

CHARLES RIVER FISH CONTAMINANT SURVEY



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Forward

This report has undergone internal and external peer review and has been found acceptable in the examination of contaminants in tissues of freshwater fish from several sections of the Charles River in Massachusetts.

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Introduction

The Charles River has been the focus of a major initiative spearheaded by USEPA in an effort to make the river a swimmable and fishable waterbody by the year 2005. Through this effort, numerous studies have been undertaken by various agencies, businesses, and organizations looking at sources of water quality impairment via the monitoring of wet weather events, dry weather ambient water quality, in-river bottom sediments, and outfall pipe discharges. In addition to physical and chemical water quality monitoring, limited biological surveys have taken place to determine the effect the river's water quality and surrounding habitat might have on the concentration of contaminants in resident fishes and ultimately, upon human health from the consumption of these fish. This report summarizes a biological monitoring component of the initiative through the monitoring and analysis of fish within the lower Charles River basin, implemented by the EPA New England Regional Laboratory in the late fall of 1999.

Purpose

Limited information is presently available on fish tissue data from a human health as well as an ecological perspective from the Charles River. In 1985, a survey comprised of three samples was completed by the Massachusetts Department of Environmental Protection (MADEP) and the Massachusetts Division of Fisheries Wildlife and Environmental Law Enforcement (MDFWELE). Fish fillet composite samples (1 species, 5 fish/composite) of brown bullhead (*Ictalurus nebulosus*), largemouth bass (*Micropterus salmoides*), and white catfish (*Ictalurus catus*) were collected and analyzed for metals concentrations and a data report published. MADEP conducted another survey in 1995 in the lower Charles River basin to test for the presence of PCBs, PAHs, metals, and gamma radiation. These samples, as with the previous 1985 collections, were analyzed by Wall Experiment Station (WES) in Lawrence, MA. The exception was gamma radiation analysis which was conducted at the Massachusetts Department of Public Health (MDPH) radiation laboratory in Jamaica Plain, MA. Analyses results from this survey were below the U.S. Food and Drug Administration's action levels (Hg-1.0 ppm, Cd - 0.3ppm), but average PCB concentrations found in carp were elevated enough to raise concern to the MDPH, who subsequently issued a fish consumption advisory for PCB's in carp (*Cyprinus carpio*). This was posted from the Hemlock Gorge dam in Needham to the Museum of Science dam in Boston.

In 1997, Charles River fish sampling upstream of the South Natick Dam took place. Mercury levels found in largemouth bass from sampling this segment prompted the MDPH to issue a consumption advisory limit for sensitive populations; pregnant women and children under the age of twelve for this species.

Information derived from EPA's 1999 survey effort will be utilized for determining if human health risks based on Food and Drug Administration action levels (USFDA Industry Activities Staff Booklet, March 1998) for consumption of fish remain the same within the lower Charles River basin, to determine if ecological health risks exist based on current literature contaminant values and associated recommendations, and to provide a cursory survey of fish populations

existing in the lower Charles River.

Sampling Design Rationale

The fish species targeted for this survey were selected to represent potential worst case scenarios for contaminant uptake in the waterbody by resident biota. They represent the species most likely to be consumed by the fishing population on the river and those species occupying different in-river habitat niches and various trophic feeding levels.

Largemouth bass were selected as a target species. They are a top-level carnivore, larger ones feeding predominantly on smaller fish, frogs, and crayfish. The species is highly sought for sport and consumption by the public at large, and has been noted as being a well established productive fishery in the Charles River basin. Their preferred habitat is weedy mud-bottomed areas with minimal current and the presence of in-stream or riparian cover or structure. They occupy a relatively small home range, making them susceptible to bio-accumulation of contaminants up through the food chain in a relatively small geographical area. There is also a largemouth bass mercury advisory for sensitive populations in effect for the upper Charles at this time. A minimum size limit of twelve inches for this species has been established in Massachusetts; for this reason the minimum size retained for this study was twelve inches. To the extent possible, all individuals within species were selected so that relative differences between individuals were less than ten percent; the same applied to the average length between composited samples.

Carp are actively sought by certain ethnic populations along the Charles River. As a benthic omnivore whose feeding habits involve the direct uptake of sediments in order to acquire desired food sources of algae and macroinvertebrates, carp have a high potential to bio-accumulate contaminants residing in the bottom sediments that are being taken up by organisms lower down the food chain. Their abundance in the Charles River system increases the likelihood of human consumption and makes them a desirable target species for collection.

Yellow perch (*Perca flavescens*) were also selected for collection as they feed within the water column and off the bottom, consuming small fish and invertebrates. They are also a source of food for the largemouth bass. This species is relatively abundant in the Charles River and is known to be consumed by the general public.

Fish sampling consisted of collecting fish from six different segments (Table I) of the Charles River basin. Five of these segments were in the lower basin, and the sixth was from the "lakes" region in Waltham/Newton. Sampling segment boundaries were delineated simply by major bridge crossings on the river with the exception of the uppermost "reference" location, and that from the Kendall Power Plant outfall down river (selected for baseline power plant/permitting issues). Field survey efforts took place at dusk and into the late evening hours utilizing EPA Lexington's electro-fishing boat. A geographic positioning system was used to track the shock path of the boat for each segment with the exception of segment 7F where the unit failed. Maps

of these shock paths are found in Appendix III. The targeted species were collected from each designated river segment with the exception of segment 1F, where not enough yellow perch could be collected to meet the required sample volume needed for analysis. Black crappie were substituted for yellow perch in this segment. Five-fish composites for each species were collected within the targeted individual size range. All fish were within a similar age class which was roughly ascertained in the field by length comparisons. Largemouth bass were selected in a size ranging between twelve and fifteen inches. The lower range is the minimum legal size (12 inches) and is representative of the consumable size slot most likely to be caught and eaten by the public. A size slot was not determined for carp other than that the fish be as close as possible in length to one another.

**TABLE I
CHARLES RIVER SAMPLING SEGMENTS**

Site #1F	From Rte 30 bridge in Newton to Woerd Ave. boat ramp in Waltham
Site #3F	From the Newton Yacht Club upstream to North Beacon Street Bridge
Site #4F	From Arsenal Street Bridge to Elliot Bridge
Site #5F	From River Street Bridge to BU Bridge
Site #7F	From Mass. Ave Bridge to Longfellow Bridge
Site #9F	From the vicinity of Kendall Power outfall and Broad Canal to Museum of Science

Fish were collected throughout a “sampling run” and retained in an on-board live well until the desired sizes and number of targeted species were collected. All other fish were returned to the waterbody. Fish were transported to the laboratory in the live well and processed within 24 hours. Upon arrival at the laboratory fish were logged in, weighed, length and sex determined, and sorted by species. Each individual fish was then filleted skin off and the fillets weighed. The remaining parts of the fish were then weighed and recorded as offal. Skin off was selected based on the Massachusetts Department of Environmental Protection’s (DEP) protocols, and to improve comparability between EPA and previous DEP sampling efforts. The Massachusetts Department of Public Health (MADPH) has established fish advisories on the Charles River using the MADEP sampling protocols. FDA protocols are based on scaled, but “skin on” fillets. This represents a worst case exposure for human consumption due to the harboring of contaminants in the fatty layer residing between and within the fish skin and muscle tissue. These samples could be considered biased low if the general public consumes or prepares these types of fish from the Charles River with the skin on.

Additional bench work-up included extraction of otoliths and scales for determining age, and the collection of bile as an experimental trial to determine PAH metabolite concentrations. Age determination and weight/length relationships were carried out by the United States Fish & Wildlife Service's Fisheries Assistance Branch in Laconia, NH (Appendix I).

