Toxic Effluents and the Effects on the Ecosystem

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Abstract:

Research was conducted by students at Coastal Carolina University to gain information on the effects of paper pulp kraft mill effluents in the aquatic environment. This issue was taken into account on a global scale as well as local and the effects were measured on organisms directly effected as well as those that are effected further down the food chain as dictated by trophic transfer.

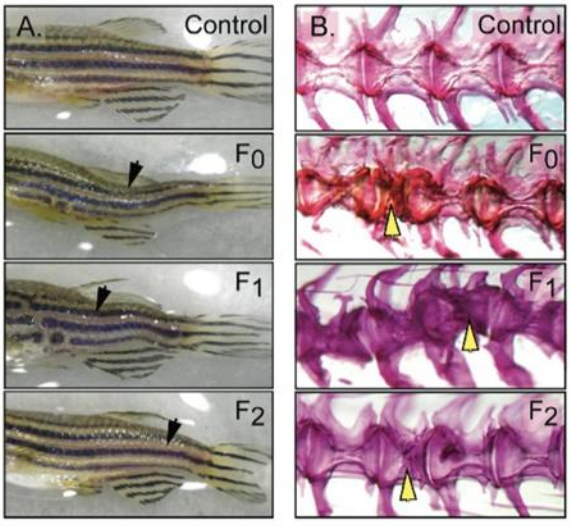
Starting with the Industrial Revolution the amount of pollutants dumped into the ocean over time have dramatically increased. The placement of an industrial plant or mill on a coastal area is ideal because they can be fueled by the uptake of the ocean or river water. Bleaching of paper by these pulp mills causes multiple chemical effluents to enter the aquatic environment at the point source of these mills. The side effects of this chemical pollution include degradation of the water quality and disturbances to the local ecosystem. Paper mills are a prime example of the industrialization that produces pollutants, in fact, they are the fifth largest contributor to water pollution (Sharma et al 2014).

Paper mills use plant fiber and raw materials such as cellulose fibers, wood, recycled paper, and agricultural residues for production (Sharma et al 2014). Once these materials have been used the mill is left with the effluents, which are the liquid waste that is discharged from a mill. Paper mills leak tons of un-treated effluents into the water system which is harmful to the environment and its inhabitants. Some of these effluents include, dioxin, β-sitosterol, PCBs, and CYP1A. These chemicals are either produced indirectly in production efforts or indirectly. For example, dioxin happens to be composed of a few chlorinated compounds and is an unintentional result of the paper mill and pulp production, while β-sitosterol is a plant based compound (Kim, et al. 2013) which comes directly from the materials used in the mills. CYP1A is a toxin that directly affects hydrocarbons which then increases the toxicity of compounds and PCB's result from electrical coolant systems and are found in copy paper. Although PCB's were banned in 1977 they may still have a long term effect on the environment due to the adaptation some fish evolved when exposed to PCBS. Furthermore, the fish that were not able to adapt,

died, therefore this toxin has already taken its extreme toll on the environment and this effect begins at the trophic level involving bacteria and fish and is carried out all throughout the food chain of the aquatic ecosystem.

In 2006 alone, the United States pulp and paper industry has generated 200 million pounds of hazardous wastes (EPA, 2013). This is an alarmingly large rate and this estimate does not even take into account the wastes that are being dumped in other bodies of water globally. Paper mill plants are located all over world, from Costa Rica to our very own International Paper mill in Georgetown, South Carolina. There are some methods of removing the effluents such as biological or chemical treatments and some agencies such as the EPA and CPCB to monitor the amounts of discharge, however, many of these attempts are mostly ineffective on a wide scale and the ecosystem continues to suffer the effects.

The chemicals found in water generated from paper mill production are linked to adverse effects in the environment, several vertebrate species and specifically fish. Coastal areas are important habitats for larval and juvenile fish and these are the same areas that are experiencing the direct exposure from paper mills. The effects of effluents on fish can be seen years after exposure and may lead to the extinction of certain species all together or disrupt early development of fish. Fish are a large food resource not only for humans but for other marine vertebrates and invertebrates. If the fish are being exposed at the larval stage and juvenile stage, they are accumulating toxin in their tissues for their entire lives and can then pass it on to their predators as well as their offspring. The following research was conducted to further explore the effects of four different effluents on the marine ecosystem and to examine how the toxins proceed to affect fish and other organisms through trophic transfer.



Dioxin is a sediment driven toxin that is released during paper mill production. In a study conducted at the University of Wisconsin, the effects of dioxin on zebrafish were examined. Three generations of fish were observed after direct or indirect exposure to dioxin and this exposure showed abnormalities, most predominately in skeletal and reproductive functions. Physically, kinks in the spine were the most shocking discovery. All three generations resulted in a bent and distorted shape due to the spinal kinks (Figure 1).

