr N Bloomfield | 392111120511301 | Tunnel | Relief Hill | 39.352952 | -120.854666 | 39° 21' 11" | -120°51' 17" | 9/1/1999, 7/30/2002 |
| SY-31 | Relief Hill Upper Pond | 392120120511801 | Pond/Pit Lake/Wetland/Reservoir | Relief Hill | 39.355833 | -120.855000 | 39° 21' 21" | -120°51' 18" | 7/30/2002 |
| SY-43 | S Yuba R at Eagle Lakes Rd nr Emigrant Gap | 391948120342201 | River | S Yuba mainstem | 39.329902 | -120.573820 | 39° 19' 48" | -120°34' 26" | 9/30/1999 |
| SY-47 | S Yuba R at Edwards Crossing nr Nevada City | 391949120585901 | River | S Yuba mainstem | 39.330172 | -120.984115 | 39° 19' 49" | -120° 59' 3" | 9/28/1999, 10/06/2000, 10/07/2001, 10/18/2001 |
| SY-52 | Blue Hole Lake at Sailor Flat Mine nr Nevada City | 391937120575301 | Pond/Pit Lake/Wetland/Reservoir | Sailor Flat / S Yuba | 39.326839 | -120.965781 | 39° 19' 37" | -120°57' 57" | 8/24/1999 |
| SY-53 | Sailor Flat Mine nr Enterprise Ck nr Nevada City | 391935120575901 | Stream | Sailor Flat / S Yuba | 39.326283 | -120.967448 | 39° 19' 35" | -120° 58' 3" | 8/24/1999 |
| SY-55 | Birchville Marsh nr French Corral | 391930121090301 | Pond/Pit Lake/Wetland/Reservoir | Western Upper Yuba | 39.325000 | -121.150833 | 39° 19' 30" | -121°° 9' 3" | 7/23/2002 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | Pond/Pit Lake/Wetland/Reservoir | Western Upper Yuba | 39.326837 | -121.151622 | 39° 19' 37" | -121°° 9' 6" | 7/23/2002 |

**Table AML-2. Mercury and methylmercury concentration data for invertebrates, South Yuba River watershed, California, 1999-2002**

[g, gram; µg/g, microgram per gram; MeHg, methylmercury; THg, total mercury; n, number of individual organisms]

