COMMONWEALTH OF MASSACHUSETTS
EXECUTIVE OFFICE OF ENERGY & ENVIRONMENTAL AFFAIRS
DEPARTMENT OF ENVIRONMENTAL PROTECTION
OFFICE OF APPEALS AND DISPUTE RESOLUTION

IN THE MATTER OF
MASSACHUSETTS DEPARTMENT OF CONSERVATION AND RECREATION
Docket No. WET-2009-039
DEP FILE #233-0641
NATICK, MA

TESTIMONY OF EMILY MONOSSON, Ph.D.

I, Emily Monosson, do hereby swear and affirm the following:

1. I am an environmental toxicologist with seventeen years experience as a research toxicologist, consultant and teacher. I received a B.A. in biology from Union College in 1983 and an M.S. and Ph.D in Veterinary Science, with a biochemical toxicology concentration, from Cornell University in 1986 and 1988 respectively.

2. From 1996-1999, I served as a consultant to the United States National Oceanographic and Atmospheric Administration ("NOAA"), researching the toxicological effects of polychlorinated biphenyls on fishes of the Hudson River. From 2001-2004, I worked with the Gloucester Fishermen's Wives Association researching and evaluating the potential for adverse effects of marine contamination on near shore and off shore cod fish. From 1999 to2008, I have taught a course on Environmental Contamination at Mount Holyoke College in South Hadley, MA, and have worked on various community based projects through this course. Most recently I have consulted for Conservation Law Foundation Ventures and Cadmus Inc. on nanotechnology related projects.

3. I have been retained by the Petitioners in this matter to evaluate the potential for adverse effects on Lake Cochituate fish and invertebrate life that may follow diquat administration as described in the Notice of Intent, as approved with conditions by the Natick Conservation Commission and the Massachusetts Department of Environmental Protection ("DEP"). I have undertaken this work on a *pro bono publico* basis. My opinions, as set forth herein, are stated to a reasonable degree of scientific certainty.

4. The materials I have reviewed in preparing this testimony include:

- Notice of Intent Application Aquatic Management Program, Lake Cochituate, Middle Pond – DCR Beach & Boat Ramp, Natick, MA prepared for the Commonwealth of Massachusetts Department of Conservation and Recreation("DCR") by Aquatic Control Technology ("ACT"), February 2009.

- Superseding Order of Conditions; DEP File number 233-0641
- Natick Conservation Commission's Order of Conditions File #233-0641
- Lake Cochituate Long Term Vegetation Management Plan, prepared for the DCR by ACT, May 2004
- Notice of Intent Application Aquatic Management Program, Lake Cochituate, Natick, MA. Aquatic Control Technology, Sutton, MA, 2003
- Eutrophication and Aquatic Plant Management in Massachusetts: Final Generic Environmental Impact Report
- Scientific literature relevant to this testimony

5. My testimony includes

a) A description of fish species residing in Lake Cochituate
b) A summary of the proposed project as described in the DCR NOI
c) A review of the toxicity of diquat to fish
d) A summary of the toxicity of diquat to invertebrate species
e) My conclusion based on the above information
f) A glossary of toxicology terminology relevant to this testimony
g) References

Fish Species and Life History Habits of Lake Cochituate Fish

6. Lake Cochituate is a popular site for sport fishing in Massachusetts. According to the Massachusetts Department of Fish and Game (DFG) (DFG 2005), Lake Cochituate is one of the "most fertile and heavily fished waters in Massachusetts." Of the species listed as "species of interest" by the DFG (Table 1), the majority are native self-sustaining populations, meaning that they reproduce successfully in the Lake. Of the species that are non-native (introduced) to the Lake, those listed in Table 1 are also considered self-sustaining populations. In addition to listing the species of interest, I have included relevant life history characteristics such as the spawning season, spawning substrate and preferred food of larval fish. Many of these species are important sport fish.

7. As detailed in Table 1, the majority of Lake Cochituate fish spawn in the spring. Of the eleven species listed below, nine species spawn in the spring or early summer. Additionally, all of the species either spawn near vegetation, or the eggs are laid in or around vegetated areas, along the lake shallows.

8. The young of all species require plankton or invertebrates (for example amphipods or cladocera) for sustenance, and many require vegetation for shelter from predators.

Table 1: Species of Interest in Lake Cochituate[a]

Species	Spawn season	Spawn location/or early life stage (ELS) location	ELS Food preference
White perch *Native*	Spring	Eggs adhere to rocks, vegetation	Plankton, insect larvae
Largemouth bass *Introduced*	Late spring-mid summer	Spawn near vegetation or roots of emergent plants	Amphipods, cladocera, invertebrates
Yellow perch *Native (?)*	Spring	Lake shallows, near rooted vegetation; egg masses adhere to vegetation on bottom	Cladocerans, ostracods, chironomid larvae
Black crappie *Introduced*	Late spring	Sediment with vegetation	Plankton, crustaceans, invertebrates
Pumpkinseed sunfish *Native*	Late spring, early summer	Shallow water, eggs may be attached to plant roots	Invertebrates, larvae
Bluegill sunfish *Introduced*	Late spring, early summer	Shallow water, eggs sink to bottom	Invertebrates, plants
Yellow bullhead *Introduced*	Late spring	Stream banks, stones or stumps; "*removal of stumps, logs or vegetation leads to decrease in numbers*"[b]	
Chain pickerel *Native*	Spring (occasionally fall)	3-10' of water; usually over flooded vegetation; eggs and newly hatched stick to vegetation	Plankton and invertebrates
White sucker *Native*	Spring	Lake margins, shallow water; eggs adhere to	Invertebrates and algae

		gravel	
Golden shiner *Native*	Summer	Eggs stick to filamentous algae, sometimes rooted plants "*aquatic vegetation is essential for spawning*" [b]	Invertebrates and algae
American eel *Native*	Do not spawn in lakes	Elvers to adults occur in fresh water	NA[c]

[a]Data on species residing in Lake Cochituate are from the Massachusetts Fish and Wildlife (http://www.mass.gov/dfwele/dfw/dfw_pond.htm); data on spawning and life history are from Scott and Crossman (1973); data on native and introduced species are from Hartell et al. (1996).
[b]Scott and Crossman (1973).
[c]Not applicable since ELS are not present in Lakes.

