

**Environmental Assessment Of The Impacts Of
Polycyclic Aromatic Hydrocarbons (PAH)
In Lake Tahoe And Donner Lake**

**A Final Report to the
California State Water Resources Control Board**

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Table of Contents

Table of Contents	ii
List of Tables	iii
List of Figures	iv
Executive Summary	v
1. Introduction	1
1.1 The chemistry of polycyclic aromatic hydrocarbons	4
1.2 Lake Tahoe and Donner Lake	8
1.2.1 Limnology of Lake Tahoe	8
1.2.2 Pelagic biological community of Lake Tahoe	9
1.2.3 Littoral biological community of Lake Tahoe	10
1.2.4 Limnology of Donner Lake	12
1.2.5 Pelagic biological community of Donner Lake	13
1.2.6 Littoral biological community of Donner Lake	14
1.3 Summary	16
2. Experimental Methods	16
2.1 Determining levels of PAH in Lake Tahoe and Donner Lake	16
2.1.1 Sampling methods and sites	16
2.1.2 Analytical methods	16
2.1.3 SPMD placement and analyses	17
2.1.4 PAH in Tahoe sediments	18
2.2 Determination of PAH emission from various marine engines	19
2.2.1 Determination of photolysis rates of marine engine PAH	21
2.3 Phototoxicity assessment	22
2.3.1 Phototoxicity of water from Lake Tahoe and Donner Lake	22
2.3.2 Determination of phototoxicity of marine engine emissions to <i>Ceriodaphnia dubia</i> in the laboratory	23
3. Results and Discussion	24
3.1 Ambient concentrations of PAH in Lake Tahoe and Donner Lake	24
3.1.1 PAH in water samples	23
3.1.2 PAH sampled with SPMD	25
3.1.3 PAH in Tahoe sediments	28
3.2 Comparison of emissions from a variety of engines	30
3.2.1 Comparison of 90 hp engines at the CARB facility	30
3.2.2 Comparison of two 9.9 hp engines	34
3.2.3 Photolysis of marine-engine PAH in sunlight	35
3.3 Phototoxicity assessment	41
3.3.1 Phototoxicity studies at Lake Tahoe and Donner Lake	41
3.3.2 Phototoxicity studies with water contaminated with marine engine exhaust	46
4. Discussion and Conclusions	52

5. Recommendations	54
6. References	56

List of Tables

Table 1.1.1	Phototoxic PAH, solubilities and partition coefficients.....	6
Table 2.1.1	PAH water quality sampling locations at Lake Tahoe and Donner Lake....	17
Table 2.2.1	Marine test engines.....	20
Table 2.2.2	Engine test cycle.....	21
Table 3.1.1	Concentrations of PAH compounds in lipid from semi-permeable membrane sampling devices emplaced in Lake Tahoe and Donner Lake...	26
Table 3.1.2	Estimated average water column concentrations of PAH compounds in Lake Tahoe and Donner Lake.....	26
Table 3.2.1	Concentrations of PAH compounds released into the tank water following a 10 minute idle period (90-4S-T10I), 10 minute run (90-42-T10R) and 30 minute run (90-4S-T30R) for the 4-stroke 90 hp engine.....	31
Table 3.2.2	Concentrations of PAH compounds released into the tank water following a 10 minute idle period (90-DI-T10I), 10 minute run (90-DI-T10R) and 30 minute run (90-DI-T30R) for the 90 hp direct fuel injected engine.....	32
Table 3.2.3	Concentrations of PAH compounds released into the tank water following 10 minute idle period (90-C-T10I), 10 minute run (90-C-T10R) and 30 minute run (90-C-T30R) for the 90 hp carbureted engine.....	33
Table 3.2.4	Comparison of PAH concentrations from the two 9.9 hp engines (ng/L)	
Table 3.2.5	Rate constants (k), R ² value, and half-lives of PAHs exposed to sunlight in water.....	35
Table 3.3.1	PAH concentrations from the two 9.9 hp Mercury engines (ng/L) for a single run.....	47
Table 3.3.2	Effect concentrations comparing UV and no-UV treatments for 2-cycle 9.9 hp Mercury engine.....	48
Table 3.3.3	Mortality concentrations comparing UV and no-UV treatments with 2-cycle 9.9 hp Mercury engine.....	49
Table 3.3.4	Effect concentrations comparing UV and no-UV treatments with 4-cycle 9.9 hp Mercury engine.....	50
Table 3.3.5	Mortality concentrations comparing UV and no-UV treatments with 4-cycle 9.9 hp Mercury engine.....	51

List of Figures

Figure 1.1.1	Chemical structures of the 15 3-ring and greater PAHs designated as priority pollutants by the U.S. EPA as part of the Clean Water Act.....	7
Figure 3.1.1	Fluoranthene and pyrene in water samples collected during the two-year period at several locations at Lake Tahoe and Donner Lake.....	25
Figure 3.1.2	Sum of PAH concentrations and number of compounds in SPMD samples collected from Lake Tahoe and Donner Lake, 2001.....	27
Figure 3.1.3	PAH compounds in sediments at Ski Run and Timber Cove (3 different locations).....	29
Figure 3.1.4	PAH compounds in sediments of Tahoe city Marina and Carnelian Marina	29
Figure 3.1.4	PAH compounds found in sediments of Bijou beach and Sunnyside Marina (2 different locations).....	30
Figure 3.2.1	Comparison of 10 minute run for each of the 90 hp engines.....	34
Figure 3.2.2	Photochemical degradation of anthracene, fluoranthene, and pyrene from 2-stroke engine contaminated water.....	36
Figure 3.2.3	Photochemical degradation of anthracene in distilled water.....	37
Figure 3.2.4	Natural log vs. concentration for pyrene in water contaminated by a 2-stroke engine.....	38
Figure 3.3.1	Fathead minnow mortality (2001).....	41
Figure 3.3.2	Redside mortality (2001).....	42
Figure 3.3.3	Final fathead minnow weight (2001).....	42
Figure 3.3.4	Fathead minnow mortality (2001 experiment 2).....	43
Figure 3.3.5	Fathead minnow final weight (2001 experiment 2).....	43
Figure 3.3.6	Dace length-weight relationship by treatment.....	44
Figure 3.3.7	Ceriodaphnia reproduction 2002.....	45
Figure 3.3.8	Ceriodaphnia mortality 2002.....	46
Figure 3.3.9	Dose-response curves of effect concentrations comparing UV and no-UV treatments with the 2-cycle 9.9 hp Mercury engine.....	48
Figure 3.3.10	Dose-response curves of mortality concentrations comparing UV and no-UV treatments with the 2-cycle 9.9 hp Mercury engine.....	49
Figure 3.3.11	Dose-response curves of effect concentrations comparing UV and no-UV treatments with the 4-cycle 9.9 hp Mercury engine.....	50
Figure 3.3.12	Dose-response curves of mortality concentrations comparing UV and no-UV treatments with the 4-cycle 9.9 hp Mercury engine.....	51

Executive Summary

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Polycyclic aromatic hydrocarbons (PAHs) are organic compounds released during combustion processes that present a significant risk to aquatic organisms, particularly those that absorb sunlight and undergo sunlight-induced reactions (phototoxicity) at the cellular level. In Lake Tahoe and other pristine recreational lakes where water clarity and ultraviolet light intensity are high, photo-enhanced toxicity of PAH is a particular concern. The specific phototoxic PAH are a particular concern to aquatic organisms because of the dramatically increased potential of toxicity when these compounds are absorbed, and sunlight induced reactions of several PAH cause damaging oxidative processes in sensitive tissues. Toxic effects have been noted at the very low concentrations of less than 0.1 µg/L of combined phototoxic PAH in water.

Motorized watercraft are known to release PAH compounds directly into water in the engine exhaust. In those areas where watercraft use is high, information on accumulation of PAH in the water and sediments is important for understanding whether these compounds are found in concentrations that may be problematic. Direct studies on the aquatic toxicity of lake water, as well as water that has received marine engine exhaust are needed to understand the potential impact of marine engine emissions. Estimating the relative contributions of each type of marine engine (e.g. two cycle engines) is also important for assessing how to manage watercraft use for reducing PAH concentrations.

The present study was conducted with the guidance of the Motorized Watercraft Technical Advisory Group (MWTAG), organized by staff from the Tahoe Regional Planning Agency and the Lahontan Regional Water Quality Control Board. The participants of this group, organized in 1997, have consisted of representatives of Lake Tahoe regulatory agencies (TRPA, Lahontan Regional Board, Nevada Division of Wildlife, Environmental Protection Agency, California Air Resources Board), universities (University of Nevada, Reno, Miami University (Ohio) and the University of California, Davis), U.S. Geological Survey, the watercraft industry, and public interest organizations. This project was funded by California Water Resources Control Board.

This study was designed to assist in planning to meet these requirements as they relate to problematic and toxic emissions from watercraft. The PAH project was divided into five tasks, as indicated below, and this list of tasks provides a description of the objectives of the project. These tasks include the following

TASK 1. Project Management and Administration. This administrative function was the responsibility of the University of Nevada, Reno.

TASK 2. Determination of Levels of PAH in Lake Tahoe and Donner Lake. Ambient water quality testing was done in both lakes at selected locations to establish the concentrations of a range of PAH that existed throughout the year. Additionally, semi-permeable membrane devices (SPMDs) were set out to collect PAH over a longer period of time (weeks) in order to get an integrated assessment of PAH present at sites in both lakes. This task was completed by UNR, UC Davis and the USGS.

TASK 3. Comparison of Emission Factors from a Variety of Engines. A variety of engines were tested to determine the relative amount of PAH released from engine types and to better understand which engines are responsible for the greatest amount of PAH release. A second aspect of this task was to determine the photochemical fate of marine engine PAH in fresh water, in order that an assessment of the photochemical fate of these compounds could be made. This task was completed by UNR.

Task 4. Phototoxicity Assessment. The toxicity of marine engine PAH to aquatic organisms required both laboratory and field studies to assess whether ambient concentrations of PAH present a particular risk to those organisms. Studies were completed on the toxicity of water from Lake Tahoe and Donner Lake to species of fish, as well as *Ceriodaphnia dubia*, a common aquatic invertebrate. In addition, laboratory studies were completed to determine if waters subjected to marine engine emissions at levels approaching those found in lakes causes toxicity to *Ceriodaphnia dubia*. These tests were the primary responsibility of Miami University (Ohio), UC Davis and UNR.

Task 5. Regulatory Assessment to evaluate management measures to control non-point pollution associated with boat emissions. Based on information developed in tasks 2-4, a risk assessment of marine-engine emissions was developed, along with recommendations for measures to protect aquatic organisms in these lakes.

Distribution and Occurrence: Both Lake Tahoe and Donner Lake receive extensive boating activity, although the intensity of use varies dramatically, depending on the season and location on the lakes. In order to determine the occurrence and distribution of PAH in these lakes, a sampling plan was implemented that included sampling at specific locations and times of the year. This sampling plan included periods when watercraft use

was intensive (July-September) as well as times when watercraft usage was very low (November-April). Of the 12 phototoxic PAH compounds examined, fluoranthene and pyrene were observed most commonly in the ambient samples. The remaining phototoxic PAH compounds were either not detected or found in a very limited number of samples.

As expected, the areas with the highest watercraft usage contained the highest number of PAH detections, as well as the highest concentrations. One site, Tahoe Keys Marina, an enclosed marina with a narrow channel that allowed access from the Lake, contained the highest PAH loadings at all times of the year. Samples taken at the Donner Boat ramp were also elevated. Alternatively, samples taken at other locations around Lake Tahoe and Donner Lake only rarely showed detections of phototoxic PAH, even during the summer months, indicating that these compounds do not accumulate in open water.

Figure 3.1.1 summarizes the occurrence and distribution of these two compounds in all of the samples examined at both lakes. If we compare these data to those obtained using SPMD's (Table 3.1.2), the results are strikingly similar. SPMD's are devices that will collect nonpolar organic compounds over a long period of time (days to months) and allow an integrated sample of the water. Fluoranthene and pyrene are found in the highest concentration. Additionally, these compounds were found predominantly in the same locations, Tahoe Keys Marina and the Donner Boat ramp. Slightly greater occurrence of PAH was also observed in other marinas, although they were less commonly observed than these two more contaminated sites. This is probably a reflection of the boat traffic and the lower mixing volume that is available at these two sites, compared to the open water of Lake Tahoe. These data offer support for the notion that PAH comes primarily from marine engines. Additionally, the samples taken during the winter months and a sampling during a forest fire in summer of 2002 did not show elevated PAH, and is also consistent with the PAH source being primarily marine engines.

A limited number of sediment samples were also taken, and indicated relatively high concentrations of PAH in the sediments, consistent with the known chemistry of these compounds. Although the data were limited in scope, higher concentrations were associated with fine grain sediments in marinas.

Engine Emissions and Photochemical Reactivity: Comparative tests on engines were conducted at the California Air Resources Facility in El Monte, California, and included 3 engines. (a 90 hp 4-cycle engine, a 90 hp direct fuel injected 2-cycle engine, a 90 hp carbureted 2-cycle engine) The results indicate that the two 2-cycle engines have approximately equivalent PAH release and the 4-cycle engine has 8-20 times lower emissions, depending on the specific PAH. It is important to note that, while the newer direct fuel injected engines release much lower amounts of gasoline than the carbureted two-stroke engines, this difference was not observed with PAH emissions. We also examined a 9.9 hp 4-cycle engine and a 9.9 hp carbureted 2-cycle engine at a test facility at UNR. Unlike the larger engines, the two small engines released very similar amounts of PAH, a finding that was inconsistent with the 90 hp engines.

Based on the same method for introducing oil and air into the DFI and carbureted system, we would not necessarily expect there to be any differences between the engine

types, assuming that PAH comes primarily from the oil. However, these data, as well as the data from the smaller engines need to be compared with a larger number of engines to make conclusive recommendations. However, at present it appears that the newer two-cycle DFI engines do not offer any benefit of lowered PAH release, compared to the older carbureted 2-cycle engines.

In tests that examined the rate of sunlight-induced degradation of the phototoxic PAH compounds, all were found to undergo rapid photolysis; most had a half-life of less than one day. Thus, for clear lakes like Tahoe and Donner, these compounds are all expected to degrade rapidly, and are unlikely to accumulate in water. The concentrations observed are likely to be a function of the total daily boat traffic, the mixing that occurs in the lake and the intensity of sunlight.

Toxicity Several toxicity studies were conducted during both summers. The results from the seven-day toxicity experiments in 2002 indicated that both the Tahoe and Donner Lake samples from high watercraft use areas exhibited phototoxicity to the water flea, *Ceriodaphnia dubia*, by altered reproduction rates as well as mortality. No effects on fish were observed. Because this toxicity in the water flea was enhanced by sunlight, it is probably a phototoxic effect, and implies involvement with PAH, although it is not possible to conclusively assign a causative agent to these results.

The two-day acute phototoxicity tests conducted at UNR indicated that water that received engine exhaust was indeed toxic to *Ceriodaphnia dubia*. With an estimated total toxic PAH concentration in the 1% diluted water of 450 ng/L, acute toxicity effects (swimming behavior) was observed for the sunlight irradiated samples, although mortality was only observed at the 10% diluted water. We note, however, that the majority of the toxic PAH load in these samples was acenaphthylene (330 ng/L), which is not considered one of the more toxic PAH compounds. Thus, it suggests caution in summing up the total weights of PAH to arrive at a comparative toxicity for a water, since the effects of each compound can be substantially different. The 0.1% dilutions (45-52 ng/L) did not show any significant effects, compared to the controls. It should also be noted that the seven-day tests are a more sensitive measure of toxicity, since they involve reproductive effects, while the two-day tests only examine effects on the parent organism.

A review of the limnological conditions at Lake Tahoe and Donner Lake also suggests that, for most of the surface area, sunlight inherently decreases the number of organisms that will exist in the top few meters. Ultraviolet light is directly harmful to most of these organisms and many come to the surface only at night. Thus, it is difficult to quantitatively establish if there is an effect from the PAH if behavioral aspects (e.g. vertical diurnal migration) are considered. However, areas that have a certain amount of shading that allow organisms to move in and out of sunlight in the same region as PAH may offer the highest risk for phototoxicity due to the presence of both PAH, as well as sunlight.

Thus, while it is apparent that some toxicity was observed in water taken from the lake, it is not clear that this is causing a significant ecological impact. Based on the PAH concentrations determined, the effect, if it occurs, is very likely restricted to high use areas.

Recommendations:

At present, phototoxic PAH compounds were not observed in any of the open water areas of Lake Tahoe at concentrations that would be expected to cause toxicity to aquatic organisms. Higher concentrations were found in areas associated with high boat traffic, that also were sheltered and had limited potential for dilution with the open water. Additionally, the water in the most impacted marina was some of the most turbid of any area at Lake Tahoe, and tended to screen sunlight that would cause loss of these compounds through photodegradation. While this area is potentially problematic, it is also unusual at Lake Tahoe, and management of boating traffic to manage PAH loadings in this marina, if needed, could best be accomplished separately from the rest of the Lake.

Phototoxic responses to fish were not observed in either Lake Tahoe or Donner Lake, although phototoxic effects were observed for the sensitive macroinvertebrate species, *Ceriodaphnia dubia* in the 2002 tests. Additionally, avoidance behavior of most aquatic species to ultraviolet light in Lake Tahoe will result in a lowered risk of effects. Because the concentrations of PAH found in the water of Lake Tahoe in 2001 and 2002 were generally very low, we do not feel there are compelling reasons, at present, to recommend changes in watercraft management at Lake Tahoe.

However, phototoxic PAH compounds were observed at several locations, particularly near marinas and high use areas, and may, at some point in the future, need to be managed as watercraft use increases. PAH loadings in sediments are also potentially a concern, again in marina areas. In order to minimize the long-term potential for PAH problems in the future, the following management issues may be considered for future implementation. These measures are proposed to address technical concerns based on the current state of the science and information gaps of understanding PAH risk.

Potential Management Actions

- Manage PAH loading into Lake Tahoe by regulating the intensity of use of 2-stroke engines, primarily by controlling the number of personal watercraft used at specific locations.
- Disperse the intensity of watercraft use by establishing a ceiling for the number of watercraft that can be operated from any single location.
- Encourage development of engine types that exhaust directly in the air, rather than the water.
- Encourage the use of non-motorized watercraft through outreach and public education. Introduce financial incentives – expand lake access for non-motorized recreational activities such as swimming, hiking and launching kayaks, canoes, inflatables, sailboards and other sail craft.

Additional information is needed in the following areas:

- Develop more complete standards for protection of sensitive species of aquatic organisms with respect to phototoxic PAH compounds.

- Improve the ability to predict PAH emissions by examination of additional marine engines of various types.
- Examine the use of alternative lubricating oils for both 2-stroke and 4-stroke engines. Since lubricating oil appears to be the primary source of PAH, additional information on PAH emissions with differing oils could provide suggestions for an inexpensive and efficient method for PAH reduction.
- Study the effect of tuning of engines on PAH emissions. If this factor is important, regulations may be considered that requires each engine to be tuned to operate efficiently at Lake Tahoe.
- Continue monitoring PAH in Lake Tahoe, with a focus on high watercraft use areas to determine PAH trends over time.
- Conduct additional studies on PAH loading in sediment to determine the source and ecological impacts.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds released during combustion processes. These compounds present a significant risk to aquatic organisms, particularly those that absorb sunlight and undergo sunlight-induced reactions (phototoxicity) at the cellular level. In Lake Tahoe and other pristine recreational lakes where water clarity and ultraviolet light intensity are high, photo-enhanced toxicity of PAH is a particular concern. Preliminary data indicate that a major source of PAHs into Lake Tahoe and Donner Lake is from marine engines, particularly in areas heavily used for recreational activity.

Based on studies by Oris and coworkers (1998), PAHs from watercraft have been identified as the single largest ecological risk factor of emissions from watercraft into Lake Tahoe. If PAH presents a risk to aquatic life, assessment of that risk requires a determination of the extent PAHs are being released from water craft, as well as the other sources. Additionally, policy makers need to know the sources of contaminants that will assist in management of the associated human and ecological risks.

Previous reports and discussions pointed toward the need for a better understanding of the sources, fate and ecological impact, as well as potential management of PAH in Lake Tahoe and Donner Lake. The present study was conducted with the guidance of the Motorized Watercraft Technical Advisory Group (MWTAG), organized by staff from the Tahoe Regional Planning Agency and Lahontan Regional Water Quality Control Board. The participants of this group, organized, in 1997, consists of representatives of Lake Tahoe regulatory agencies (TRPA, Lahontan Regional Board, Nevada Division of Wildlife, Environmental Protection Agency, California Air Resources Board), universities (University of Nevada, Reno, Miami University of Ohio and the University of California, Davis), U.S. Geological Survey, the watercraft industry, and public interest organizations. This project was funded by the California State Water Resources Control Board.

The impacts of motorized watercraft have been studied by members of the MWTAG over the past six years, primarily investigating the release of unburned hydrocarbons present in gasoline. Several two-cycle engines and four-cycle engines were tested for gasoline emissions, as well as extensive monitoring of Lake Tahoe from 1997 to present. Following these studies, the Governing Board of the Tahoe Regional Planning Agency took action based on those results to reduce the overall hydrocarbon load to surface water within the Lake Tahoe Basin. In 1997, the Governing Board passed a ruling that largely banned the use of carbureted 2-stroke engines from Lake Tahoe Basin effective June 1, 1999, although direct fuel-injected engines were permitted, since the amount of unburned hydrocarbons emitted was relatively low. However, recent information from a California Air Resource Study (Kado, et al., 2000) suggests that the newer fuel-injected two-stroke engines emit similar or even larger amounts of PAH than the older carbureted engines. Although PAH emission from watercraft, particularly two-cycle watercraft, has been firmly established,

(Mastran, et al., 1994; U. Tjarnlund, et al., 1996) the relative amounts of these emissions that remain in the water from different watercraft has not been investigated.

