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Altered gill morphology in benthic macroinvertebrates from mercury enriched streams in the Neversink Reservoir Watershed, New York

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Abstract Aquatic macroinvertebrates were collected from five sites in the Neversink Reservoir Watershed in Sullivan County, New York: Aden Brook, Biscuit Brook, Main Branch, Tison and Winnisook, and examined for gill abnormalities. The Neversink Reservoir is part of the New York City water supply system and is located in the Catskill Mountains. Total mercury and methylmercury concentrations were measured by the New York State Department of Environmental Conservation (NYSDEC) in composite samples of macroinvertebrates at the five sites and ranged from 13.6 to 20.9 ng/g total mercury and 2.4–9.8 ng/g methylmercury. Gill deformities in the organisms were evident from each sampling site. These were observed as puckering or dimpling of the gill lamellae and interior spotting. The greatest percentage of gill morphological abnormalities were from invertebrates at the Main Branch site where 28% of invertebrate gills exhibited abnormalities. This site had the highest mercury/methylmercury concentration in composite invertebrate samples. Macroinvertebrates from a reference location showed little evidence of gill abnormalities. Other factors may have contributed to the abnormalities such as dissolved oxygen, pH, temperature, other contaminants, and/or stream profiles.

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Introduction

The gills of an aquatic macroinvertebrate are one of the most impacted structures on the body of the organism when the environment in which it lives is altered. They are especially sensitive due to their large surface area and their ability to accumulate compounds and gases (LaPorte et al. 2002). Heavy metals, especially mercury and its methylated form, and acidic pH are two factors that are often present in streams in the Northeastern United States due to the burning of fossil fuels elsewhere (EPA 2001).

Inorganic mercury is methylated in aquatic environments primarily in sediments enhanced by sulfate-reducing bacteria (Jeremiason et al. 2006). Methylmercury is the most toxic form of mercury in the food web, and it biomagnifies as trophic order increases and with the age of an organism (LaPorte et al. 2002; Trembly et al. 1998). There are two ways in which aquatic organisms accumulate mercury: through injested food and directly from the water. According to LaPorte et al. (2002), methylmercury is transported in organisms via amino acid channels in membranes.

Previous studies by Block et al. (1997), Boening (2000), and LaPorte et al. (2002) have demonstrated that mercury/methylmercury uptake is primarily via the gills in herbivorous fish, crab, and lobster. However, there are few studies regarding aquatic macroinvertebrates from mercury impacted streams and gill morphology. Pennuto et al. (2005) studied the mode of mercury accumulation in crayfish but not specifically the gills of the organisms.

It is well established that pH affects metal uptake in aquatic organisms (Baldigo and Murdoch 1997). The Neversink Reservoir tributaries in the Catskill Mountains, New York is an area that represents one of the highest acidic deposition rates from acid rain in the Northeast United States (Miller et al. 2005).

The New York State Department of Health has issued fish consumption advice for the Neversink Reservoir region due to high mercury concentrations in selected fish species (NYSDOH 2004). In particular, Loukmas and Skinner (2005) determined that yellow perch and smallmouth bass from acidic Catskill reservoirs had high levels of mercury. It is, therefore, possible that the macroinvertebrates, a major food source for these fish, would also exhibit higher levels of mercury which could impact gill structure (Hall et al. 1998).

The purpose of the present study was to investigate the morphology of macroinvertebrate gills from the Neversink Reservoir Watershed, to characterize possible associations of gill structure with mercury/methylmercury concentrations in the animals, and to consider other possible impacts on gill structure such as pH, or the presence of other contributing conditions.