Summary of NOI as it relates to diquat application

9. According to the NOI, ACT proposes to treat up to 5 acres (near the swimming area, kayak launch, and boat ramp) of Middle Pond with Reward (diquat dibromide, referred to as diquat in this testimony) (ACT 2009). Earlyanalysis of the Lake, as described in the Long Term Vegetation Management Plan (ACT 2004), indicates that milfoil coverage in Middle Pond was greater than originally thought, and most recent measures which included fragment barriers were apparently not fully effective. As indicated in the NOI, ACT recommends application of diquat toshoreline areas of Middle Pond.

10. Diquat is a nonselective contact herbicide, used to control broadleaf and grassy weeds. Because diquat works through contact (adherence to leaves rather than uptake by the roots), it injures only the parts of the plant with which it comes in contact. Since diquat is unlikely to translocate to the roots, it may not kill the whole plant, requiring repeated treatment. In a letter sent to Rachel Freed, an Environmental Analyst with the DEP, Peter Webber of the DCR stated, "[I]t can be expected that Reward-treated plants would regrow to some extent the year following treatment, albeit with a lower cover and density," (Webber 2003).

11. As currently proposed, the application will occur in early-mid May, prior to bathing season, and contingent upon water temperature and milfoil emergence. The rate of application of diquat will range from 1.0-1.5 gallons/acre, and the herbicide will be applied underwater through weighted spray hoses. It is estimated that an application rate of 2.0 gallons/acre concentration of diquat ion (the active form of diquat) results in

approximately 0.37 parts per million (ppm) (ACT 2003; ACT 2004; ACT NOI 2009). However it is important to note 0.37 is a calculated target concentration rather than an actual measured concentration in the water body. In some cases, actual concentrations of diquat have been found to exceed the intended target concentrations, depending on the method of application and the location of water sampling.

12. For example, following the application of diquat to a freshwater impoundment (with a target concentration of 0.11 ppm diquat), Berry et al. (1975) reported a "minimum of 0.03 ppm in the deep and 0.73 ppm in the shallow area (by) twelve hours after application (Berry et al., 1975)". Shaw and Hamer (1995) also note that "it is not uncommon for 'hot spots' to occur immediately following application…..," and that field studies have shown complete mixing may take at least twenty four hours (Shaw and Hamer 1995).

13. Following application, diquat is cleared from the water as a result of its affinity for plants and sediments (where it persists once absorbed,[1]), although reports of residence time of diquat in the water column can vary. For example, detectable concentrations have been reported in the water 10 days after pond applications (Gilderhaus, 1967).

14. Thus, concentration of diquat in the water column depends upon the application rate, application method and the biological and physical factors of the system to which it is applied (Simonin et al., 1995; Shaw and Hamer 1995; Langeland et al., 1994; Gilderhaus 1967), and cannot be determined solely through calculations based on application concentration and water body area.

Effect of diquat on fish habitat

15. Diquat is nonspecific in that it will kill not only the target species (in this case, Eurasian watermilfoil[2]) but any other broadleaf or grassy plant species with which it comes in contact. This attribute of diquat can result in areas of oxygen depletion, which can lead to fish kills. Syngenta, the manufacturer of diquat pesticides such as Reward, states that "fish can be killed by oxygen depletion when very heavy weed populations are all killed at once," (ACT 2003, Attachment C, p. no page number).

16. Both because of the adverse effects on fish of low dissolved oxygen caused by decaying vegetation, and because many species require vegetation for survival, the US EPA states that for aquatic application of diquat, only "one-third or one-half of the dense weed area in a water body" should be treated at any one time, leaving the rest as refuge

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[1] [1] Because of this tendency (diquat dibromide is considered very persistent with a "high" persistence hazard) and its acute toxicity, diquat dibromide recently received a failing grade by the Thurston County Health Department in Washington State (http://www.co.thurston.wa.us/health/ehipm/pdf_aqua/aquatic%20actives/diquat%20dibromide.pdf)

[2] Although resistance is not yet reported in milfoil, at least one species of duckweed was found to have developed resistance following years of repeated diquat application (Koschnikc et al., 2006.)

(US EPA 1995, p. 69). The US EPA further states that additional application should "not be made for a further two weeks," (US EPA 1995, p.69).

17. Greater adverse effect on the ecosystem by repeated applications is acknowledged by ACT. According to the Long Term Management Plan, other herbicides such as SONAR are suggested rather than diquat, since "There is less disturbance to the lake's ecosystem when larger scale treatments are not being repeated each year,"(ACT 2004, p. 33).

18. While adult fish may be able to seek refuge in areas of the Lake to which diquat is not applied, the proposed application in May and June will be detrimental to the freshly laid eggs expected to be present during treatment. Larval fish will most likely die in the areas of treatment, as they will be unable to relocate to refuge areas.

19. Writing about the role of submerged aquatic vegetation (including non-native invasive species) to fish in Minnesota Lakes, Valley et al. (2004), states that "vegetated, nearshore habitat is critical for fish recruitment. Any removal should be viewed as habitat loss, and efforts should be made to minimize this loss," (Valley et al., 2004, p.2).