Compositing of same species/same location samples consisted of weighing out equal weights of edible tissue from each individual fish of a species and homogenizing them together. These samples were then freeze dried and blended prior to extraction and analyses. A similar procedure was used for the offal as well as fillets. Separate analyses of offal and filleted samples allows for post-analytical calculation of reconstructed "whole body" contaminant burdens, which can be utilized for making determinations regarding ecological health and risk. Optimizing sampling effort and use of analytical results were primary goals of this survey.

Analyses

The interpretation of contaminant residues in humans and wildlife is, at a minimum, a complex science. Contaminants may have drastically different effects on an organism depending on the species it interacts with, its life stage, exposure times and concentrations, the synergistic effects of other contaminants, the degradation state of the contaminant in question, the medium in which it resides and the chemical characteristics of the medium (ie. redox potential, pH). Contaminants may also demonstrate no visible effects on an organism, but be deleterious to its progeny, passing contaminants on to unborn fetuses or eggs. Most of the limits or action levels established for human health have been established based on substantial data, often collected worldwide and extensively peer reviewed prior to establishing a threshold level, with an added margin of safety.

Interpretation of wildlife tissue concentrations often involves more uncertainty due to many of the factors listed in the preceding paragraph. Interpretation of results usually necessitate extensive literature searches to compile data on similar organisms at similar life stages in order to make reasonable assessments or determinations of risk and health. Threshold levels established for many contaminants in wildlife are based on what is deemed the most sensitive species and life stage (ie. lake trout sac fry), but in most cases there is uncertainty associated with what the contaminant residue levels may mean to the present organism or its offspring. These factors should always be considered when interpreting wildlife contaminant data.

The analytical laboratory services for this survey consisted of separate muscle tissue and offal analysis for PCBs and organochlorine pesticides, PAHs, metals including total mercury, % lipids, and dioxins. Extracted fish liver bile was originally targeted for experimental analysis of PAH metabolites but was found problematic and was discontinued. The following are brief synoptic reviews of the analyte data sets and some information on the target compound. Data tables for the various analyses can be found in Appendix II. All methods followed USEPA approved standard laboratory methods. Analytical details may be found in the approved quality assurance plan at the USEPA Region 1 laboratory in Lexington, Massachusetts.

Metals

Analyses for total mercury were completed on all the fish collected from the Charles River. Total mercury analysis rather than methyl mercury analysis was performed as a cost effective approach, based on widespread findings that 85% or more of the mercury found sequestered in fish tissue is of the more toxic methylated form (Wren, Harris, Harttrup 1995). All fish fillet samples from the Charles River survey were below the FDA's action level of 1.0 ppm wet weight, ranging from 0.07 to 0.48 ppm. Some states, including Massachusetts, use an action limit of 0.5 ppm for issuing advisories for sensitive populations. These concentrations fall within what are considered natural background concentrations (0.01 - 0.5 ppm) for most fish by the FDA. The FDA considers the 1.0 ppm action level to be ten times lower than the lowest mercury concentration where toxic effects to humans have been observed. Although the differences are small, Figure 1 shows the distribution of mercury in the edible fillet samples by species and location. Largemouth bass show slightly higher concentrations than the carp or perch, which appear to have quite similar body burdens. Mercury is known to bioaccumulate and biomagnify up the food chain, and largemouth bass are the dominant top level predator species in the lower Charles River system. This species would be expected to have higher tissue concentrations.

One of the major sources of mercury in the Northeast is atmospheric deposition. The inorganic mercury deposited from the atmosphere into aquatic systems is transformed by resident bacteria to methyl mercury. The methyl mercury form is readily transferred across gill membranes as well as by the consumption of other fish in the food chain. Mercury is concentrated in organisms as it binds to proteins in the muscle tissues. Toxic effects of mercury have been observed in fish occurring in the range of 10 to 30 ppm whole body wet weight concentrations. However, mercury is not as acutely toxic to fish as other metals such as cadmium, lead, copper, or zinc.

Cadmium is one of the more toxic metals to fish and has demonstrated adverse effects including high mortality, reduced growth, and reduced reproductive success at ambient water concentrations in the 10 ppb range. Whole body wet weight concentrations in fish exceeding 2.0 ppm should be considered as evidence of cadmium contamination (Eisler 1985). Cadmium was not detected above the analytical reporting limits (0.09 - 0.2 ppm) in any of the Charles River fish fillet or offal samples. Primary sources of cadmium in the environment are fuel combustion and metal smelting (Lymburner 1974).

Lead levels in edible fillets are considered hazardous to human health at or above 0.3 ppm wet weight (Schmitt et al. 1984). None of the fish fillets exceeded this level, but a high proportion of the fish offal did. Lead is concentrated in the hard tissues of organisms, mostly residing in the bones and teeth. It is not known to biomagnify in aquatic food chains. Sources of lead include historical deposition from automobile exhausts and urban runoff, smelting and refining, sewage sludge, historic pesticide use, and lead artifacts from fishing and hunting. The highest wildlife risk presently appears to be for waterfowl and piscivorous birds and mammals. As with many of the metals, bioavailability in aquatic systems is regulated by many factors, including water hardness, pH, alkalinity, and in stream organic content.

The FDA has established action levels for chromium in crustaceans and molluscs at 12 and 13 ppm wet weight, respectively. No action level has been established for fin fish, but tissue levels in excess of 4.0 ppm dry weight total chromium should be viewed as evidence of contamination (Eisler 1986). Largemouth bass fillets from station 4F and yellow perch from station 5F exceeded this total chromium concentration. Offal samples in several instances also exceeded this value.

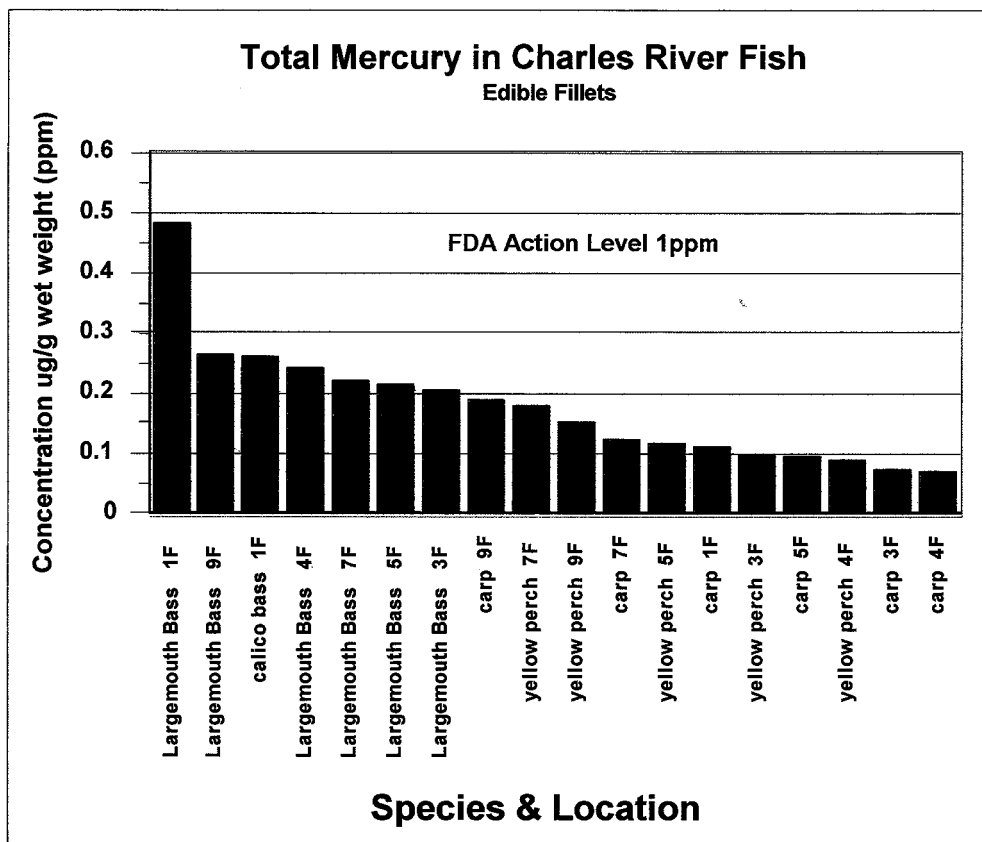


Figure 1

There is high variability among species of toxic effects of chromium, but chromium concentrations observed among fish from the Charles River fell within these concentrations. It appears likely that there may be some chromium contamination in these fish.

