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The Beauty and Sorrow of a Tropical Paradise: Anthropogenic Pollution in Coastal Water of Kenting National Park, Taiwan

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Abstract

Kenting National Park (KNP) is a very popular tourist area in Taiwan, attracting millions of visitors every year. Activities of tourists and the local population create tremendous pressure on nearby environment. Coastal development has caused urbanization, deforestation, and soil erosion. Runoff during heavy rainfall carries terrestrial contaminants into the receiving streams and can severely affect water quality. Excess nutrients in the water can cause eutrophication and change coral reef community structure. Oil spill from grounded ships causes catastrophic consequences to the coastal ecosystems. Domestic wastewater is another important issue in KNP. Treated and untreated municipal wastewater is discharged directly into the local river and ditch systems and flows into the estuaries. The wastewater contains a complex mixture of contaminants, including excess nutrients, pesticides, PAHs, dioxins, pharmaceuticals and personal care products (PPCPs). These compounds may cause carcinogenic, teratogenic, neuroactive, and/or endocrine disrupting effects to fish. This paper aims to discuss the pollution in coastal water of KNP, with an emphasis on toxicity to fish and PPCPs in the surface water of KNP. Our research found that wastewater discharges in KNP contain high levels of ammonia, nitrite and PPCPs, resulting in lethal and behavioral toxicities in developing fish.

Keywords: Kenting, Taiwan, ecotoxicology, behavior, PPCPs

Introduction

Kenting National Park (KNP) is located on the southernmost coast of Taiwan. KNP is one of the most popular tourist attractions in Taiwan, attracting millions of visitors every year due to its beautiful beaches, coral reefs, and abundant tropical biodiversity. The movie Cape No. 7 (海角七號) filmed here in 2008 was so popular that more and more domestic and international tourists are attracted to visit KNP. According to the latest statistics, the number of tourists visiting KNP has reached a record high of almost seven million in 2015. During holidays, summer vacation, and special events, hundreds of thousands of tourists rush into KNP, creating tremendous pressure on the environment.

To accommodate so many tourists, the coastal area of KNP has been under intensive development for hotels, restaurants, and other recreational facilities. Ill-conceived developments has caused coastal urbanization, deforestation, and soil erosion (Meng et al., 2008). Runoff during heavy rainfall also carries terrestrial contaminants into the receiving streams and can severely affect water quality. These anthropogenic wastes eventually flow to the estuaries and drain into nearby coasts and coral reefs (Lin et al., 2007; Liu et al., 2012). Indeed, previous reports have shown that streams and ditches in KNP are the major point sources of nutrients, ammonia, and suspended solids for the nearby coastal waters (Lin et al., 2007; Meng et al., 2008). Suspended solids and sedimentation resulting from runoffs can cause bleaching, smothering or even direct death of the corals in the reefs.

Domestic wastewater is discharged directly into the local streams and ditch systems. Excess nutrients in the water can cause eutrophication and consequent blooming of macroalgae and anemones. Competition between these creatures and stony corals can cause deterioration of local coral reef ecosystem, turning healthy coral reefs into ecosystems dominated by macroalgae and anemones (Liu et al., 2009). Unlike stony

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corals, macroalgae and anemones do not form complex physical structure so they cannot provide diverse microhabitats for other reef creatures. Therefore, eutrophication causes not only decline of coral populations but also changes in community structure in the coral reefs.

Oil spill is another serious environmental threat. Although not often, oil leakage from grounded ships may cause catastrophic consequences to the coastal ecosystems. The most severe oil spill incident which has occurred in KNP was the Amorgos oil spill in January of 2001 (Chiau, 2005). Approximately 1300 tons of fuel oil leaked from the grounded Greek merchant vessel *Amorgos,* covering ~5 km KNP shoreline. Spilled oil in the sea or on the shore can smother wildlife to death. Feathers of seabirds covered by oil lost their capability for flight and thermal insulation. Spilled oil can cause acute lethal and chronic sublethal toxicities to marine life such as fish and corals. The toxic effects include death, tissue necrosis, developmental abnormality, growth retardation, reproductive impairment, and coral bleaching. Many studies have shown that the dispersants used to clean spilled oil are even more toxic than the oil (Negri and Heyward, 2000; Shafir et al., 2003, 2007). Oil also contains complex mixture of polycyclic aromatic hydrocarbons (PAHs); many PAH compounds are carcinogenic or teratogenic. In additional to oil leakages, combustion of fuels by vehicles is another important source of PAHs for the air and water in KNP (Cheng et al., 2013; Ko et al., 2014).