Diquat toxicity

20. Diquat is toxic to both adult and early life stages (ELS) of fish (from egg through to the juvenile stage), with the ELS generally the more sensitive stage.

21. Since average target concentrations with applications of 1.0 to 1.5 gallons per acre could range from 0.19-0.28 ppm (based on the estimated concentration of 0.37 ppm with 2.0 gallons per acre), I will consider these concentrations as the primary concentrations of concern in assessing risk to fish in Lake Cochituate. The U.S. Environmental Protection Agency has accepted a concept referred to as the acute Risk Quotient (or "RQ") in determining ecological risk. The acute RQ is the ratio of the effective concentration of the subject chemical (in this case, diquat) to the concentration of that chemical that will kill 50% of the exposed population. The shorthand term for this lethality factor is "LC50." The LC50 is determined by experimentation, which, in this case, would determine the concentration of diquat that causes death to 50% of an exposed population of an aquatic species in a 96 hour period.

22. The RQ reflects how lethal the effective concentration can be expected to be. For example, if the effective concentration of a chemical is 5 ppm and the LC50 is 10 ppm then the RQ would equal 0.5 (5ppm/10ppm). The greater the difference between the effective concentration and the LC50 the smaller the risk quotient. The smaller the RQ, the lower the risk.

23. Risk associated with diquat is categorized by comparing the acute RQs to predetermined Levels of Concern (LOC). According to the US EPA, LOC are criteria that "indicate potential risk to nontarget organisms. The criteria indicate that a chemical, when used as directed, has the potential to cause undesirable effects on nontarget

organisms (US EPA 1995)." Acute RQs are compared to acute LOCs. An acute LOC of 0.5 (or a two-fold difference between the effective and lethal concentrations) indicates Acute High Risk; an LOC of 0.1 (a ten-fold difference) indicates "risk that may be mitigated through restricted use;" and an LOC of 0.05 indicates endangered species may be affected acutely (US EPA 1995 p. 48).

24. The US EPA Reregistration Eligibility Decision (RED) for diquat reported the RQ for acute toxicity in fish to be 0.02 below any LOC (US EPA 1995). When calculating the acute RQ, US EPA listed eight different studies which it finds acceptable for use. In 1995, when US EPA last calculated the RQ for diquat, it apparently used the LC50 for the bluegill sunfish, which at the time was considered the standard test species (US EPA, personal communication). However, the current US EPA guidelines for RQ calculation indicate that the LC50 of the most sensitive species be used (US EPA 2004). Of the eight LC50's found as acceptable by US EPA in 1995, yellow perch was the most sensitive, with an LC50 of 4.4. If the RQ were determined today using the LC50 reported in the US EPA RED for yellow perch, a resident species in Lake Cochituate, the RQ for diquat would be 0.05, equal to the LOC for acute endangered species. A recent analysis of the impact of aquatic herbicides on California's aquatic ecosystems concluded that "based on a number of LOC exceedences (for aquatic plants in addition to the fathead minnow and Delta smelt) as well as some toxicity, additional risk characterization of diquat dibromide applications are warranted" (Siemering et al., 2008[3].)

25. It is important to note that US EPA's derivation of the acute RQ is based on lethality. It does not consider sublethal impacts of chemicals on aquatic organisms such as behavioral or physiological impacts on fish, such that RQ's below a given LOC do not guarantee absence of harm. Furthermore, US EPA's derivation of the acute RQ is based on lethal effects observed in juvenile and adult fish. It does not consider impacts on the more sensitive early life stages (ELS) of fish.

26. In gathering information on toxicity of diquat to fish I have reviewed primary peer reviewed literature. In addition, I have reviewed the US EPA RED for Diquat (US EPA 1995); the Final Risk Assessment for Diquat Bromide prepared for the Washington State Department of Ecology (WA SEIS 2002); and the Opinion of the Scientific Committee on Plants Regarding the Inclusion of Diquat. (E.U. 2000). These sources provide either data summaries or reference lists useful for cross referencing.[4]

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[3] The authors acknowledge additional input of diquat by terrestrial use may be an issue in addition to aquatic application.

[4] The Long Term Vegetation Management Plan (ACT, 2004) states that "according to Madsen (June/July 2000) currently no product can be labeled for aquatic use if it poses more than a one in a million chance of causing significant damage to human health, the environment or wildlife resources." (pp. 23-24) I find this quote misleading, and because it is an important statement I researched its origins. First, the reference is not included in the citation list (Madsen June/July 2000) of the Long Term plan. I did find it through a Google search. The reference is to LakeLine, a magazine of the North American Lake Management Society. The US EPA is the agency responsible for the registration of aquatic herbicides and I was unable to find any indication that decisions are based on a 1 in a million risk to "wildlife resources." In fact, the US EPA states that when a herbicide is approved for registration the US EPA has found "it does not pose

Adult Fish

27. There is a considerable database on the impacts of diquat on fish. The majority of studies (roughly 21 studies out of 32) have been conducted in adult or juvenile fish (e.g. WA SEIS 2002). Generally the difference between a juvenile and an adult fish is that the juvenile is a fully formed yet immature version of the adult. The great majority of these studies are acute studies, with a reported endpoint of lethality (e.g. 96 hour LC50 tests). Testing with juvenile fish is in accordance with US EPA's Ecological Effects Test Guidelines (US EPA 1996). According to the US EPA, such short term lethality tests are used to: "establish acute toxicity levels…, compare toxicity information with measured or estimated pesticide residues…, [and] indicate whether further laboratory and/or field studies are needed," (US EPA 2005).