There are two toxic forms of chromium, hexavalent chromium and trivalent chromium; the former being known as the more toxic of the two. Sources of chromium come from historic tannery wastes, metal plating operations, steel production, municipal wastewater sludges, and many chemicals.

The FDA has no established action levels for copper, and information on copper body burdens in fish tissue is scarce. Copper has been recognized as abnormally affecting fish behavior, metabolism and growth at water concentrations ranging between 4 and 10 ug/L. Further

literature searches need to be done to determine implications of the copper body burden of Charles River fish.

PCBs

EPA's New England Regional Laboratory analyzed thirty-six fish tissue fillet and offal samples for polychlorinated biphenyls, (PCBs), and organochlorine pesticides. PCB samples were analyzed for non-congener specific Aroclors.

The analyses revealed that some carp fillet samples exceed the U.S. Food and Drug Administration's (FDA) tolerance limit for PCBs in fish tissue of 2.0 parts per million. This limit is used by the FDA to trigger removal of food products from the market. The tolerance limit does not necessarily represent acceptable levels for consumption. However, some states use this limit to trigger fish consumption advisories for a water body or segment of a water body.

In May of 1996, based upon FDA tolerance limits, the Massachusetts Department of Public Health (MDPH) issued a carp consumption advisory for the lower Charles River system between the Hemlock Gorge Dam in Needham/Newton to the Museum of Science Dam in Boston. The advisory was directed toward sensitive populations, specifically for children under the age of twelve and for pregnant and nursing mothers. Some of the individual carp fillet samples from the 1995 survey exceeded the U.S. FDA tolerance level of 2.0 parts per million and fall within the same range as those collected for this 1999 survey.

Figure 2 shows the concentrations of PCBs in fillet samples among species and across sampling segments of the Charles River. Carp have the highest PCB concentrations of all species in offal and fillets, followed by largemouth bass, yellow perch, and calico bass, respectively. Carp from station 9F are well above the FDA tolerance limit and are the oldest fish collected in this survey. High lipids and high PCB concentrations correlate relatively well, as expected considering the lipophilic nature of PCBs. This is especially true for the fillets, $r = 0.85$, which are a much more homogeneous mixture of tissue than the offal (ie. scales, skin, and bone) and therefore less variable.

In aquatic environments, PCBs commonly bind to fine sediments in river bottoms, providing a PCB "sink" where they may remain until they are disturbed or re-suspended, or in the case of carp, ingested. The bottom feeding nature of carp and their high fat content provide a pathway for PCB bioaccumulation into carp tissue. Additionally, carp in this survey are the oldest in age on average of all the species collected and provide another factor potentially contributing to higher PCB body burdens (longer exposure time) than the other species.

Unlike the bottom feeding nature of carp, largemouth bass are considered top-level predators in the Charles River, feeding on smaller bait/forage fish and bottom dwelling organisms, such as

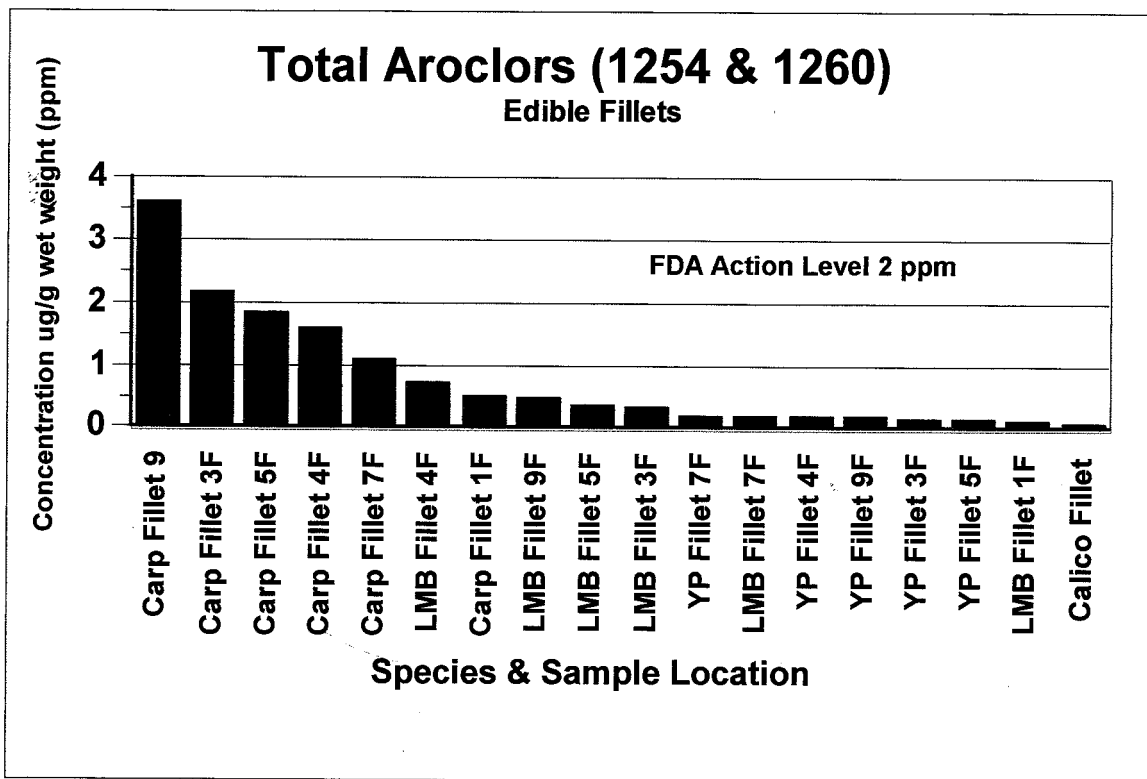


Figure 2

crayfish. Inspection of largemouth bass stomach contents revealed both of these food sources to be common. Crayfish are bottom scavengers and are known to accumulate PCBs, providing a potential biomagnification link up the food chain. Yellow perch and calico bass (*Pomoxis nigromaculatus*) are considered generalist feeders, feeding off small forage fish, bottom dwelling insects, egg masses, and larvae. These species are on average younger than the other species caught in this study, and contain significantly less % lipids than the carp and to a lesser extent, the largemouth bass. The PCB concentrations reflect these factors.

The trophic status, age, and lipid content are all factors likely to be contributing to the concentration of PCBs in Charles River fish. Sediment data excluded from this study with the exception of dioxin analysis, might provide additional insight into the uptake potential of contaminants into fish tissue (see Breault et al. 2000).