Ecotoxicology of wastewater to fish: whole effluent toxicity testing

Runoff and wastewater discharges contain a complex mixture of contaminants including excess nutrients, industrial chemicals, pesticides, PAHs, dioxins, and pharmaceuticals and personal care products (PPCPs) (Notch and Mayer, 2009). The major economic activity in KNP is tourism. Therefore, we can expect that chemicals resulting from human daily life such as PPCPs would be significant in the wastewater. PPCPs in the surface water of KNP have never been formally characterized. However, previous studies have shown that human and veterinarian pharmaceuticals are commonly detected in waste streams and surface waters in other areas of Taiwan (Lin and Tsai, 2009; Lin et al., 2008). It has been shown that wastewater discharges in KNP may lead to the drop of coral coverage (Meng et al., 2008). A study conducted along the coast of Malta of Italy in the Mediterranean found that sewage discharges can alter rocky-reef fish community assemblages (Azzurro et al., 2010). However, the effects on fish of sewage discharges in KNP have never been studied.

Traditionally, regulation of wastewater effluent discharges relied heavily on chemical analyses of water quality. Normally, only a few items, e.g., biological oxygen demands (BODs), chemical oxygen demands (CODs), and suspended solids (SS), are routinely regulated by the wastewater discharge standards. In some cases, specific chemicals such as some heavy metals and organic contaminants would be measured. However, it is infeasible to identify and quantify all the chemicals in wastewater due to the high cost of money, time, and labor. Even if one can characterize most, if not all, the chemicals in the wastewater, the complex interactions of multiple compounds (e.g., synergism, antagonism, addition, and potentiation) in the mixture can result in toxic effects that are not observed in single-chemical exposures (Belz et al., 2008). Therefore, a more holistic approach is needed to characterize the toxic effects of wastewater on the organisms in the receiving waters.

To address this issue, U. S. Environment Protection Agency (U. S. EPA) started to formalize whole effluent toxicity (WET) testing in 1985 and keeps revising their protocols afterwards (USEPA, 2002a, b). Some other countries including Canada, Germany, and Netherlands had also come up with similar approaches for monitoring and regulating wastewater discharges. Indeed, WET testing has become an important tool in the discipline of ecotoxicology in the last couple decades. Taiwan EPA has also developed some methods to evaluate acute toxicity of effluents on freshwater organisms. WET testing is a holistic approach that integrates interactions among complex mixtures of contaminants in the wastewater. WET tests measure the total toxicity regardless the chemical composition. Therefore, WET testing can be done before the chemistry information in the water is identified. Compared to comprehensive chemical analyses, WET testing can be relatively simple and cost effective. However, it still has some disadvantages. WET testing by itself is not diagnostic, i.e., it cannot identify the causative chemical(s) of the toxic effects. The testing methods are not necessarily environmentally realistic, and they cannot test everything (Chapman, 2000). In fact, there is no single, perfect, universal tool available in ecotoxicology. Therefore, WET tests should not be used alone. When combined with other approaches, such as chemical analysis and risk assessment, WET testing is an effective tool to identify and characterize the toxic effects of discharges on aquatic organisms (Chapman, 2000).