28. As previously noted, the US EPA's focus on lethality for derivation of RQs precludes consideration of any other endpoint which might impact fish survival such as behavioral, reproductive or neurological effects. For example, studies with fairly low concentrations of diquat (0.5 ppm) found reduced swimming speeds in rainbow trout and in fathead minnows. (Dodson and Mayfield, 1979; de Peyster and Long 1993). Indeed, de Peyster and Long (1993) reported a reduction in swimming performance at diquat concentrations as low as 12% of the LC50.

29. Additionally, LC50 tests are a statistical point estimate of a chemical concentration, around which there is a range of uncertainty. For example, Tapp et al., (1989), report the 4 day LC50 for rainbow trout as 6.1 ppm of diquat, with a 95% confidence interval of 3.9-8.9, meaning that they are 95% confident that the actual LC50 lies within this range. The LC50 could be 4.2 ppm or it could be 8.0 ppm.

30. The lowest reported LC50 in adult or juvenile fish in the WA SEIS (WA SEIS 2002) was 4.2 ppm reported for large mouth bass fingerlings (64 mm in length) in "very soft" water. Consideration of hardness (ppm $CaC0_3$) is highly relevant when discussing diquat toxicity, as toxicity is greatly altered by water hardness, with greater resistance to toxicity in hard (up to ten-fold) compared with soft water (WA SEIS 2002). For example, changing water hardness from 40 ppm to 300 ppm resulted in decreased toxicity to some species of fish by up to 50% (Johnson and Finley 1980). Information from the 1976 – 1980 time frame indicates that Lake Cochituate water ranges from very soft to moderate hardness (Data and Summary Report for Lake Cochituate Apr. 1976-1980).

31. The US EPA classifies sensitivity to toxicants (in terms of lethality) using LC50 measurements as follows:

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an unreasonable risk to public health and the environment" and is based on a risk-benefit analysis (http://www.epa.gov/pesticides/health/risk-benefit.htm), rather than any kind of ecological risk guideline such as less than 1 in a million. This is an important distinction, and the use of such a quote suggests at best a misunderstanding of the pesticide regulation process and at worst an intention to downplay the hazards of pesticide application by ACT.

Aquatic Organisms: Acute Toxicity (US EPA 2004).

Concentration (ppm)	Toxicity Category
<0.1	very highly toxic
0.1 - 1	highly toxic
>1 - 10	moderately toxic
>10 - 100	slightly toxic
>100	practically nontoxic

32. The attached Figure 1, "Distribution of sensitivity of adult and juvenile stages to diquat toxicity for different species of fish," summarizes data from the WA SEIS (WA SEIS 2002) review tables using the US EPA classification for acute toxicity. Species are characterized as either most sensitive (e.g. diquat is highly toxic), moderately sensitivity (diquat is moderately toxic) or least sensitive (diquat is slightly toxic). In addition to species reviewed in the WA SEIS (2002), I have included the species of interest in Lake Cochituate (from Table 1) for comparison.

33. Several observations can be drawn from Figure 1:

a) Adults and juveniles from a wide range of species have been tested for diquat toxicity.

b) Of the ten species for which data were available (those species surrounded by colored boxes in Figure 1), only four species are included as species of interest in Lake Cochituate. I was unable to find data on diquat toxicity (in adults or juveniles) for seven of the eleven species known to inhabit Lake Cochituate.

c) Of the ten species for which data were available, eight of the ten were categorized as least sensitive, and two were moderately sensitive.

Early Life Stages of Fish

34. The remainder of this section focuses on diquat toxicity to early life stages ("ELS") of fish. As discussed above these stages are often more sensitive to toxicants than are adults or juveniles.

35. The data discussed in this section have been extracted primarily from the peer-reviewed literature, in addition to the grey (unpublished) literature. As with the adult testing, the majority of tests with ELS have focused on acute (96 hour or less) toxicity tests. A few chronic tests (lasting over a week) have been conducted as well. Most studies report LC50's while some reported No Observed Effect Concentrations (NOEC) and Low Observed Effect Concentrations (LOEC), the latter often referring to the lack of lethality. In some cases, more sensitive endpoints such as growth are observed.

36. The results of these studies are summarized in Table 2, and in the attached Figure 2. LC50's for ELS ranged from 0.43-110 ppm, indicating that at least for some species diquat can be highly toxic to ELS fish. Because the US EPA does not calculate RQs for ELS fish, I have calculated them based on these ELS fish studies and the estimated target diquat concentration of the proposed application (i.e., 0.28 ppm.) Those which exceed a US EPA LOC are in bold. Of the seven species for which I could calculate an ELS RQ, four had RQs above 0.1, which according to US EPA's characterization of RQ's (which was derived for generally less sensitive adult fish and not ELS), would exceed the "restricted use" category, and one (the fathead minnow) exceeds the acute high risk category. If one were to apply the current US EPA risk assessment methodology for freshwater fish to ELS, and base the risk to ELS fish on the RQ of the most sensitive ELS fish (i.e., the fathead minnow larvae), the risk analysis would produce an RQ of 0.63. This indicates a very small margin (less than twofold) between the target application concentration and the concentration that would result in lethality to 50% of ELS fish.

37. Again, it should be noted that assessing risk in this manner fails to account for sublethal effects in ELS fish exposed to diquat. For example, in a study of Fundulus heteroclitus, the author reported abnormal development in ELS exposed continuously from egg through fry, at diquat concentrations as low as 0.01 ppm (Crawford and Guarino 1985). This indicates a very high degree of sensitivity to diquat which may not be accounted for by reliance upon RQs calculated with data from 96 hour LC50 tests.

38. The attached Figure 2, "Distribution of Sensitivity of Early Life Stages to Diquat Toxicity for Different Species," summarizes data obtained from the primary literature and in some cases from grey literature.