Field studies have shown different biological responses in fish associated with aquatic habitats contaminated with PCBs, including egg and fry mortality, decreased length and weight, presence of hepatic neoplasms and lesions, fin erosion, and increased ah hydroxylase activity. Eisler proposed 0.4 ppm total PCB whole body wet weight of fish as an appropriate criteria for the

protection of aquatic life (Eisler 1986). Since this time there has been broad agreement that significantly more testing and evaluation should take place in order to determine species-specific toxic effects of planar and non-planar PCBs. These apparent toxicological responses are often difficult to directly attribute to PCB concentrations from the surrounding habitat or organism body burdens because of the presence of other contaminants which potentially act synergistically to bring about the observed effects. In addition, PCBs are found in many different forms with varying levels of associated toxicity which make determinations of their effects difficult. In spite of this, controlled laboratory studies on aquatic organisms do indicate that PCBs can bring about toxicological responses at low ambient water concentrations and/or low body burden levels (Niimi 1996). The PCB concentrations found in the Charles River fish warrant the continuation of human health fish advisories and should raise some question as to the ecological health of the species surveyed. Based on a comparison of the previous contaminant data with this latest survey, it does not appear that there have been any changes in the PCB tissue concentrations in the carp of the Charles River.

Manufacturing of polychlorinated biphenyl mixtures initially started in 1929 and shortly thereafter were being produced globally. In the United States, PCB mixtures were commercially manufactured under the trade name Aroclors. They were characterized by a four digit numbering system of which the last two digits represented the percentage of chlorine contained in the mix. Aroclor 1260 for example, is approximately 60% chlorine, Aroclor 1254, is approximately 54% chlorine. Mixtures varied from sixteen to sixty-eight percent chlorine. The more chlorine associated with the mixture, the greater the molecular weight and the more viscous it became. Higher viscosities made it more resistant to environmental degradation or "weathering" processes such as photolysis, dissolution, and biodegradation. These characteristics make PCBs pervasive in the environment and they are commonly found today in aquatic habitats and organisms.

From an industrial perspective, PCBs were extremely useful. They are essentially electrically non-conductive and inert, and are an excellent heat dissipating medium. They can withstand temperatures in the 250 to 360°C range which make them desirable for flame resistant manufacturing products and for use in such things as electrical transformers and fluorescent light ballasts. The down side is that they are suspected carcinogens, and when they become thermally unstable or combust they produce highly toxic dioxins and furans. Because of the desirability of these compounds, historical production was high and indiscriminate waste discharges into aquatic systems common. Today PCBs are ubiquitous in the environment and can be found virtually anywhere from inner city to the remotest of areas; all upper trophic organisms are likely to have some level of PCBs residing within them. The increasing evidence that PCBs could cause health effects and burgeoning public concern led to the discontinuance of PCB manufacturing in 1979, yet twenty years later their presence is still apparent.

Organochlorine Pesticides

Analytical method 8081A was run to assess the concentration of organochlorine pesticides. Only those compounds that were detected are discussed. These compounds represent some of the

more common historical pesticides used until the late 1980s. Many of these compounds are known to cause central nervous system disorders, organ damage, reduced reproductive viability, teratogenic effects, and mortality. They are lipophilic and many are very persistent in the environment, degradation products of the parent compound often lasting decades. Table II lists the compounds detected and their respective concentrations.

Following the advent of its commercial production in the late 1930's (originally synthesized in 1874), Dichlorodiphenyl-trichloroethane, commonly known as DDT, was produced along with other organochlorine pesticides at unprecedented rates until emerging evidence revealed their indiscriminate and acute lethality to wildlife other than the organisms they were targeted for exterminating. Even with mounting scientific evidence and public concern, production only declined after the organisms for which DDT was designed demonstrated increasing tolerances to the pesticide. Banning of many of these pesticides from sales and production did not occur until much later. Due to the persistent nature of many of these compounds, metabolites and degradation products of the parent compounds are still found lingering in the environment.

DDT was known for its acute toxic effects early on but the sublethal effects such as eggshell thinning and reproductive decline or failure were not ascertained until analytical techniques were developed that could recognize the compound and its metabolites in environmental samples (Blus 1996).

DDT showed up in only one carp sample at a concentration of 4 ppb. Its metabolites however, DDE and DDD, were present in all of the samples and ranged from 0.01 to 0.25 ppm in fillets and .08 to 0.6 ppm in offal for DDE. DDD concentrations ranged from 0.01 to 0.22 ppm in fillets and 0.03 to 0.4 ppm in offal. Concentrations showed a very distinct relationship with the lipid content in these fish, as would be expected based on its lipophilic affinity. All samples were well below the FDA action level of 5 ppm DDT for human consumption of edible fillets.

Chlordane, or technical chlordane, was used as a pesticide for home use on lawns and gardens and commercially for crops such as corn and citrus fruits. Being a very persistent compound consisting of chlordane and a mixture of related chemicals, it can reside for decades in the environment. Manufacturing took place from 1948 until 1988. All applications of chlordane were banned in 1983 with the exception of termite control, which was subsequently banned in 1988. Human exposure to chlordane was predominantly from the consumption of contaminated foods, with high exposures resulting in nervous system and or liver damage. The FDA action level for technical chlordane is 0.1 ppm in edible fish fillets. Technical chlordane was not detected in the fish survey for the Charles River, but alpha and gamma chlordane were detected in trace amounts. The action level for technical chlordane or the sum of individual (ie. alpha & gamma) components, with the exception of heptachlor epoxide, is 0.3 ppm in edible fillets (USFDA 2000). Compositated fillets from Charles River fish did not exceed 0.13 ppm total chlordane.

Aldrin is a pesticide historically used as a rodenticide and for controlling insects and birds. This

TABLE II
Pesticides in Charles River Fish
mg/kg wet weight (ppm)

Station	Sample type	% solids	% Lipids	Alpha Chlordane	Gamma Chlordane	Total Chlordane	DDD	DDE	DDT	Dieldrin	Endrin
1F	Calico Bass Fillet Composite	20	0.6	0.0039	0.0011	0.0050	0.0066	0.0239	ND	0.0013	ND
1F	Carp Fillet Composite	27	5.5	0.0457	0.0237	0.0694	0.0715	0.1234	ND	0.0177	ND
3F	Carp Fillet Composite	24	5.9	0.0075	0.0237	0.0312	0.2141	0.2163	ND	0.0127	ND
4F	Carp Fillet Composite	23	3.9	0.0138	0.0297	0.0435	0.1598	0.1457	ND	0.0122	ND
5F	Carp Fillet Composite	23	3	0.0132	0.0229	0.0361	0.1197	0.1340	ND	0.0103	ND
7F	Carp Fillet Composite	24	5	0.0162	0.0220	0.0382	0.1071	0.1067	ND	0.0187	ND
9F	Carp Fillet Composite	27	7.3	0.0845	0.0431	0.1276	0.2241	0.2573	ND	0.0335	0.0274
1F	LMB Fillet Composite	21	0.3	0.0019	0.0006	0.0025	0.0048	0.0226	ND	0.0012	ND
3F	LMB Fillet Composite	22	0.9	0.0030	0.0026	0.0056	0.0187	0.0470	ND	0.0028	ND
4F	LMB Fillet Composite	22	1.1	0.0033	0.0041	0.0074	0.0388	0.0875	ND	0.0037	ND
5F	LMB Fillet Composite	23	0.7	ND	0.0007	0.0007	0.0138	0.0435	ND	0.0027	ND
7F	LMB Fillet Composite	22	0.3	0.0030	0.0022	0.0052	0.0122	0.0283	ND	0.0027	ND
9F	LMB Fillet Composite	22	1	0.0013	0.0021	0.0034	0.0228	0.0440	ND	0.0036	ND
3F	YP Fillet Composite	20	0.4	0.0018	0.0023	0.0041	0.0102	0.0175	ND	0.0019	ND
4F	YP Fillet Composite	20	0.5	0.0011	0.0027	0.0038	0.0123	0.0194	ND	0.0021	ND
5F	YP Fillet Composite	20	0.3	0.0074	0.0014	0.0088	0.0057	0.0134	ND	0.0017	ND
7F	YP Fillet Composite	21	0.5	0.0023	0.0017	0.0040	0.0076	0.0146	ND	0.0027	ND
9F	YP Fillet Composite	20	0.4	0.0029	0.0014	0.0043	0.0079	0.0124	ND	0.0017	ND
1F	Calico Bass Offal Composite	30	2	0.0161	0.0045	0.0206	0.0330	0.0822	ND	0.0059	ND
1F	Carp Offal Composite	37	12.5	0.0877	0.0443	0.1320	0.1473	0.2739	ND	0.0130	ND
3F	Carp Offal Composite	34	12.8	0.1704	0.0893	0.2597	0.4893	0.3917	ND	0.0611	ND
4F	Carp Offal Composite	37	5	ND	0.0360	0.0360	0.2200	0.2400	ND	0.0162	ND
5F	Carp Offal Composite	33	11.7	0.0433	0.0841	0.1274	0.4334	0.6094	ND	0.0439	ND
7F	Carp Offal Composite	34	12	0.0168	0.0380	0.0548	0.2641	0.2905	ND	0.0370	ND
9F	Carp Offal Composite	32	12.8	0.0258	0.0844	0.1102	0.6075	0.4375	ND	0.0476	ND
1F	LMB Offal Composite	27	3.1	0.0219	0.0057	0.0276	0.0698	0.2972	0.0047	0.0080	ND
3F	LMB Offal Composite	31	5.5	0.0091	0.0169	0.0260	0.1604	0.3135	ND	0.0183	ND
4F	LMB Offal Composite	32	4.7	0.0094	0.0135	0.0229	0.1998	0.5589	ND	0.0162	ND
5F	LMB Offal Composite	32	4.9	0.0200	0.0210	0.0410	0.1522	0.3368	ND	0.0218	ND
7F	LMB Offal Composite	29	4.8	0.0321	0.0198	0.0519	0.1521	0.2604	ND	0.0242	ND
9F	LMB Offal Composite	30	5.6	0.0510	0.0159	0.0669	0.1869	0.2498	ND	0.0406	ND
3F	YP Offal Composite	31	3.7	0.0116	0.0286	0.0402	0.1800	0.1843	ND	0.0198	ND
4F	YP Offal Composite	29	4.3	0.0231	0.0250	0.0481	0.1644	0.1882	ND	0.0236	ND
5F	YP Offal Composite	24	3.2	ND	0.0038	0.0038	0.0799	0.1389	ND	0.0137	ND
7F	YP Offal Composite	30	3.6	0.0285	0.0186	0.0471	0.1202	0.1568	ND	0.0352	ND
9F	YP Offal Composite	30	3.5	0.0088	0.0178	0.0266	0.1656	0.1837	ND	0.0246	ND