As discussed above, one of the MWTAG studies that raised additional concerns was the project of Dr. James Oris from Miami University (Ohio), working with the Tahoe Research Group (Oris, et al., 1998). Dr. Oris and his cooperators found that water from Lake Tahoe that received significant watercraft usage was toxic to fathead minnows (*Pimephales promelas*) and a zooplankton (*Ceriodaphnia dubia*). The results generated concerns, and suggested that certain emissions from watercraft were responsible, particularly the products of incomplete combustion, polycyclic aromatic hydrocarbons (PAH). These compounds, which are composed of multiple aromatic rings, are known to be toxic and carcinogenic. They also possess the characteristic of "photo-enhanced toxicity." Because of their very low solubility in water, these compounds tend to partition into biological material, and when exposed to ultraviolet sunlight, result in reactions that increase the toxicity of the compounds dramatically. Giesy (1997) has indicated that enhancement of toxicity is as much as 50,000 times greater in sunlight than in dark conditions. From studies such as these, the No-Observed Effect-Concentrations (NOEC) may be as low as 0.01 µg/L (parts per billion) in Lake Tahoe (Landrum, et al., 1987; Oris, et al., 1998). Indeed, Lake Tahoe, and other high elevation ultraoligotrophic lakes may be the most susceptible to these phototoxic effects. The penetration of ultraviolet light is maximized and the amount of ultraviolet light that strikes the water body is generally greater than for lower elevation lakes.

Planning for protection of the biological integrity of Lake Tahoe and other high elevation recreational lakes requires an understanding of the sources of potentially toxic contaminants. Regulatory prohibitions for release of toxic contaminants into Lake Tahoe exist, and include the following:

The Water Quality Control Plan for the Lahontan Region contains the following language:

"The discharge of any waste or deleterious material to surface waters of the Lake Tahoe HU is prohibited" (page 5.2-1 WQCP for the Lahontan Region).

The Water Quality Management Plan for the Lake Tahoe Region (208 Plan) states that

"To control wastewater releases from vessels...controls on boat and marina maintenance practices are necessary to protect Lake Tahoe from the addition of toxic substances from this source."

The Regional Plan for the Lake Tahoe Basin Code of Ordinances states that

“The discharge of toxic or hazardous waste to Lake Tahoe, other lakes in the Region,...is prohibited.” (Subparagraph 81.2.D)

This study was designed to assist in planning to meet these requirements as they relate to problematic and toxic emissions from watercraft. The PAH project was divided into five tasks, as indicated below, and this list of tasks provides a description of the objectives of the project. These tasks include the following

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TASK 5. Regulatory Assessment to evaluate management measures to control non-point pollution associated with boat emissions. Based on information developed in tasks 2-4, a risk assessment of marine-engine emissions was developed, along with recommendations for measures to protect aquatic organisms in these lakes.

This report begins with a background discussion on the sources, chemistry and toxicology of PAH, followed by a discussion on the physical characteristics and ecology of Lake Tahoe and Donner Lake.

1.1 The Chemistry of Polycyclic Aromatic Hydrocarbons

PAHs consist of a large class of organic (i.e., carbon- and hydrogen-containing) compounds comprised of two or more fused benzene rings (cf. Diagram 1). They are formed as the result of the incomplete decomposition or combustion of organic materials (Neff, 1979, Blumer, 1976). PAHs can be derived through natural processes such as the formation of coal and oil, forest fires, and volcanic activity (Neff, 1979, Blumer, 1976). The majority of PAH releases into the environment, however, is the direct result of human-related activities (Blumer, 1976; Suess, 1976; Neff, 1979; Schmeltz, et al., 1976; Laflamme and Hites, 1978; Malins, et al., 1985; Moore, et al., 1987; Roberts, et al., 1989; Vizayakumar and Mueller, et al., 1991; Mohapatra, 1992; Mastran, et al., 1994, Braunstein, et al., 1998,). Important sources of PAHs in the environment include spillage and release of oil and fuel (Neff, 1979), combustion of coal and oil (Suess, 1976; Tancell, et al., 1995), run-off from urban areas (Schmeltz, et al., 1976; Laflamme and Hites, 1978; Braunstein, et al., 1998;), runoff from coal storage facilities (Vizayakumar and Mohapatra, 1992), release from creosote derived wood preservatives (Malins, et al., 1985; Roberts, et al., 1989; Mueller, et al., 1991; Vizayakumar and Mohapatra, 1992), and release of fuel, oils and combustion exhaust associated with the operation of motorized watercraft (Moore, et al., 1987; Cosmacini, 1987; Mastran, et al., 1994;).

This latter source has only recently been identified. In a comprehensive review of polycyclic aromatic hydrocarbons and their effect on aquatic organisms (Eisler, 1987) emissions from watercraft were not mentioned. However, during the present study it has become apparent that watercraft are probably a main source of PAH into freshwater lakes, particularly those that exist in comparatively pristine settings and have only limited transportation corridors in the watershed. Such is the case for Lake Tahoe.

Most recreational watercraft use a “wet” exhaust process in which the engine exhaust is emitted directly into the water. This reduces noise from the engine, as well as reducing the odor from two-cycle engines. However, this mode of exhaust forces hot gases directly into the lake, and provides the most efficient mechanism for transfer of PAH, as well as other hydrocarbons, into the water. Alternatively, “dry” exhaust systems vent directly into the atmosphere. Although a percentage of these substances can also settle onto the lake’s surface, the total amount entering the water body will be lower because much of the atmospheric loading will remain suspended in the air and will be either carried away from the lake or be degraded in the atmosphere.

PAHs have extremely low solubilities in water, and vary depending on the ring structure and molecular size and weight of the compounds. Generally, the solubility of PAHs range from tens of milligrams per liter (mg/L) for 2-ring compounds down to tens of nanograms per liter (ng/L) for 6- and 7-ring compounds (Neff, 1979). Compared to their solubility in water, PAHs are 100 to >1,000,000 times more soluble in organic materials

such as proteins, oils, and fats (Klaassen, 1996). Therefore, PAHs in aquatic systems tend to accumulate primarily in sediments, particulate organic matter, and living organisms (Rand and Petrocelli, 1985). Sediments taken from marinas and harbors around the world have been shown to contain enriched amounts of PAH (Malins, et al., 1985). Examinations of fish collected from areas known to have sediments contaminated with PAH have demonstrated tissue accumulations of PAH and related compounds, as well as a high rate of tumors and lesions (Malins, et al., 1988, Bauman, et al., 1982).

When aquatic organisms are concurrently exposed to PAH and near ultraviolet radiation (285-400nm) the toxicity of PAH is greatly enhanced (Oris, et al., 1984, 1985, 1987, 1990; Landrum, et al., 1985, Hall, et al., 1991, McCloskey, et al., 1991, McCloskey, et al., 1993, Diamond, et al., 1995, Weinstein, et al., 1997, Holst and Giesy, 1989, Mekenyan, et al., 1994, Ankley, et al., 1995, Anklet, et al., 1997). This photo induced toxicity is a result of absorption of energy from the UV-A (320-400nm), and UV-B (290-320 nm) portions of the solar spectrum. This energy is passed on to oxygen, creating reactive oxygen molecules. Reactive oxygen molecules cause damage to gill and skin membranes in a process very similar to sunburn. The disruption of gill and skin membranes results in respiratory stress and loss of osmotic regulation. These effects are manifested in aquatic organisms in reduced growth, decreased reproduction rates, and ultimately, death. Near ultraviolet radiation penetrates surface waters to a considerable depth (Smith and Baker, 1979), and is dependent on the productivity and turbidity of a particular water body (Smith and Baker, 1979). For example, in eutrophic Park Lake, MI, 99% of incident UV-A is attenuated in the upper 2 meters, while in offshore Lake Michigan 1% of incident UV-A penetrates to about 10 meters (Oris and Giesy, 1984, Holst and Giesy, 1989). Measurements taken at mid-lake in Lake Tahoe indicate a 1% UV-A level of approximately 20 meters depth (Oris, 1997). Thus near ultraviolet radiation penetrates to ecologically significant depths.

Not all of the PAH compounds exhibit photo enhanced toxicity. This is due to either a lack of absorbance of sunlight, or a lack of photo reactivity. The compounds generally assumed to be phototoxic and some of their properties are included in Table: 1.1.1.

In previous work, Dr. Oris and coworkers have conducted extensive studies on the photoinduced toxicity of PAHs in aquatic organisms (Oris, et al., 1984, Oris, et al., 1984, Oris, et al., 1985, Landrum, et al., 1985, Oris, et al., 1987, Oris, et al., 1990, Hall, et al., 1991, McCloskey, et al., 1991, McCloskey, et al., 1993, Diamond, et al., 1995, Weinstein, et al., 1997). These studies have demonstrated that the level of toxicity expressed is determined quantitatively by the combination of UV intensity and amount of PAH absorbed into the organism. Laboratory experiments at relatively high PAH concentrations and low UV intensities are thus predictive of field conditions of low PAH concentration and high UV intensities. Conditions exist for phototoxicity of PAHs to occur in oligotrophic lakes such as Lake Tahoe and Donner Lake. Due to low levels of dissolved organic matter and particulate matter, conditions that are favorable for bioavailability of PAH and increased penetration of UV.

Table 1.1.1. P

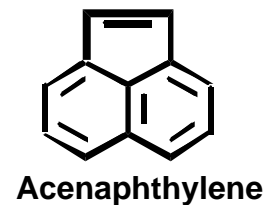
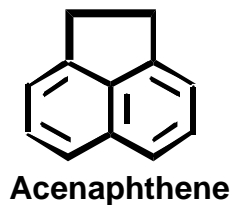
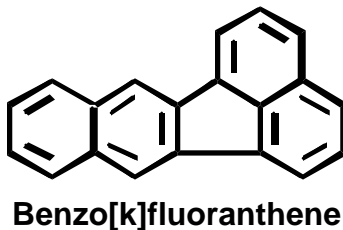
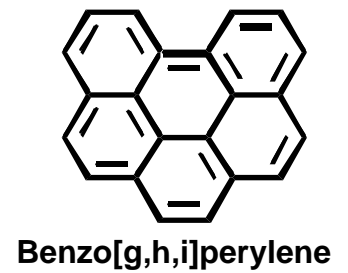
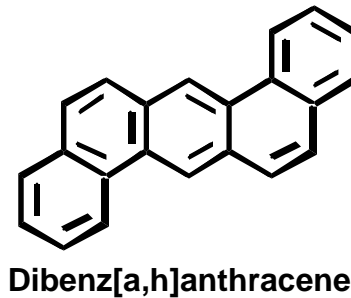
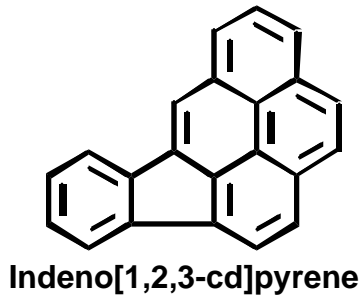
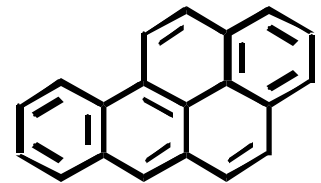
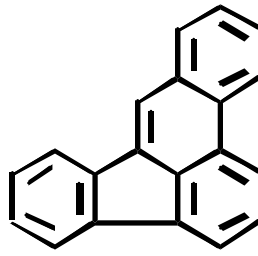
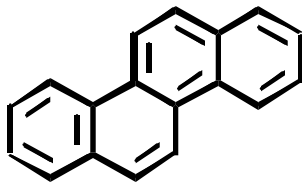
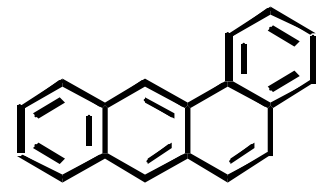
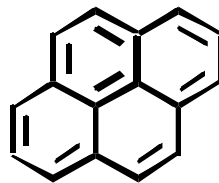
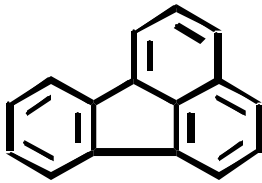
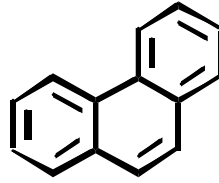
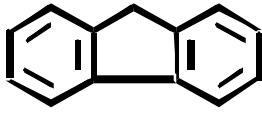
polycyclic aromatic hydrocarbon solubilities, vapor pressures and partition coefficients*

<u>Compound</u>	Molecular weight	Water Solubility (mg/L)	Vapor Pressure (mmHg)	Log K _{ow}
Acenaphthene	154.2	3.57 - 4.24	0.0025	3.92
Acenaphthylene	152.2	16.1	0.000912	4.07
Fluorene	166.2	1.89 - 1.98	0.000633	4.18
Anthracene	178.2	0.0434 - 0.0748	0.00000267	4.45
Phenanthrene	178.2	1.15 - 1.29	0.000112	4.46
Fluoranthene	202.3	0.206 - 0.26	1.23E-08	4.95
Pyrene	202.3	0.135 - 1.35	0.00000459	4.88
Chrysene	228.3	0.002 - 0.0063	6.23E-09	5.66
Benz(a)anthracene	228.3	0.0094 - 0.014	0.000000105	5.66
Benzo(a)pyrene	252.3	0.00162 - 0.0038	5.49E-09	5.97
Benzo(b)fluoranthene	252.3	0.0015	0.0000005	6.12
Benzo(k)fluoranthene	252.3	0.0008	0.000000002	6.12
Benzo(g,h,i)perylene	276.3	0.00026	1.01E-10	6.58
Indeno (1,2,3-cd)pyrene	276.3	0.000022	1E-10	6.58
Dibenzo(a,h)anthracene	278.4	0.00249 - 0.0249	1E-10	6.5

*These data were obtained primarily from a Syracuse Research Corporation website:
<http://esc.syrres.com/>

Figure 1.1.1 Chemical structures of the 15 3-ring and greater PAHs designated as priority pollutants by the U.S. EPA as part of the Clean Water Act, Section 301(h) [EPA TSD for 01(h).]

U.S. EPA Priority Polycyclic Aromatic Hydrocarbons



1.2 Lake Tahoe and Donner Lake

1.2.1 Limnology of Lake Tahoe

Lake Tahoe is a very deep (500m), oligotrophic, subalpine lake with a large surface area (501 Km²) compared to its watershed (812 Km²). It lies in a graben fault at an elevation of 1898 meters above sea level. The optical clarity and algal growth rate (phytoplankton) in the lake has been measured continually since the early 1960s by the University of California -Tahoe Research Group. This monitoring has chronicled a three fold increase in primary production, and a 30% loss in clarity (-0.25m/Yr, measured by secchi disk) (Goldman and Byron 1986, Jassby et al. 1999). This eutrophication, or change as a result of nutrient loading, has been linked to human development in the Tahoe Basin (Goldman 1988). While current work continues to refine the specific contribution from various sources, contributing to diminished clarity, both algae and mineral suspensoids are believed to be significant (Jassby et al. 1999).

The large volume of Lake Tahoe (156 km³), is responsible for much of the lakes temperature characteristics. The lake does not develop ice coverage, even during the harshest Sierra winters. This is due to the large pool of relatively warm water (5⁰ C) that compensates for short durations of sub-zero surface temperatures. Lake Tahoe is monomycitic (mixes once each year), however, the depth of annual mixing varies with winter storm intensity (100m – >450m) (Lake Tahoe Watershed Assessment 2000). The maximum depth of mixing usually occurs during mid-winter (February) (Pearl et al. 1975). Thermal stratification begins in the spring (April) and persists through the fall (November) with a thermocline well developed near 20 meters during the summer months. Summer winds cause mixing of the surface water (above the thermocline), and may result in short periods of upwelling, but do not significantly breakdown thermal stratification (Richards, Personal communication, Hook unpublished data).

The optical clarity of Lake Tahoe is primarily affected by small particles in the water column that either absorb or scatter incident light. These particles can be algal cells, autochthonous (generated in the lake) dissolved (DOC) and particulate (POC) organic matter, and allochthonous (land generated) material in the form of mineral suspensoids, and dissolved and particulate organics (Jassby et al. 1999). The component having the greatest affect on the light penetration depth, changes with season. Chlorophyll based particles dominate the light scattering properties during the fall as a result of the deepening of the thermocline to the depth of the chlorophyll maximum (60-120m). This allows phytoplankton to reenter the depth of optical measurements (0-40m) resulting in a decrease in measured water clarity. During the spring, small (<2 µm) suspended particles, carried into the lake with snow melt, are thought to be most responsible for the loss of clarity (Jassby et al. 1999).

Despite the measurable loss of Tahoe's clarity, the depth of one percent of photosynthetically active radiation (PAR) is 50-60m (Huovinen and Goldman 2000). This level of transparency has been documented to inhibit shallow (< 2m) primary

production as a result of UV-B radiation, with possible effects as deep as 10m (Huovinen and Goldman 2000). The suppression of primary productivity in the surface waters may also be linked to the limited day time zooplankton abundance at depths shallower than 10m, which was described by Burgii et al. (1993).

Dissolved organic carbon (DOC) can be ecologically important in aquatic systems that are subjected to PAH pollution. This is especially true when photo-induced toxicity of aquatic biota is considered. DOC concentrations of 1.5-2.0 $\mu\text{g/L}$ can limit the toxic effect of photo activated PAH (Weinstein and Oris, 1999). This is due in part to the light scattering effect of DOC and its ability to adsorb PAH molecules. DOC concentrations have been measured in both the pelagic and littoral zones of Lake Tahoe. Samples collected from the pelagic zone, during the spring (April and May) and late summer (September), from all depths and locations (15-400m), ranged from 0.3 to 3 $\mu\text{g/L}$, with only one measurement exceeding 1 $\mu\text{g/L}$ (400m) (Chang et al. 1992). Generally, concentrations decreased with depth over the top 200m and were slightly higher near shore (2 km) than they were in the middle of the lake (Chang et al. 1992). Shallow samples (15m) ranged from 0.4-0.8 $\mu\text{g/L}$ regardless of location.

DOC samples collected during the summer of 2001, from 3m depth, in the littoral zone, at Tahoe City and Sand Harbor were 0.6 $\mu\text{g/L}$ and 0.5 $\mu\text{g/L}$ respectively (Oris, Unpublished data). Concentrations measured in the Tahoe Keys Marina, a man made lagoon, isolated from the main body of Lake Tahoe, were 1.1 $\mu\text{g/L}$. The higher values from the Tahoe Keys are indicative of the more productive environment that has developed in the marina.

1.2.2 Pelagic Biological Community of Lake Tahoe

The pelagic zone of Lake Tahoe is defined for these purposes as the open lake environment, where shore zone and bottom substrate are no longer a factor in determining habitat utilization by aquatic species. In Tahoe the pelagic zone can be quite near shore due to the very steep littoral zone, or may begin several kilometers offshore, where shelf areas exist (Kings Beach, Tahoe City, South Lake Tahoe). The biota of the pelagic zone is comprised of zooplankton and fish.

The pelagic zooplankton community is dominated by two native copepods, *Diaptomus tyrelli* and *Epischura nevadensis*, which are able to coexist with the introduced, omnivorous *Mysis relicta*. *Mysis* was introduced to the lake between 1963 and 1965 (Linn and Frantz 1965) in an effort to provide greater food resources for non-native lake trout (*Salvelinus namaycush*) and kokanee salmon (*Oncorhynchus nerka*). The resulting ecological change, including the disappearance of three cladocerans (*Daphnia rosea*, *D. pulicaria*, and *Bosmina longirostris*) and the altered feeding habits of both lake trout and kokanee has been well documented (Richards et al. 1975, Rybock 1978, Goldman et al. 1979, Morgan 1979, Threlkeld et al. 1980, Sawyer 1985, Richards et al. 1991, Beauchamp et al. 1994a, Beauchamp 1994b, Van Tassell 2000). During periods of low *Mysis* abundance and high primary productivity, *Bosmina longirostris* and *D. pulicaria* are able to make brief appearances in the pelagic zone of Lake Tahoe (Richards et al.

1991, Van Tassell et al. 2000). However, for discussion purposes, the zooplankton community will be limited to *Mysis*, and the two native copepods.

Studies of pelagic zooplankton distribution in Lake Tahoe indicate that there are very few individuals of any species present in the shallow surface water (<5m) during daylight conditions (Morgan et al. 1978, Morgan and Threlkeld 1982, Byron et al. 1983, Burgii et al. 1993). Fewer than one copepod (*D. tyrelli*) per cubic meter was reported above 10m during the daytime in July (Byron et al. 1983). Burgii et al. (1993) found daily vertical migrations of copepods (*D. tyrelli* and *E. nevadensis*) with the adults and juveniles absent from the surface layer until nightfall. This is consistent with findings from other lakes, where zooplankton species are known to make vertical migrations away from light (Wetzel 2001). Even more dramatic vertical migrations, away from light, have been described for *Mysis* (>300m) in Lake Tahoe by Morgan et al. (1978).

The absence of zooplankton in the shallow pelagic zone of the lake has implications for the distribution of fish species. Introduced kokanee salmon are the primary pelagic species in Lake Tahoe. A small portion of the adult rainbow trout population also exists in the open water, but they tend to be more closely associated with the littoral zone, where forage fish abundance is higher. Kokanee salmon are planktivores throughout their three-year life cycle in Lake Tahoe (Van Tassell et al. 2000, Moyle 2002), indicative of their pelagic existence. However, little is known about the specific depth distribution of juveniles and adults. The limited information available from Tahoe studies indicates that adults inhabit the open water near the thermocline depth during the summer and fall (Beauchamp et al. 1994a, Moyle 2002, Brant Allen, unpublished data), while juveniles inhabit shallow water (<10m) upon emerging from their natal stream (Taylor Creek) in the spring (Gemperle 1998). Unfortunately little effort was given to searching for juveniles in deeper water (>10m) during the 1998 study, leaving their selected habitat essentially unknown.