Figure 1. Image from study at University of Wisconsin showing physical abnormalities due to Dioxin exposure

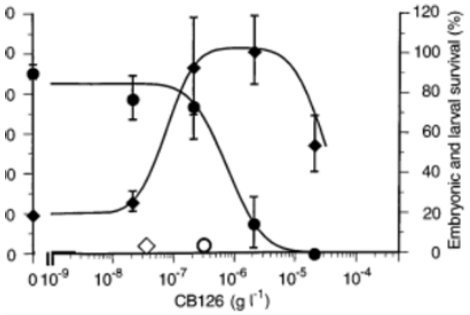
A healthy zebrafish typically has a smooth, thin, and streamlined shape and are small fast swimmers, therefore a zig-zag shape to their spine is likely to create much more drag than normal. Not only is this physical abnormality detrimental to the health of the fish but it also is likely to make them more susceptible to prey. In addition to the spinal deformities the individual vertebrae of the exposed lost their natural shape as well. Naturally, each vertebrae should appear similar to an hour glass shape. After exposure to dioxin, the vertebrae became distorted and some even completely collapsed. Vertebral and spinal deformities can occur naturally, however less than 2% of the population observed in the wild exhibit this deformity. The reproductive capabilities were also hindered, resulting in a lack of ovarian organization, the presence of testes in females and a decrease in fertilization success. This allows for good insight as to what one specific chemical of paper mill pollution has on fish, although there are many more harmful effluents entering the ecosystem as well.

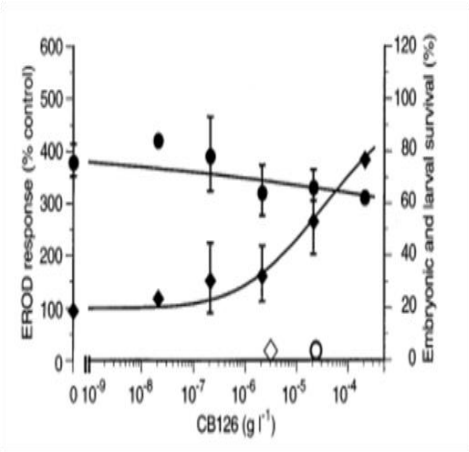
CYP1A is a dioxin like compound that is another of these chemicals affecting fish reproduction and sexual tendencies. Costa Rica is one place in particular where this occurrence can be observed. The *Parachromis dovii* and *Poecilia gillii* commonly known as the Guapote and the Short Fin Mollie. RNA biomarkers were used to detect dioxin like compounds and other harmful chemicals in the water. Palo Verde National Park and aquaculture laboratory of the Universidad Nacional at Heredia, Costa Rica studied the *P. dovii* and the *P. gilli* finding that they are both applicable candidates for RNA biomarkers.

After these fish were obtained they were prepared for the experiments and had to be acclimated to certain conditions before they could be used, which would usually take around one month. The fish obtained in the field were kept in the laboratory at 24° C in filtered, de-chlorinated UV treated water. After exposing the fish, the next step of the experiment was to sequence DNA and to isolate RNA from the livers of the fish. The RNA was then treated with DNase to remove any genomic containments. After all the genetic work was done, the fish were transported to sites in aerated containers. One control group was always present consisting of ten fish. Young fish exposed to CYP1A can have problems with heart growth as it affects the layered cells around the heart as well as sex change of the fish observed.

β- sitosterol is another chemical effluent linked to paper mill pollution with long lasting effects and reduction in the reproduction of fish. β- sitosterol a plant sterol that is found in heightened concentrations at the sites of bleached kraft pulp mills. It occurs naturally in legumes, wood and plant oils. Depressed hormone levels and later age of reaching sexual maturity are among the most common of adverse effects linked to exposure of β- sistosterol. A study was conducted in the Hagen Aqualab facility of the University of Guelph in Canada which measured the effects of β-stiosterol on sexually immature rainbow trout. Fish were obtained from a local fish farm and exposed to 34 liters of two different test strains of β-sitosterol from two Canadian mill locations, for 21 days, and each of the treatment groups contained 12 fish equally distributed in two tanks (Tremblay et al 1999) which were compared to a control group of white sucker fish. When the exposure period was over, the fish were anesthetized and blood samples were taken and many measurements on gonad and liver size were recorded. The overall findings were that the testosterone levels in the exposed rainbow trout were 30 percent less than that of the control group. Pregnenolone is a steroid hormone that is the building block of many other hormones such as testosterone, cortisone and estrogen (MacLatchy et al 1995). The pregnenolone levels observed in the rainbow trout were also significantly lower in the exposed fish as well as total plasma cholesterol levels significantly reduced.