| Station Map ID | Collection date | Common name | Order | Family | Sample size (n) | Live weight (g) | Moisture (percent) | Total mercury (µg/g wet) | Methylmercury (µg/g wet) | MeHg/THg (percent) | Age |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SY-04 | 9/2/1999 | Banana slugs | Gastropoda | Ariolimacidae | 3 | 44.70 | 87.6 | na | 0.0034 | na | Undetermined |
| SY-14 | 7/31/2002 | Banana slugs | Gastropoda | Ariolimacidae | 3 | 47.18 | 84.4 | 0.009 | 0.0031 | 36.1 | Undetermined |
| SY-15 | 7/31/2002 | Banana slugs | Gastropoda | Ariolimacidae | 3 | 13.56 | 88.0 | 0.053 | 0.0120 | 22.5 | Undetermined |
| SY-16 | 7/31/2002 | Banana slugs | Gastropoda | Ariolimacidae | 1 | 17.86 | 82.9 | 0.018 | 0.0070 | 38.5 | Undetermined |
| SY-22 | 9/3/1999 | Banana slugs | Gastropoda | Ariolimacidae | 3 | 22.85 | 86.1 | na | 0.0021 | na | Undetermined |
| SY-25 | 8/20/1999 | Banana slugs | Gastropoda | Ariolimacidae | 2 | 21.55 | 76.7 | na | 0.0027 | na | Undetermined |
| SY-27 | 8/20/1999 | Banana slugs | Gastropoda | Ariolimacidae | 2 | 18.47 | 77.1 | na | 0.0033 | na | Undetermined |
| SY-47 | 10/6/2000 | Banana slugs | Gastropoda | Ariolimacidae | 3 | 36.77 | 87.9 | na | 0.0074 | na | Undetermined |
| SY-04 | 9/2/1999 | Dobsonflies | Megaloptera | Corydalidae | 5 | 2.70 | 78.9 | na | 0.0549 | na | Immature |
| SY-08 | 9/2/1999 | Dobsonflies | Megaloptera | Corydalidae | 1 | 0.85 | 81.7 | na | 0.0547 | na | Immature |
| SY-08 | 7/29/2002 | Dobsonflies | Megaloptera | Corydalidae | 4 | 2.45 | 76.7 | 0.095 | 0.0920 | 97.3 | Immature |
| SY-10 | 7/29/2002 | Dobsonflies | Megaloptera | Corydalidae | 8 | 3.39 | 79.9 | 0.066 | 0.0597 | 90.5 | Immature |
| SY-14 | 7/31/2002 | Dobsonflies | Megaloptera | Corydalidae | 4 | 3.37 | 76.5 | 0.063 | 0.0353 | 56.0 | Immature |
| SY-16 | 7/31/2002 | Dobsonflies | Megaloptera | Corydalidae | 3 | 2.70 | 75.1 | 0.088 | 0.0403 | 46.0 | Immature |
| SY-18 | 5/15/2002 | Dobsonflies | Megaloptera | Corydalidae | 3 | 1.63 | 77.0 | 0.090 | 0.0580 | 64.1 | Immature |
| SY-27 | 8/20/1999 | Dobsonflies | Megaloptera | Corydalidae | 1 | 1.91 | 68.8 | na | 0.1529 | na | Immature |
| SY-29 | 9/1/1999 | Dobsonflies | Megaloptera | Corydalidae | 1 | 0.86 | 80.3 | na | 0.0526 | na | Immature |
| SY-43 | 9/30/1999 | Dobsonflies | Megaloptera | Corydalidae | 1 | 0.99 | 78.4 | na | 0.0311 | na | Immature |
| SY-47 | 10/18/2001 | Dobsonflies | Megaloptera | Corydalidae | 3 | 2.14 | 78.5 | 0.078 | 0.0621 | 79.8 | Immature |
| SY-08 | 9/2/1999 | Dragonflies | Odonata | Cordulegastridae | 5 | 2.43 | 77.1 | na | 0.0449 | na | Immature |
| SY-08 | 7/29/2002 | Dragonflies | Odonata | Aeshnidae | 2 | 2.01 | 76.4 | 0.046 | 0.0455 | 99.5 | Immature |
| SY-08 | 7/29/2002 | Dragonflies | Odonata | Cordulegastridae | 4 | 2.31 | 78.7 | 0.046 | 0.0426 | 92.2 | Immature |
| SY-10 | 7/29/2002 | Dragonflies | Odonata | Aeshnidae | 1 | 1.08 | 77.5 | 0.055 | 0.0511 | 93.4 | Immature |
| SY-10 | 7/29/2002 | Dragonflies | Odonata | Cordulegastridae | 9 | 1.35 | 83.9 | 0.092 | 0.0660 | 71.9 | Immature |
| SY-12 | 7/29/2002 | Dragonflies | Odonata | Aeshnidae | 6 | 7.59 | 79.7 | 0.035 | 0.0349 | 100.6 | Immature |
| SY-13 | 8/23/2000 | Dragonflies | Odonata | Aeshnidae | 15 | 1.19 | 80.3 | na | 0.0121 | na | Immature |
| SY-13 | 10/18/2001 | Dragonflies | Odonata | Aeshnidae | 10 | 1.71 | 86.1 | 0.009 | 0.0073 | 82.2 | Immature |
| SY-13 | 10/18/2001 | Dragonflies | Odonata | Aeshnidae | 10 | 1.60 | 86.5 | 0.007 | 0.0057 | 77.9 | Immature |
| SY-13 | 7/29/2002 | Dragonflies | Odonata | Aeshnidae | 2 | 1.27 | 89.1 | 0.013 | 0.0106 | 82.4 | Immature |
| SY-16 | 7/31/2002 | Dragonflies | Odonata | Aeshnidae | 1 | 0.91 | 75.0 | 0.087 | 0.0695 | 80.1 | Immature |
| SY-18 | 5/15/2002 | Dragonflies | Odonata | Cordulegastridae | 1 | 1.40 | 72.1 | 0.113 | 0.1133 | 100.5 | Immature |
| SY-18 | 5/15/2002 | Dragonflies | Odonata | Gomphidae | 5 | 1.55 | 73.4 | 0.109 | 0.0944 | 86.4 | Immature |
| SY-20 | 8/20/1999 | Dragonflies | Odonata | Aeshnidae | 5 | 6.94 | 78.2 | 0.124 | 0.1339 | 107.5 | Immature |
| SY-20 | 8/20/1999 | Dragonflies | Odonata | Aeshnidae | 5 | 6.35 | 81.3 | 0.109 | 0.1288 | 118.6 | Immature |
| SY-25 | 8/20/1999 | Dragonflies | Odonata | Cordulegastridae | 2 | 2.37 | 81.4 | na | 0.0493 | na | Immature |
| SY-27 | 8/20/1999 | Dragonflies | Odonata | Cordulegastridae | 9 | 1.22 | 80.9 | na | 0.0554 | na | Immature |

**Table AML-2. Mercury and methylmercury concentration data for invertebrates, South Yuba River watershed, California, 1999-2002**