Several observations on diquat toxicity in ELS can be drawn from Figure 2.

a) As with adults, diquat toxicity has been tested in a broad range of species, including seven different families of fishes.

b) Unlike adult fish, ELS sensitivity to diquat, based on 96 hour LC50 testing, ranges from Most Sensitive to Least Sensitive.

c) Of all the species tested, two were least sensitive, seven were moderately sensitive, and four were most sensitive.

d) There appears to be no relationship between toxicity and family (for example, the Cyprinidae family includes ELS of all sensitivities.)

e) Of the 13 species for which ELS 96 hour toxicity data were found, only two species are known to inhabit Lake Cochituate.

f) The ELS of the two Lake Cochituate species are moderately sensitive to diquat toxicity.

g) No data on the toxicity of diquat to ELS could be found for nine of eleven fish species that inhabit Lake Cochituate.

39. Studies on the toxicity of diquat to ELS of resident species indicate that a lack of data does not indicate lack of toxicity. Prompted by concern about the impacts of diquat administration on ELS of resident species in New York and California water bodies, Paul et al. (1994) and the California Department of Fish and Game (Riley and Finlayson 2004) conducted their own ELS testing in species of interest. In both cases, testing revealed species (walleye, delta smelt and fathead minnow) in which diquat can be characterized as highly toxic.

40. Furthermore, it is highly likely that ELS of species found in Lake Cochituate will be exposed to diquat, given the common association of ELS with highly vegetated areas that serve both as nursery habitat and as protection from predators.

Table 2. Diquat exposure in fish: summary of toxicity endpoints.

Species	Test	LC50 (ppm)	NOEC	LOAC	Other	Citation
Walleye 8-10 day post hatch[a]	Static non-renewal 96 hour toxicity test	24 hr: 2.9 48 hr: 1.6 72 hr: 1.0 96 hr: 0.75 **RQ=0.37**	24 hr: 0.93 48 hr: 0.93 96 hr: 0.48	24 hr: 2.0 48 hr: 2.0 96 hr: 0.93		Paul et al. 1994[c]
Walleye 41-43 day post hatch	Same	24 hr: 3.1 48 hr: 1.9 72 hr: 1.6 96 hr: 1.5 **RQ=0.19**	24 hr: 0.90 48 hr: 0.90 96 hr: 0.90	24 hr: 1.9 48 hr: 1.9 96 hr: 1.9		Paul et al. 1994
Walleye 84-86 day post hatch	Same	24 hr: 7.8 48 hr: 4.9 72 hr: 4.9 96 hr: 4.9 RQ=0.06	24 hr: 4.4 48 hr: 2.6 96 hr: 2.6	24 hr: 8.4 48 hr: 4.4 96 hr: 4.4		Paul et al. 1994
Smallmouth Bass[a] 6-8 day post swim-up	Same	24 hr: 110 48 hr: 28 72 hr: 10 96 hr: 3.9 RQ=0.06	24 hr: 68 48 hr: 14 96 hr: 1.6	24 hr: 130 48 hr: 34 96 hr: 3.4		Paul et al. 1994
Largemouth Bass[a] 9-13 day post swim-up	Same	24 hr: 15 48 hr: 11 72 hr: 8.0 96 hr: 4.9	24 hr: 7.1 48 hr: 7.1 96 hr: 1.8	24 hr: 18 48 hr: 18 96 hr: 3.6		Paul et al. 1994

		RQ=0.04				
Delta smelt Larvae [b]	96 hour static renewal at 48 hr	96 hr:1.1 **RQ=0.25**				Riley and Finlayson 2004
Sacramento splittail Larvae [b]	Same	96 hr: 3.7 RQ=0.06				Riley and Finlayson 2004
Fathead minnow Larvae [b]	7-day test; daily renewal	96 hr: 0.43 **RQ=0.63**				Riley and Finlayson 2004
Fathead minnow larvae	30 days post hatch continued exposure				Larval growth unaffected by $\leq$ 0.58 ppm	Suprenant 1987
Striped bass Larvae	96 hour static, no renewal?	24 hr: 1.0[d] 48 hr: 1.0 96 hr: 1.0 **RQ=0.22**		All larvae survived 0.1 ppm		Hughes 1973
Fundulus heteroclitus Embryo through fry;	Static renewal	Not reported			Abnormal fry reported for all doses from 0.01-10ppm	Crawford and Guarino 1985
Channel catfish Yolk sac	Static				Fry survived up to 111 hours with maximum concentrat ion of 10 ppm	Jones 1961
Bluegill fry (19.5 mm)	Static				Fry survived 96 hours with concentrat ions up to 5 ppm	Jones 1961
Black bass fry	Static				0.5 ppm the highest 'safe'	Jones 1961

					concentrat ion	

[a]Water hardness=132 ppm $CaC0_3$ for walleye; 81 ppm $CaC0_3$ for small mouth bass; and 65 ppm $CaC0_3$ for large mouth bass.
[b]Water hardness=68 ppm $CaC0_3$ for delta smelt; 69 ppm $CaC0_3$ for splittail; and 78 ppm $CaC0_3$ for fathead minnow.
[c] Paul et al. values expressed as cation; CA Dept Fish and Game
[d]It is unclear why all three measurements are the same; although interestingly the author notes that the LC0 (where no deaths occur)=0.1 ppm.