Materials and methods

Aquatic macroinvertebrates were collected from several sites in the Neversink Reservoir system in the Delaware River Basin in the Catskill Mountains, New York, including Biscuit Brook, Aden Brook, Winnisook, Tison and the Main Branch (Fig. 1). The Neversink watershed consists of over 166 km^2 of which 95% is forested primarily representing mixed hardwood species. At elevations of between 480–1280 m (Winnisook the highest), the slopes down to the streams are steep, 40% (Lawrence et al. 2001). The sites were similar in that they exhibited similar aquatic vegetation, light availability, and stream width at sample site. All samples were collected in riffle zones with sand and gravel stream bottoms. Minimal leaf litter was evident at the time of collection. All samples were collected at times of relatively slow current. At the time of collection, the Quanta Hydrolab (Hach, Inc., Colorado) was used to measure temperature, specific conductance, dissolved oxygen and pH. All of these measurements were taken between 11:00 AM and 2:00 PM on several days in June, July, August, and September of 2003.

Fig. 1 Map of experimental sites on the Neversink Reservoir system in the Catskill Mountains, New York State, USA.The sampling region is indicated on the state map

Organisms were collected in cooperation with the New York State Department of Environmental Conservation (NYSDEC) by the kick sampling method in riffle zones (Bode et al. 2002). The samples were randomly sorted into two groups: one destined for total mercury and methylmercury analysis and another for gill examination. The samples were placed in glass jars and refrigerated. The organisms for mercury analysis were identified by NYSDEC personnel and composite samples of 1.0 g or greater were prepared. The samples were frozen and analyzed for total mercury and methylmercury concentrations at CEBAM Analytical, Inc. (USEPA Method 1631 2001 EPA2001; USEPA Method 1630 2001 EPA, 2001).

The samples held for gill examination were identified at the New York State Department of Environmental Conservation Biomonitoring Unit Laboratory, North Greenbush, NY. All macroinvertebrates in this study were identified to genus. After identification, the specimens were placed in glass vials with either a 70, 80 or 95% ethanol solution dependent on the macroinvertebrate order determined from Peckarsky et al. (1990). To study the gill morphology, the organism's gills were removed with forceps at their proximal points on the body, either on the abdomen, as for mayflies (Ephemeroptera) and caddisflies (Tricoptera) or on the thorax, as for stonefly (Plecoptera) specimens. The gills from each specimen were mounted on glass slides with euparol mounting medium, ASCO

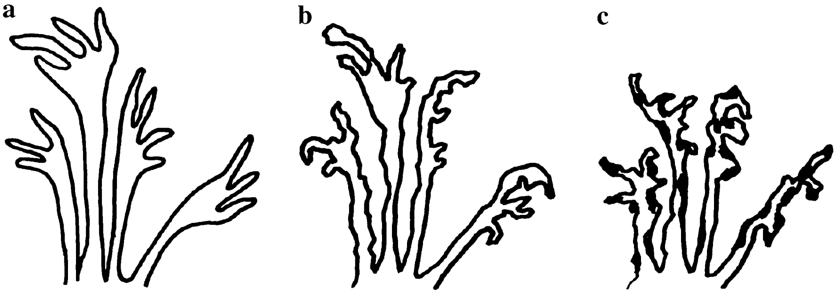

Fig. 2 Comparison of normal macroinvertebrate gill profiles: (**a**) Normal gill profile, not impaired, Long, phalanged gills. Strong stalks and branches. Periphery of gills without membrane perturbations or puckering. No discoloration.(**b**) Impaired gill profile. Many branches exihibit peripheral abnormality and membrane puckering. Slight discoloration/spotting.(**c**) Severely impaired gill profile. Many branches exihibit peripheral deterioration and puckering. Discoloration/spotting is prevalent. Gills atrophy

Laboratories. The presence or absence of gill abnormality, and if present, the relative severity of the abnormality was recorded (Fig. 2).

Reference animals were collected by kick sampling from the Poesten Kill, a tributary of the Hudson River in Rensselaer County, New York. The Poesten Kill site represents a stream similar to the Neversink tributaries but approximately 140 km north of the Catskill sites, and less impacted by acid rain and mercury deposition. Gills from twenty-one macroinvertebrates were examined for abnormalities using the procedure explained above.