product rapidly broke down in the environment to Dieldrin, which is equally as toxic as the parent compound Aldrin. The Charles River fish samples showed no detectable level of Aldrin, but Dieldrin was found in all samples, the highest concentrations being found in the offal as with the other pesticides (highest lipid content). The FDA action level for Aldrin/Dieldrin is 0.3 ppm in edible fillets; all fillet samples were at least an order of magnitude below this level. Human exposure to Aldrin/Dieldrin is usually through consumption of foods, producing nervous system damage when years of exposure result in toxic levels in the body. In wildlife, ring-necked pheasant egg production ceased by ten weeks on 1 to 2 ppm per day diet (Genelly & Rudd 1956) Dieldrin has shown a lethal level of 5 ppm in experimental studies and from investigative studies on waterfowl and other birds that were found dead in the field (Stickel & Spann 1969). This pesticide was used from 1950 until a ban in 1970, with the exception of its use for termite control. Total banning of the product by the USEPA occurred in 1987.

Dioxin

Sixteen edible tissue and offal composite samples and six sediment samples were sent to EPA's Region 7 laboratory in Kansas City, Kansas for dioxin analysis. Samples consisted of carp and largemouth bass from sampling reaches 1F, 4F, 5F, and 7F, and one yellow perch fillet and one offal sample from segment 5F. Sediment samples were only analyzed for dioxin in this survey.

Results of the dioxin analysis revealed its presence in the sediment samples, fillet and offal samples of carp, and in some largemouth bass. However, the concentrations detected were extremely low, being found only in trace quantities, and falling within known background levels in the United States, which range from four to fourteen parts per trillion (U.S. EPA 1994).

These concentrations in fish and sediment present a low risk based on current scientific literature (EPA/600/R-93/055 March 1993); low risk being defined as "the highest concentration that is unlikely to cause significant effects to sensitive organisms." Sediment samples held the highest concentrations. Dioxin was found in carp from all locations and in largemouth bass at locations 5F and 7F. Highest concentrations in fish were found in the offal of carp and largemouth bass. Carp fillets were also found to contain dioxin. All other samples of largemouth bass and yellow perch revealed no detectable dioxin concentrations.

Although dioxin is present at background levels in these samples, the nature of the analytical results make it worthy of some discussion. Table III displays the dioxin data. The table illustrates that dioxin concentrations in carp offal are the highest of all the fish collected and can be partially attributed to the presence of dioxin-laden sediment within the intestinal tract. Carp take up sediment as they feed, sorting out palatable organisms and materials and subsequently rejecting less desirable coarse sediments and detritus. Not being the most efficient process, much of the finer materials involuntarily make their way into the carp's intestinal tract. Visual inspections of these fish during processing revealed the prevalence of fine sediments throughout the gut. Carp offal contains a high lipid content compared to filleted muscle tissue, which attracts these lipophilic and hydrophobic dioxin-like compounds, sequestering them within the more fatty body

tissues. Offal samples are comprised of the entire fish less the fillets, and not just limited to the gut and visceral organs. Bone, skin, and scales comprise the offal sample as well. Had analyses been done exclusively on the internal gut cavity organs, these values would likely be significantly higher, considering dioxin compounds have such a high affinity for concentrating in target organs (ie. liver). This may underestimate the concentrations and fish health effects of dioxin contaminants from an ecological perspective.

Differences of dioxin concentrations in carp among the various segments as well as samples may be due to differences in age among the composited fish samples. The highest concentration of 2,3,7,8- TCDD, the most toxic and bioaccumulative of the seventeen dioxin-like congeners assessed was found at site 4F where the average age of the fish was eight years compared to site 1F where the average age was four years. The 2,3,7,8 -TCDD molecule does not metabolize easily, residing in fish tissue and bioaccumulating more readily than the non 2,3,7,8 congeners. Fish have the ability to transform many of the other congeners from nonpolar to polar metabolites which allows them to pass easily through and out the body of the fish.

Several of the fish samples reveal 2,3,7,8 - TCDD concentrations, yet sediment samples from the same river segments do not. One possible explanation is that levels in the sediment are at concentrations below the detection limits of the analytical instrument, yet since 2,3,7,8-TCDD is bioaccumulative, it has concentrated in the fish over time, resulting in detectable body burdens. Sources contributing to the presence of dioxin within the Charles River are not easily discernable. Historical and present atmospheric deposition from industrial emissions, proximity of roadways and associated vehicle emissions, historic discharges to the river, and residual artifacts from PCB contaminated sediments could all be potential sources.

Dioxins and furans can be by products of industrial processes and manufacturing. Produced from a variety of sources, they are associated with waste incineration and paper manufacturing, cigarette smoke, diesel exhausts, barbecuing of meats, chimney soot, and sewage sludge. With the exception of the latter, these compounds are often attached to particulates that are emitted by these processes and transported great distances prior to settling into particular environmental "compartments." The environmental relevance of dioxins is that they can be extremely toxic in certain forms and to certain organisms, producing mortality, carcinogenicity, and teratogenic effects. Dioxin is a known human carcinogen, endocrine disrupter, and ranks highly as one of the most toxic chemicals regarding human health. Their toxicity is highly species dependent and can vary widely depending on the life stage of the organism.