A second study done with β- sitosterol was also conducted in Canada in which goldfish that were obtained from a local fishery were injected with either β-sitosterol or a placebo sesame oil to measure the effects of this effluent on the reproductive systems of the goldfish. The fish were exposed for a total of four days and steroid levels in the males and females were monitored daily. Male fish were also subjected to oxidized forms of β-sitosterol as this is another form of the effluent that they would be exposed to if inhabiting environments near the point source of the paper mills. The results of this study concluded that exposed fish have a delayed age of sexual maturation, in addition to decreased gonadal size and reduction of secondary sexual characteristics, and a reduction in plasma levels of the reproductive steroids (MacLatchy et al 1995).





PCBs and dioxins were tested on the estuarine fish Fundulus heteroclitus, commonly refered to as mummichogs, during the early development stages of embryos and larvae to determine the contaminants they were being exposed to in their environment. The pollutants found in the highly contaminated sites were dioxin-like compounds (DLCs). There has been evidence of adaptation of invertebrates to environmental contaminant exposure, and “this change in genetic structure is an evolutionary mechanism by which populations adapt to local conditions” (Nacci et al. 1999). The population of F. heteroclitus that lived in high DLC concentrated environments would display an adaptation (Fig. 3) to

Top: Figure 2. Survival and EROD responses to DCL of West Island Fish (Reference Fish) fish embryos

Bottom: Figure 3. Survival and EROD responses to DCL of New Bedford Harbor fish embryos

the high exposure of the DLCs which are toxic to the early development of many fish including F. heteroclitus. These populations have showed to have a tolerance built against the local contaminants. The reference fish that are not exposed to these dioxins tend to not survive after the exposure (Fig. 2). Without an adaptation of high tolerance to the exposed levels of DCLs and PCBs, the fish ultimately die. This resulted in a larger number of fish dying as not all fish can adapt to these PCBs. This study compared the mean responsiveness of the different contaminated areas, individuals who showed variation, if adaptation of DLC was inherited and if these adaptations occurred to the environment exposure. When considering the effects of paper mill effluents on fish, the effects it has on the environment must also be considered. Since fish are a main source of food for not only humans but other vertebrae the toxins stored in the tissue of the fish can be passed on to the secondary consumers of the fish. This trophic transfer creates the toxins to expand far beyond the local environment of exposure to the neighbors of paper mills. The fish that are initially exposed at the mill sites may migrate and transport toxins to other fresh water or salt water systems. When these “toxic fish” are eaten they can not only affect the predator that eats the fish, but females will transfer the toxin to their placenta and affect a further generation of their offspring.

The transfer between predator and prey could specifically effect birds and mammals more, because fish have the ability to eliminate dioxins through the gills while mammals and birds can only do so through by less efficient means such as metabolism and through feces. (Loonen et al. 1996). The transfer of paper mill effluents to mammals can result in mortality, reproductive failure, reduced kit survival and increased liver weight (Loonen et al.1996). The mink is an example of a marine mammal that is very sensitive to the exposure of toxins through indirect exposure. While minks cannot be found in South Carolina this species is a good indicator of the trophic effects on a larger scale in other locations. A popular native animal to South Carolina however is the alligator. Alligators have been shown to possess concentration of toxins in their eggs in sites that are more remote and lacking the presence

of a mill. Because many of the toxins such as dioxin, are sediment driven, it is unlikely that the exposure came from the mill itself and exposure is likely due to trophic transfer.

Further studies to explore the harmful effects of the release of toxins should be conducted because the conservation and management of our water is critical. The toxic wastes that are being disposed into the waters are harming the marine life, their stages of development and their reproductive capabilities. These compounds are such a threat because of their ability to bioaccumulate and persist under certain conditions. There are also several more compounds being discharged into the water to consider although the above only focused on a few of these toxic compounds.

Not only do the toxins harm the fish which are at the base of the food chain for many predators but in turn they harm the predators through trophic transfer. Many coastal organisms face enough stress through habitat loss and natural changes in water quality. Adding harmful substances into the environment in which they live can only increase the rate of mortality. To understand the decline in populations is very important. The naturally existing food chain is very sensitive and if key prey and predators are being lost in the environment, then the entire ecosystem dynamics will change or collapse. The challenge presented by the entrance of these effluents into the aquatic environment is that they have so heavily made their mark and take such a long time to cycle through the environment, therefore continued study and efforts to reduce amount of toxins released into the environment must be done before this problem becomes any more severe.