Invertebrate toxicity

41. Diquat is known to be slightly to highly toxic to invertebrate species including Daphnia and Hyallela, with the amphipod Hyallela demonstrating the greatest sensitivity (US EPA 1995, Hilsenhoff 1965; Gilderhaus 1967; Williams et al., 1984; Wilson and Bond 1969). In a pond treated with 1ppm diquat, Hilsenhoff (1965) found a decline in both amphipods and in four different types of snails, and suggested that the changes may have resulted from destruction of shoreline plants (although at the time there were little toxicity data available.) The 48 hour EC50 (equivalent of the LC50 in fish, only immobilization rather than death is used as the endpoint) for the Amphipod Hyalella azeteca has been reported as ranging from 0.12 to 3.4 ppm depending on the study (Wilson and Bond 1969; Williams et al., 1984; US EPA 1995).

42. The US EPA has calculated the acute RQ for freshwater invertebrates (using data from Daphnia, which was the standard test species) as 0.3 which is between the LOC for acute high risk and risk that may be mitigated through controlled use and which exceeds the LOC for endangered species. However, in the US EPA RED (US EPA 1995) the EPA concluded that freshwater invertebrates are not likely to be affected by the use of diquat dibromide. The rationale for this conclusion, given their own calculations, is unclear. It is also noteworthy that if the RED assessment were conducted today, using the most sensitive species (e.g. Hyallela), the RQ would likely be higher, possibly resulting in a classification of acute high risk for invertebrates.

43. Diquat is toxic to at least two species of aquatic snails. A single dose of 0.222 ug/L diquat (as Reglone) was found to affect adult growth, and reduce hatch rate and lengthen embryo development time of the pond snail (*Lymnaea stagnalis*) (Coutellac et al., 2008) in both mesocosms and in laboratory bioassays. . Diquat was also found to be moderately to highly toxic to the Apple snail with a 96 hour LC50 of 1.8 ppm (Mayer and Ellersieck 1986) and a 48 hour EC50 of 0.34 reported in the EPA RED (EPA 1995). If an RQ for the Apple snail were calculated using data reported in the EPA RED (using 0.28 ppm as a target concentration) the RQ would be 0.82, exceeding the acute high risk LOC.

44. It is evident from Table 1 that ELS of fish species in Lake Cochituate are dependent upon several different kinds of invertebrates (e.g. cladocera, amphipods, phytoplankton) as a primary food source. The proposed diquat application is likely to result in a reduction in available prey species, at a critical time in fish development, that could adversely affect ELS of several different species, potentially impacting recruitment.

45. Studies of other aquatic species of invertebrates generally, and of two species of snails in particular, indicate that diquat can be highly toxic to some species of snails (US EPA 1995). According to the US EPA RED (US EPA 1995), the RQ for invertebrates exceeds the LOC for endangered species, and is defined as a situation in which "endangered species may be affected acutely" (US EPA 1995). Additionally, as discussed above, the RQ calculated for the Apple snail is above the LOC for acute high risk in addition to exceeding the LOC for endangered species.

46. Snails can also be adversely affected by destruction of aquatic vegetation in their habitat (Hilsenhoff 1965). As noted above, the nonspecific character of diquat means that it will kill not only the target species (in this case, Eurasian watermilfoil), but any other broadleaf or grassy plant species with which it comes in contact. Since snails often live and breed in association with aquatic vegetation, killing the vegetation will likely kill any associated snails. .

Conclusion

47. I have concluded that the application of diquat as proposed by the DCR and approved by the Natick Conservation Commission and DEP is likely to adversely affect the young fish that inhabit the Lake, and the important fishery and habitat functions served by the land under the Lake. I base this conclusion on the following.

48. Lake Cochituate supports a diversity of self-sustaining fish populations, many of which are important game fish. The majority of Lake Cochituate species spawn in the spring or early summer. Almost all of the species that spawn in Lake Cochituate rely upon either vegetation, or sediment locations near vegetation, for either spawning or nursery grounds.

49. The application of diquat will cause removal of the target and non-target vegetation. The Spring application of this nonspecific pesticide is likely to adversely impact both spawning habitat and protective habitat for early life stages of most resident species. The proposed application also has the potential to cause low dissolved oxygen in the spawning or nursery areas, and is likely to reduce available food resources. As a result of this alteration of habitat, the proposed application to the heavily weed- infested areas during or just after spawning will likely kill eggs and young larvae unable to seek refuge.

50. The diquat is also likely to adversely affect the population of ELS fish in the Lake due to its toxicity. Early Life Stages of fish are more sensitive to diquat toxicity than adult or juvenile fish. Data are available for diquat toxicity in the ELS of only two species of ten species known to spawn in Lake Cochituate (largemouth bass and bluegill sunfish), and these species may be classified as moderately sensitive to diquat. While there are few studies of toxicity to ELS for resident species in Lake Cochituate, the distribution of diquat sensitivity across species indicate that it is unlikely that the two resident species for which data are available are the most sensitive species residing in Lake Cochituate.

51. Furthermore, the available studies utilize primary endpoints of 50% lethality. This is an insensitive endpoint, which does not consider sublethal effects such as behavioral changes or physiological changes which may adversely impact ELS survival rendering larvae more vulnerable to prey or less likely to feed. The LC50s are also point estimates, around which there can be a range of uncertainty. Even taking the

shortcomings of the US EPA's method of risk assessment into account, an assessment performed consistent with US EPA's methodology reveals an RQ of 0.63 for the most sensitive species of ELS fish. This indicates a very small margin (less than twofold) between the target application concentration and the concentration that would result in lethality to 50% of ELS fish. This is certainly considered acute High Risk.

52. Although target concentrations of diquat based on an application rate of 1.0-1.5 gallons/acre range from approximately 0.19-0.28 ppm, this is only a theoretical value, and the potential for higher concentrations in some locations, particularly shallow areas, exists. In addition, water hardness can greatly impact toxicity, with softer water often resulting in lower LC50's. If the water hardness of Lake Cochituate has remained relatively unchanged since the most recent years for which I found data, this Lake may pose a "worst case" scenario with respect to the toxicity of diquat.