Results

Table 1 provides the average number of macroinvertebrates (as a percentage of the total animals examined) exhibiting gill impairment from each site sampled. Impairment was recorded if the gills of an organism exhibited puckering or dimpling at the membrane margins and/or if discolorations or spots, that often appeared red, were evident in the interior structure of the gills. A normal gill compared to an impaired gill is shown in Fig. 3. The Aden Brook site where twenty-two aquatic macroinvertebrates were examined for gill abnormalities, and the Winnisook site, where nineteen animals were screened, exhibited the lowest levels of gill impairment at the Neversink sites, 15 and 16% respectively. This correlates with 16.9 and 13.6 ng/g average mercury and 7.0 and 2.4 ng/g average methylmercury in composite samples of macroinvertebrates.

Macroinvertebrate gills from Biscuit Brook, Tison, and the Main Branch locations had 23, 25, and 28 percent abnormalities, respectively, and total mercury concentrations in composite samples of 20.9, 15.4, and 20.6 ng/g. The methylmercury concentrations from animals at Biscuit Brook, Tison, and the Main Branch were 7.7, 5.1, and 9.8 ng/g respectively.

Table 1 Percent gill impairment as it relates to temperature, dissolved oxygen, pH, mercury, and methylmercury. Percent impairment represents the number of animals affected as a function of the total number of animals at that site. The percents were rounded to the nearest whole number

Site	Temp(°C)	D. O. (mg/l)	PH	Number of macro-invertebrates	Number of taxa collected	Total mercury[a] (ng/g)	Methylmercury[a] (ng/g)	% Impairment
Poesten kill (Reference)	11.00	8.33	7.01	21	5	200 (ng/l)[b]	N.A.	5
Aden brook	10.20	9.95	6.20	22	4	16.9	7.0	15
Winnisook	9.40	9.56	4.97	19	2	13.6	2.4	16
Biscuit Brook	9.40	9.60	6.80	13	6	20.9	7.7	23
Tison	10.40	9.41	5.38	32	2	15.4	5.1	25
Main branch	11.80	7.82	6.58	25	2	20.6	9.8	28

[a] Determined in composite samples of macroinvertebrates collected by NYSDEC

[b] Represents water column data (NYSDEC, 2003). Methylmercury was not analyzed. The mercury detection limit was 200 ng/l. N.A. represents not available

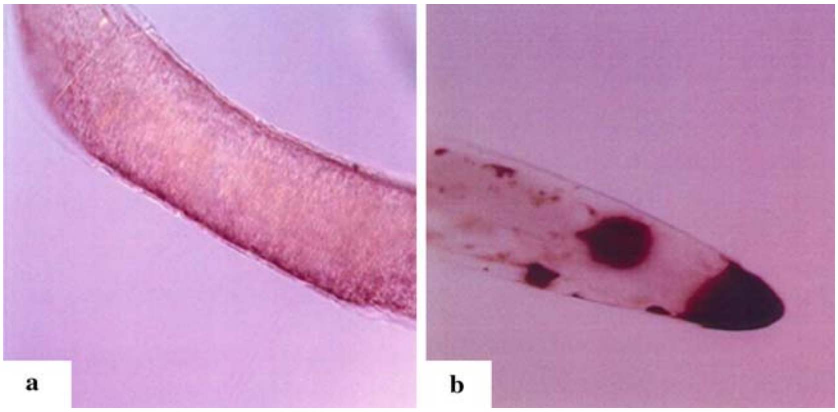

Fig. 3 (**a**) Non impaired gill from Northern case maker (*Pycnopsyche* sp.) (**b**) Impaired gill from Northern case maker (*Pycnopsyche* sp.). Gill exihibits internal discolorations