Annotated Bibliography

Baker, T.T., Peterson, R.E, Heideman, W. 2014. Using Zebrafish as a Model System for Studying the Transgenerational Effects of Dioxin. Toxicological Sciences, 138 (2), 403-411

This article is regarding a controlled study that was done at the University of Wisconsin. The article reviews the direct effect of dioxin on zebrafish. It provides as a good insight to what one "specific" toxin is capable of doing to a small fish like the zebrafish.

Cobb, G. P., Houlis, P. D., & Bargar, T. A. (2002). Polychlorinated biphenyl occurrence in american alligators (alligator mississippiensis) from louisiana and south carolina. Environmental Pollution, 118(1), 1-4.

The effects on dioxin-like pollutants on the environment are clearly noticeable, even in South Carolina waters. In one study eggs of the American Alligator were taken from multiple sites around the Bear Island and Winyah Bay. The goal of the study was to determine the concentrations of PCBs in the egg tissues of the collected alligator eggs. The results were conclusive that this dioxin-like compound was present in this new generation of alligators.

Environmental Protection Agency Newsletter. 2014.Transforming Papermill pollution intoCommercial Resource http://www.epa.gov/sciencematters/june2011/papermill.htm

Environmental Protection agency newsletter containing data on amounts of dioxin released into the environment and discusses the findings of researchers that may result in the decrease of toxins released from mills.

Loonen, H., Van, d. G., Parsons, J. R., De Voogt, P., & Govers, H. (1996). Ecological hazard assessment of dioxins: Hazards to organisms at different levels of aquatic food webs (fish- eating birds and mammals, fish and invertebrates). Science of the Total Environment,182(1-3), 93-103.

In the next study researches looked at the hazards of dioxins at different levels of the food web. The study was done on marine invertebrates, fish, mammals that feed on fish, and fish eating birds to determine the effects of dioxin on each food web level. Dioxins have extensive effects on fish, mammals, birds, and invertebrates in this study. The side effects range from reproductive failure to death. In conclusion of this article it was stated that the effects were greater for mammals and birds in a food web than any of the other organisms mentioned.

MacLatchy, L. D. & Van der Kraak, J. G. (1995). The Phytoestrogen B- Sitosterol Alters theReproductive Endocrine Status of Goldfish. Toxicology and Applied Pharmacology, 134, pp 305-312.

This article explains the methods and results of a study conducted at the University of Guelph in which male and female goldfish were exposed to a placebo sesame oil or B- sitosterol for four days and measured for reproductive function.

Nacci, D., Coiro, L., Champlin, D., Jayaraman, S., McKinney, R., Gleason, T.R., Munns, W.R., Specker, J.L., Cooper, K.R. (1999) Adaptations of wild populations of estuarine fish Fundulus heteroclitus to persistent environmental contaminants. Marine Biology 134, 9-17

This article describes an experiment that was tested on the estuarine fish Fundulus heteroclitus to analyze the effectiveness of dioxin-like compounds (DLCs) on their early development. There was two different sites that the samples were taken from two sites then compared data to determine the survival rate of these fish exposed to the DLCs. This article gives more background information on the effects of dioxins on fish and marine organisms.

Navarro, A., Mena, F., Cansado, M., Pinnock, M., De La Cruz, E., Barata, C., and Pina, B.2014. "Development of Quantitative RNA Biomarkers for detecting Dioxin-like and Estrogenis Pollutants in Costa Rican Native Fish Species." Journal of Environmental Biology35 pp 99-105

The article was about a controlled study, whether or not RNA biomarkers in specific Costa Rican fish would be feasible. The RNA biomarkers are a part of the fish's genetic strands that can be used to detect dioxin and other toxic chemicals in the water. After preparation and experimentation itwas shown that the fish are feasible as a RNA biomarkers for detecting dioxin and other toxic chemicals. The monitoring of environmental impact is crucial for the correct management of an ecosystem.

Sharma, R. Chandra, S., Singh, A., and Singh, K. 2014. Degradation of Pulp and Paper MillEffluents. IIOAB Journal, 5(3), pp 6-12.

This article discusses paper mill effluents and conducts a study in China. The article provides detailed background on the production in mills, to include what is used to make paper and what byproducts are produced as a result. This is a good source for understanding the production in paper mills.

Tremblay, L. & Van der Kraak, J. G. (1999). Comparison between the Effects of the Phytosterol B-Sitosterol and Pulp and Paper Mill Effluents on Sexually Immature Rainbow Trout. Environmental Toxicology and chemistry 18(2), pp 329-336.

This article describes the methods and results of a study conducted at the University of Guelph in which sexually immature rainbow fish were exposed to B- sitosterol for a 21 day period in vivo and measured for reproductive weight and gonads.