53. Because existing data regarding the toxic effects of diquat on adult or juvenile fish do not accurately reflect the toxicity to ELS fish, I strongly recommend that pre-application studies be performed regarding the toxic effects of diquat on ELS of fish species residing in Lake Cochituate, such as was done with respect to New York and California water bodies (Paul et al. 1994; Riley and Finlayson 2004)). As noted above, in both the New York and California cases, testing revealed species in which diquat can be characterized as highly toxic at the Early Life Stage.

54. There are insufficient data to support the conclusion that the proposed diquat application will not have an adverse impact on adult or juvenile fish species inhabiting the Lake. The majority of studies that are available for fish are in species other than Lake Cochituate fish. The insensitivity of the endpoint in these studies, i.e., death, affords no basis for determining other impacts from toxicity at lower concentrations. There is data that indicates that such impacts exist (e.g., reduced swimming speed have been reported in adult and juvenile fish at concentrations well below a species LC50).

55. Finally, there are insufficient data to support the conclusion that the proposed diquat application will not have an adverse impact on snails species living in Lake Cochituate. The potential for diquat to harm endangered invertebrate species, based on the RQ calculations in addition to the disruption of its habitat through destruction of aquatic vegetation, suggests that the use of diquat will adversely affect both adult and newly hatched snail species if any are present during application.

Glossary of toxicology terminology:

Acute: generally refers to a short period of time, in this context, four days (96 hours) or less.

Adverse effect (US EPA definition): "A biochemical change, functional impairment, or pathologic lesion that affects the performance of the whole organism, or reduces an organism's ability to respond to an additional environmental challenge," (http://www.epa.gov/iris/gloss8.htm)

Bioavailable: The extent to which a chemical in the environment is available to be taken up either through contact, ingestion or inhalation by a living organism.

Chronic: Most often greater than seven days depending on the species; usually describes an exposure 28 days or longer.

Diquat: Reference to Diquat may include either the salt or the cation. The distinction is important when comparing Diquat concentrations in relation to toxicity. Most often reference to Diquat concentrations imply Diquat ion. Also note that most often Diquat is tested as a 35% solution.

ELS: Early life stage (ELS) generally refers to the life stages from fertilized egg, through the embryo and larval stages of fish. Sometimes this includes juvenile stages as well.

Juvenile: The juvenile stage in fish usually refers to an immature (not yet spawning) fish. Juveniles look just like the adult, in contrast to larval fish.

Endpoint: All toxicology tests are designed with a specific outcome such as lethality, change in reproductive capacity or biochemical change. These are all endpoints.

Half-life: Refers to the amount of time required to reduce the concentration of a specific chemical by one half. If the half life of a chemical present at 10 parts-per-million (ppm) is 24 hours, then after 24 hours 5 ppm remains. After 48 hours 2.5 ppm remains. Half life may be dependent upon environmental conditions such as organic matter in the water, sunlight etc.

Larval: The larval stage encompasses all life stages from post-hatch to juvenile stages.

LC50: The concentration of a chemical required to kill half the test organisms (it is a statistically derived number). Given an initial test population of 20 animals, an LC50 of 10 indicates that 10ppm will kill (approximately) 10 of those animals.

LOC: Level of concern; US EPA's LOC's for acute toxicity are as follows: Acute High Risk=0.5; Acute Restricted Use=0.2; Acute Endangered Species=0.1.

NOEC: No observed effect concentration. A concentration derived from chronic toxicity tests.

PPM; PPB: Parts-per-million (PPM) and parts-per-billion is used to refer to concentrations of chemicals in the environment or test system. Most often PPM =milligrams solute per liter of solvent (mg/L); PPB=micrograms of solute per liter of solvent (ug/L).

RQ, Acute Risk Quotient: The acute risk quotient may be used by the US EPA for ecological risk assessment. The quotient is a ratio of the peak water concentration of a particular chemical (EEC) and the LC50 (EEC/LC50).

Sublethal: refers to any adverse impact.

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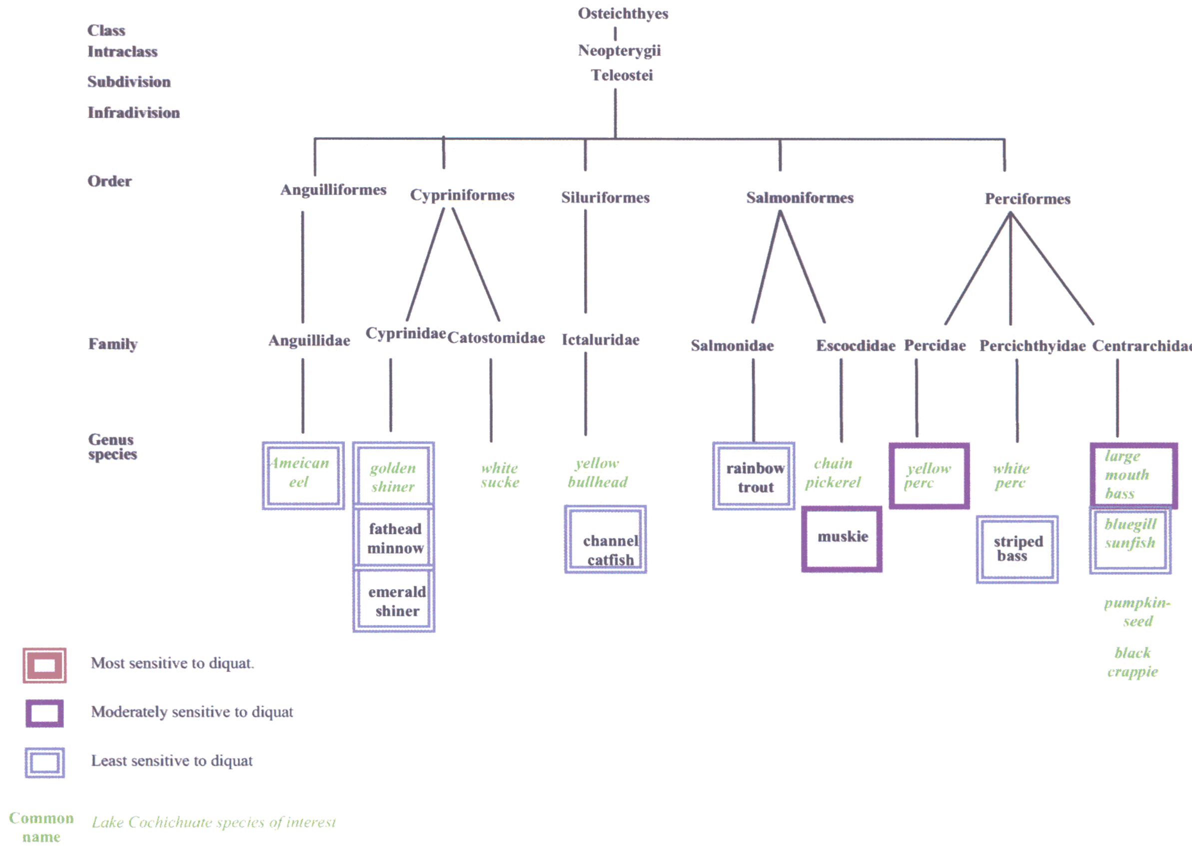
Signed under the pains and penalties of perjury this 30 day of October 2009.