1.2.3 Littoral Biological Community of Lake Tahoe

The littoral zone of Lake Tahoe is defined as the region around the lake shore, extending from high water down to the depth of the metalimnion (20-25m). This zone comprises a large variety of habitats from gently sloping, open sand to very steep boulder drop offs. The biological community associated with this zone of the lake includes benthic invertebrates, zooplankton, and fish.

The most complete study of Tahoe's macro invertebrate benthic community was conducted by the California Department of Fish and Game and the Nevada Department of Wildlife from 1962-1963 (Frantz and Cordone 1996). Eckman grab samples were collected from about 1m to the deepest part of the lake (500m). Collected benthos was sieved and species were identified. Over one hundred samples from 1-10m revealed that 9% (4.95 g/m²) of the whole lake standing crop was located in less than 10m of water (weighted estimate adjusted for bathymetric surface area). Additionally, species diversity decreased with increasing depth. During both sampling years (1962 and 1963) all the major taxonomic groups were represented above the 30m depth contour (Frantz and

Cordone 1996). Extensive snorkel surveys around the littoral zone of Lake Tahoe have not revealed the same abundance of benthic macro invertebrates (Brant Allen, unpublished data). This is believed to be due to the cryptic behavior of the benthic organisms. When shore zone rocks or sediment is excavated, many invertebrates can be located. It is possible that this behavior is an adaptation to avoid both predation and the effects of UV radiation in the very clear waters of Lake Tahoe.

The early collections by the state wildlife agencies, described above, did not include crayfish in their estimates of benthic standing crop. This is likely due to the inability of the sampling device to capture crayfish. The signal crayfish (*Pacifastacus leniusculus*) was introduced to Lake Tahoe in 1895 (Abrahamsson and Goldman 1970). Whole lake density estimates have been calculated at 1.4 crayfish/m² (Abrahamsson and Goldman 1970) and 3.8 crayfish/m² (Chandra, unpublished data) while site specific densities have been measured as high as 10 crayfish/m² (Flint 1975). While a large variation exist in the standing crop estimates, it is well accepted that crayfish are abundant in the littoral zone, occupying the shallowest depths during the summer months. Like other benthic invertebrates, in the shallow waters, the crayfish are seldom seen during daylight hours. They remain hidden under rocks and woody debris on the bottom, emerging at night to feed. The nocturnal behavior is presumably an adaptation to avoid predation, but has an added benefit of limiting exposure to UV radiation.

Relatively little is known about the littoral zooplankton in Lake Tahoe. Far more scientific effort has been placed on the pelagic zooplankton, due to the small percentage of the lake that is represented by the littoral zone, and the direct implications pelagic zooplankton have on water quality and the lakes' ecology. While littoral species surely exist in the Tahoe shore zone, it has been shown in other lakes that many species are associated with submerged aquatic vegetation, or are sessile, remaining in the bottom substrate (Wetzel 2001). There is very little aquatic vegetation in Lake Tahoe due to coarse granitic sands and low nutrient levels in the substrate. For this reason, it may be more appropriate to view pelagic zooplankton abundance in the littoral zone as representative of this community.

While pelagic zooplankton, especially copepods and cladocerans, have been shown to avoid the shore zone (Wetzel 2001), exceptions have been found in Lake Tahoe during the spring and summer months. Swarms of the copepod, *D. tyrelli*, were found among the shoreline rocks in depths less than 1m, presumably to increase reproductive success (Byron et al. 1983). Swarm densities were highly variable and exceeded 11,000 individuals per liter in one instance. The dense swarms do not appear to be an annual event and the specific mechanisms affecting their formation remains unknown. It is important to note that *D. tyrelli* is heavily pigmented. The dark red coloration of this species has been shown to increase their vulnerability to predation (Byron 1982) but may provide significant protection from UV radiation while at the surface in Lake Tahoe. Levels of carotenoid pigment have been shown to increase in populations exposed to higher intensity ambient light (clearer water and increased altitude) (Wetzel 2001), and within individuals during daily photoperiods (Ringleberg 1981).

The fish community in the littoral zone of Lake Tahoe is dominated by native, non-game fish species, including Lahontan redbside shiner (*Richardsonius egregious*), Lahontan speckled dace (*Riynichthys osculus*), Tui chub (*Gila bicolor*), Tahoe sucker (*Catostomus tahoensis*), and Piute sculpin (*Cottus beldingi*) (Miller 1951, Beauchamp et al. 1994c). Only adult and larval rebsides and speckled dace, and larval Tahoe suckers are common at depths less than 5m during daylight hours (Miller 1951, Baker 1967, Evans 1969, Beauchamp et al. 1994c). The adults of these species are heavily pigmented and can be seen feeding in the near shore waters, during the summer months. Larval and juvenile life stages of these species are often found in very shallow (<0.5m), protected areas of the lake, including marinas and lagoons, from June through October (Baker 1967, Evans 1969, Beauchamp et al. 1994c). The “young of year” (YOY) fish, those that have emerged from spawned eggs during the early summer, have very little pigmentation and are therefore able to take advantage of shallow open sandy areas, with minimal risk of predation. As the fish develop coloration (late summer), they tend to form schools or move to more complex habitat (rocks) to decrease predation risk (Beauchamp et al. 1991).

Shore zone spawning in Lake Tahoe takes place after the onset of darkness, during the summer, when shallow lake temperatures remain above 11⁰C (Allen 1996). Lahontan rebsides and speckled dace deposit eggs over gravel substrate at depths less than 20cm (Miller 1951, Evans 1969, Allen 1996). The eggs settle into the substrate such that they are no longer visible from the surface. One of the most productive spawning sites was located immediately adjacent to a marina, receiving heavy boat traffic and supporting a personal watercraft concession. During a shore zone spawning study, no evidence of toxicological effects could be determined in eggs collected from the substrate (Allen 1996). It is important to note that this study was conducted prior to a Tahoe Regional Planning Agency (TRPA) regulation prohibiting 2-stroke motors on Lake Tahoe.

1.2.4 Limnology of Donner Lake

Relatively few studies have been published on the limnology and biology of Donner Lake (Dong 1975, Dong and Averett 1977, Morgan 1981, Reuter et al. 1999). Perhaps the most detailed, long term work was conducted by Gary Vinyard with the University of Nevada, Reno, between 1991 and 1996. Vinyard’s information is contained in unpublished technical reports. Additional published studies have investigated Donner Lake in relation to other lakes of the Sierra Nevada range, but did not focus on ecological interactions between species or how limnological properties may affect biological communities (Iwatsubo et al. 1972, Morgan and Threlkeld 1982).

Donner Lake is located in California, approximately 20 km northwest of Lake Tahoe, at an elevation of 1809m. The lake surface elevation is enhanced by a 2m dam, which controls a reservoir volume of 9,500 acre feet. Water is held in the reservoir during the summer months with a small decrease (8cm/d) in surface elevation being attributed to evaporation and minimal, seasonal precipitation (UC Davis 2003). Donner Lake has a maximum depth of 70m and an average depth of 33m. With a surface area of 3.9 km² and

a drainage basin of 36 km², the watershed to lake ratio is 9.2 (Dong 1975, Vinyard 1994, Reuter et al. 1999).

Donner Lake becomes thermally stratified during the summer months. A thermocline develops at about 10m by mid-June, with shallow surface temperatures exceeding 20⁰C during July and August (Dong 1975, Reuter et al. 1999, UC Davis 2003). Water temperatures below 20m can reach 8⁰C during the suppression of the thermocline in October, but are generally less than 7⁰C. Lake mixing occurs in December or January with complete ice cover possible during winter (Dong 1975, Vinyard 1994).

Water transparency in Donner Lake is significantly less than that of Lake Tahoe. Spring secchi depths of 8-9m increase during July to 14m. It is believed that particulate matter carried into the lake with seasonal snow melt, increased primary production, and deposition of pine pollen is responsible for the poor spring clarity (Dong and Averett 1977). The summer clearing is likely due to few additional nutrient inputs and a removal of algae by zooplankton grazing. Chlorophyll maximums have been recorded around the thermocline depth, and were associated with peaks in dissolved oxygen concentration (Vinyard 1994).

Transmission of incident surface light has been measured numerous times in Donner Lake (Dong 1975, Dong and Averett 1977, Vinyard 1994) and follows the trend in secchi depth measurement. The depth of one percent photosynthetically active radiation changes with season. Mean values, for all years measured, are lowest in the spring (16.1m, May), and increase during the summer (23.0m and 23.9m respectively for July and August).

Dissolved organic carbon concentrations were measured in the shallow surface water (0.5m) and deep water (>35m) during July, from 2000 through 2002 (Oris, Unpublished data). There was little difference in concentration between years or depth with values ranging from 1.4 µg/l to 1.9 µg/L.

1.2.5 Pelagic Biological Community of Donner Lake

The pelagic zone of Donner Lake is large in relation to its total volume. This is due to the steep sided basin, which is evidenced by the average depth (33m) equaling half of the maximum depth (70m). While its overall size is significantly smaller than Lake Tahoe, the same relationship exists with a narrow littoral zone and a vast pelagic volume.

The zooplankton community has undergone significant changes as a result of the intentional *Mysis* shrimp introduction in 1965 (Hansen 1966). *Daphnia rosea* and *Diaptomus pallidus*, identified by Dong (1975), were found to be missing from the lake in 1978 (Morgan 1981). Their disappearance is believed to be the result of *Mysis* predation (Morgan 1981). The current zooplankton assemblage is being studied in a cooperative research project involving the same universities in this study, but preliminary data are not yet available. Therefore, the best information is provided by Dong (1975), noting the exceptions discovered by Morgan (1981). Based on this assumption, the

zooplankton community of Donner Lake includes one cladoceran species, *Bosmina longirostris*, one copepod, *Epischura nevadensis*, and *Mysis relicta*.

Little is known about the vertical distribution of these zooplankton species. Net tows to the surface from 5, 10, and 40m in 1973 suggest that significant portions of the population may have been present in the top 5m of the water column during daylight hours (Dong 1975). However, the small net size (0.25m²), lack of replicate tows, and expected zooplankton patchiness (Burgii 1993), do not allow detailed interpretation of the data. A study of the vertical distribution of zooplankton at Castle Lake, California, indicates that zooplankton species avoided the 0-5m depth strata during daylight hours (Burgii et al. 1993). This may be a good surrogate for Donner Lake since light transparency in the two water bodies is similar during the summer months (12 – 14m secchi depth).

The pelagic fish community in Donner Lake is believed to be dominated by kokanee salmon and seasonally stocked, adult rainbow trout. Both species are expected to be concentrated around the thermocline depth (10m). This is due to the relatively warm surface temperatures in Donner Lake (>20⁰C) during the summer months, and the species preference for cool water (10-15⁰C) (Moyle 2002).

1.2.6 Littoral Biological Community of Donner Lake

The littoral zone of Donner Lake is defined as the region around the lake shore, extending from high water down to the depth of the metalimnion (10m). The habitats most commonly found in this zone of the lake are gently sloping, open sand and cobble, to steep drop offs of silt and sand substrate. Significant areas of aquatic macrophytes can be found along the gently sloping contours between 2m and about 6m. Near the outlet, at the east end of the lake, a shallow lagoon provides protected water and fosters dense macrophyte growth. The biological community associated with the littoral zone of the lake includes benthic invertebrates, zooplankton, and fish.

Dong (1975) collected benthic invertebrates from 30 locations in Donner Lake covering a wide range in depths, during the summer of 1973. Species collections were identified to family, and the percentages of the whole community were reported. Unfortunately, no information was made available regarding which organisms were collected from the shallow zone (littoral) and which were collected from the deep zone (profundal). Dipterans (*Tendipedidae*, larvae), dominated the benthic collections, comprising more than 74% of the organisms collected.

Signal crayfish (*P. leniusculus*) are also known to be abundant in Donner Lake, but were not collected during the 1975 study, due to the sampling methods that were used. As in Lake Tahoe, crayfish are known to inhabit the shallow benthos and seek protective cover during daylight hours. They have been observed foraging along the bottom substrate at night, presumably when predation risk has been diminished. Crayfish have also been observed in holes excavated into the soft sediment, where limited benthic structure exists (Allen, Unpublished data).

The literature on Donner Lake does not suggest that any studies have evaluated the near shore zooplankton community, in relation to the rest of the lake. Since zooplankton have been shown to display an avoidance of near shore areas (Wetzel 2001), it is suspected that their density in the littoral zone is diffuse. This is likely to be enhanced in Donner Lake, compared to Lake Tahoe. The shore zone avoidance is a response to incident light angles in the water column. In small, alpine lakes, where mountains often elevate the surrounding horizon, the shore zone avoidance affect is more pronounced (Wetzel 2001). Based on our literature review and an understanding of zooplankton behavior, for the purposes of this study, the near shore zooplankton community will be assumed to be similar to that of the pelagic zone.

The littoral fish community in Donner Lake is characterized by native non-game fish. The species assemblage is the same as that of Lake Tahoe. For this reason, the discussion will focus on those species, and age classes, already shown to be potentially vulnerable to the toxic effects of UV enhanced PAH toxicity, based on their habitat selection.

Larval and juvenile life stages of Lahontan redbside shiner (*R. egregious*) and Tui chub (*G. Bicolor*) utilize the very warm (30⁰C), shallow lagoon area at the east end of Donner Lake. The habitat provides protection from wave energy and is too shallow for most piscivorous predators. This coupled with warm temperatures and a productive environment, allows rapid growth rates that could not be realized in the open lake. The nursery lagoon was not sampled for PAH concentrations during this study. The very shallow depth prevents motorized craft from entering, and isolates the water in the lagoon from the regular circulation patterns that exist in the lake. It is suspected that young-of-year fish leave the lagoon in late summer or fall, before lake levels drop.

Shore zone spawning by redb sides, chubs, and Lahontan speckled dace (*R. osculus*) is expected to occur in the spring and early summer, as it does in Lake Tahoe. Redside and dace egg deposition is likely to be associated with shallow (<20cm) shore zone gravel substrate, where eggs settle into the interstitial spaces. Tui chub spawning has been described on both gravel substrate, and on aquatic vegetation (Moyle 2002). Based on the presence of larval tui chubs in the lagoon area described above, it is likely chubs are spawning on vegetation in Donner Lake.

Spawning by deep living lake trout (*S. namaycush*) has been observed in the littoral zone of Donner Lake during the fall (Allen Unpublished data). In 2000, gravel was added to the deepest portion of the public boat ramp, located on the northeast shore of the lake. Nighttime observations in October revealed large adult lake trout spawning over the newly established substrate, at a depth of 1-3m. Since boating traffic decreases dramatically after the Labor Day weekend in September (UC Davis 2003), disturbance to the incubating eggs is thought to be diminished, compared to summertime conditions.

1.3. Summary

The aquatic organisms that are most likely to be affected by photo induced toxicity of PAH, are those living in the shallow waters that are exposed to sunlight. The existing literature on Lake Tahoe and Donner Lake, suggest that very few organisms occupy this habitat. Pelagic zooplankton display daily migrations away from light, with few organisms present shallower than 10m in Lake Tahoe (Byron et al. 1983). Based on studies in Castle Lake (Burgii et al. 1993), zooplankton would not be expected above 5m during daylight conditions in Donner Lake. The littoral zone fish, specifically the larval stages with limited pigmentation, may be the most susceptible to exposure, since they spend the daylight hours in very shallow water, benefiting from the effects of thermal heating. It is important to consider the ambient concentrations of PAH in these areas when evaluating the risk assessment. Therefore, the following monitoring and laboratory experiments have been conducted.

2. Experimental Methods

2.1 Determining Levels of PAH in Lake Tahoe and Donner Lake (Task 2)

2.1.1 Sampling methods and sites

Samples were collected throughout 2001 and 2002 at various sites at Lake Tahoe and Donner Lake. The site locations are listed in Table 2.1.1. Samples were collected in 2.6 L amber bottles that were opened below the surface of the water in order to minimize the amount of surface film that was introduced into the bottles. Samples were placed in a cooler and transported to UNR for analyses. Samples were extracted within 7 days, and stored in a -20°C freezer until analyzed by gc-ms.

2.1.2 Analytical

The water samples were extracted by passing the 2.6 liters of water through an Empore® sample extraction disk (using the procedure provided with the disks), that allowed the water to pass through but retained non-polar compounds. Sample disks were placed in vacuum desiccators until dry and then extracted with 5 ml acetonitrile followed by two 5 ml portions of methylene chloride. Sample extracts were collected in 40 ml amber vials and stored at -20 °C until evaporated, and analyzed. Each sample was then passed through anhydrous sodium sulfate to remove any excess water and concentrated under a stream of nitrogen in an N-EVAP to 2 ml. Deuterated compounds served as internal standards and were obtained from AccuStandard (New Haven, CT) as were calibration reference standards. Samples and standards were analyzed using a HP 6890 Plus series Gas chromatograph (GC) coupled with a HP 5973N mass selective detector (MSD). The GC was equipped with a 30 m x 0.25 mm i.d. DB5 ms (HP model 19091S-433) capillary column and was run in constant flow mode at 1.0 ml/min. The MSD was run in selected ion monitoring mode (SIM).

Table 2.1.1. – PAH water sampling locations at Lake Tahoe and Donner Lake

Lake Tahoe Locations	LATITUDE	LONGITUDE	ABBR.
Incline Beach	N39 14 15	W119 56 49	
Mid-Lake Buoy (TR-2)	N39 08 09	W120 04 52	
Zephyr Cove	N39 00 26	W119 57 06	
Ski Run Marina	N38 57 04	W119 57 30	
Tahoe Keys Homeowner Lagoon	N38 56 06	W120 04 40	TKM HO
Tahoe Keys Marina, East Channel	N38 56.253	W 120 00.277	
Tahoe Keys Marina (Nun Buoy)	N38 56 31	W120 00 57	TKM NUN Buoy
Kiva Beach	N38 56 31	W120 03 20	
Emerald Bay (South side of Bay)	N38 57 08	W120 05 31	EM. Bay
Tahoe City	N39 10 06	W120 08 01	TCM
Sand Harbor			
Camp Richardson	N 38 56.523	W 120 02.469	Camp Rich.
Sugar Pine			
Donner Lake Locations			
Donner Lake Boat Ramp	N39 19 43	W120 17 02	Donner Ramp
Mid-Lake West End (STA-4)	N39 19 17	W120 16 22	Donner Mid
Mid-Lake East End (STA-5)	N39 19 47	W120 15 22	Donner Mid

2.1.3. SPMD placement and analyses

Detection of PAHs in water samples is problematic because of their low concentrations and transient nature. Semipermeable membrane sampling devices (SPMDs) were used to sample these organic compounds in the water column. SPMDs consist of a flat low-density polyethylene tube filled with triolein (lipid) and are deployed in stainless-steel canisters suspended in the water column (Huckins et al., 1990 and 1993). Typically, these devices are left in the water for 1 or 2 months. These devices are effective in sequestering hydrophobic organic compounds from water and are useful in assessing the bioavailability of these compounds (Bevans et al., 1996). Semipermeable membrane sampling devices (SPMDs) were emplaced at seven locations in Lake Tahoe and two locations in Donner Lake. The SPMDs were emplaced by SCUBA in areas of high boating activity with the exception of a control site located centrally in Lake Tahoe. The SPMDs deployed in Donner Lake also were in areas of high boating activity. The SPMDs were attached to buoy chains or dock supports at 3 meters depth. For quality-assurance purposes, a blank (SPMD transported to the sites and opened to the atmosphere at the sites) was collected during SPMD deployment and retrieval. Duplicate samples were collected at three sites during the 2001 SPMD deployment to determine reproducibility of sample collection and analytical procedures. However, vandals destroyed the duplicate sample from the Tahoe City location during its deployment. When the SPMDs were retrieved, they were placed on ice and shipped overnight to Environmental Sampling Technologies for processing. Compounds were recovered from the SPMDs by dialysis, gel-permeation chromatography, extracted into hexane, and sealed in glass ampoules. The hexane extracts were analyzed by gas chromatography-mass spectrometry at the University of Nevada, Reno laboratory.

2.1.4 PAH in Tahoe Sediments

Sediment samples were collected from 12 sites in Lake Tahoe on September 12, 2002. The sites contained a variety of sediment textures from fine grained mud to coarse grained sand. A stainless steel Ponar grab sampler (15 cm x 18 cm) was used in sample collection. Samples were transferred to polyethylene bags (zip-loc) and stored at 4⁰C before analysis.

In the laboratory, the sediment samples were thoroughly mixed with a stainless steel spatula and 10.0 ± 0.1g samples were transferred to 120ml amber glass screw cap bottles with Teflon lid liners. Anhydrous sodium sulfate (approximately 30g dried overnight at 120⁰C) was added to the sample. Samples were spiked with 3.2µl of a 50ng/µl of surrogate compounds in methanol (listed in US EPA method 8270C), (includes PAH internal standards, anthracene d₁₀, Pyrene d₁₀ purchased from AccuStandard, Inc, New Haven, CT). A 1:1 (v/v) mixture of dichloromethane:acetone (HPLC grade) was added to the bottle followed by immediate capping and vigorous shaking to break up the solids. The contents were sonicated in an ultrasonic bath for 30min and left to stand overnight. The following day the samples were vigorously shaken and sonicated for 20mins. A 10ml portion of each extract was transferred to a 15ml glass concentrator tube and concentrated to 2ml by gently blowing a stream of nitrogen onto the surface of the solvent. When the sample volume was approximately 2ml, 5ml of ethyl acetate was added to the tube and then evaporated down to 2ml once again. A 1.2µl of 50ng/µl p-

Terphenyl d₁₄ (AccuStandard, Inc, New Haven, CT) in ethyl acetate was added to the contents. The contents were transferred to 2ml amber glass vials with 200µl glass inserts and analyzed using GC/MS (Agilent Model 6890 GC with Agilent 5973 Mass selective Detector and Agilent 7683 autosampler). A textural analysis of these sediments has not been completed.