"Dioxin-like" refers to a class of compounds that can include dioxins, furans, and polychlorinated biphenyls depending on the nomenclature used. The USEPA uses a nomenclature that addresses only dioxins and furans, while the World Health Organization (WHO) terminology includes dioxin-like coplanar polychlorinated biphenyls. These compounds are similar structurally and toxicologically, and vary only by the number and position of chlorine atoms on the molecule.

Compounds with the same number of chlorine atoms, but located in different positions are termed congeners. There are 210 known dioxin and furan isomers in the environment, and of these a total of seventeen (seven dioxin congeners and ten furan congeners) are known to bioaccumulate. These particular congeners contain chlorine atoms located in the 2,3,7,8 positions on the molecule and are coplanar. Since these compounds can exist in the environment in complex and often innumerable combinations and concentrations, all varying in their levels of toxicity, an approach has been established by the World Health Organization to compare all dioxin-like compounds found in a sample to the most toxic and well known dioxin, 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD). Each of the seventeen recognized dioxin-like congeners has an established Toxic Equivalency Factor (TEF) assigned to it based on extensive peer review of scientific databases and consensus among world scientists. Each concentration of a congener found in a mixture is multiplied by the TEF and summed with the other congeners in the mixture to derive a toxic equivalent, or TEQ. The TEQ can then be used to compare relative toxicities among multiple samples within a specific medium.

The USEPA established low and high risk dioxin concentrations for sensitive fish by exposing lake trout eggs to the toxicant and measuring embryo and sac fry mortality. Lake trout are considered highly sensitive to dioxin, and embryo and sac fry the most sensitive life stages of the species. A tissue concentration of fifty parts per trillion is considered the low risk concentration for fish and the threshold level at which no reproductive effects are observed within the species. The established high risk fish tissue concentration of 80 parts per trillion is derived from dioxin doses expected to cause 50 to 100% mortality in embryos and young of sensitive species (EPA 1993). Concentrations of dioxin in fish of the Charles River collected from this survey are well below these established values.

Summary & Conclusions

Analyses of over one hundred fish collected from the lower Charles River system and representing three different trophic groups were found to be within accepted USFDA action limits established for protection of human health through consumption; the one exception being Polychlorinated biphenyls. PCBs exceeded the established action level at three locations and were elevated in the other river segments. Based on the present data, PCB concentrations in edible fillets do not appear to have diminished when compared to previous studies undertaken in 1985 and 1995. Metals concentrations did not exceed any of the established action levels, and mercury was below the Massachusetts state trigger level. Pesticide levels and dioxin levels were also low and did not exceed any established limits for human health concerns. Noticeable internal anomalies were observed in many of the fish however, especially carp, and the concentration of many of these analytes may be cause for concern from an ecological perspective. The majority of contaminant analytes were found to be elevated to a much greater degree in the offal than the edible fillets portions, with the exception of mercury. Analytical results typically followed predictable bioaccumulative pathways with the sequestering of contaminants in target tissues and species dependent upon lipid content and trophic status. Largemouth bass revealed the highest mercury concentrations localized in muscle tissue, whereas carp had the highest concentrations of PCBs

and % lipids. Despite the low levels of contaminants detected in most cases, the fact that they are still present years after production has ceased highlights their persistence and tells us they will be with us for some time to come. Continued opportunities should be sought to gain more insight into the possible ecological impacts resulting from these contaminants as well as the acknowledgment that continued diligence is warranted for the protection of human health from consumption of Charles River fish.

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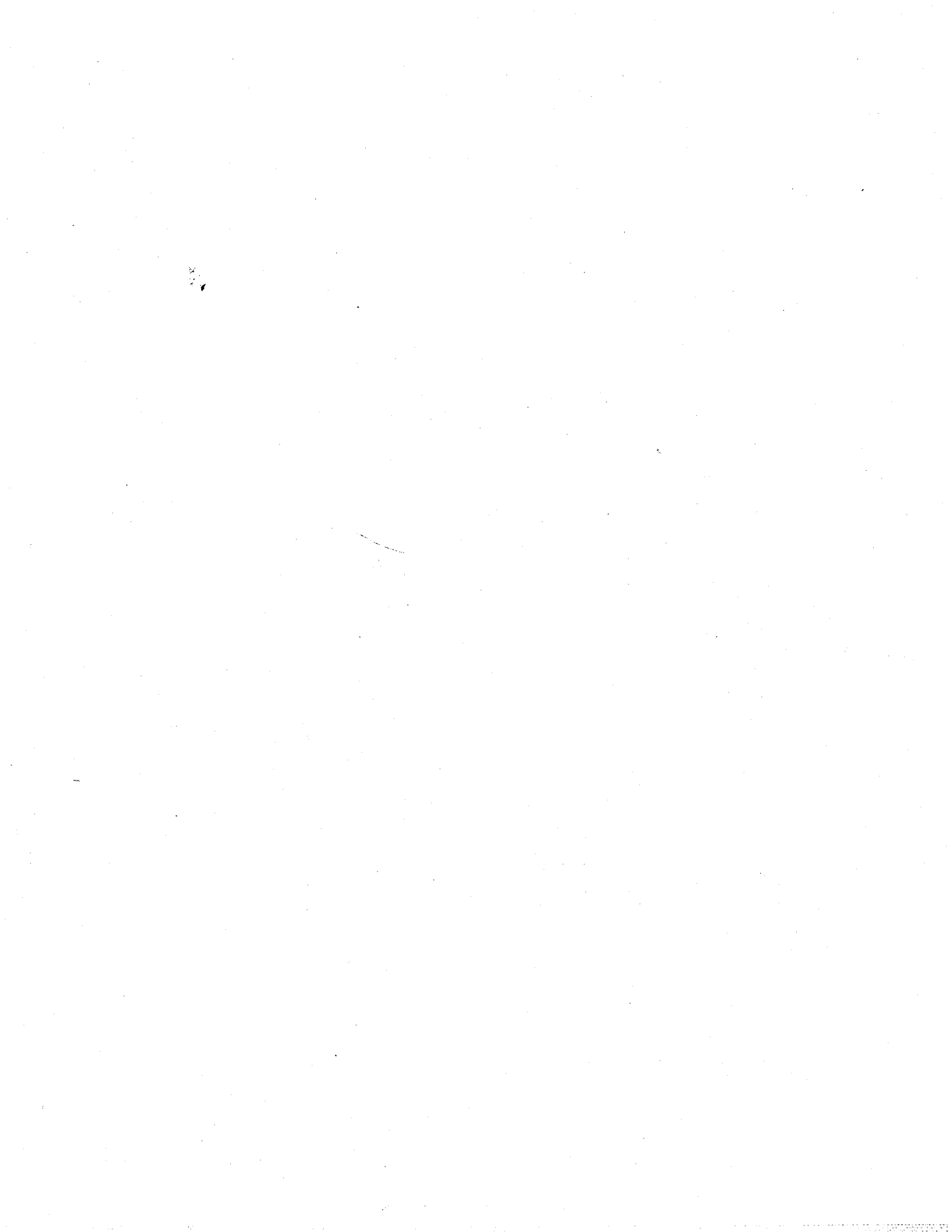
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APPENDIX I
USFWS AGE DETERMINATION REPORT



**Age Determination of Fish Species Sampled
from the
Charles River, Massachusetts**

December 1999

Office of Fishery Assistance
U.S. Fish and Wildlife Service
Federal Building, Room 124
Laconia, NH 03246

**under:
U.S. Environmental Protection Agency Interagency Agreement
Number DW-14-94022501-0**

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APPENDIX A. Comparison of the length-weight relationship and length-age relationship of largemouth bass captured from sites 1F, 3F, 4F, 5F, 7F, and 9F, and comparisons of individual sites to a composite of largemouth bass captured from all sites on the Charles River, November 1999. 11

APPENDIX B. Comparison of the length-weight relationship and length-age relationship of common carp captured from sites 1F, 3F, 4F, 5F, 7F, and 9F, and comparisons of individual sites to a composite of largemouth bass captured from all sites on the Charles River, November 1999. 17

Introduction

This report provides the results of the age analysis of fish captured in the Charles River, Middlesex County, Massachusetts between November 1 and November 18, 1999 under the direction of the U.S. Environmental Protection Agency. Following sampling, representatives of the U.S. Environmental Protection Agency delivered otoliths and/or scales to the U.S. Fish and Wildlife Service for age analysis. The age analysis and final report was completed by the Office of Fishery Assistance, U.S. Fish and Wildlife Service, Laconia, New Hampshire.