Emily Monosson, Ph.D.

Figure 1. Distribution of sensitivity of *adult and juvenile* stages to diquat toxicity for different species of fish

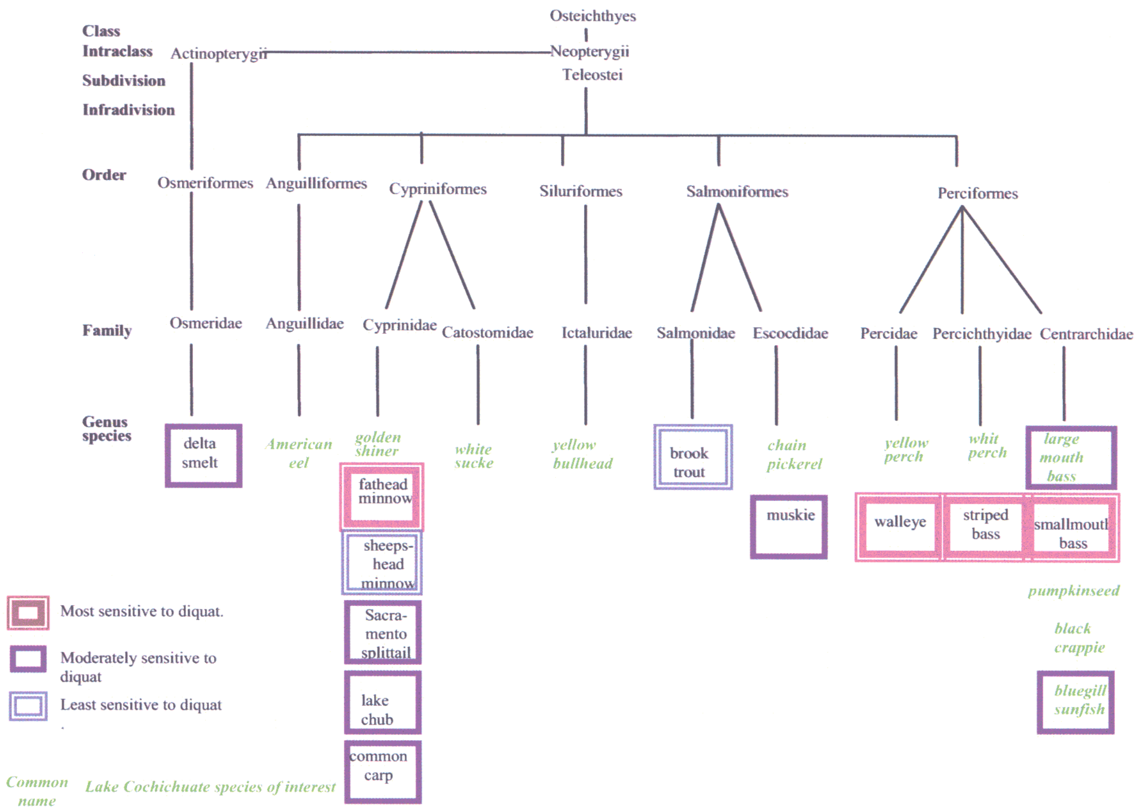


Adults or fingerlings were selected from literature where either life stage was reported or the size of the fish was small large enough to be considered past early life stage.

LC50 data are from 24-96 hour LC50s. The lowest reported LC50s were used to categorize toxicity.

Toxicity data summarized from: Washington State SEIS Risk Assessment of Aquatic Herbbicides, Appendix 1 (WA SEIS 2004).

Figure 2. Distribution of sensitivity of *early life stages* to diquat toxicity for different species of fish



ELS (Early life stage) tests were selected from literature where either life stage was reported or the size of the fish was small enough to be considered an early life stage. Fingerlings were not considered to be ELS.

LC50 data are from 24-96 hour LC50s. The lowest reported LC50s were used to categorize toxicity.

Toxicity data summarized from: Washington State SEIS Risk Assessment of Aquatic Herbbicides, Appendix 1 (WA SEIS 2004); primary and gray literature.