2.2 Determination of PAH emission from various marine engines (Task 3)

As initially proposed, this task was to be conducted using an in-lake method, in which a grid was established in a calm and clean portion of Lake Tahoe. A variety of engine types would then pass over the grid and samples appropriately taken to determine PAH concentrations. However, it became apparent that the concentrations of PAH in the water column following a single pass of an engine would be less than the analytical detections limits, even using the more sensitive gc-ms method. In addition, the cost and time requirements for determining PAH in water was sufficiently greater than for volatiles, that it became clear that this method was unlikely to provide useful results.

Three options were explored at that point. The first was to utilize samples taken at the California Air Resources Board facility (CARB) in El Monte, California. This site contained a tank for conducting emission testing of marine engines. While the CARB group was primarily interested in atmospheric emissions, the tank proved invaluable for the testing that we required, and this option was utilized.

The second option (also utilized) was to examine the relative emission of two small 9.9 hp engines (one a carbureted 2-cycle engine and the other a 4-cycle engine) in a smaller 500 L tank system at UNR.

The third option was to participate in a study conducted by the Southwest Research Institute in San Antonio, Texas that was funded by the California Air Resources Board. The study was designed to comparatively test emissions from a variety of marine engines. This option proved highly attractive, since the testing would be conducted under a rigorous protocol and would examine a variety of engines under identical conditions. The tests were to be initiated in June of 2002. Sample bottles were sent and procedures to get samples transported back to UNR were developed. Several delays occurred associated with getting the equipment at Southwest Research Institute operational. One test was conducted on one engine in August of 2002, and staff from UNR traveled to San Antonio and samples were taken, and analyzed. However, this was the only test conducted, and because the results were meant to compare several engines, a single test was not useful for comparison of emissions. Although the tests are unlikely to be conducted prior to the termination of this project, we plan to continue with these tests if they are indeed conducted. Thus, the comparative tests that are available include 5 engines, three at the CARB facility and two at UNR.

Test performed at the California Air Resources Board facility (CARB) in El Monte, CA proved to be very useful. Previously, Kado and coworkers, (2000) conducted similar tests to determine the contribution from marine engines to airborne particulates and associated toxic pollutants such as PAH. Since normal operation of marine engines typically results in significant exhaust of these pollutants into the water column, we examined how much total PAH was contributed by each of these types of marine engines. We utilized the same test facility and marine engines as reported by Kado, *et al* (2000). To facilitate discussion, the test facility and engine models tested are summarized in the materials and methods section.

The test facility consisted of a specially designed tank filled with approximately 2600 gallons of tap water. Engines (Table 2.2.1) were mounted near one end of the tank and connected via cables to a console located near the test tank. Each test engine was fitted with a special test propeller to provide adequate load while minimizing wake. Tank design included baffles to prevent water loss from agitation due to propeller action. A typical test cycle is describe in Table 2.2.2 and was designed to simulate actual use. At the end of each test interval, samples were collected by pulling tank water directly through prepared Empore C-18 disks under vacuum until approximately 1 liter had been sampled, and the volume measured. The sample apparatus was protected during immersion into the tank water to prevent sampling of surface film and samples were taken from near mid depth. The tank was assumed to be well mixed due to the vigorous churning achieved during all test cycles.

All engines tested were commercially available outboard engines from 1998 to 1999 model years. A summary of engine specifications is provided in Table 2.2.1. Engine tests were conducted according to the following schedule: 3/20 am 4-cycle Honda, 3/20 pm 4-cycle Honda duplicate test; 3/20 pm 2-cycle DFI, 3/21 am 2-cycle DFI duplicate test; 3/21 am 2-cycle carbureted, 3/21 pm 2-cycle carbureted duplicate test. A third test for the 2-cycle carbureted engine was conducted on 3/21 because a low load propeller was inadvertently used for the first test cycle. Results from the low-load propeller are not presented here.

Table 2.2.1. Marine test engines

	<i>Test Engine</i>		
	2-stroke	2-stroke	4-stroke
Fuel system	Carburetor	Direct fuel injection (FICHT)	Carburetor
Make	Johnson	Evinrude	Honda
Model year	1998	1999	1998
Rated max HP	90	90	90
Max RPM	5200	5500-6000	5000-6000

From Kado *et al*, 2000

All engines were fueled with summer-grade unleaded (87 octane) fuel from the same batch. Oil was added to the two-cycle engines according to manufacturers' specifications.

Table 2.2.2. Engine test cycle

Sample Interval		
T10I	Idle -- 10 minutes	
	High Idle	2 minutes
	Low Idle	8 minutes
Engine Off	Sample	10 minutes
T10R	60% Full Throttle	10 minutes
Engine Off	Sample	15 minutes
T30R	60% Full Throttle	20 minutes
Engine Off	Sample	15 minutes
Drain/Clean/Re-fill		~ 2 hours

Following each portion of the test cycle, PAH from the water samples were collected on the Empore sample disks and placed in vacuum desiccators until dry and then extracted with 5 ml acetonitrile followed by two 5 ml portions of methylene chloride. Sample extracts were collected in 40 ml amber vials and stored at -20 °C until returning to the University for analysis. Each sample was then passed through anhydrous sodium sulfate to remove any excess water and concentrated under stream of nitrogen in an N-EVAP to 2 ml. Deuterated compounds served as internal standards were obtained from AccuStandard (New Haven, CT) as were calibration reference standards. Samples and standards were analyzed using a HP 6890 Plus series Gas chromatograph (GC) coupled with a HP 5973N mass selective detector (MSD). The GC was equipped with a 30 m x 0.25 mm i.d. DB5 ms (HP model 19091S-433) capillary column and was run in constant flow mode at 1.0 ml/min. The MSD was run in selected ion monitoring mode (SIM).

2.2.1 Determination of photolysis rates of PAH generated by marine engines

Engine test tanks were filled with tap water the night prior to the experiment to allow any chlorine in the water to volatilize. The engine (either a 9.9 hp Mercury 2-stroke carbureted engine or 9.9 hp Mercury 4-stroke engine) was placed in the tank and run at idle for 5 minutes then at approximately 90% wide open throttle (WOT) for 15 minutes. The water in the tank was allowed to settle for a couple of minutes and the water was collected in 2.63 L amber sample bottles. Sample bottles were submerged in the tank prior to removing the cap to prevent sampling the oily surface layer. Twelve 500 mL volumetric flasks were filled to exactly 500 mL with the water collected from the tank. Four of the volumetric flasks were wrapped in foil to serve as dark controls and all flasks were placed in direct sunlight. Exposed and dark control samples were collected and extracted (using EPA method 525) at prescribed intervals throughout the 8-hour experimental day. The samples were then analyzed for PAH concentrations using a gas chromatograph equipped with a mass selective detector. For individual PAH compounds and the PAH mixture the volumetric flasks were filled with distilled water and then spiked with 1 mL of 2 ng/μL of either an individual compound or the PAH mixture. Samples were then exposed to sunlight and analyzed as described above.

2.3 Phototoxicity Assessment (Task 4)

2.3.1 Phototoxicity of water from Lake Tahoe and Donner Lake

Experiments were conducted during the summer months of 2001 and 2002. The original intent of the study was to use native species of fish and zooplankton to determine whether waters of Lake Tahoe and Donner Lake exhibited photoinduced toxicity to aquatic organisms. The following organisms were used in attempts to characterize toxicity of lake waters: Lahontan redbreast minnows *Richardsonius egregius* (juveniles), speckled dace, *Rhinichthys osculus* (juveniles), rainbow trout, *Oncorhynchus mykiss* (embryo-larval), fathead minnow, *Pimephales promelas* (embryo-larval), *Daphnia spp.* (collected from Topaz Lake), and *Ceriodaphnia dubia* (neonate to adult). Native species included the redbreast minnows, dace, and *Daphnia* species, and were collected from local waters. Rainbow trout embryos were obtained from the State of Nevada Department of Wildlife. Fathead minnows were obtained from a culture maintained in the aquatics facility at Miami University (Oxford, OH), and *Ceriodaphnia dubia* were obtained from a culture maintained at the University of Nevada-Reno in the G. Miller laboratory.

Experiments with fish were conducted to determine possible effects on survival or growth. Experiments with zooplankton were conducted to determine possible effects on survival (*Daphnia* and *Ceriodaphnia dubia*) or reproduction (*Ceriodaphnia dubia*).

Study sites were located at the Tahoe City Marina and at the public boat ramp at the northwest end of Donner Lake. The basic procedure for the experiments was to collect water in 4 L glass bottles from each location on a daily basis (at 1500 hours) during experiments and transport the water to the Tahoe Research Group Laboratory near Tahoe City for use in the tests. In 2001, water was collected at a depth of 1m directly from the dock at both sites. In 2002, water was collected from a depth of 3m at both sites. Water was still collected from the dock at Donner Lake in 2002, but water from Tahoe City was collected from 600' offshore the marina channel to mimic the near shore collection site of Dr. Oris' 1997 studies. Tests were initiated by pipetting water from the glass bottles into test chambers (250 ml for fish, 50 ml for zooplankton), and adding organisms as described below. Chambers were held inside the lab overnight. In the morning, water in the chambers was refreshed from an unopened 4 L bottle, and chambers were placed into an outdoor constant temperature bath by 0900 hours. One half of the chambers were placed beneath a UV-transparent filter and one half of the chambers were placed beneath a UV-opaque filter for UV and no-UV treatments. Temperature and UV radiation readings were monitored every hour throughout the day. Chambers were brought back into the laboratory at 1800 hours, water was refreshed in the chambers using newly collected water, and the chambers were left in the lab overnight. This procedure was repeated over a seven day period for all experiments with all organisms, following standard U.S. Environmental Protection Agency effluent toxicity test methods for zooplankton and fish larvae [48]. In 2002, a 0.5 µg/L addition of fluoranthene to aerated, aged Lake Tahoe water served as a positive, phototoxicity control.

Water collected from each site on a daily basis was also extracted and analyzed for PAHs to determine concentration and make-up of possible exhaust components in water. Data

for survival and reproduction (zooplankton) and for survival and growth (fish) were analyzed for differences between UV and no-UV treatments.

Data on survival were collected 2 times daily. *Ceriodaphnia dubia* reproduction was assessed each day during a test. Fish growth was assessed at the end of a test and was measured as total wet weight per individual fish. Reproductive output and fish growth during the tests were compared among treatments using an analysis of variance (ANOVA) approach implemented with the Statistical Analysis System (SAS) GLM Procedure.

2.3.2 Determination of phototoxicity of marine engine emissions to *Ceriodaphnia dubia* in the laboratory.

Laboratory bioassay experiments with *Ceriodaphnia dubia* were set-up on campus at the University of Nevada (UNR). Data were collected on the direct and UV-enhanced toxicity of PAH compounds associated with watercraft engine emissions. Two 9.9 hp Mercury outboard engines, a 2-cycle and a 4-cycle, were run in 500 L test tanks to generate water containing watercraft engine exhaust. The engines were run several times during August, September, October and November of 2002. PAH-contaminated water was then collected in 1 L bottles and refrigerated until it was used for bioassay experiments comparing the toxicity of waters from the two engines.

Bioassay experiments followed the U. S. EPA protocol, *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fourth Edition* (August 1993). Treatment waters were prepared using 10.0%, 1.0%, 0.2% and 0.1% engine exhaust water mixed with control water. Control water was a mixture of 80% deionized local water and 20% Perrier mineral water, aerated for at least 24 hours. A static-renewal type test was used with complete water changes twice a day for the duration of each experiment. *C. dubia* neonates were isolated and placed in 30 mL glass beakers, each containing treatment or control water, then floated in Styrofoam frames inside one of two 30-gallon aquaria. The temperature of the water in the aquaria was maintained at 25 +/- 2 °C by running chillers with heat exchangers during the warm-weather experiments. Once ambient temperatures fell to approximately 10 °C in October and November, the chillers were no longer run. One aquarium was covered with a UV-screening sheet of clear acrylic (ACRYLITE OP-3), and the other aquarium was covered *with a sheet of clear acrylic without UV-screening (ACRYLITE OP-4). All *C. dubia* were acclimated for at least one day in direct sunlight with a light mesh screen over the acrylic covers at the beginning of each experimental run.

3. Results and Discussion

3.1 Ambient Concentrations of PAH in Lake Tahoe and Donner Lake (Task 2)

3.1.1 PAH in water samples

Water sampling was conducted at various times in Lake Tahoe and Donner Lake to determine PAH concentrations during several times of the year. Concentrations of PAH found Lake Tahoe and Donner Lake during 2001 and 2002 were low, and most of the larger PAH compounds (MW >228) were rarely detected. With some exceptions, the higher concentrations were found associated with marinas (and watercraft) and observed during the more active boating summer season. The complete data set is found in the appendix.

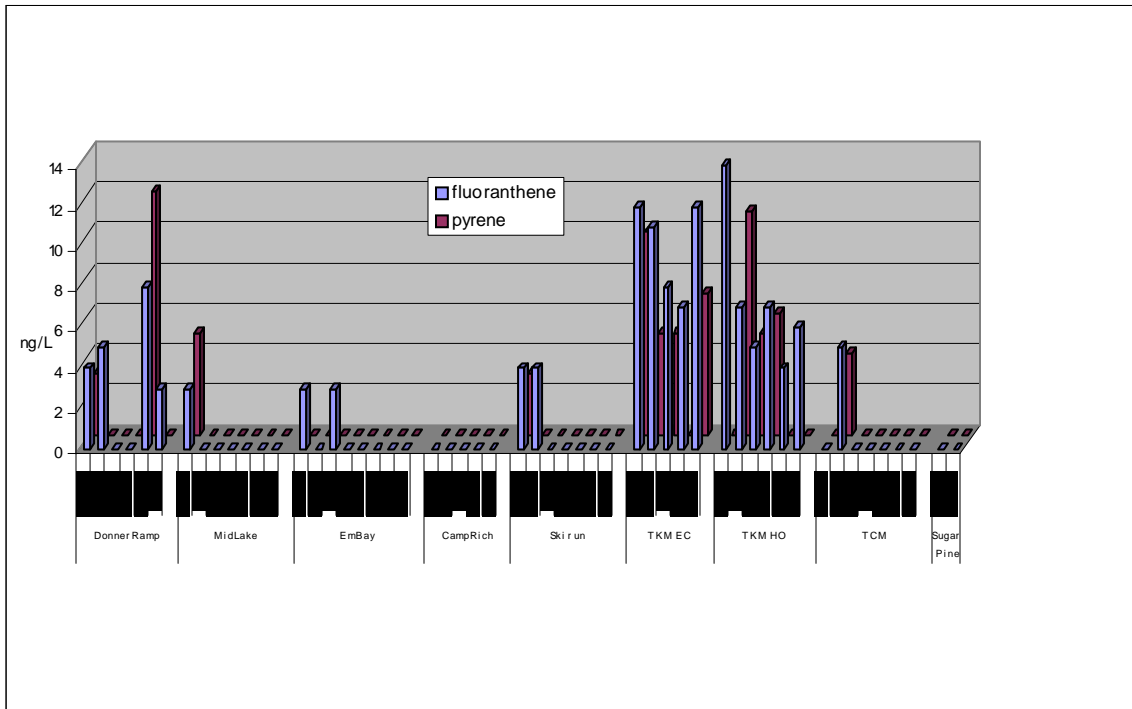
The only two photoactive compounds found commonly were pyrene and fluoranthrene. Infrequent detection of other photoactive compounds was observed, although the concentrations again were relatively low (<10 ng/L). Figure 3.1.1 shows the rate of detection at various sites during the sampling periods. As can be observed, even the mid lake sites showed some detections of pyrene and fluoranthrene. The greatest frequency and the largest concentrations were observed in the Tahoe Keys Marina area, although even in these samples, the concentrations were generally low.

Data presented in the appendix also indicate a common detection of phenanthrene and fluorene. These are commonly laboratory contaminants, and lack significant phototoxicity. They are thus not included in the discussion as potential contaminants of ecological interest. However, both compounds are emitted in relatively large amounts from engines, and increases in water concentrations are generally consistent with finding phototoxic PAH compounds.

Two sampling periods are noteworthy, since they captured times of the year when other sources of PAH might be expected. The first sample, on August 29, 2001, was during an intense forest fire in the Sierra. Smoke was prevalent in the Tahoe Basin and over Donner Lake. Samples were collected to see if elevated PAH would be present in the surface water. However, the samples did not show any elevated PAH, particularly in the open water samples, where smoke would be expected to impact the Lake.

Winter sampling for PAH would capture times when fireplace use was prevalent, and when photolysis is reduced due to reduced ultraviolet light. However, neither the November, sample or the March, sample showed elevated concentrations. All three of these sampling events are consistent with the suggestion that the majority of PAH does not come from atmospheric deposition from wood burning or forest fire smoke.

Figure 3.1.1. Fluoranthene and pyrene in water samples collected during the two-year period at several locations at Lake Tahoe and Donner Lake.



3.1.2 PAH sampled with SPMD

The PAH concentrations in SPMD samples collected during July-August 2001 are reported in tables 3.1.1 and 3.1.2. The concentrations of detected PAH compounds in the lipid material of the SPMDs are listed in Table 3.1.1 as micrograms of PAH per kilogram of lipid ($\mu\text{g PAH/kg lipid}$). These concentrations were calculated from the nanograms of each PAH compound in the extract reported by the UNR laboratory. Three SPMDs were extracted from each sample with a total lipid mass of 2.745 grams. The average concentrations of PAH in the water column (Table 3.1.2) were calculated from the data in table 3.1.1 ($\mu\text{g PAH/kg lipid}$) using the models described by Huckins et al. (1990 and 1993). Input to the models include the average water temperature during deployment, the sampling rate of the SPMD, concentration of PAH compound in the lipid, exposure time of the SPMD to the water, octanol-water partition coefficient, and the biofouling of the membrane.

PAH compounds were detected at all sites in Lake Tahoe and Donner Lake where SPMDs were deployed in 2001. Extracts from the SPMDs had a wide range of total PAH concentration and the number of compounds detected (Tables 3.1.1 and 3.1.2). The most commonly detected PAH compound was phenanthrene, which was present in all samples collected. Benzo(b,k)fluoranthene (sum of benzo(b)fluoranthene and benzo(k)fluoranthene) was the next most commonly detected PAH and was found in nine of 11 samples. Other PAHs present in the samples were acenaphthene, benz(a)anthracene, benzo(a)pyrene, benzo(g,h,i)perylene, fluoranthene, naphthalene, and

pyrene. Indeno(1,2,3-cd)pyrene was found in one sample from Camp Richardson. The PAHs chrysene, dibenzo(a,h)anthracene, and fluorene were not detected in any samples.

TABLE 3.1.1. Concentrations of PAH compounds in lipid from semi-permeable membrane sampling devices emplaced in Lake Tahoe and Donner Lake. [Concentrations are expressed in micrograms of PAH / kilogram of lipid. ND, compound not detected]

Compound	Site										
	Tahoe City	Mid-Lake	Sugar Pine Point	Emerald Bay	Ski Run Marina	Camp Richardson	Tahoe Keys East Channel	Tahoe Keys East Channel	Donner Lake Boat Ramp	Donner Lake Boat Ramp	Donner Lake Buoy
Acenaphthene	ND	ND	ND	ND	ND	ND	3.64	4.37	ND	ND	ND
Acenaphthylene	18.2	ND	ND	2.19	30.6	8.74	38.6	54.6	36.4	37.2	9.47
Anthracene	ND	ND	ND	ND	ND	ND	22.6	28.4	ND	ND	ND
Benz(a)anthracene	ND	ND	ND	ND	ND	ND	147	147	283	252	136
Benzo(a)pyrene	ND	ND	ND	ND	ND	ND	52.5	64.1	246	212	ND
Benzo(b,k)fluoranthene	10.2	ND	ND	0.73	39.3	7.29	85.2	84.5	168	145	91.8
Benzo(g,h,i)perylene	ND	ND	ND	ND	ND	ND	251	244	641	503	ND
Chrysene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dibenzo(a,h)anthracene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fluoranthene	ND	ND	ND	4.37	ND	ND	1240	1280	621	508	180
Fluorene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Indeno(1,2,3-cd)pyrene	ND	ND	ND	ND	ND	99.8	ND	ND	ND	ND	ND
Naphthalene	ND	ND	ND	5.10	ND	5.83	7.29	13.8	48.8	99.8	2.91
Phenanthrene	106	35.0	35.7	45.2	127	61.9	121	140	157	143	52.5
Pyrene	ND	ND	ND	ND	ND	ND	1020	1060	772	430	ND

TABLE 3.1.2. Estimated average water column concentrations of PAH compounds in Lake Tahoe and Donner Lake. [Concentrations are expressed in nanograms of PAH / liter. ND, compound not detected]

Compound	Site										
	Tahoe City	Mid-Lake	Sugar Pine Point	Emerald Bay	Ski Run Marina	Camp Richardson	Tahoe Keys East Channel	Tahoe Keys East Channel	Donner Lake Boat Ramp	Donner Lake Boat Ramp	Donner Lake Buoy
Acenaphthene	ND	ND	ND	ND	ND	ND	0.05	0.06	ND	ND	ND
Acenaphthylene	0.36	ND	ND	0.04	0.61	0.18	0.61	0.86	0.65	0.67	0.17
Anthracene	ND	ND	ND	ND	ND	ND	0.14	0.17	ND	ND	ND
Benz(a)anthracene	ND	ND	ND	ND	ND	ND	1.2	1.2	1.7	1.5	0.83
Benzo(a)pyrene	ND	ND	ND	ND	ND	ND	0.35	0.43	1.3	1.1	ND
Benzo(b,k)fluoranthene	0.05	ND	ND	0.004	0.19	0.04	0.72	0.71	1.1	0.93	0.59
Benzo(g,h,i)perylene	ND	ND	ND	ND	ND	ND	3.0	2.9	5.8	4.6	ND
Chrysene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Dibenzo(a,h)anthracene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Fluoranthene	ND	ND	ND	0.01	ND	ND	7.7	8.0	3.0	2.4	0.86
Fluorene	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Indeno(1,2,3-cd)pyrene	ND	ND	ND	ND	ND	0.39	ND	ND	ND	ND	ND
Naphthalene	ND	ND	ND	0.52	ND	0.59	0.74	1.4	4.9	10	0.29
Phenanthrene	1.0	0.32	0.34	0.42	1.2	0.58	0.91	1.1	1.3	1.2	0.45
Pyrene	ND	ND	ND	ND	ND	ND	5.6	5.8	0.30	1.8	ND

PAH concentrations found in samples from Donner Lake were among the highest observed during this study. Only the sample from the Tahoe Keys Marina East Channel had more compounds and higher concentrations of PAHs than those from Donner Lake. Both sites where samples were collected at Donner Lake had relatively high boating use, including two-stroke carbureted engines, which had been banned at Lake Tahoe in 1999.