[g, gram; µg/g, microgram per gram; MeHg, methylmercury; THg, total mercury; n, number of individual organisms]

| Station Map ID | Collection date | Common name | Order | Family | Sample size (n) | Live weight (g) | Moisture (percent) | Total mercury (µg/g wet) | Methylmercury (µg/g wet) | MeHg/THg (percent) | Age |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SY-31 | 7/30/2002 | Dragonflies | Odonata | Aeshnidae | 3 | 2.28 | 82.0 | 0.018 | 0.0178 | 101.1 | Immature |
| SY-31 | 7/30/2002 | Dragonflies | Odonata | Aeshnidae | 3 | 1.74 | 83.6 | 0.016 | 0.0130 | 82.0 | Immature |
| SY-47 | 9/28/1999 | Dragonflies | Odonata | Cordulegastridae | 3 | 1.30 | 84.6 | na | 0.0571 | na | Immature |
| SY-47 | 10/6/2000 | Dragonflies | Odonata | Gomphidae | 7 | 2.49 | 80.2 | na | 0.0275 | na | Immature |
| SY-47 | 10/18/2001 | Dragonflies | Odonata | Aeshnidae | 9 | 2.63 | 83.6 | 0.130 | 0.1333 | 102.3 | Immature |
| SY-47 | 10/18/2001 | Dragonflies | Odonata | Cordulegastridae | 2 | 1.82 | 86.1 | 0.138 | 0.1446 | 104.6 | Immature |
| SY-47 | 10/18/2001 | Dragonflies | Odonata | Gomphidae | 6 | 2.51 | 80.1 | 0.065 | 0.0593 | 90.6 | Immature |
| SY-56 | 7/23/2002 | Dragonflies | Odonata | Aeshnidae | 4 | 4.16 | 78.8 | 0.027 | 0.0290 | 108.7 | Immature |
| SY-56 | 7/23/2002 | Dragonflies | Odonata | Aeshnidae | 4 | 2.18 | 82.3 | 0.021 | 0.0234 | 111.9 | Immature |
| SY-56 | 7/23/2002 | Dragonflies | Odonata | Libellulidae | 2 | 0.72 | 79.5 | 0.044 | 0.0301 | 69.0 | Immature |
| SY-08 | 9/2/1999 | Giant water bugs | Hemiptera | Belostomatidae | 1 | 3.27 | 70.5 | 0.267 | 0.1590 | 59.6 | Adult |
| SY-12 | 7/29/2002 | Giant water bugs | Hemiptera | Belostomatidae | 3 | 8.42 | 83.1 | 0.148 | 0.1690 | 114.2 | Adult |
| SY-13 | 8/23/2000 | Giant water bugs | Hemiptera | Belostomatidae | 1 | 4.17 | 75.0 | na | 0.2105 | na | Adult |
| SY-13 | 8/23/2000 | Giant water bugs | Hemiptera | Belostomatidae | 1 | 4.69 | 72.1 | na | 0.3292 | na | Adult |
| SY-13 | 10/18/2001 | Giant water bugs | Hemiptera | Belostomatidae | 1 | 3.87 | 72.1 | 0.278 | 0.2818 | 101.3 | Adult |
| SY-13 | 10/18/2001 | Giant water bugs | Hemiptera | Belostomatidae | 1 | 4.02 | 77.6 | 0.358 | 0.3226 | 90.0 | Adult |
| SY-13 | 7/29/2002 | Giant water bugs | Hemiptera | Belostomatidae | 3 | 11.15 | 77.0 | 0.057 | 0.0738 | 130.5 | Adult |
| SY-13 | 7/29/2002 | Giant water bugs | Hemiptera | Belostomatidae | 3 | 7.75 | 81.5 | 0.047 | 0.0422 | 89.4 | Adult |
| SY-52 | 8/24/1999 | Giant water bugs | Hemiptera | Belostomatidae | 1 | 3.19 | 73.1 | 0.066 | 0.0468 | 71.0 | Adult |
| SY-04 | 9/2/1999 | Predaceous diving beetles | Coleoptera | Dytiscidae | 13 | 1.20 | 54.3 | na | 0.0599 | na | Adult |
| SY-13 | 8/23/2000 | Predaceous diving beetles | Coleoptera | Dytiscidae | 15 | 3.19 | 63.4 | 0.127 | 0.1109 | 87.3 | Adult |
| SY-13 | 10/18/2001 | Predaceous diving beetles | Coleoptera | Dytiscidae | 15 | 3.47 | 68.4 | 0.100 | 0.0932 | 93.7 | Adult |
| SY-13 | 10/18/2001 | Predaceous diving beetles | Coleoptera | Dytiscidae | 15 | 3.12 | 68.0 | 0.125 | 0.1043 | 83.4 | Adult |
| SY-13 | 10/18/2001 | Predaceous diving beetles | Coleoptera | Dytiscidae | 30 | 1.23 | 63.8 | 0.147 | 0.1549 | 105.4 | Adult |
| SY-13 | 7/29/2002 | Predaceous diving beetles | Coleoptera | Dytiscidae | 20 | 4.58 | 65.2 | 0.071 | 0.0609 | 85.4 | Adult |
| SY-19 | 5/15/2002 | Predaceous diving beetles | Coleoptera | Dytiscidae | 16 | 1.01 | 52.1 | 0.087 | 0.0810 | 93.4 | Adult |
| SY-31 | 7/30/2002 | Predaceous diving beetles | Coleoptera | Dytiscidae | 10 | 2.42 | 64.0 | 0.057 | 0.0533 | 93.7 | Adult |
| SY-52 | 8/24/1999 | Predaceous diving beetles | Coleoptera | Dytiscidae | 19 | 4.10 | 60.1 | 0.078 | 0.0818 | 105.1 | Adult |
| SY-55 | 7/23/2002 | Predaceous diving beetles | Coleoptera | Dytiscidae | 3 | 5.81 | 56.5 | 0.014 | 0.0153 | 111.8 | Adult |
| SY-56 | 7/23/2002 | Predaceous diving beetles | Coleoptera | Dytiscidae | 3 | 6.26 | 52.8 | 0.006 | 0.0076 | 118.2 | Adult |
| SY-04 | 9/2/1999 | Stoneflies | Plecoptera | Perlidae | 14 | 1.52 | 77.1 | na | 0.0417 | na | Adult |
| SY-05 | 9/2/1999 | Stoneflies | Plecoptera | Perlidae | 13 | 1.67 | 75.7 | na | 0.0474 | na | Immature |
| SY-08 | 9/2/1999 | Stoneflies | Plecoptera | Perlidae | 14 | 3.39 | 69.7 | na | 0.0688 | na | Immature |
| SY-08 | 7/29/2002 | Stoneflies | Plecoptera | Perlidae | 8 | 1.79 | 69.4 | 0.065 | 0.0367 | 56.6 | Immature |
| SY-08 | 7/29/2002 | Stoneflies | Plecoptera | Perlidae | 8 | 1.09 | 72.7 | 0.072 | 0.0317 | 44.1 | Immature |
| SY-10 | 7/29/2002 | Stoneflies | Plecoptera | Perlidae | 10 | 2.06 | 73.8 | 0.072 | 0.0600 | 83.0 | Immature |