The purpose of the collections was to measure body burden of chemicals or contaminants in fish tissue for the use in Ecological Risk and Human Health Risk Assessments. Fish were captured at six sites on the Charles River, and scales and/or otoliths were removed or extracted from these specimens from collection sites as described in Table 1. Otolith(s) and scales from five largemouth bass (*Micropterus salmoides*), scales from five common carp (*Cyprinus carpio*), and scales from one composite sample of either black crappie (*Pomoxis nigromaculatus*) or yellow perch (*Perca flavescens*) were obtained and catalogued for each of the six sites (from EPA IAG DW-14-94022501-0).

This report is divided into three sections, Section One contains the age analysis for largemouth bass, Section Two contains the age analysis for common carp, and Section Three contains the age analysis for the composite samples of either yellow perch or black crappie. Each section is further broken down into three Subsections: Methods, Data, and Discussion.

The Methods Subsection contains specific steps used for preparing and analyzing the otoliths and/or scales used to age the specimens of the specific species being analyzed.

The Data Subsection contains a master table which includes the sample code, length, weight, gender, age, method of age determination, and comments for specific species being analyzed. In addition, in Sections One (largemouth bass) and Two (common carp), the Data Subsections contain composite graphs for both the length-weight relationships and the length-age relationships of all sampled specimens for that species from all sampling sites. The appendices contain graphs which show the length-weight and length-age relationships of the fish specimens caught at individual sites to a composite of fish for that species caught in all sites. This additional information is given to help the reader visually compare the characteristics of fish sampled from one site to those sampled from all other sites.

The Discussion Subsection contains an overview of observations made during the aging process, and an explanation of the limitations in determining the ages of the specimens using the structures employed. This subsection also explains quality control measures that were used in determining the ages of fish specimens for that species.

Table 1. Description of Sampling Sites on the Charles River, November 1999.

Site Identification Code	Site Description
1F	From Woerd boat launch in Newton, MA upstream to Route 128 Bridge
3F	From Newton Yacht Club upstream to North Beacon Street Bridge
4F	From Arsenal Street Bridge upstream to Elliot Street Bridge
5F	From River Street Bridge upstream to Boston University Bridge
7F	From Longfellow Bridge upstream to Mass Avenue Bridge
9F	From Museum of Science upstream to Longfellow Bridge

Section 1. Largemouth Bass Age Determination

A. Methods

Five to six largemouth bass specimens were sampled from each of the six sites. With only three exceptions, both sagittal otoliths and scales were collected from each specimen. For specimens in which otoliths were obtained, this structure was used exclusively for age determination. In two cases where otoliths were not obtained, scales were used to determine the age of the specimens. For one specimen, neither scales nor otoliths were obtained and no age determination was made.

Otoliths were cleaned and dried using a method of bleach soaking, distilled water rinsing, and ethanol soaking followed by air drying. Cleaned otoliths were embedded in epoxy resin and thin sectioned (15-20 microns) through the transverse plane using an Isomet low speed saw with a diamond cutting blade. The sectioned wafers were clarified using clove oil, and permanently mounted on microscopic slides with Cytoseal 280 mounting medium. The number of annuli contained on the otoliths were determined using a dissecting scope, compound microscope, or overhead projector with transmitted light. Each dark band was considered a yearly mark (annulus). The age of the specimen was given as being equal to the number of annuli counted (see discussion for explanation).

Scales were cleaned and mounted between microscopic slides, and read using an overhead projector with 50X magnification. Three scales containing the most distinct annuli were identified and the positions of their focus, annuli, and margin were recorded on specially designed data sheets. Annuli were determined by the signatures formed by the circuli, specifically, the constriction and expansion patterns in intercirculi spacing, cutting over of the circuli, and the

extension of circuli into the posterior field (Lager 1952).

B. Data

Table 2: Total lengths, weights and ages of largemouth bass captured from the Charles River, November 1999.

Fish Identification Code	Site	Length (cm)	Weight (g) ¹	Sex	Age ²	Method	Comments
LB01-1F	1F	36.1	678	F	4	O	
LB02-1F	1F	NA	1068	M	8	O	
LB03-1F	1F	38.1	815	F	5	O	
LB04-1F	1F	35.6	738	F	6	O	
LB05-1F	1F	36.8	713	M	8	O	
LB01-3F	3F	37.5	803.7	M	5	O	
LB02-3F	3F	37.8	837.0	F	4	O	
LB03-3F	3F	37.8	764.4	M	4	O	
LB04-3F	3F	43.2	1306.0	M	6	O	
LB05-3F	3F	39.4	971.6	M	5	O	
LB01-4F	4F	37.5	933.9	F	4	O	
LB02-4F	4F	37.3	884.5	M	6/6	O	
LB03-4F	4F	38.3	845.3	F	5	O	
LB04-4F	4F	36.5	735.6	M	6/6	O	
LB05-4F	4F	37.2	718.1	M	7/7	O	
LB01-5F	5F	34.9	662.7	F	4/4	O	
LB02-5F	5F	38.1	958.1	M	6/6	O	
LB03-5F	5F	36.2	720.3	M	4	O	
LB04-5F	5F	35.9	628.1	M	3	O	
LB05-5F	5F	33.0	525.9	F	3	O	
LB01-7F	7F	37.1	762.1	M	5	O	
LB02-7F	7F	40.6	1054.0	M	6	S	
LB03-7F	7F	36.8	827.0	M	5	S	
LB04-7F	7F	37.5	864.2	M	5	O	
LB05-7F	7F	39.1	889.8	M	5	O	

Table 2. (Cont'd).

Fish Identification Code	Site	Length (cm)	Weight (g) ¹	Sex	Age ²	Method	Comments
LB06-7F	7F	38.4	886.4	M	4	O	
LB01-9F	9F	42.5	1183.6	F	5/5	O	
LB02-9F	9F	34.3	566.8	F	4	O	
LB03-9F	9F	45.1	1595.7	M	6	O	
LB04-9F	9F	34.9	738.2	M			no samples delivered
LB05-9F	9F	38.7	899.2	M	4	O	

Note- Aging Method, O= otolith, S=scale

¹ Different levels of precision were used on different sampling dates

² When two ages are given, it is the result of the aging of both otoliths independently.