Fish is one of the most important test organisms due to its ecological and economic significance. Routine WET testing on fish only focuses on acute mortality effect and sometimes 7-d growth effect (USEPA, 2002a). However, the WET concept has been widely adapted by researchers to address many other toxicological endpoints in fish, especially in the three major small teleost model species, i.e., fathead minnow (*Pimephales promelas*), zebrafish (*Danio rerio*), and Japanese medaka (*Oryzias latipes*). For example, a WET test was conducted to study the developmental effects of a municipal wastewater effluent on two generations of the fathead minnow (Sowers et al., 2009). Negative reproductive effects were found in male fathead minnows exposed to wastewater treatment plant effluent (Barber et al., 2007). Physiological, growth, reproduction, and molecular endpoints have been incorporated in WET studies using zebrafish (Notch and Mayer, 2009; Smolders et al., 2002). An effluent from a banknote printing plant was found to delay hatching, decrease survival and growth, and alter sex ratio in embryo-larval Japanese medaka (Zha and Wang, 2006). The effluent from a large sewage treatment plant in Beijing induced expression of biochemical biomarkers (VTG and CYP450), inhibited growth of gonads, and reduced reproductive success in Japanese medaka (Ma et al., 2005). Survival, behavioral, and biochemical biomarkers have also been used to evaluate the short-term responses of Japanese medaka to municipal and pharmaceutical effluents (Gerhardt et al., 2002).

Wastewater may contain neurotoxic compounds such as some pesticides, heavy metals, and PPCPs. However, neurotoxicity is generally not assessed in routine WET tests. Zebrafish has also been proposed as a potential model for developmental neurotoxicity testing (de Esch et al., 2012). Quantification of locomotor activity of larval zebrafish has been successfully applied to evaluate the neurobehavioral toxicity of chemicals (Chen et al., 2014; Chen et al., 2011b; Irons et al., 2010; Kienle et al., 2009; Selderslaghs et al., 2013). However, this behavioral approach is rarely applied in ecotoxicology studies of field water. Combined with WET tests, locomotor activity analysis in larval zebrafish has the potential to become a useful tool for assessing the developmental neurotoxicity of complex environmental matrices such as effluents and surface waters (García-Cambero et al., 2012).

Fish toxicity and PPCP analysis in KNP wastewater: a case study

In one of our recently published study, we utilized the embryo-larval zebrafish model to assess the lethal and sublethal toxicities of sewage-polluted stream waters in KNP. In this study, besides conventional endpoints such as hatchability, survival rate, heart rate, and morphological deformity, larval locomotor activity was also digitally quantified to evaluate the possible neurobehavioral toxicity resulting from the mixture of contaminants in the water. Additionally, several groups of PPCPs were also analyzed in the field water samples to better characterize the impact of anthropogenic pollution on the aquatic environment (Chen et al., 2015). The following content will use this paper as a case study to address toxicity to fish and anthropogenic contaminants in the surface water of KNP.

In this study, water samples were collected from three creeks or ditches in KNP from May to October in 2010. During the monthly bioassays, zebrafish embryos were exposed to the water samples for 144 h. Hatchability, embryonic heart rate, larval survival rate and deformities were recorded. Larval swimming behavior was also digitally quantified at the end of exposure. Lethal toxicities such as significant decreases in hatchability and larval survival rate were observed at all sites. Both hatchability and larval survival rate were negatively correlated with nitrite and ammonia concentrations in the water. The field water had little effect on embryonic heart rate and morphology. Interestingly, lower swimming speeds and activity levels were observed in the morphologically normal larvae, suggesting neurobehavioral toxicity of the surface waters.

For the zebrafish embryo-larval bioassay results, the most sensitive sublethal endpoint is locomotor behavior of the larvae. Exposure to the sewage-polluted field water caused lower swimming speed and activity level without affecting morphological development in the larvae. Behavioral endpoints of fish are valuable tools to characterize the effects of environmental stress because they integrate endogenous and exogenous factors that can link biochemical and physiological processes (Kane et al., 2004). Additionally, any interruption to fish behaviors may negatively affect normal fish functions such as foraging, predator-avoidance, reproduction, and social interaction, consequently decreasing the fitness of the fish (Little and Finger, 1990; Scott and Sloman, 2004). With the aid of video-based animal movement tracking software, high-throughput quantification of locomotor behavior of larval zebrafish can be easily achieved in toxicological bioassays for chemicals (Chen et al., 2014; Chen et al., 2011a; Kane et al., 2004; Selderslaghs et al., 2013; Wang et al., 2014). Reduced swimming speed and activity level suggest neurological toxicity and/or metabolic disruption in the exposed larvae (García-Cambero et al., 2012; Scott and Sloman, 2004; Selderslaghs et al., 2013). Given the sensitivity and biological/ecological significance, we suggest that locomotor activity analysis can be more broadly utilized in embryo-larval zebrafish bioassays for effluents or surface waters (García-Cambero et al., 2012).