**Table AML-2. Mercury and methylmercury concentration data for invertebrates, South Yuba River watershed, California, 1999-2002**

[g, gram; µg/g, microgram per gram; MeHg, methylmercury; THg, total mercury; n, number of individual organisms]

| Station Map ID | Collection date | Common name | Order | Family | Sample size (n) | Live weight (g) | Moisture (percent) | Total mercury (µg/g wet) | Methylmercury (µg/g wet) | MeHg/THg (percent) | Age |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SY-14 | 7/31/2002 | Stoneflies | Plecoptera | Perlidae | 4 | 1.39 | 72.1 | 0.061 | 0.0480 | 78.9 | Immature |
| SY-15 | 7/31/2002 | Stoneflies | Plecoptera | Perlidae | 6 | 1.68 | 71.2 | 0.073 | 0.0922 | 126.0 | Immature |
| SY-16 | 7/31/2002 | Stoneflies | Plecoptera | Perlidae | 10 | 2.65 | 73.3 | 0.041 | 0.0323 | 78.1 | Immature |
| SY-18 | 5/15/2002 | Stoneflies | Plecoptera | Perlidae | 5 | 1.90 | 73.7 | 0.062 | 0.0560 | 90.3 | Immature |
| SY-27 | 8/20/1999 | Stoneflies | Plecoptera | Perlidae | 11 | 1.09 | 71.9 | na | 0.0318 | na | Immature |
| SY-43 | 9/30/1999 | Stoneflies | Plecoptera | Perlidae | 10 | 1.33 | 74.2 | 0.025 | 0.0323 | 128.7 | Immature |
| SY-47 | 9/28/1999 | Stoneflies | Plecoptera | Perlidae | 8 | 1.71 | 72.8 | 0.068 | 0.0726 | 106.8 | Immature |
| SY-47 | 10/6/2000 | Stoneflies | Plecoptera | Perlidae | 10 | 2.16 | 76.4 | 0.062 | 0.0597 | 95.8 | Immature |
| SY-47 | 10/18/2001 | Stoneflies | Plecoptera | Perlidae | 10 | 1.97 | 73.4 | 0.111 | 0.0971 | 87.3 | Immature |
| SY-04 | 9/2/1999 | Water striders | Hemiptera | Gerridae | 13 | 0.72 | 59.2 | na | 0.1167 | na | Adult |
| SY-05 | 9/2/1999 | Water striders | Hemiptera | Gerridae | 23 | 1.23 | 64.8 | na | 0.0736 | na | Adult |
| SY-08 | 9/2/1999 | Water striders | Hemiptera | Gerridae | 32 | 1.81 | 63.8 | 0.083 | 0.1097 | 132.9 | Adult |
| SY-08 | 7/29/2002 | Water striders | Hemiptera | Gerridae | 25 | 1.46 | 66.2 | 0.091 | 0.0848 | 93.0 | Adult |
| SY-10 | 7/29/2002 | Water striders | Hemiptera | Gerridae | 25 | 1.58 | 67.5 | 0.140 | 0.1482 | 106.0 | Adult |
| SY-12 | 7/29/2002 | Water striders | Hemiptera | Gerridae | 40 | 1.19 | 66.7 | 0.086 | 0.0736 | 85.3 | Adult |
| SY-13 | 7/29/2002 | Water striders | Hemiptera | Gerridae | 13 | 0.35 | 68.2 | 0.121 | 0.0525 | 43.5 | Adult |
| SY-14 | 7/31/2002 | Water striders | Hemiptera | Gerridae | 25 | 1.51 | 66.1 | 0.180 | 0.0783 | 43.5 | Adult |
| SY-16 | 7/31/2002 | Water striders | Hemiptera | Gerridae | 25 | 1.77 | 54.4 | 0.119 | 0.1254 | 105.4 | Adult |
| SY-18 | 5/15/2002 | Water striders | Hemiptera | Gerridae | 25 | 1.48 | 58.3 | 0.189 | 0.1355 | 71.7 | Adult |
| SY-19 | 5/15/2002 | Water striders | Hemiptera | Gerridae | 25 | 1.59 | 56.3 | 0.150 | 0.1342 | 89.2 | Adult |
| SY-25 | 8/20/1999 | Water striders | Hemiptera | Gerridae | 21 | 1.12 | 72.3 | na | 0.0379 | na | Adult |
| SY-29 | 9/1/1999 | Water striders | Hemiptera | Gerridae | 10 | 0.60 | 58.3 | na | 0.0942 | na | Adult |
| SY-29 | 7/30/2002 | Water striders | Hemiptera | Gerridae | 25 | 1.46 | 67.4 | 0.060 | 0.0629 | 104.3 | Adult |
| SY-30 | 9/1/1999 | Water striders | Hemiptera | Gerridae | 27 | 1.85 | 55.3 | 0.048 | 0.0469 | 98.1 | Adult |
| SY-30 | 7/30/2002 | Water striders | Hemiptera | Gerridae | 25 | 1.47 | 63.0 | 0.060 | 0.0429 | 72.0 | Adult |
| SY-31 | 7/30/2002 | Water striders | Hemiptera | Gerridae | 25 | 1.51 | 58.2 | 0.105 | 0.1062 | 101.2 | Adult |
| SY-43 | 9/30/1999 | Water striders | Hemiptera | Gerridae | 26 | 1.42 | 55.2 | na | 0.0502 | na | Adult |
| SY-47 | 9/28/1999 | Water striders | Hemiptera | Gerridae | 25 | 1.27 | 56.8 | na | 0.0881 | na | Adult |
| SY-47 | 10/6/2000 | Water striders | Hemiptera | Gerridae | 30 | 1.65 | 63.5 | 0.088 | 0.0752 | 85.8 | Adult |
| SY-47 | 10/18/2001 | Water striders | Hemiptera | Gerridae | 25 | 1.40 | 62.2 | 0.153 | 0.1021 | 66.8 | Adult |
| SY-53 | 8/24/1999 | Water striders | Hemiptera | Gerridae | 17 | 1.17 | 58.3 | na | 0.0909 | na | Adult |
| SY-56 | 7/23/2002 | Water striders | Hemiptera | Gerridae | 14 | 0.47 | 60.9 | 0.071 | 0.0630 | 88.5 | Adult |

**Table AML-3. Mercury concentration data for frogs, South Yuba River watershed, California, 1999-2002**

[N, number of individuals; g, gram; mm, millmeter; µg/g, microgram per gram]