PAH concentrations and distribution in Lake Tahoe and Donner Lake are linked directly to boating activities in the waters of the lakes. The results from Lake Tahoe collected during this study parallel those found by Lico and Pennington (1999). Lico and Pennington (1999) found the number of PAH compounds and their concentrations were highest where motorized boating activity is high. Lakes where boating activity was nonexistent or areas in Lake Tahoe where boating activity was low had the fewest number of PAHs and the lowest concentrations found during their study.

PAH concentrations and distribution in Lake Tahoe are similar before and after the ban of two-stroke engines on the Lake. Lico and Pennington (1999) found between two and 13 PAH compounds having a range of total PAH concentrations between nine and 228 $\mu\text{g}/\text{kg}$ of lipid in SPMD samples collected before the ban of carbureted two-stroke engines in Tahoe Basin waters. SPMD samples collected in 2001 after the ban (this study) had between one and 11 PAH compounds and concentrations of total PAH between 36 and 3,060 $\mu\text{g}/\text{kg}$ of lipid (Fig. 3.1.2.). If the sample from the Tahoe Keys Marina East is not considered, the number of compounds in the 2001 samples is reduced to a range of one to five and have total PAH concentrations between 36 and 197 $\mu\text{g}/\text{kg}$ of lipid. Lico and Pennington (1999) did not sample the Tahoe Keys Marina East site during their study, so a comparison excluding that site is valid. In low boat-use areas, Lico and Pennington (1999) found one PAH compound in three SPMD samples. It had a concentration of 5 $\mu\text{g}/\text{kg}$ of lipid in one of the samples. In 2001, one site (mid-lake) was sampled that had low boat use and it had a detection of one PAH compound with a concentration of 35 $\mu\text{g}/\text{kg}$ of lipid, although it is not phototoxic and may be an artifact.

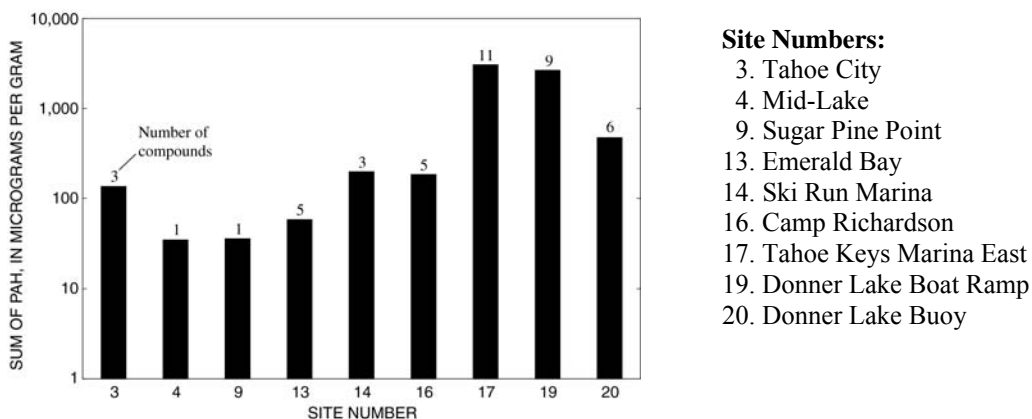


Figure 3.1.2. Sum of PAH concentrations (in $\mu\text{g}/\text{kg}$) and number of compounds in SPMD samples collected from Lake Tahoe and Donner Lake, 2001.

Two areas sampled with SPMDs had much higher concentrations of PAHs than the other sites in Lake Tahoe. These sites were located in the Tahoe Keys Marina East and at

Donner Lake Boat Ramp and are characterized by very heavy boat use. Also, boats travel at low speeds in these areas due to speed limits and congestion caused by heavy traffic, thus their engines do not run at an efficient level. Each of these two sites had the greatest number of compounds and the highest concentrations of PAH observed during the 2001 sample collection. An additional factor that may contribute to the high concentrations at Donner Lake is that carbureted two-stroke engines are not banned from the lake. Thus, oils burned during the combustion within these engines may contribute more PAH to the water than the newer technology engines.

Two sites, Tahoe Keys Marina East and Donner Lake boat ramp, had concentrations of PAH high enough to potentially cause toxicity to zooplankton and fish larvae. Oris et al. (1998) found the “no observable effect concentration” of total phototoxic PAH in Lake Tahoe waters to be 6.5 ng/L and 9.0 ng/L for zooplankton and fish larvae, respectively. Phototoxic PAH compounds include anthracene, benz(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, fluoranthene, and pyrene. The average concentrations of total phototoxic PAH at Tahoe Keys Marina East and Donner Lake boat ramp during the deployment period were 16 and 7.6 ng/L, respectively. All of the other sites in Lake Tahoe and Donner Lake had average concentrations of phototoxic PAH well below the “no observable effect levels” defined by Oris et al. (1998).

The paucity of PAH in areas available for atmospheric deposition but where no boating occurs indicates a non-atmospheric source for PAH in Tahoe Basin waters. Water samples were collected and analyzed at the mid-lake site by the University of Nevada, Reno (Glenn Miller, University of Nevada, Reno, oral commun., 2002) during the winter of 2001 and 2002 and during a period when heavy smoke filled the Tahoe Basin from nearby forest fires. These samples had no PAH compounds present at detectable concentrations indicating smoke from wood stoves or forest fires is not a major source of PAH to Lake Tahoe. Automobile exhaust is a constant source of PAH but the low concentrations of PAH at the mid-lake site and in the other lakes where no boating occurs indicates it is a minor source to surface waters of the Basin. Lico and Pennington (1999) demonstrated that spring runoff and base flow of six tributary streams to Lake Tahoe were not a major source of PAH to the lake.

3.1.3 PAH in Tahoe Sediments

PAH concentrations in sediments are given on a dry weight basis. Levels of PAH in sediments in Lake Tahoe were found to vary widely from <1ng/g to 400ng/g dry weight. The coarse, sandy sediments had relatively low PAH concentrations compared to samples that were qualitatively more fine-grained. Nearly all sediment sites were found to have a similarity in the relative abundance of individual PAH compounds. A relatively high abundance of higher molecular weight compounds, including, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(a)pyrene, benzofluoranthenes, benzo(g,h,i)perylene was observed at these the sites. As expected, these compounds will sorb more strongly to sediments than the lower molecular weight compounds due to a generally much lower water solubility and higher octanol-water partition coefficient (see table 1.1.1).

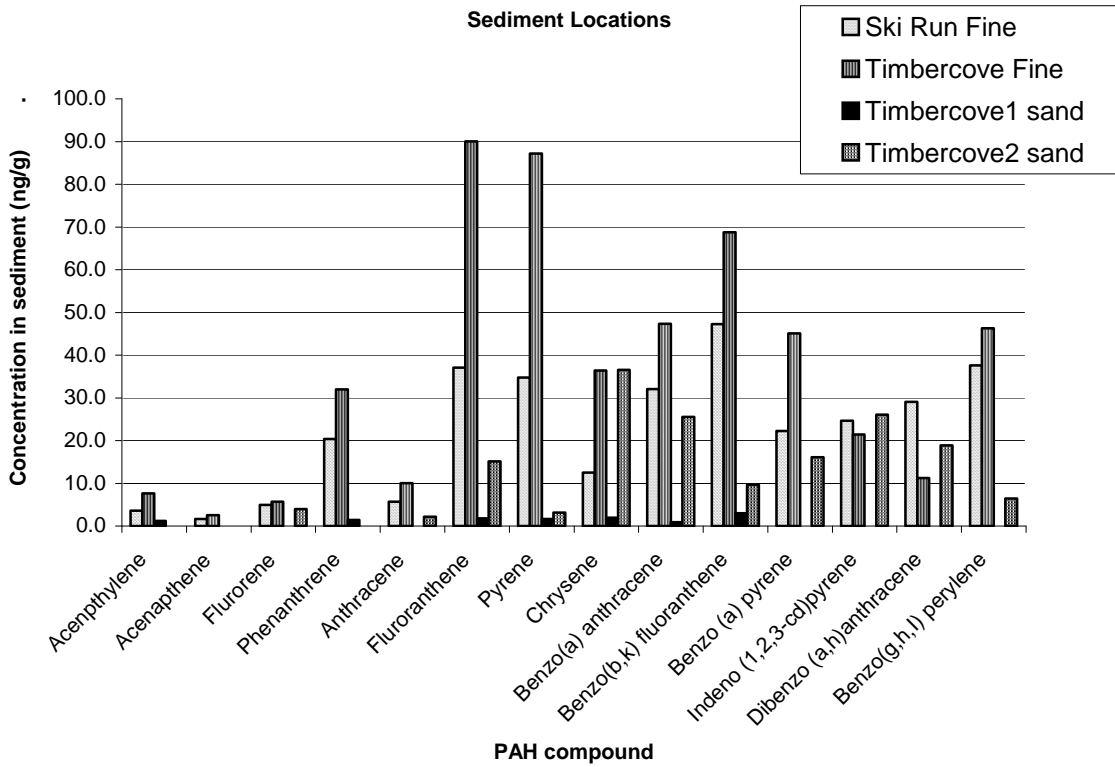


Figure 3.1.3. PAH compounds in sediments at Ski Run and Timber Cove (3 different locations)

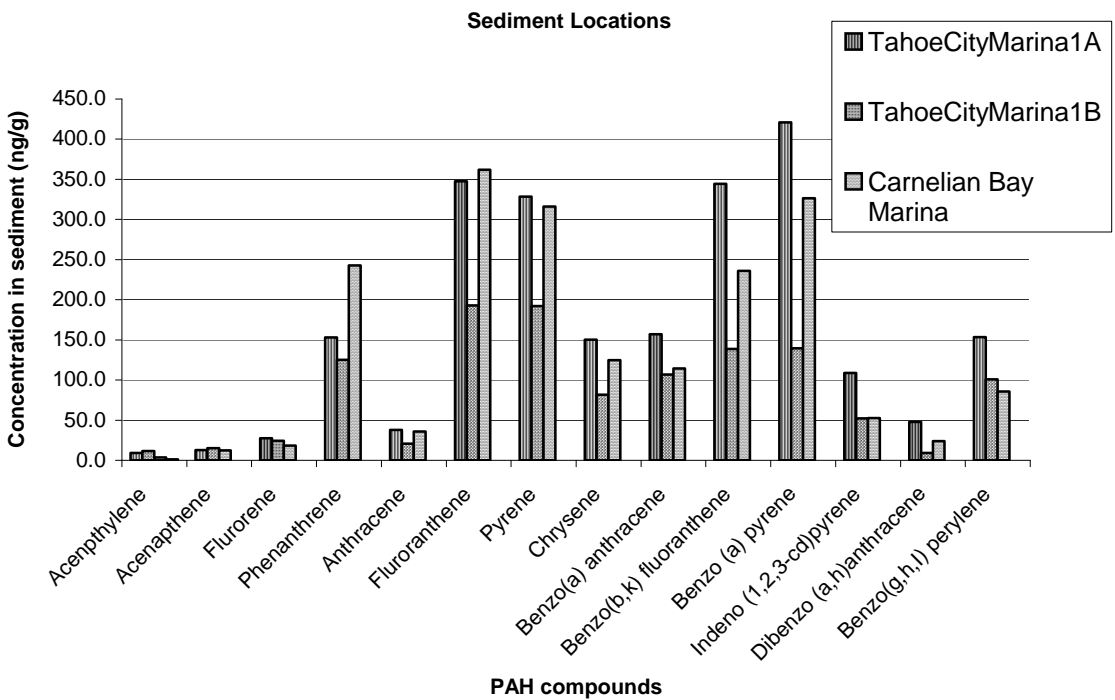


Figure 3.1.4. PAH compounds in sediments of Tahoe city Marina and Carnelian Marina

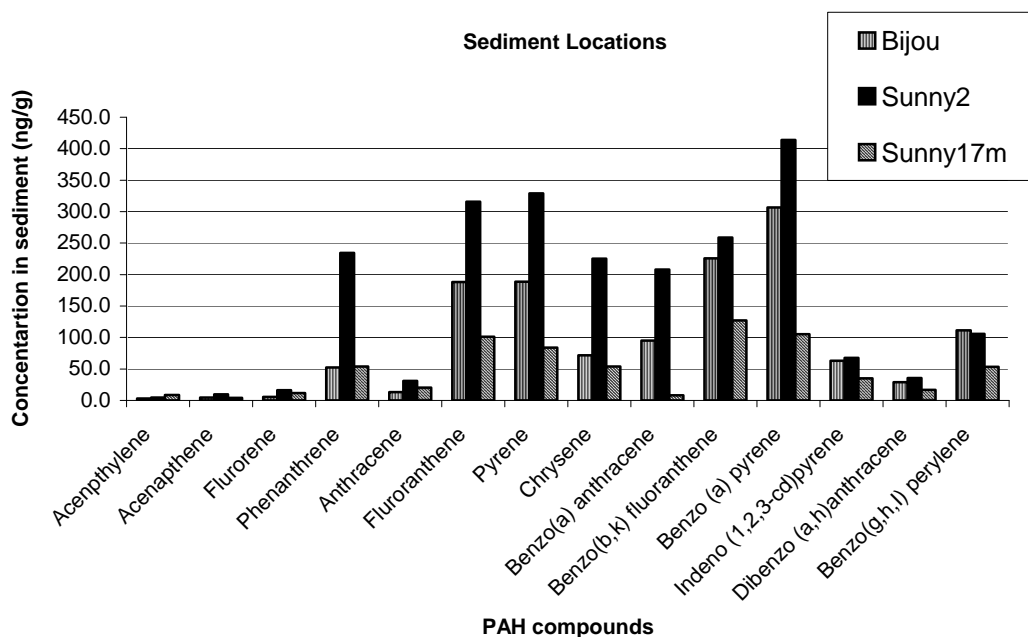


Figure 3.1.4. PAH compounds found in sediments of Bijou beach and Sunnyside Marina (2 different locations).

The sediments that are listed in Table 3.1.3 were taken at locations that were distant (0.3-1 km) from marinas, and showed generally lower PAH concentrations (note that the y axis in Table 3.1.3 is compressed by a factor of 4 compared to the other two tables). Sediments collected at marinas showed elevated concentrations, consistent with the source of PAH coming from marine engines. In Table 3.1.5 the Sunny2 sample was taken inside the enclosed marina, while Sunny17m was taken approximately 30 m from the marina from a depth of approximately 17 meters. These data, however, cannot exclude sediment PAH originating from surface runoff, however. The relative abundance of PAH compounds at each site were remarkably similar, again, suggesting a common source.

The predominant high molecular weight PAH compounds with three rings or more, indicates that high temperature combustion processes were the source of the PAH contamination (McCready et al., 2000). The same pattern of PAH contamination in sediments has been observed in Sydney Harbor, Australia (McCready et al., 2000) and sediments around the world (Simpson et al., 1996; Benlahcen et al., 1997). However, very high PAH levels at a few sites can also suggest the influence of a discrete point source of pollution.

3.2. Comparison of emissions from a variety of engines.

3.2.1 Comparison of 90 hp engines at the CARB facility

Each engine was operated twice and the data averaged for each engine. The results of the 10 minute idle, 10 minute operations and 30 minute operation sampling periods are presented in Tables 3.2.1, 3.2.2 and 3.2.3. For the operating periods, each engine was run at 60% full throttle. Although the concentrations observed in these tank test are much greater than expected in a natural water body, the comparisons do provide an indication of the relative amount of PAH released by each engine.

As mentioned previously, the test engines included a 90 HP Honda 4-Stroke rated for 2006 EPA Standards, a 90 HP Evinrude Direct Inject (Ficht) engine, and a 90 HP Johnson carbureted engine. The results presented below represent a combination of samples taken over two tests for each engine. In general, the compounds did not appear to volatilize substantially during the 10 versus the 30 minute operation, since the concentrations of these two test are approximately linear, with some exceptions.

Table 3.2.1. Concentrations of PAH compounds released from a **4-stroke 90 hp engine** into the tank water following a 10 minute idle period (90-4S-T10I), 10 minute run (90-42-T10R) and 30 minute run (90-4S-T30R).

Analyte	90-4S-T10I (ng/L)	90-4S-T10R (ng/L)	90-4S-T30R (ng/L)
Acenaphthylene	80	440	778
Acenaphthene	12	48	105
Fluorene	85	244	400
Phenanthrene	110	304	535
Anthracene	BLOQ	84	385
Fluoranthene	25	88	140
Pyrene	8	57	85
Chrysene	38	50	253
Benz(a)anthracene	20	43	168
Benzo[b/k]fluoranthene	BLOQ	3	5
Benzo(a)pyrene	BLOQ	BLOQ	BLOQ
Indeno(1,2,3-cd)pyrene	BLOQ	BLOQ	BLOQ
Dibenzo(a,h)anthracene	BLOQ	BLOQ	BLOQ
Benzo(g,h,i)perylene	BLOQ	BLOQ	BLOQ

Table 3.2.2 . Concentrations of PAH compounds released by the 90 hp direct fuel injected, 2-stroke engine into the tank water following a 10 minute idle period (90-DI-T10I), 10 minute run (90-DI-T10R) and 30 minute run (90-DI-T30R).

	90-DI-T10I (ng/L)	90-DI-T10R (ng/L)	90-DI-T30R (ng/L)
Acenaphthylene	190	8500	29000
Acenaphthene	37	620	2000
Fluorene	170	2200	6600
Phenanthrene	270	2900	8100
Anthracene	27	610	2100
Fluoranthene	62	1200	3100
Pyrene	18	1700	4200
Chrysene	17	70	320
Benz(a)anthracene	12	270	920
Benzo[b/k]fluoranthene	BLOQ	240	960
Benzo(a)pyrene	60	410	2200
Indeno(1,2,3-cd)pyrene	BLOQ	BLOQ	BLOQ
Dibenzo(a,h)anthracene	BLOQ	BLOQ	BLOQ
Benzo(g,h,i)perylene	BLOQ	BLOQ	BLOQ

As is evident from these data, the 4-stroke engine water contained about 8-20x less of each PAH than the direct injected engine or the carbureted engine comparing the results for the 10 minute run of each engine. The direct injected engine and the carbureted engines are similar in the total amount of PAH emitted (Figure 3.2.1). The larger amount of acenaphthylene emitted by the carbureted engine is either an analytical artifact for the 10-minute run, or due to the fact that it is a component of gasoline, and may be released in greater amounts than in the direct fuel injected engine because of the much greater amount of gasoline released unburned by the carbureted two-cycle engine, compared to the direct fuel injected 2-stroke engine.

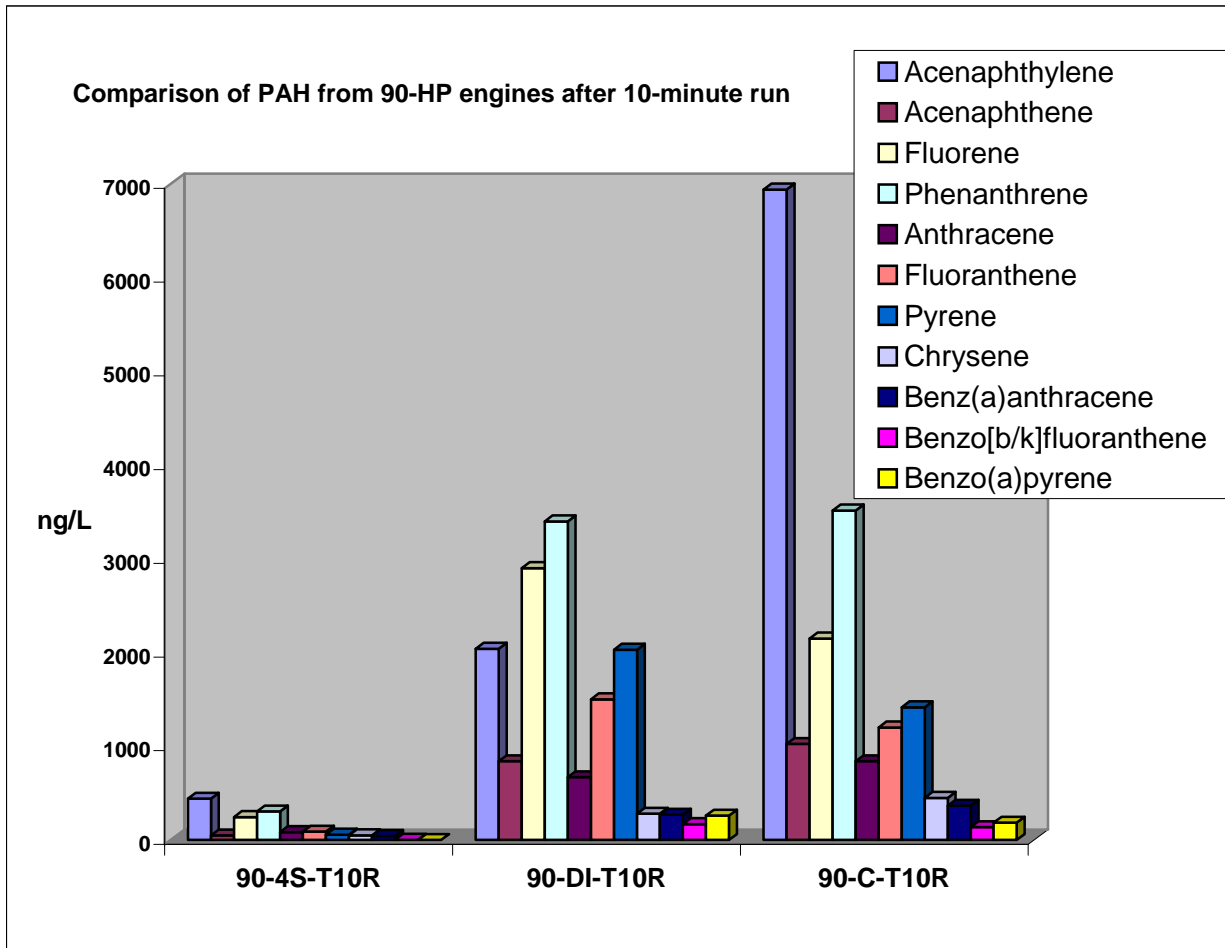
On examination of the ratios of compounds in the samples, it is apparent that the relative distribution of compounds collected in the water samples is approximately equivalent. Thus, to a first approximation, there does not appear to be an engine signature of the 2-stroke would allow a determination of the type of engine that generated the contaminants.