We also analyzed PPCPs in the water samples collected from the field. We targeted 28 common ingredients of PPCPs in seven categories: analgesics, hormones, suntan lotions, repellents, stimulants, detergents/disinfectants, and preservatives in cosmetics. Of the 28 target PPCPs, 21 compounds (75%) were detected at least once during this study. For the detected compounds, maximum concentrations ranged from a few ng/l to approximately 15 μg/l. Three compounds, i.e., DEET (N,N-diethyl-3-methylbenzamide), caffeine, and NP (nonylphenol), were the most frequently detected (with 100% detection frequency at all three sites) and had the highest measured concentrations (up to μg/l levels). Other compounds such as analgesics, hormones, organic UV filters, and paraben preservatives were also frequently detected, but usually at lower levels (ng/l). Our data indicate that the streams in KNP are heavily affected by anthropogenic pollution associated with domestic wastewater discharges.

It is notable that DEET, caffeine, and NP were the most frequently detected and had highest concentrations. DEET is the most common active ingredient in commercial insect repellents, frequently detected in surface waters throughout the world (Costanzo et al., 2007; Glassmeyer et al., 2005). The major pathway of DEET entry into aquatic environments is via sewage effluent following washing off and absorption/excretion by humans (Costanzo et al., 2007). DEET has been shown to be neurotoxic to insects, birds, and mammals (Abou-Donia et al., 2004; Abou-Donia et al., 1996; Sfara et al., 2013; Swale et al., 2014). Caffeine is the most consumed stimulant by humans contained in coffee, tea, chocolate, and soft drinks and is one of the most commonly detected PPCPs in effluents and surface waters in Taiwan and around the world (Lin et al., 2008; Moore et al., 2008). Actually, caffeine is considered an indicator of domestic wastewater because it is ubiquitous and almost entirely human-related (Chen et al., 2002; Sankararamakrishnan and Guo, 2005; Sauvé et al., 2012). Previous studies have shown that caffeine can cause neurotoxicity in developing vertebrates including zebrafish (Capiotti et al., 2011; Chen et al., 2008; Rodriguez et al., 2014). NP in the environment is a degradation product of nonylphenol ethoxylates, which are the most common surfactants used in domestic detergents, pesticides, and industrial products (Ying et al., 2002). Although most NP ecotoxicology studies have focused on its endocrine disrupting effects, NP has also been reported to cause neurobehavioral toxicity in animals including zebrafish (Chandrasekar et al., 2011; Jie et al., 2013; Xia et al., 2010). The behavioral effects observed in our study may result from neuroactive PPCPs and their mixtures in the stream water. However, it is difficult to establish a clear cause-effect relationship between chemical analysis and biological response data because of complex composition/interactions and notable spatial/temporal variation of compounds in the sewage. Further research is needed to elucidate the mechanisms of the behavioral effects observed herein.

Conclusions

Our study shows that surface waters in KNP have been strongly impacted by human activities, resulting in lethal and behavioral toxicities in developing fish. We for the first time characterized fish ecotoxicity and analyzed PPCPs in the aquatic environment of KNP. Herein we demonstrate that the fish embryo-larval bioassay incorporated with behavioral and chemical analyses is a sensitive tool to assess the lethal and sublethal effects of wastewater on fish. In addition to the embryo-larval toxicities, endocrine disrupting effect of PPCPs and other contaminants in wastewater is another important issue in ecotoxicology. More research is needed to address the effect of wastewater on fish reproduction, behavior, and endocrine physiology.

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