| Station Map ID | Collection Date | Common Name | Species | Sample size (N) | Live weight (g) | Sample weight (g) | Total Length (mm) | Moisture (percent) | Total mercury (µg/g wet) | Age | Gender |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SY-20 | 8/20/1999 | Bullfrog | *Lithobates catesbeianus* | 1 | 206.2 | 200.90 | 115.36 | 78.9 | 0.121 | Adult | Female |
| SY-20 | 9/15/1999 | Bullfrog | *Lithobates catesbeianus* | 1 | 377.31 | 372.23 | 130.21 | 76.1 | 0.181 | Adult | Male |
| SY-20 | 8/20/1999 | Bullfrog | *Lithobates catesbeianus* | 1 | 135.72 | 132.69 | 128.11 | 80.2 | 0.188 | Adult | Male |
| SY-20 | 9/15/1999 | Bullfrog | *Lithobates catesbeianus* | 1 | 303.96 | 286.16 | 129.47 | 78.1 | 0.272 | Adult | Male |
| SY-22 | 9/15/1999 | Bullfrog | *Lithobates catesbeianus* | 1 | 77.04 | 74.26 | 87.68 | 74.6 | 0.052 | Adult | Female |
| SY-26 | 9/3/1999 | Bullfrog | *Lithobates catesbeianus* | 1 | 448.46 | 397.62 | 138.40 | 73.2 | 0.112 | Adult | Female |
| SY-56 | 7/23/2002 | Bullfrog | *Lithobates catesbeianus* | 1 | 8.66 | 7.98 | 44.50 | 80.5 | 0.048 | Juvenile | Unknown |
| SY-56 | 7/23/2002 | Bullfrog | *Lithobates catesbeianus* | 1 | 11 | 10.04 | 51.60 | 83.5 | 0.102 | Juvenile | Unknown |
| SY-56 | 7/23/2002 | Bullfrog | *Lithobates catesbeianus* | 1 | 13.35 | 13.14 | 54.30 | 81.5 | 0.117 | Juvenile | Unknown |
| SY-5 | 9/2/1999 | Foothill yellow-legged frog | *Rana boylii* | 1 | 41.62 | 39.53 | 58.06 | 74.6 | 0.034 | Adult | Female |
| SY-5 | 9/2/1999 | Foothill yellow-legged frog | *Rana boylii* | 1 | 42.39 | 40.01 | 61.81 | 77.1 | 0.041 | Adult | Female |
| SY-5 | 9/2/1999 | Foothill yellow-legged frog | *Rana boylii* | 1 | 39.2 | 36.68 | 56.70 | 76.0 | 0.059 | Adult | Female |
| SY-8 | 9/2/1999 | Foothill yellow-legged frog | *Rana boylii* | 1 | 34.84 | 33.51 | 65.33 | 79.6 | 0.077 | Adult | Female |
| SY-8 | 7/29/2002 | Foothill yellow-legged frog | *Rana boylii* | 1 | 26.25 | 24.65 | 61.70 | 76.3 | 0.068 | Adult | Female |
| SY-10 | 7/29/2002 | Foothill yellow-legged frog | *Rana boylii* | 1 | 31.52 | 29.44 | 64.90 | 79.9 | 0.052 | Adult | Female |
| SY-10 | 7/29/2002 | Foothill yellow-legged frog | *Rana boylii* | 1 | 22.99 | 21.72 | 57.50 | 79.1 | 0.074 | Adult | Male |
| SY-10 | 7/29/2002 | Foothill yellow-legged frog | *Rana boylii* | 1 | 29.27 | 26.83 | 61.50 | 79.8 | 0.103 | Adult | Male |