PAH in all of these samples was determined on the total amount present in the water and included both particulate and dissolved PAH. Particularly for those larger compounds that have very low water solubility, partitioning onto particulate may be important with respect to availability (and thus toxicity) to aquatic organisms.

Table 3.2.3. Concentrations of PAH compounds released by the **90 hp carbureted 2-stroke engine** into the tank water following a 10 minute idle period (90-C-T10I), 10 minute run (90-C-T10R) and 30 minute run (90-C-T30R).

	90-C-T10I (ng/L)	90-C-T10R (ng/L)	90-C-T30R (ng/L)
Acenaphthylene	70	6940	23250
Acenaphthene	16	1025	3700
Fluorene	75	2150	7650
Phenanthrene	155	3515	9450
Anthracene	50	840	2450
Fluoranthene	45	1200	3000
Pyrene	108	1415	3250
Chrysene	30	450	1750
Benz(a)Anthracene	25	365	1350
Benzo[b/k]fluoranthene	BLOQ	138	180
Benzo(a)pyrene	60	185	1500
Indeno(1,2,3-cd)pyrene	BLOQ	BLOQ	BLOQ
Dibenzo(a,h)anthracene	BLOQ	BLOQ	BLOQ
Benzo(g,h,i)perylene	BLOQ	BLOQ	BLOQ

Figure 3.2.1. Comparison of 10 minute run for each of the 90 hp engine, four cycle (4S); 2-cycle, direct fuel injected (DI); 2-cycle, carbureted (C).



3.2.2 Comparison of two 9.9 hp engines.

These tests were conducted at UNR using the protocol provided in section 2.2.1, and the results are presented in Table 3.2.4. As observed in the CARB tank samples, the concentrations of PAH were high in both samples. The concentrations of PAH from the 2-stroke engine are the average of 4 runs, which generated concentrations that were repeatable with a relative standard deviation of less than 25% for the individual PAH concentrations. For the 4-stroke engine, we were initially surprised to observe that the concentrations of PAH from the 4-stroke engine were similar (and sometimes larger) than in the 2-stroke engine. Following the first observation of this, and also because the 4-stroke engine did not appear to be running well (difficult to start), we had the engine tuned up at a local marine engine shop. Additionally, the engine had been recently overfilled with oil and operated briefly. Following the tune-up we ran it sequentially

over the next few weeks, and these are the data presented in Table 3.2.4. As can be observed, the concentrations of PAH decrease for each subsequent operation of the engine. For the final run on Nov. 4, 2002, the concentrations of PAH released into the water were about 3-fold lower than either the 2-stroke average or the 4-stroke sample taken on October 16, 2001. Because we have not since operated the engine, we did not establish whether further reductions in PAH would be observed with additional operation of the engine. However, the results indicate a general consistency with the 90-hp engine tests, in that the 4-stroke engine releases less PAH than the 2-stroke engine. The results also suggest that the source of PAH is probably incomplete combustion of motor oil.

Table 3.2.4. Comparison of PAH concentrations from the two 9.9 hp engines (ng/L).

	2-Stroke Average	4-Stroke 10/16/02	4-Stroke 10/23/02	4-Stroke 11/04/02
Acenaphthylene	32000	41000	21000	12000
Acenaphthene	4600	1500	920	660
Fluorene	7500	6800	4100	2600
Phenanthrene	7600	9800	5600	4000
Anthracene	2300	3200	1700	1300
Fluoranthene	2500	5200	2800	1800
Pyrene	4100	6700	3500	2200
Chrysene	1200	1200	600	400
Benzo(a)anthracene	550	960	490	400
Benzo[b/k]fluoranthene	500	750	340	170
Benzo(a)pyrene	890	1300	560	310
Indeno(1,2,3-cd)pyrene	990	1200	640	370
Dibenzo(a,h)anthracene	0	280	240	220
Benzo(g,h,i)perylene	1400	1500	750	360
Total PAH	66130	81390	43240	26790
Total Phototoxic PAH	45230	62090	32020	19130

3.2.3 Photolysis of marine-engine PAH in sunlight.

Photolysis of PAH usually involves oxidation of the parent molecule to a variety of compounds that are oxygenated. Some of these compounds completely lose any phototoxicity (e.g. carboxylic acids) while others retain a high degree of phototoxicity (anthroquinone). Thus, it is important to understand both the rate of transformation of these compounds, as well as the products that are produced.

After PAH concentrations were determined for all of the samples, the data were plotted to give a graph of concentration in ng/L versus time in hours. These graphs show the decline in concentration presumed to be due to the photolysis. Figure 3.2.2 shows the graphs for some representative compounds in water contaminated by a 2-stroke engine, and Figure 3.2.3 shows the graph for anthracene in distilled water.

Figure 3.2.2. Photochemical degradation of anthracene, fluoranthene, and pyrene from 9.9 hp 2-stroke engine contaminated water.

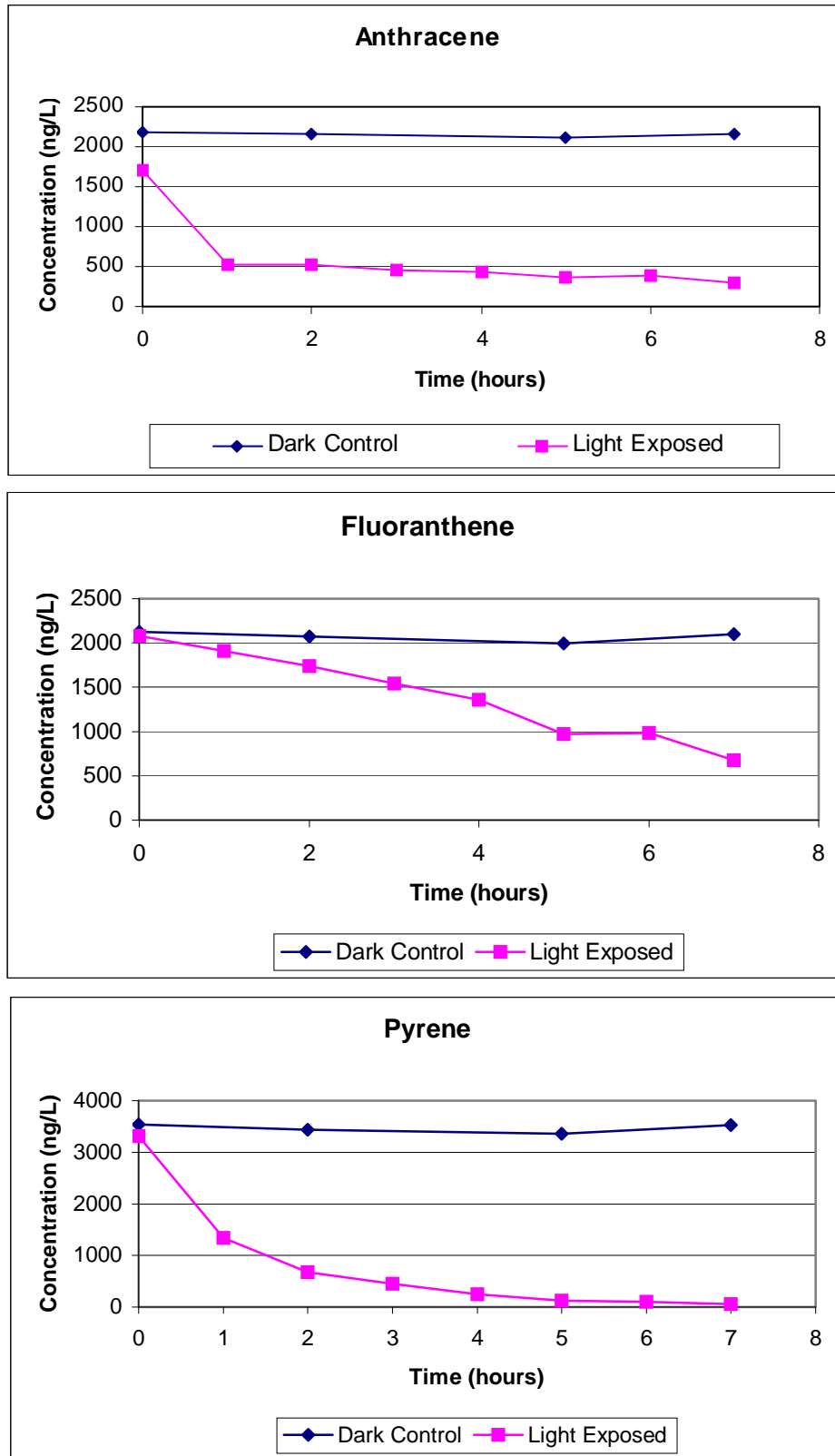
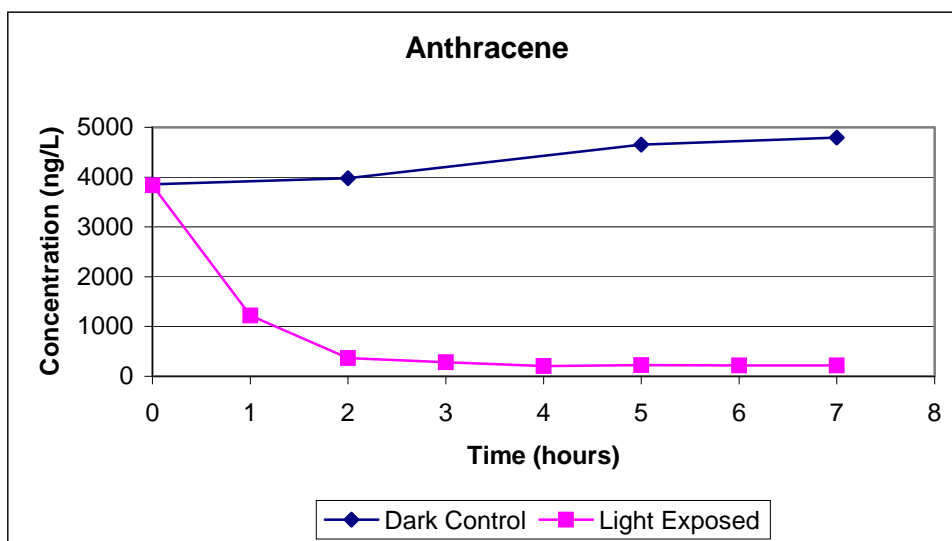


Figure 3.2.3. Photochemical degradation of anthracene in distilled water.



The concentration of the dark control remained fairly constant throughout the course of the experiment as was expected. The concentration of anthracene, pyrene, chrysene, benzo(a)pyrene, and benzo(g,h,i)perylene in both the engine generated water and the distilled water leveled off and remained fairly constant after 1 to 2 hours of sunlight exposure. This is presumed to be due to sorption of the compounds to the glass surface of the flask in both the engine water and the distilled water and sorption to the particulate matter generated by the engine in the sample taken from the water contaminated by the engines. The additional particulate matter in the water generated by the engine accounts for the much higher concentration of the compounds that were photolytically inactive in the engine samples.

The half-life of each compound was determined by graphing the natural log of the concentration versus time (hours). The negative slope of the resulting line is the rate constant (k) for the reaction of the compound with sunlight. The half-life ($t_{1/2}$) is given by the equation:

$$t_{1/2} = \ln 2/k$$

In cases where the concentration levels off due to sorption, only the points showing degradation were included in the graph of \ln concentration versus time to determine the rate constant. Figure 3.2.4 shows a \ln concentration vs time graph for pyrene in water contaminated by a 2-stroke engine.

Figure 3.2.4. First order loss of pyrene in water contaminated by a 2-cycle engine.

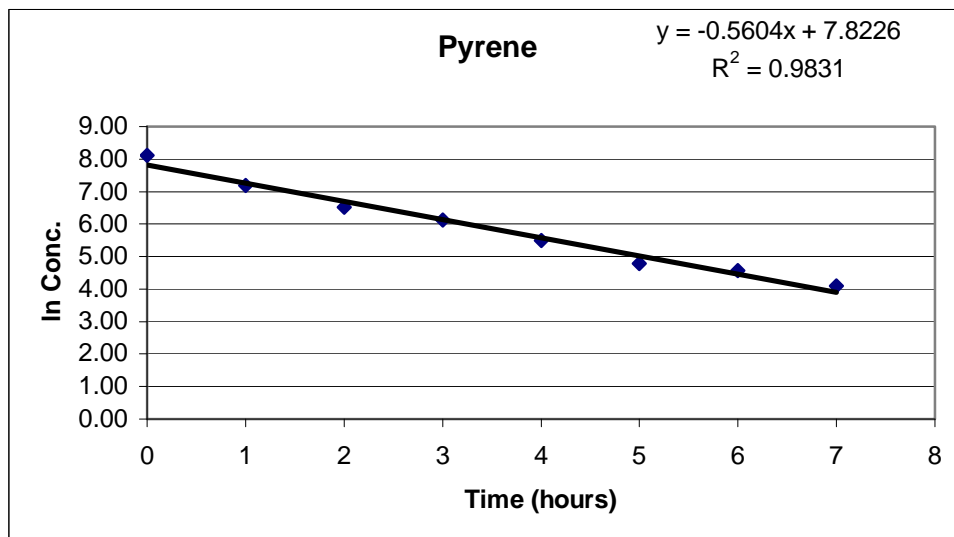


Table 3.2.5 includes the half-lives of the compounds detected in water contaminated by a 2-stroke engine and a 4-stroke engine, as well as the half-life of the compounds in a mix of PAHs in distilled water and individual compounds in distilled water. The table also includes the rate constants determined for each compound and the R^2 value of the line used to determine the rate constant. The half-life in water for most of the compounds is less than 24 hours of sunlight exposure.

Comparing the half-life of the compounds in engine contaminated water versus the compounds in distilled water shows that the half-lives are very similar for most compounds, with the exception of fluoranthene. For most of the compounds the photolysis rate was slightly slower in the water generated by the engines than in the distilled water. This could be a result of the cloudiness of the engine water that may have resulted in a small reduction in the amount of sunlight penetrating the water. Conversely fluoranthene degraded more rapidly in the engine water than in the distilled water. This may indicate that there is another compound released from the engines that increases the rate of reaction of fluoranthene with sunlight using indirect photolysis. Sources of uncertainty include the fact that some of the compounds had very short half-lives so there were not many points included in the determination of the rate constant and the variability of the GCMS, as well as the fact that all of the 500 mL volumetric flasks were not identical. Since these experiments were done outside in natural sunlight there is also some variability in the amount of sunlight from day to day since the experiments were run from August until October.

Under any circumstances, however, all of the phototoxic PAH compounds degraded relatively rapidly in sunlight, with most half-lives of less than one day of summer sunlight. With sunlight intensity even greater at Lake Tahoe than Reno, the photolysis rates of these compounds would be even greater.

Table 3.2.5. Rate constants (k), R^2 value, and half-lives of PAHs exposed to sunlight in water.

Acenaphthylene			
Run	k (h^{-1})	R^2	$T_{1/2}$ (h)
2-Stroke Engine #1	0.486	0.99	1.43
2-Stroke Engine #2	0.560	0.99	1.24
2-Stroke Engine #3	0.523	1.00	1.33
4-Stroke Engine #1	0.369	0.99	1.88
4-Stroke Engine #2	0.102	0.94	6.82
PAH Mix #1	0.435	0.93	1.59
PAH Mix #2	0.612	0.96	1.13
Acenaphthene			
Run	k (h^{-1})	R^2	$T_{1/2}$ (h)
2-Stroke Engine #1	0.157	0.95	4.42
2-Stroke Engine #2	0.254	0.96	2.73
2-Stroke Engine #3	0.290	0.99	2.39
4-Stroke Engine #1	0.173	0.99	4.02
4-Stroke Engine #2	0.067	0.94	10.39
PAH Mix #1	0.371	0.99	1.87
PAH Mix #2	0.371	0.99	1.87
Anthracene			
Run	k (h^{-1})	R^2	$T_{1/2}$ (h)
2-Stroke Engine #1	1.131	1.00	0.61
2-Stroke Engine #2	1.184	1.00	0.59
2-Stroke Engine #3	1.040	0.90	0.67
4-Stroke Engine #1	0.936	0.96	0.74
4-Stroke Engine #2	1.003	1.00	0.69
PAH Mix #1	3.311	1.00	0.21
PAH Mix #2	5.387	1.00	0.13
Anthracene #1	1.178	1.00	0.59
Anthracene #2	0.928	0.99	0.75
Fluoranthene			
Run	k (h^{-1})	R^2	$T_{1/2}$ (h)
2-Stroke Engine #1	0.094	0.92	7.37
2-Stroke Engine #2	0.148	0.87	4.68
2-Stroke Engine #3	0.103	0.90	6.76
4-Stroke Engine #1	0.098	0.99	7.09
4-Stroke Engine #2	0.078	0.98	8.91
PAH Mix #1	0.031	0.90	22.65
PAH Mix #2	0.041	0.95	17.03
Fluoranthene #1	0.026	0.89	27.08
Fluoranthene #2	0.032	0.70	21.94
Pyrene			

Run	k (h ⁻¹)	R ²	T _{1/2} (h)
2-Stroke Engine #1	0.374	0.97	1.85
2-Stroke Engine #2	0.526	0.99	1.32
2-Stroke Engine #3	0.616	1.00	1.13
4-Stroke Engine #1	0.959	0.98	0.72
4-Stroke Engine #2	0.595	0.99	1.16
PAH Mix #1	1.176	0.99	0.59
PAH Mix #2	0.740	0.99	0.94
Pyrene #1	0.596	0.96	1.16

Chrysene

Run	k (h ⁻¹)	R ²	T _{1/2} (h)
2-Stroke Engine #1	0.427	0.97	1.63
2-Stroke Engine #2	0.325	0.94	2.13
2-Stroke Engine #3	0.273	0.99	2.54
4-Stroke Engine #1	0.303	0.99	2.29
4-Stroke Engine #2	0.338	0.99	2.05
PAH Mix #1	1.014	1.00	0.68
PAH Mix #2	0.750	0.93	0.92

Benzo(a)anthracene

Run	K (h ⁻¹)	R ²	T _{1/2} (h)
2-Stroke Engine #1	0.036	0.79	19.36
2-Stroke Engine #2	0.042	0.91	16.54
2-Stroke Engine #3	0.036	0.99	19.31
4-Stroke Engine #1	0.140	0.95	4.96
4-Stroke Engine #2	0.044	0.85	15.61
PAH Mix #1	0.047	0.88	14.91
PAH Mix #2	0.034	0.57	20.21

Benzo[b/k]fluoranthene

Run	k (h ⁻¹)	R ²	T _{1/2} (h)
2-Stroke Engine #1	0.067	0.70	10.33
2-Stroke Engine #2	0.086	1.00	8.07
2-Stroke Engine #3	0.020	0.98	34.48
4-Stroke Engine #1	0.098	1.00	7.07
4-Stroke Engine #2	0.063	0.98	10.95
PAH Mix #1	0.103	0.97	6.72
PAH Mix #2	0.091	0.91	7.60

Benzo(a)pyrene

Run	k (h ⁻¹)	R ²	T _{1/2} (h)
2-Stroke Engine #1	0.987	1.00	0.70
2-Stroke Engine #2	0.687	1.00	1.01
2-Stroke Engine #3	0.386	0.88	1.79
4-Stroke Engine #1	0.431	0.95	1.61
4-Stroke Engine #2	0.517	0.99	1.34
PAH Mix #1	0.811	0.88	0.85
PAH Mix #2	0.756	0.86	0.92

Benzo(g,h,i)perylene			
Run	k (h^{-1})	R^2	$T_{1/2}$ (h)
2-Stroke Engine #1	0.278	0.93	2.50
2-Stroke Engine #2	0.261	0.99	2.65
2-Stroke Engine #3	0.265	1.00	2.61
4-Stroke Engine #2	0.140	0.99	4.96
PAH Mix #1	0.267	0.82	2.60
PAH Mix #2	0.319	0.94	2.17

3.3. Phototoxicity assessment

3.3.1 Phototoxicity studies at Lake Tahoe and Donner Lake

Results from the 2001 Experiments: The first set of experiments were conducted from 5 July - 11 July, 2001. In these experiments, fathead minnows and lahontan redbreast minnows, and daphnia were tested. Due to problems with collecting and transferring daphnia, significant laboratory mortality occurred and the daphnia tests failed. There were no significant differences among treatments for fathead minnow mortality during the first experiments (Fig. 3.3.1). In addition, there were no significant differences among treatments for redbreast mortality (Fig. 3.3.2.). Growth was significantly greater in Tahoe City water under the UV treatment compared to the no-UV treatment (Fig. 3.3.3.). There were no significant differences in growth for the other treatments or for redbreast (Fig. 3.3. growth data for redbreast not shown).