Table 2 Macroinvertebrates examined for gill abnormalities at each sampling site

Site	Macro-invertebrate		Number
	Family/Genus	Common name	
Aden Brook	Heptageniidae/*Stenacron*	Flatheaded mayfly	7
	Hydropsychidae/*Hydropsyche*	Net-spinning caddisfly	1
	Limnephilidae/*Pycnopsyche*	Northern case maker caddisfly	1
	Pteronarcidae/*Pteronarcys*	Giant stonefly	13
Winnisook	Heptageniidae/*Stenacron*	Flatheaded mayfly	1
	Siphlonuridae/*Siphlonurus*	Primitive minnow mayfly	18
Biscuit Brook	Baetidae/*Baetis*	Small minnow mayfly	1
	Ephemerellidae/*Ephemerella*	Spiny crawler mayfly	3
	Heptageniidae/*Stenacron*	Flatheaded mayfly	1
	Hydropsychidae/*Hydropsyche*	Net-spinning caddisfly	2
	Limnephilidae/*Pycnopsyche*	Northern case maker caddisfly	3
	Pteronarcidae/*Pteronarcys*	Giant stonefly	3
Tison	Heptageniidae/*Stenacron*	Flatheaded mayfly	27
	Siphlonuridae/*Siphlonurus*	Primitive minnow mayfly	5
Main Branch	Ephemerellidae/*Ephemerella*	Spiny crawler mayfly	20
	Limnephilidae/*Pycnopsyche*	Northern case maker caddisfly	5
Poesten Kill (reference)	Ephemerellidae/*Drunella*	Spiny crawler mayfly	5
	Glossosomatidae/*Glossosoma*	Saddlecase maker caddisfly	2
	Heptageniidae/*Stenonema*	Flatheaded mayfly	2
	Limnephilidae/*Pycnopsyche*	Northern case maker caddisfly	6
	Siphlonuridae/*Siphlonurus*	Primitive minnow mayfly	6

Table 2 contains the genus analyzed at each site, including the reference site. It was not possible to maintain absolute consistency of genus type in sufficient numbers at all sites because of the lack of availability of some animals at some sites. Giant stoneflies, flatheaded mayflies, Northern case maker caddisflies, and net-spinning caddisflies were found at Aden Brook. At Winnisook, only the primitive minnow mayfly and the flatheaded mayfly were available.

The greatest diversity of species collected occurred at Biscuit Brook with the giant stonefly, the Northern case maker caddisfly, the net-spinning caddisfly, the flatheaded mayfly, the spiny crawler mayfly, and the small minnow mayfly. At Tison, only the primitive minnow mayfly and the flatheaded mayfly were available for examination. The Main Branch, the collection site nearest to the Neversink Reservoir, yielded Northern case maker caddisflies and spiny crawler mayflies.

Twenty-one reference macroinvertebrates from the Poesten Kill, a tributary of the Hudson River in Rensselaer County, New York, were examined and one animal exhibited gill abnormalities, representing 5% of the animals examined.

Discussion

Gill abnormalities were present in aquatic macroinvertebrates with detectable tissue mercury and methylmercury from the Neversink Watershed. Alternatively, the gills examined from twenty-one macroinvertebrates collected from the Poesten Kill, a stream located outside the Catskill region, showed little evidence of gill abnormalities. Although levels of total mercury and methylmercury have not been measured in samples of macroinvertebrates from this site, total mercury in the water column has been measured as non-detectable (NYSDEC–Statewide Waters Monitoring Section, 2003).

Other factors, alone or in combination with mercury, may have contributed to the abnormal gill morphology. Acidic conditions may mobilize deposited inorganic mercury to be converted to methylmercury which can be taken up by biota. Block et al. (1997) demonstrated an increase in methylmercury concentrations in minnows when exposed to a pH of 3.9. It is possible that the combination of acidic pH, mercury/methylmercury concentrations in water and sediments and other factors such as varying mercury loading rates, dissolved organic carbon and temperature caused an increase in the bioaccumulation of methylmercury in aquatic organisms (Simonin et al. 1998; Watras et al. 1998).