| SY-19 | 5/15/2002 | Foothill yellow-legged frog | *Rana boylii* | 1 | 15.13 | 14.66 | 56.41 | 85.1 | 0.025 | Adult | Unknown |
| SY-19 | 5/15/2002 | Foothill yellow-legged frog | *Rana boylii* | 1 | 25.29 | 23.75 | 60.50 | 76.1 | 0.118 | Adult | Female |
| SY-26 | 9/3/1999 | Foothill yellow-legged frog | *Rana boylii* | 1 | 32.57 | 28.39 | 60.95 | 73.3 | 0.015 | Adult | Female |
| SY-26 | 9/3/1999 | Foothill yellow-legged frog | *Rana boylii* | 1 | 38.47 | 33.44 | 59.06 | 74.5 | 0.081 | Adult | Female |
| SY-28 | 9/3/1999 | Foothill yellow-legged frog | *Rana boylii* | 1 | 10.64 | 9.81 | 42.76 | 80.8 | 0.001 | Adult | Male |
| SY-28 | 8/20/1999 | Foothill yellow-legged frog | *Rana boylii* | 1 | 36.43 | 33.64 | 60.81 | 79.9 | 0.082 | Adult | Female |
| SY-47 | 9/28/1999 | Foothill yellow-legged frog | *Rana boylii* | 1 | 17.21 | 16.72 | 49.28 | 74.3 | 0.105 | Adult | Male |
| SY-47 | 9/28/1999 | Foothill yellow-legged frog | *Rana boylii* | 2 | 4.47 | 3.70 | na | 82.3 | 0.025 | Juvenile | unknown |
| SY-47 | 10/6/2000 | Foothill yellow-legged frog | *Rana boylii* | 1 | 13.94 | 12.50 | 49.07 | 77.0 | 0.047 | Adult | Female |
| SY-47 | 10/6/2000 | Foothill yellow-legged frog | *Rana boylii* | 1 | 39.83 | 36.17 | 64.67 | 76.3 | 0.173 | Adult | Female |
| SY-47 | 10/6/2000 | Foothill yellow-legged frog | *Rana boylii* | 1 | 36.16 | 34.37 | 66.12 | 74.0 | 0.175 | Adult | Female |
| SY-47 | 10/17/2001 | Foothill yellow-legged frog | *Rana boylii* | 1 | 29.51 | 27.27 | 65.12 | 79.4 | 0.062 | Adult | Female |
| SY-47 | 10/17/2001 | Foothill yellow-legged frog | *Rana boylii* | 1 | 21.58 | 20.19 | 56.02 | 80.5 | 0.066 | Adult | Female |
| SY-47 | 10/17/2001 | Foothill yellow-legged frog | *Rana boylii* | 1 | 52.25 | 50.78 | 77.16 | 72.3 | 0.153 | Adult | Female |
| SY-12 | 7/29/2002 | Sierra chorus frog | *Pseudacris sierra* | 3 | na | 1.08 | na | 84.6 | 0.037 | Juvenile | Unknown |
| SY-13 | 7/29/2002 | Sierra chorus frog | *Pseudacris sierra* | 3 | na | 0.64 | na | 81.1 | 0.027 | Juvenile | Unknown |
| SY-19 | 5/15/2002 | Sierra chorus frog | *Pseudacris sierra* | 1 | 4.77 | 4.66 | 39.42 | 74.9 | 0.088 | Adult | Female |
| SY-19 | 5/15/2002 | Sierra chorus frog | *Pseudacris sierra* | 1 | 4.45 | 4.15 | 42.60 | 78.8 | 0.095 | Adult | Female |
| SY-25 | 8/20/1999 | Sierra chorus frog | *Pseudacris sierra* | 1 | 8.22 | 7.09 | 38.04 | 77.2 | 0.052 | Adult | Female |