Figure 3.3.1. Fathead minnow mortality (2001)

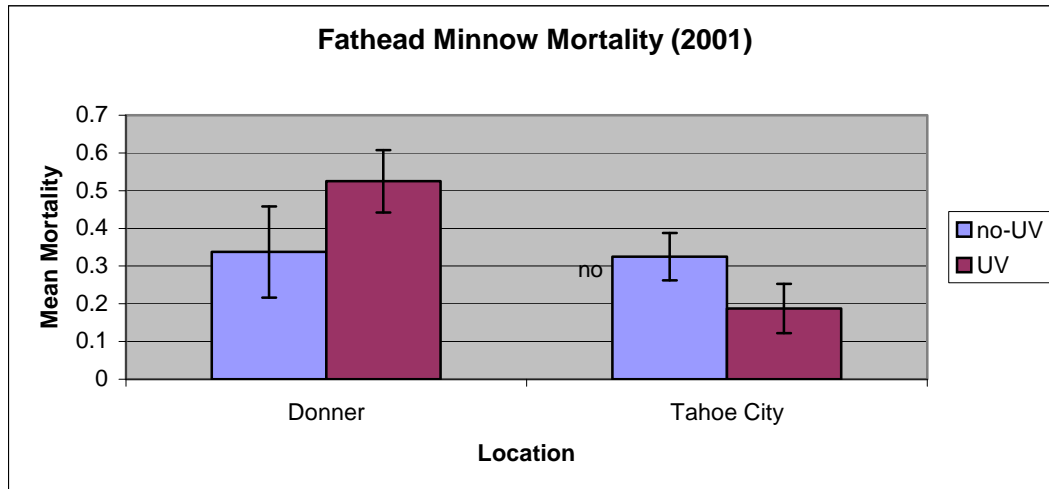


Figure 3.3.2. Redside mortality (2001)

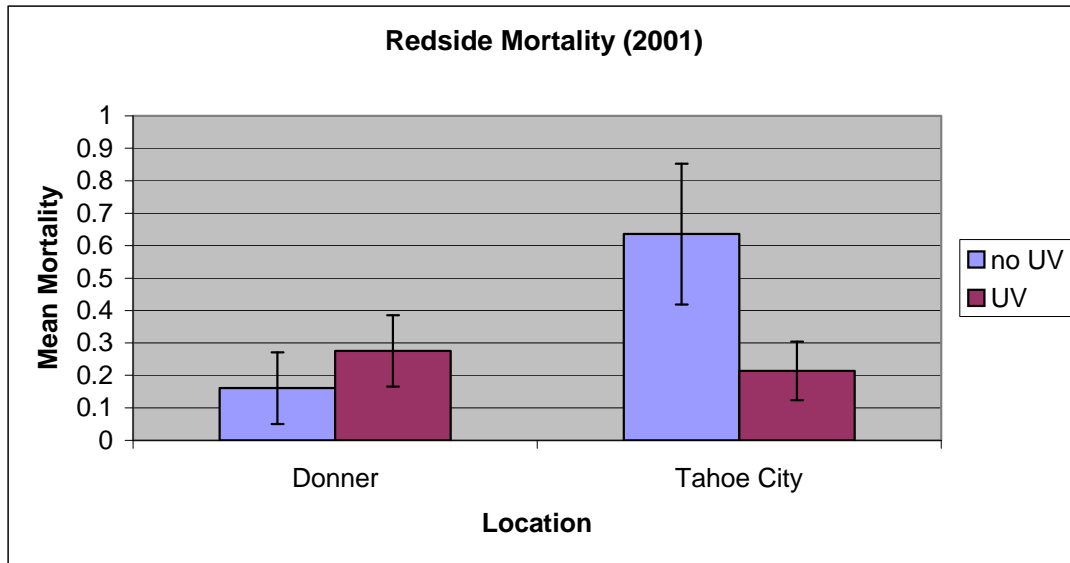
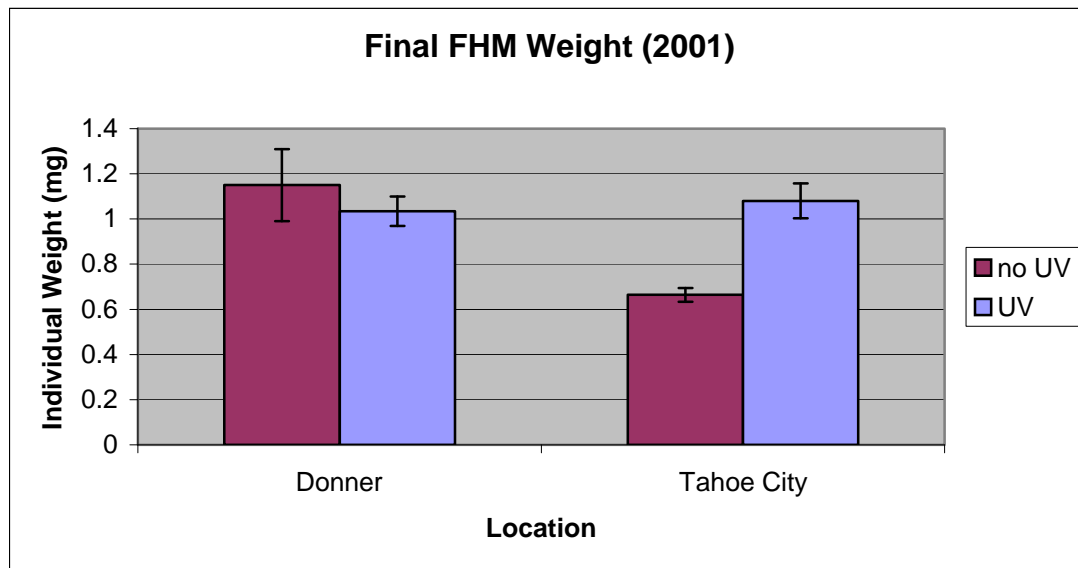


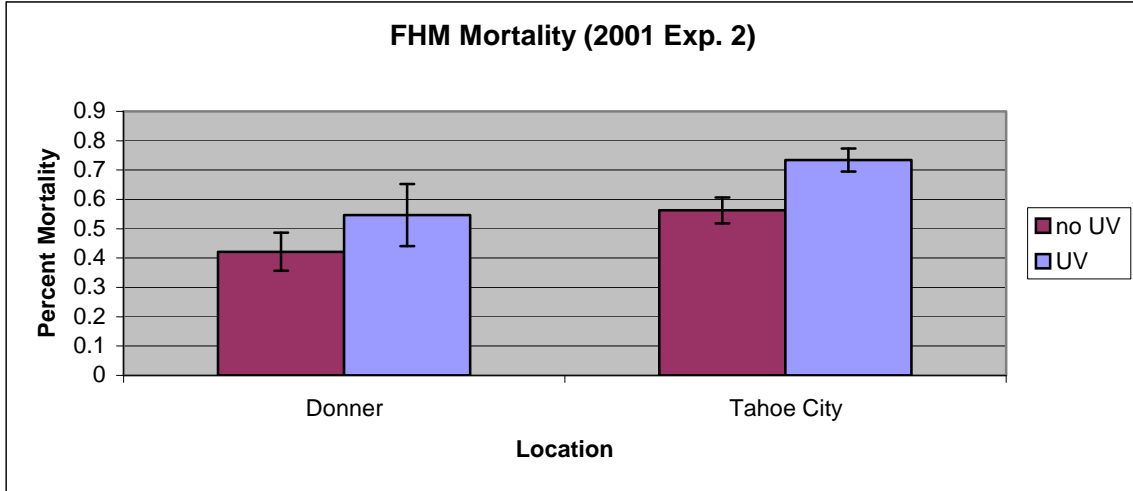
Figure 3.3.3. Final fathead minnow weight (2001)



The second set of experiments was conducted from 20 July - 27 July, 2001. In these experiments, fathead minnow, speckled dace, and daphnia were tested. For fathead minnows, there was enhanced mortality in all treatments, with the UV treatment from Tahoe City significantly greater than the no-UV treatment (Fig. 3.3.4.). These deaths

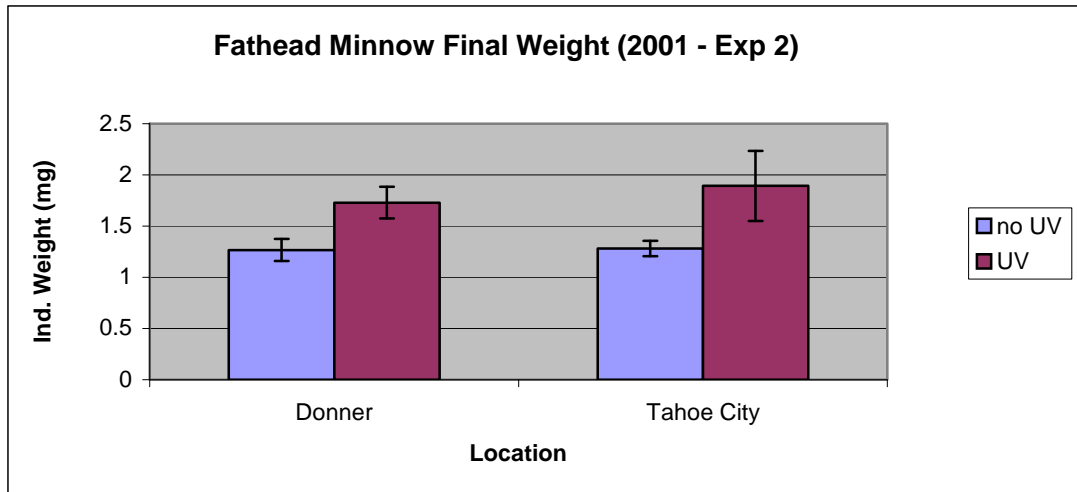
were attributed to handling stress, however, and this test was declared unacceptable for further interpretation.

Figure 3.3.4. Fathead minnow mortality (2001 Experiment 2)



Fish growth, based on the mass of fish measured at the end of the experiment was enhanced in both lake waters in the UV treatments (Fig. 3.3.5.). We have observed this phenomenon in previous experiments, where because of inhibition of fungal growth due to UV, fish perform slightly better in UV conditions. This result is indicative of "control" or non-toxic conditions.

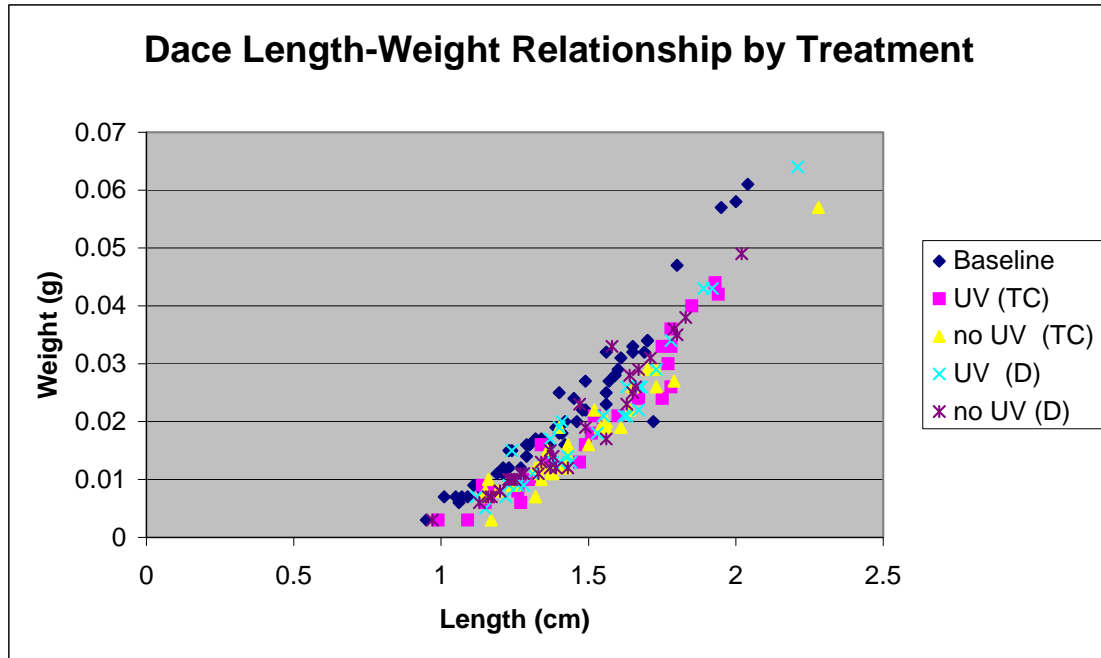
Figure 3.3.5. Fathead minnow final weight (2001 Experiment 2)



Since dace were collected from natural populations and consisted of a range of initial sizes, we measured lengths and weights of fish from a sub-sample of the collected population prior to experimentation ("baseline" in Fig. 3.3.6.). After exposure to the different lake waters and UV treatments, lengths and weights were measured in fish from

the different treatments (Fig. 3.3.6.). There were no significant differences between any of the treatments or between any of the treatments and the baseline condition.

Figure 3.3.6. Dace length-weight relationship by treatment for both Tahoe City (TC) and Donner Lake (D) samples.



For the daphnia tests, only enough daphnia were available to conduct tests using Donner Lake water. In these tests, there was 100% mortality in both the UV and no-UV treatments within the seven-day test period. Results of this test suggested that water collected from the boat launch ramp dock at Donner Lake was possibly toxic to daphnia.

Results from 2002 Experiments:

In 2002, experiments were conducted with *Ceriodaphnia dubia* and rainbow trout. Three sets of experiments were performed. Experimental period 1 was 04 July - 10 July, 2002. Experimental period 2 was 17 July - 23 July, 2002. Experimental period 3 was 29 July - 04 August, 2002. *Ceriodaphnia dubia* were tested in all three experiments. Because of timing of availability, trout were tested only during experiment 2.

During the first two experiments we rediscovered that *Ceriodaphnia dubia* are sensitive to bright light and need to be acclimatized to the UV conditions at Tahoe. During the first two experiments, mortality in all treatments was unacceptably high, and thus were not useable. As a result, *Ceriodaphnia dubia* were acclimatized to outdoor conditions by placing the cultures in full sunlight over a period of two weeks prior to experimentation. Therefore, we only report the results of experiment three with *Ceriodaphnia dubia*. In the three water types, the UV treatment organisms had significantly lower reproduction compared to no-UV treatments (Fig. 3.3.7.). Comparing across water types, there was

decreased reproduction in Donner Lake water compared to Tahoe City or Control for the no-UV treatment, indicating direct toxicity of Donner Lake water. In the UV treatments, both Tahoe City and Donner Lake water resulted in significantly lower reproduction, indicating a phototoxic effect. In terms of mortality, Donner Lake water resulted in elevated mortality in the no-UV treatment, again indicating direct toxicity, and both Tahoe City and Donner Lake waters resulted in elevated mortality in UV treatments as compared to controls, again indicating photoinduced toxicity (Fig. 3.3.8.).

For the trout experiments, no significant effects were observed in any of the treatments in either mortality or growth. However, we did note that the larval fish were agitated by the bright light and by day six of the experiment, fish were actually jumping out of their test chambers to escape the light, and the test was terminated on day 6. Regardless of the irritation due to light, no toxic effects attributable to water type were observed to fish in this experiment.

Results of experiments from 2001 and 2002 with a variety of species indicates that photoinduced toxicity, most likely due to PAH present in the water from motorized watercraft activity, is present for zooplankton in both Tahoe City and Donner Lake water. In addition, Donner Lake appears to have sufficient other contamination to cause toxicity in the absence of UV radiation. There was no indication in any of our experiments that larval or juvenile fish were affected by either source of water or by UV or no-UV treatment. Compared to results obtained in 1997 for Tahoe City, this is an improvement in conditions. In the earlier studies, we observed toxicity both to zooplankton (mortality and reproduction) and to fish (growth). There is no earlier reference for toxicity of Donner Lake water.

Figure 3.3.7. *Ceriodaphnia dubia* reproduction 2002

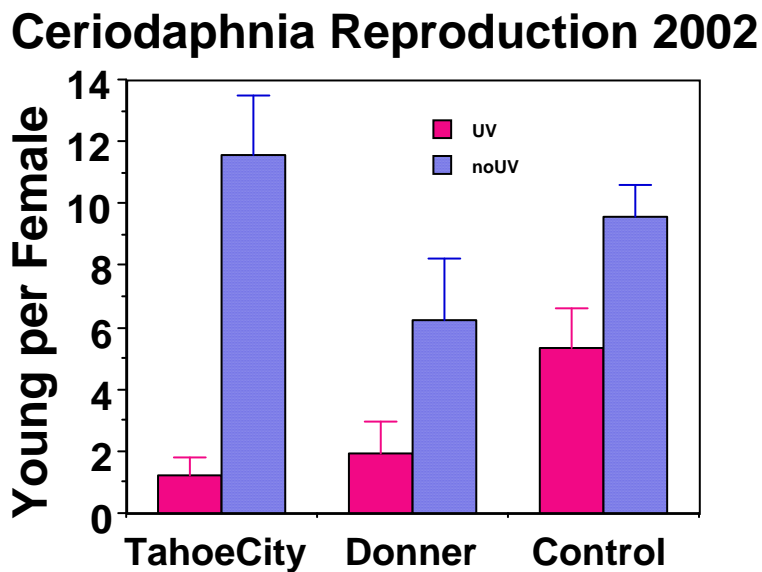
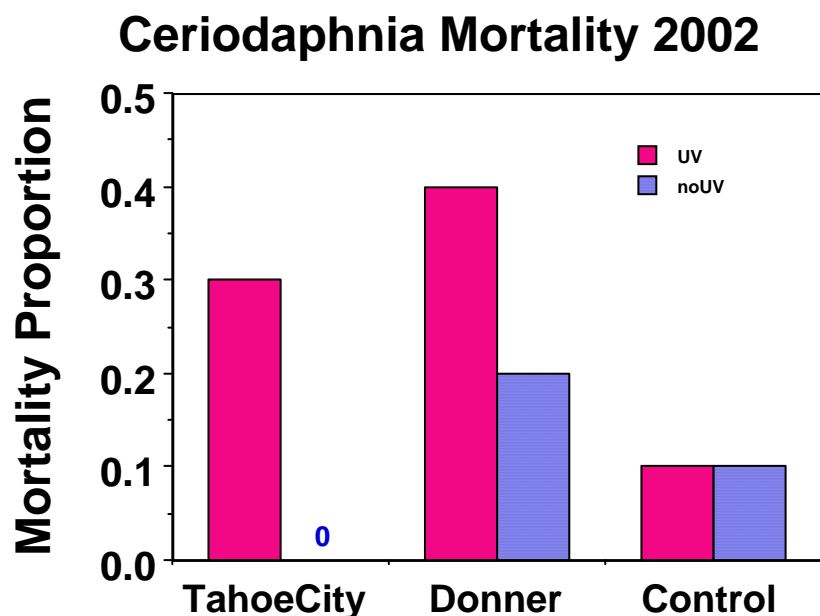


Figure 3.3.8. *Ceriodaphnia dubia* mortality 2002



3.3.2. Phototoxicity studies with water contaminated with marine engine exhaust

The concentrations of PAH generated by operation of the 9.9 hp engines are presented in Table 3.3.1. As discussed previously, the 2-cycle engine tests gave concentrations of PAH that were reproducible with a relative standard deviation of less than 25%. An average of these concentrations is presented for the 2-cycle data. However, for the 4-cycle engine tests, the concentrations decreased for subsequent runs of this engine, as excess oil was slowly eliminated from the engine as a result of overfilling this engine with lubricating oil. The PAH concentrations in these samples were sufficiently different (an decreasing with each operation of the engine) that it was necessary to indicate those concentrations (Table 3.3.1), and which exhaust water was used for each test.

Results of the experiments with 2-cycle engine water (9.9 hp Mercury) suggest that the 10% treatment with UV screening yielded sublethal toxic effects to *C. dubia*, (Table 3.3.2; Figure 3.3.9) and the 10% treatment with UV exposure is acutely toxic (100% mortality) (Table 3.3.3; Figure 3.3.10). The effects included swimming erratically in circles, swimming more slowly than controls or not at all until moved into new treatment water and absence of eggs, but no mortality. The effects of the 1.0%, 0.2% and 0.1% treatments included erratic swimming and fewer neonates produced, although the differences between the daphnids in the control and 0.1% treatment waters were not significant. The UV effect observed was for the 10% concentration (100% mortality vs 83% mortality for the no-UV exposure) and the 1% concentration where 54% of the *C. dubia* showed effects while the no-UV group showed no effects.

Results of the experiments with 4-cycle engine water (9.9 hp Mercury) suggest that the 10% treatment with UV exposure (conducted on 10/23/02) was the most toxic of the 4-

cycle waters (Table 3.3.4 and 3.3.5; Figure 3.3.11 and 3.3.12). The mortality rate of 41.6% was the highest of these treatment waters but much lower than that of the 2-cycle water at 100% mortality. The effects seen in the 1.0% treatment water included erratic swimming but no mortalities. The 0.2% and 0.1% treatment waters resulted in very low to no differences between treatments and controls. As can be observed from Table 3.3.1, the PAH concentration was highest for the 4-cycle engine used in the 10/23/02 experiment, and lower, by a factor of 2-3 for the subsequent toxicity test.

In addition to the variable 4-cycle engine PAH concentrations, a direct comparison of the relative toxicity determined for the 2-cycle engine and the 4-cycle engine should consider two additional factors. The 2-cycle engine runs and bioassays were carried out during August and September when ambient temperatures and UV intensities were greater. The 4-cycle engine runs and bioassays were carried out during October and November when ambient temperatures and UV intensities were lower. Metabolic processes are certainly affected by temperature.

Table 3.3.1. PAH Concentrations from the two 9.9 hp Mercury engines (ng/L) for a single run. These concentrations represent undiluted samples. 10% and 1% concentrations, for example are 0.1 and 0.01 times the concentrations listed in this table.