The pH of the five experimental sites varied from 4.97 at Winnisook to 6.80 at Biscuit Brook, and was measured at 7.01 at the Poesten Kill reference site. Although the sites on the Neversink tributaries all exhibited pH values below seven, the Winnisook site, with the lowest pH did not yield the highest percentage of gill abnormalities. However, this site had the lowest total mercury and methylmercury concentrations in macroinvertebrate composite samples, 13.6 and 2.4 ng/g. It is unclear from the present study why the relationship between pH and mercury concentrations in animals from the experimental sites is not direct. It is possible that since mercury/methylmercury levels were elevated at all Neversink sites sampled, pH may not have played a major distinguishing role because all measured pH was acidic. Stream profile and processes may have, instead, have played a larger role. For example, the Neversink Watershed Study established that there was a connection between acid deposition, loss of soil calcium, and stream water acidification (Lawrence et al. 2001).

Braeckmann et al. (1998) found that as temperature increased, the concentration of methylmercury in the burrowing mayfly *(Hexagenia rigida)* increased. In the present study, at temperatures in the range of 9.4–11.8°C, it is unlikely that the gill abnormalities seen were caused by temperature alone.

Dissolved oxygen measurements at each site sampled in the present study ranged from 9.95 mg/l at Aden Brook to 7.82 mg/l at the Main Branch. Since these levels all represent acceptable conditions for macroinvertebrate survival, it is probable that lack of dissolved oxygen was not responsible for gill impairment.

The largest percentage of gill impairments occurred at the Main Branch site, the location nearest the Neversink Reservoir (Fig. 1). This site is correlated with the highest mercury/methylmercury concentrations in composite macroinvertebrate samples collected by NYSDEC at the five Neversink tributaries examined in this report (Loukmas et al. 2006). However, the number of animals available at the higher impacted sites was not limited by the concentration of mercury or methylmercury.

Other studies have demonstrated an impact on gill morphology due to contaminants other than mercury or methylmercury. Damage to aquatic insect gills was observed by Simpson (1980) in macroinvertebrates from streams exposed to chlorinated or crude oil wastes. He documented tar like spots and membrane abnormalities in the gills of some stoneflies and caddisflies. Ribeiro et al. (2000) found that the bioaccumulation of inorganic mercury caused fish gill lamellae abnormalities. An increase in aluminum impacted Atlantic salmon gills in another study (Fivelstad et al. 2003). Lawrence et al. (2001) established that aluminum concentrations in the Neversink watershed were elevated.

The discolorations that were viewed in the altered gills of the present study are consistent with burst tracheoles or thinned cuticular areas, similar to changes in stonefly nymph gills seen by Lechleitner et al. (1985). Additionally, it is known that insects have special plasma proteins within their hemolymph which function in the immune response, especially against bacterial and fungal infections. These proteins respond to the infected site by releasing peptides, specifically prophenoloxidase, which produce quinones that form melanin which alter the color of the infected site (Kanost 2003). If the macroinvertebrates in the present study were stressed due to the presence of mercury, methylmercury, or other factors, bacteria may more easily have infected the gill lamellae to cause a release of melanin resulting in the discolorations seen.

It is interesting that only one animal out of twenty-one exhibited dimpling or puckering of gill membrane margins from the Poesten Kill despite the similarity of the physical characteristics of the Neversink sites to those in the Poesten Kill, particularly the gentle current. Therefore, it is unlikely that physical damage due to swift current caused the gill abnormalities seen in the Neversink organisms.

No significant correlations, at $p = 0.05$, were found between percent gill impairment and total mercury, methylmercury, temperature, dissolved oxygen, or pH using the data in Table 1. The only significant correlation, $p < 0.01$, was between total mercury and methylmercury in the macroinvertebrate samples. Therefore, in the present study, although a greater percentage of gill impairments were found in animals with detectable mercury/methylmercury concentrations, no significant causative relationship can be assigned without further study.

Additional field and laboratory experiments will be done on macroinvertebrates from other locations to determine if mercury/methylmercury concentrations are associated with gill abnormalities.

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