**Table AML-3. Mercury concentration data for frogs, South Yuba River watershed, California, 1999-2002**

[N, number of individuals; g, gram; mm, millmeter; µg/g, microgram per gram]

| Station Map ID | Collection Date | Common Name | Species | Sample size (N) | Live weight (g) | Sample weight (g) | Total Length (mm) | Moisture (percent) | Total mercury (µg/g wet) | Age | Gender |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SY-26 | 9/3/1999 | Sierra chorus frog | *Pseudacris sierra* | 1 | 2.63 | 2.35 | 29.69 | 80.9 | 0.017 | Adult | Male |
| SY-27 | 8/20/1999 | Sierra chorus frog | *Pseudacris sierra* | 1 | 5.7 | 4.80 | 38.16 | 77.0 | 0.091 | Adult | Male |
| SY-28 | 9/3/1999 | Sierra chorus frog | *Pseudacris sierra* | 1 | 4.37 | 3.59 | 31.27 | 81.9 | 0.020 | Adult | Male |
| SY-31 | 7/30/2002 | Sierra chorus frog | *Pseudacris sierra* | 1 | 0.68 | 0.61 | 22.40 | 78.6 | 0.054 | Juvenile | Unknown |

## Table AML-4. Mercury concentration data for fish, South Yuba River watershed, California, 2002

[g, gram; mm, millmeter; mg/kg, milligram per kilogram; Hg, mercury; %, percent; na, not available]

| Station Map ID | Station name | Station number | Collection Date | Common name | Species | Number | Type | Total length (mm) | Total weight (g) |
|---|---|---|---|---|---|---|---|---|---|
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | channel catfish | *Ictalurus punctatus* | 1 | fillet | 452 | 759 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | channel catfish | *Ictalurus punctatus* | 1 | fillet | 202 | 53 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | channel catfish | *Ictalurus punctatus* | 1 | fillet | 180 | 36 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | channel catfish | *Ictalurus punctatus* | 1 | fillet | 176 | 34 |
| SY-16 | Poorman Ck bl Ancho Erie Mine drainage nr Washington | 392513120453301 | 7/31/2002 | rainbow trout | *Oncorhynchus mykiss* | 5 | whole body | 183 | 75.2 |
| SY-16 | Poorman Ck bl Ancho Erie Mine drainage nr Washington | 392513120453301 | 7/31/2002 | rainbow trout | *Oncorhynchus mykiss* | 5 | whole body | 160 | 46.1 |
| SY-16 | Poorman Ck bl Ancho Erie Mine drainage nr Washington | 392513120453301 | 7/31/2002 | rainbow trout | *Oncorhynchus mykiss* | 5 | whole body | 125 | 19.2 |
| SY-14 | Ancho Erie drainage at Tailings Dam nr Washington | 392512120453101 | 7/31/2002 | rainbow trout | *Oncorhynchus mykiss* | 5 | whole body | 205 | 97.1 |
| SY-14 | Ancho Erie drainage at Tailings Dam nr Washington | 392512120453101 | 7/31/2002 | rainbow trout | *Oncorhynchus mykiss* | 5 | whole body | 172 | 57.5 |
| SY-14 | Ancho Erie drainage at Tailings Dam nr Washington | 392512120453101 | 7/31/2002 | rainbow trout | *Oncorhynchus mykiss* | 5 | whole body | 149 | 37.5 |
| SY-10 | Scotchman Ck bl Falls nr Washington | 392119120470201 | 7/29/2002 | rainbow trout | *Oncorhynchus mykiss* | 1 | fillet | 252 | 147.6 |
| SY-10 | Scotchman Ck bl Falls nr Washington | 392119120470201 | 7/29/2002 | rainbow trout | *Oncorhynchus mykiss* | 1 | fillet | 243 | 162.5 |
| SY-10 | Scotchman Ck bl Falls nr Washington | 392119120470201 | 7/29/2002 | rainbow trout | *Oncorhynchus mykiss* | 1 | fillet | 240 | 143.2 |
| SY-10 | Scotchman Ck bl Falls nr Washington | 392119120470201 | 7/29/2002 | rainbow trout | *Oncorhynchus mykiss* | 1 | fillet | 222 | 119.8 |
| SY-10 | Scotchman Ck bl Falls nr Washington | 392119120470201 | 7/29/2002 | rainbow trout | *Oncorhynchus mykiss* | 1 | fillet | 185 | 68.3 |
| SY-10 | Scotchman Ck bl Falls nr Washington | 392119120470201 | 7/29/2002 | rainbow trout | *Oncorhynchus mykiss* | 1 | fillet | 243 | 162.5 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | *Micropterus dolomieu* | 1 | fillet | 210 | 122 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | *Micropterus dolomieu* | 1 | fillet | 182 | 47 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | *Micropterus dolomieu* | 1 | fillet | 177 | 63 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | *Micropterus dolomieu* | 1 | fillet | 174 | 40 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | *Micropterus dolomieu* | 1 | fillet | 168 | 55 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | *Micropterus dolomieu* | 1 | fillet | 165 | 57 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | *Micropterus dolomieu* | 1 | fillet | 159 | 41 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | *Micropterus dolomieu* | 1 | fillet | 140 | 32 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | *Micropterus dolomieu* | 1 | fillet | 95 | 9 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | *Micropterus dolomieu* | 1 | fillet | 84 | 7 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | *Micropterus dolomieu* | 1 | fillet | 210 | 122 |