Engine type	2-cycle	4-cycle	4-cycle	4-cycle
Date of engine operation	<i>Average</i>	10/16/02	10/23/02	11/4/02
Date of toxicity test		10/23/02	10/31/02	11/6/02
PAH				
Acenaphthylene	32000	41000	21000	12000
Acenaphthene	4600	1500	920	660
Fluorene	7500	6800	4100	2600
Phenanthrene	7600	9800	5600	4000
Anthracene	2300	3200	1700	1300
Fluoranthene	2500	5200	2800	1800
Pyrene	4100	6700	3500	2200
Chrysene	1200	1200	600	400
Benzo(a)anthracene	550	960	490	400
Benzo[b/k]fluoranthene	500	750	340	170
Benzo(a)pyrene	890	1300	560	310
Indeno(1,2,3-cd)pyrene	990	1200	640	370
Dibenzo(a,h)anthracene	0	280	240	220
Benzo(g,h,i)perylene	1400	1500	750	360
Total PAH	66130	81390	43240	26790
Total Phototoxic PAH	45230	62090	32020	19130

Table 3.3.2. Effect concentrations comparing UV and No-UV treatments for 2-cycle engine

2-cycle UV			2-cycle No-UV		
Date	Dose %	Effect %	Date	Dose %	Effect %
8/23/2002	0	0	8/23/2002	0	0
9/19/2002	0.1	33	9/19/2002	0.1	33
9/19/2002	0.2	16	9/19/2002	0.2	16
8/23/2002	1	54	8/23/2002	1	0
9/15/2002	10	100	9/15/2002	10	100

Figure 3.3.9. Dose-response curves of effect concentrations comparing UV and no-UV treatments with the 2-cycle engine.

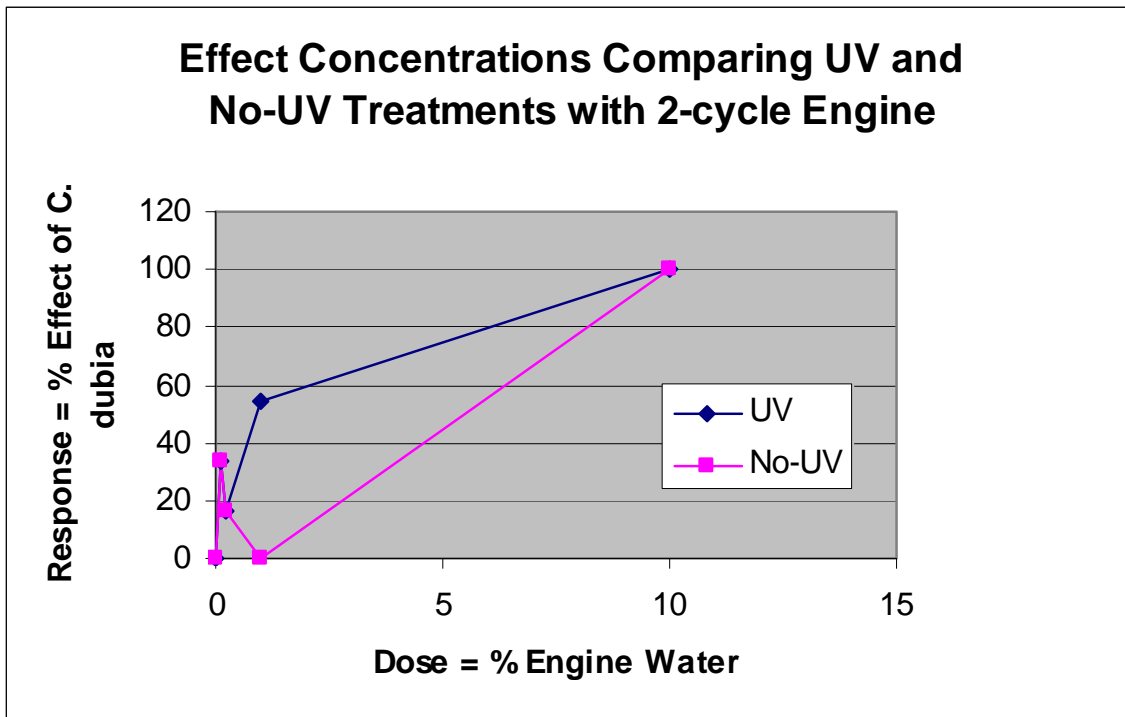


Table 3.3.3. Mortality concentrations comparing UV and No-UV treatments with 2-cycle engine

2-cycle UV			2-cycle No-UV		
Date	Dose %	Mortality %	Date	Dose %	Mortality %
8/23/2002	0	0	8/23/2002	0	0
9/19/2002	0.1	0	9/19/2002	0.1	0
9/19/2002	0.2	0	9/19/2002	0.2	0
8/23/2002	1	0	8/23/2002	1	0
9/15/2002	10	100	9/15/2002	10	83

Figure 3.3.10. Dose-response curves of mortality concentrations comparing UV and no-UV treatments with the 2-cycle engine.

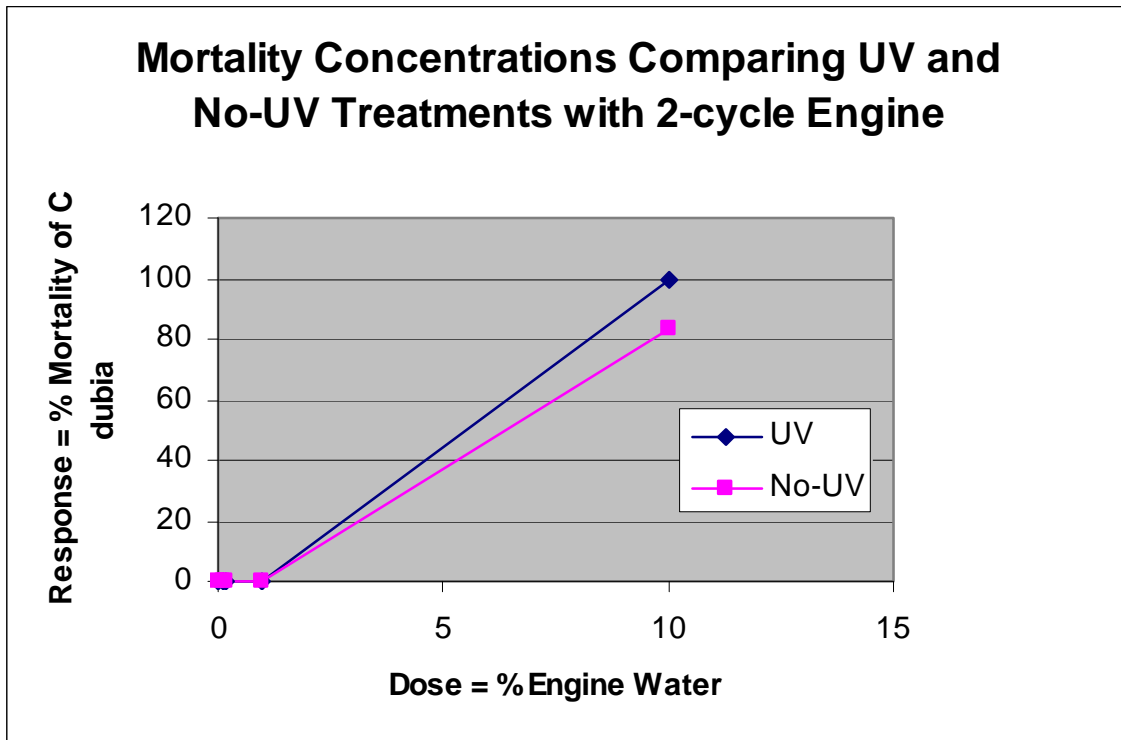


Table 3.3.4. Effect concentrations comparing UV and No-UV treatments with 4-cycle engine

4-cycle UV			4-cycle No-UV		
Collected	Dose	Effect	Collected	Dose	Effect
	%	%		%	%
10/23/02	0	0	10/23/02	0	0
11/6/02	0.1	8	11/6/02	0.1	8
11/6/02	0.2	0	11/6/02	0.2	0
10/31/02	1	33	10/31/02	1	0
10/23/02	10	100	10/23/02	10	0

Figure 3.3.11. Dose-response curves of effect concentrations comparing UV and no-UV treatments with the 4-cycle engine.

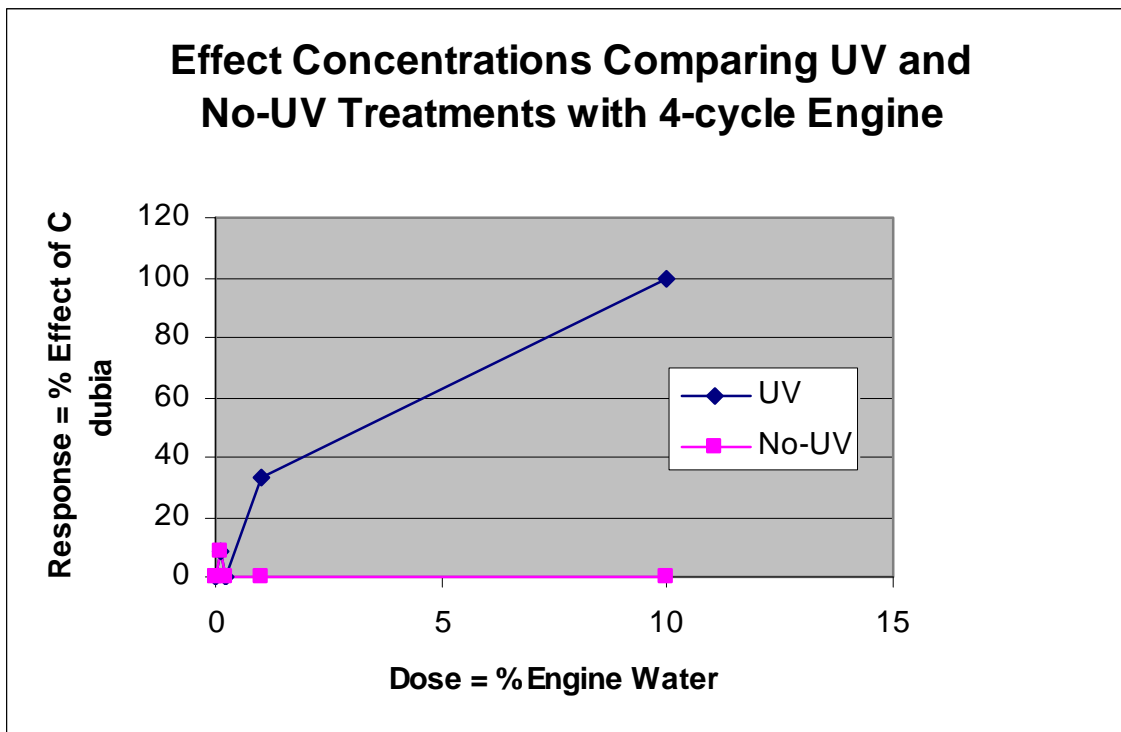
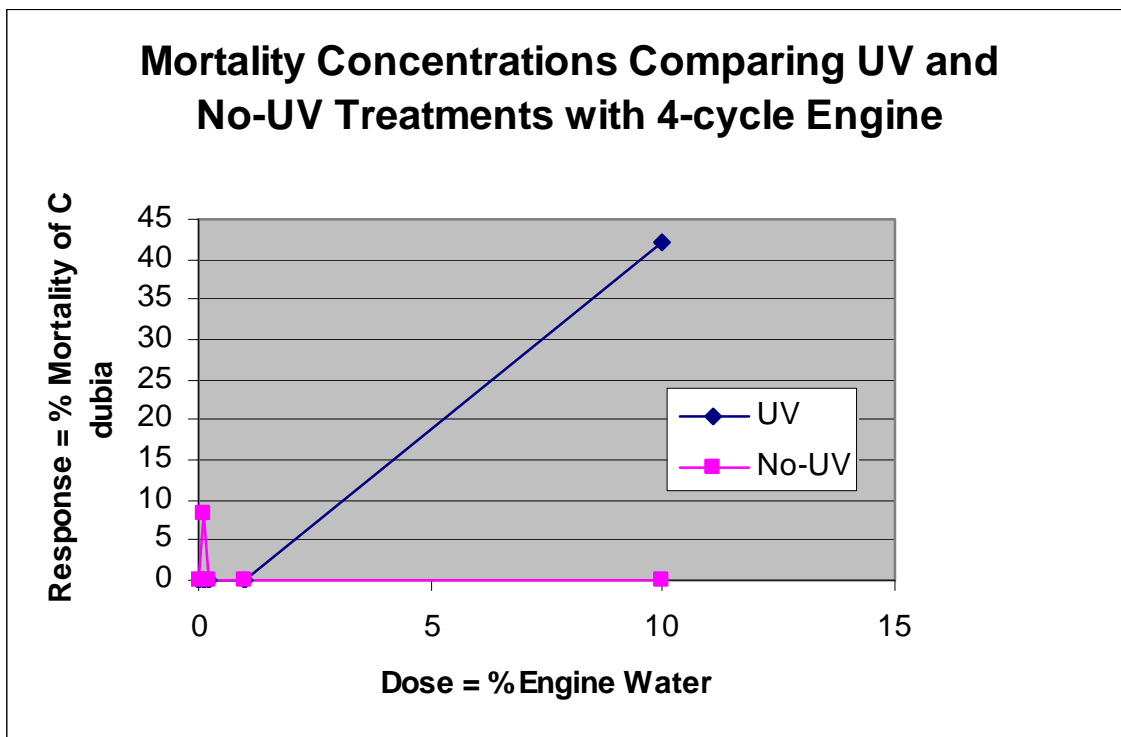


Table 3.3.5 Mortality concentrations comparing UV and No-UV treatments with 4-cycle engine

4-cycle UV			4-cycle No-UV		
Date	Dose %	Mortality %	Date	Dose %	Mortality %
10/23/02	0	0	10/23/02	0	0
11/6/02	0.1	0	11/6/02	0.1	8
11/6/02	0.2	0	11/6/02	0.2	0
10/31/02	1	0	10/31/02	1	0
10/23/02	10	42	10/23/02	10	10

Figure 3.3.12. Dose-response curves of mortality concentrations comparing UV and no-UV treatments with the 4-cycle engine.



4. Discussion and Conclusions

This study has investigated the occurrence, sources, fate and ecological risk of PAH derived from marine engines. The combined results of the several investigators allow a more complete understanding of PAH in Lake Tahoe and Donner Lake.

Distribution and Occurrence: Although a low number of detections of several PAH compounds was observed, fluoranthrene and pyrene are the compounds most commonly observed in the ambient samples. Figure 3.1.1 summarizes the occurrence and distribution of these two compounds in all of the samples examined at both lakes. Fluoranthrene and pyrene were found in the highest concentration. Additionally, these compounds were found predominantly in the same locations, Tahoe Keys Marina and the Donner Boat ramp. Slightly greater occurrence of PAH was also observed in other marinas, although they were less commonly observed than in these two more contaminated sites. This is probably a reflection of the boat traffic and the lower mixing volume that is available at these two sites, compared to the open water of Lake Tahoe. These data offer support for the notion that PAH comes primarily from marine engines. Additionally, the winter months and the sampling during the forest fire did not show elevated PAH and is also consistent with the PAH source being primarily marine engines.

If we compare these data to those found in the SPMD's (Table 3.1.2), the results are remarkably similar. Again, fluoranthrene and pyrene were found in the highest concentrations. Because the SPMD's accumulate PAH compounds over time, they also indicated the presence of some of the larger compounds that were sufficiently low in concentration to detect in the ambient water samples. These include benz(a)anthracene, benzo{b/k}fluoranthrene and benzo(a)pyrene. These compounds were observed as significant compounds in marine engine exhaust in all of engine emission tests.

Except for the Tahoe Keys Marina, there were very few PAH detections in late fall or winter, and the predominant PAH detections were in the summer. The Tahoe Keys Marina had elevated concentrations in most of the samples taken.

Even for the Tahoe Keys Marina, the concentrations were very low for these sampling periods, and do not appear to exceed the no effect concentration that was observed in the laboratory studies that were conducted at UNR.

Engine Emissions: The tests we had hoped to have completed at the Southwest Research Institute have not occurred, and the data that are available include 5 engines. (a 90 hp 4-cycle engine, a 90 hp direct fuel injected ((DFI) 2-cycle engine, a 90 hp carbureted 2-cycle engine, a 9.9 hp 4-cycle engine and a 9.9 hp carbureted 2-cycle engine). The data on the 90 hp engines are the most useful, since these engines are common in both lakes (except for the carbureted 2-cycle engine, which has been banned at Lake Tahoe). The results indicate that the two 2-cycle engines have approximately equivalent PAH release and the 4-cycle engine has 8-20 times lower emissions, depending on the specific PAH. The data on the two 9.9 hp engines are generally consistent with the 90 hp engines,

although a probable problem of overfilling the 4-cycle engine with oil appears to have substantially increased the amount of PAH that was released. Over 3 runs, however, the amount of PAH had decreased by a factor of 3, indicated that the residual oil was being removed.

Oil and air are introduced similarly into the direct-fuel-injected and carbureted 2-cycle engines, and we would not expect there to be any differences between PAH emission from these engine types, assuming that PAH comes primarily from the oil. However, these data, as well as the data from the smaller engines need to be compared with a larger number of engines to make conclusive recommendations. However, at present it appears that the newer two-cycle DFI engines do not offer any benefit of lowered PAH release, compared to the older carbureted 2-cycle engines.

All of the phototoxic PAH compounds undergo rapid photolysis, most within one day. Thus, for clear lakes like Tahoe and Donner, these compounds are all expected to turn over rapidly, and the phototoxic effects are probably transient (on the order of 1-3 days). The concentrations observed are likely to be a function of the total boat traffic, the mixing that occurs and the amount of open sunlight present.

Toxicity Several toxicity studies were conducted during both summers. The results from 7 day toxicity experiments in 2002 again indicated that both the Tahoe and Donner Lake samples exhibited phototoxicity to *Ceriodaphnia dubia* by altered reproduction rates as well as mortality. No effects on fish were observed. Because this toxicity was enhanced by sunlight, it is probably a phototoxic effect, and implies involvement with PAH, although it is not possible to conclusively assign a causative agent to these results.

The two-day acute phototoxicity tests conducted at UNR indicated that engine emission water was indeed toxic to *Ceriodaphnia dubia*. With an estimated total toxic PAH concentration in the 1% diluted water of 450 ng/L, acute toxicity effects (swimming behavior) was observed for the sunlight irradiated samples, although mortality was only observed at the 10% diluted water. We note, however, that the majority of the toxic PAH load in these samples was acenaphthylene, which is not considered one of the most toxic PAH compounds. Thus, it suggests caution in summing up the total weights of PAH to arrive at a comparative toxicity for a specific contaminated water, since the effects of each compound can be substantially different. The 0.1% dilutions (45-52 ng/L) did not show any significant effects, compared to the controls.

A review of the limnological conditions at Lake Tahoe and Donner Lake also suggests that, for most of the surface area, sunlight decreases the number of organisms that will exist in the top few meters. Ultraviolet light is harmful to most of these organisms and at least a portion of these organisms come to the surface only at night. However, it is difficult to quantitatively establish if there is an effect from the PAH if behavioral aspects (e.g. vertical diurnal migration) are considered. However, areas that have a certain amount of shading, that allow organisms to move in and out of sunlight in the same region as PAH may offer the highest risk for phototoxicity due to the presence of both PAH, as well as sunlight.

5.0 Recommendations:

At present, phototoxic PAH compounds were not observed in any of the open water areas of Lake Tahoe at concentrations that would be expected to cause toxicity to aquatic organisms. Higher concentrations were found in areas associated with high boat traffic, that also were sheltered and had limited potential for dilution with the open water. Additionally, the water in the most impacted marina was some of the most turbid of any area at Lake Tahoe, and tended to screen sunlight that would cause loss of these compounds through photodegradation. While this area is potentially problematic, it is also unusual at Lake Tahoe, and management of boating traffic to manage PAH loadings in this marina, if needed, could best be accomplished separately from the rest of the Lake.

Phototoxic responses to fish were not observed in either Lake Tahoe or Donner Lake, although phototoxic effects were observed for the sensitive macroinvertebrate species, *Ceriodaphnia dubia* in the 2002 tests. Additionally, avoidance behavior of most aquatic species to ultraviolet light in Lake Tahoe will result in a lowered risk of effects. Because the concentrations of PAH found in the water of Lake Tahoe in 2001 and 2002 were generally very low, we do not feel there are compelling reasons, at present, to recommend changes in watercraft management at Lake Tahoe.

However, phototoxic PAH compounds were observed at several locations, particularly near marinas and high use areas, and may, at some point in the future, need to be managed as watercraft use increases. PAH loadings in sediments are also potentially a concern, again in marina areas. In order to minimize the long-term potential for PAH problems in the future, the following management issues may be considered for future implementation. These measures are proposed to address technical concerns based on the current state of the science and information gaps of understanding PAH risk.

Potential Management Actions

- Manage PAH loading into Lake Tahoe by regulating the intensity of use of 2-stroke engines, primarily by controlling the number of personal watercraft used at specific locations.
- Disperse the intensity of watercraft use by establishing a ceiling for the number of watercraft that can be operated from any single location.
- Encourage development of engine types that exhaust directly in the air, rather than the water.
- Encourage the use of non-motorized watercraft through outreach and public education. Introduce financial incentives – expand lake access for non-motorized recreational activities such as swimming, hiking and launching kayaks, canoes, inflatables, sailboards and other sail craft.

Additional information is needed in the following areas:

- Develop more complete standards for protection of sensitive species of aquatic organisms with respect to phototoxic PAH compounds.
- Improve the ability to predict PAH emissions by examination of additional marine engines of various types. Examine the use of alternative lubricating oils for both 2-stroke and 4-stroke engines. Since lubricating oil appears to be the primary source of PAH, additional information on PAH emissions with differing oils could provide suggestions for an inexpensive and efficient method for PAH reduction.
- Study the effect of tuning of engines on PAH emissions. If this factor is important, regulations may be considered that requires each engine to be tuned to operate efficiently at Lake Tahoe.
- Continue monitoring PAH in Lake Tahoe, with a focus on high watercraft use areas to determine PAH trends over time.
- Conduct additional studies on PAH loading in sediment to determine the source and ecological impacts.

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