**Table AML-4. Mercury concentration data for fish, South Yuba River watershed, California**,

[g, gram; mm, millmeter; mg/kg, milligram per kilogram; Hg, mercury; %, percent; na, not available]

| Station Map ID | Station name | Station number | Collection Date | Common name | Total Hg-Dry (mg/kg) | Total Hg-Wet (mg/kg) | Moisture (%) |
|---|---|---|---|---|---|---|---|
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | channel catfish | 0.955 | 0.161 | 83.1 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | channel catfish | 1.4 | 0.248 | 82.3 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | channel catfish | 1.03 | 0.190 | 81.6 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | channel catfish | 0.962 | 0.181 | 81.2 |
| SY-16 | Poorman Ck bl Ancho Erie Mine drainage nr Washington | 392513120453301 | 7/31/2002 | rainbow trout | 0.305 | 0.084 | 72.3 |
| SY-16 | Poorman Ck bl Ancho Erie Mine drainage nr Washington | 392513120453301 | 7/31/2002 | rainbow trout | 0.311 | 0.088 | 71.8 |
| SY-16 | Poorman Ck bl Ancho Erie Mine drainage nr Washington | 392513120453301 | 7/31/2002 | rainbow trout | 0.198 | 0.051 | 74.2 |
| SY-14 | Ancho Erie drainage at Tailings Dam nr Washington | 392512120453101 | 7/31/2002 | rainbow trout | 0.297 | 0.086 | 71.2 |
| SY-14 | Ancho Erie drainage at Tailings Dam nr Washington | 392512120453101 | 7/31/2002 | rainbow trout | 0.266 | 0.075 | 71.7 |
| SY-14 | Ancho Erie drainage at Tailings Dam nr Washington | 392512120453101 | 7/31/2002 | rainbow trout | 0.206 | 0.060 | 71.1 |
| SY-10 | Scotchman Ck bl Falls nr Washington | 392119120470201 | 7/29/2002 | rainbow trout | 0.433 | 0.091 | 79 |
| SY-10 | Scotchman Ck bl Falls nr Washington | 392119120470201 | 7/29/2002 | rainbow trout | 0.724 | 0.159 | 78 |
| SY-10 | Scotchman Ck bl Falls nr Washington | 392119120470201 | 7/29/2002 | rainbow trout | 0.775 | 0.157 | 79.7 |
| SY-10 | Scotchman Ck bl Falls nr Washington | 392119120470201 | 7/29/2002 | rainbow trout | 0.413 | 0.085 | 79.3 |
| SY-10 | Scotchman Ck bl Falls nr Washington | 392119120470201 | 7/29/2002 | rainbow trout | 0.507 | 0.097 | 80.9 |
| SY-10 | Scotchman Ck bl Falls nr Washington | 392119120470201 | 7/29/2002 | rainbow trout | 0.628 | 0.139 | 77.9 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | 0.724 | 0.153 | 78.8 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | 0.627 | 0.133 | 78.8 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | 0.648 | 0.133 | 79.5 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | 0.647 | 0.144 | 77.8 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | 0.652 | 0.140 | 78.6 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | 0.728 | 0.159 | 78.2 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | 0.727 | 0.161 | 77.8 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | 0.788 | 0.174 | 77.9 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | 0.334 | 0.077 | 77 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | 0.4 | 0.085 | 78.7 |
| SY-56 | Birchville Pond nr French Corral | 391937121090201 | 7/23/2002 | smallmouth bass | 0.719 | 0.152 | 78.9 |