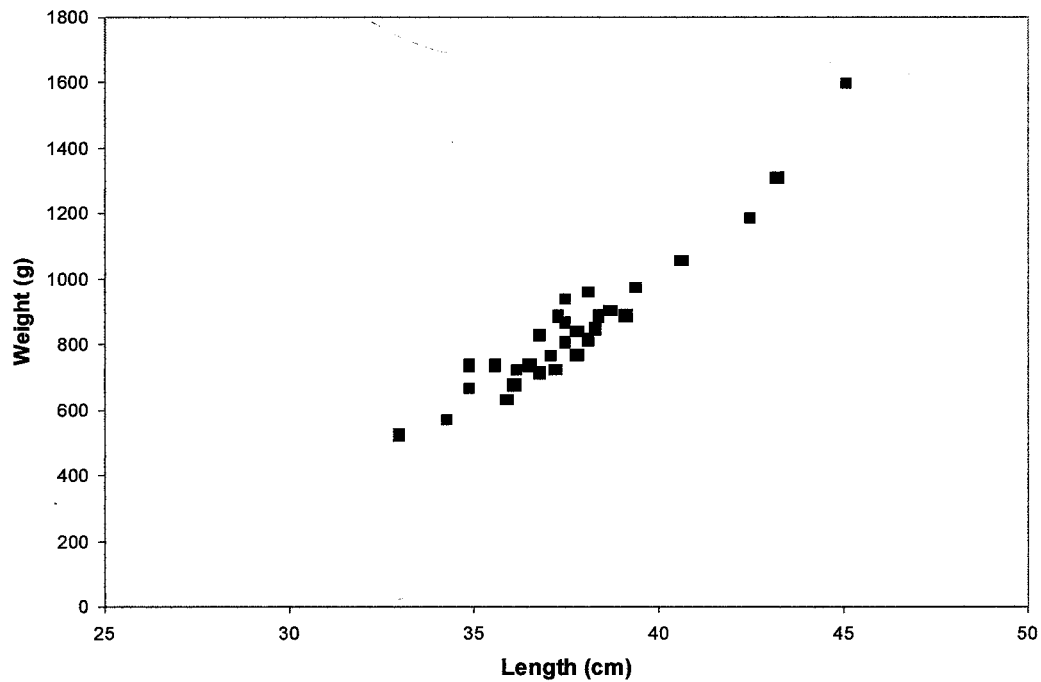


Figure 1. Length-weight relationship of largemouth bass captured from all sites on the Charles River, November 1999.

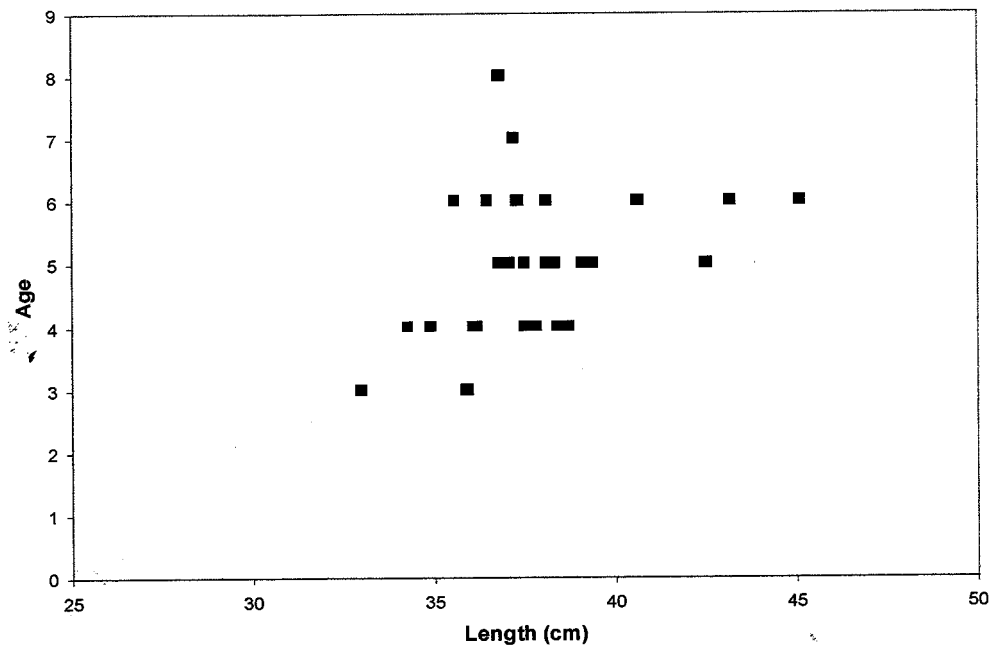


Figure 2. Length-age relationship of largemouth bass captured from all sites on the Charles River, November 1999.

C. Discussion

By convention, all northern hemisphere fish are considered to have their birthdays on January 1. Therefore, a bass born in June of one year will be considered to be one year old after January 1 of the following year (DeVries and Frie 1996). Compounding this situation is the fact that New England largemouth bass typically do not form current year annulus until late spring or early summer (Chandler, 1977, Vol.2). Therefore a bass sampled in March with three annuli would be aged as a four year old fish, with the assumption that the fourth annulus had not yet formed. However, due to the late sampling date (November), it was assumed that the current year annulus was completely formed. This assumption is supported by the fact that the most distal annulus contained additional growth towards the margin of the scale. As a result, the age assigned to each specimen is given as being equal to the number of annuli contained on the otolith or scale.

Three quality control methods were used to determine the precision of the age determination methods used in this section (precision defined as the repeatability of measurement). In the first method, a second otolith (otoliths occur in pairs) from six specimens was prepared and aged independently from the first otolith. The primary reader then determined the age of the second otolith which was compared to the age determined for the first otolith. In all six cases the second otolith was determined to have the identical age as those ascertained for the first otolith. In the second method, ten previously aged and randomly selected otoliths were aged a second time by the same primary reader. All ten otoliths were aged identically during both readings. In the third method, a secondary experienced reader determined the age of ten randomly selected largemouth bass samples, nine of the ten samples contained otoliths. For these samples, both

readers determined identical ages for all

samples. The tenth sample was one of the two samples for which only scales were provided, and age determination for this sample differed between readers by one year. A consensus age is given for this sample.

Section 2. Common Carp Age Determination

A. Methods

Between two and five common carp specimens were sampled from each of the six sampling sites. Between two and ten scales were prepared for each specimen by soaking and cleaning them in a diluted soap solution and gently brushing the scales with a bristle hobby brush. Clean scales were rinsed and mounted between two microscopic slides. Scales were read using a dissecting scope set between 7X and 30X magnification and were frequently read under the scope while submerged in water in a partially filled watch glass prior to mounting. This technique often gave clearer visibility of the circuli patterns than did viewing dried scales that were sandwiched between microscopic slides.

The scales analyzed in this study were all from older, sexually mature fish. The typical scale analyzed contained two to three inner annuli that were formed during the juvenile stage of life. These inner juvenile annuli were often obscure due to erosion of their circuli or surficial deposits that could not be removed. Distal to the inner annuli were a series of outer annuli that were distinctly different. These annuli contained interruption in their circuli typically associated with what are considered to be spawning marks. For the most part, these outer annuli were distinct and non-overlapping, therefore, when inner annuli were not apparent, the first annulus (most proximal to the focus) with spawning mark characteristics was considered to be the third year annulus. The rationale for this assignment is given in the discussion section below.

B. Data

Table 3. Total lengths, weights and ages of common carp captured from the Charles River, November 1999.

Fish Identification Code	Site	Length (cm)	Weight (g)	Sex	Age	Method	Comments
Carp01-1F	1F	57.4	2737	F	3	S	spawning checks 3 rd year
Carp02-1F	1F	62.0	3908	F	5	S	3 spawning marks
Carp01-3F	3F	62.5	5171	F	7	S	
Carp02-3F	3F	65.5	3724	F	6(4-8)	S	very indistinct annuli
Carp03-3F	3F	71.5	4123.2	F	6	S	indistinct annuli

Table 3. (Cont'd).

Fish Identification Code	Site	Length (cm)	Weight (g)	Sex	Age	Method	Comments
Carp04-3F	3F	63.5	3111.6	F	4	S	
Carp05-3F	3F	66.0	4422.5	F	8	S	indistinct annuli
Carp01-4F	4F	62.5	3285.7	F	5	S	
Carp02-4F	4F	65.5	3801.7	M	9	S	4-5 spawning marks
Carp03-4F	4F	71.5	5443.1	F	9	S	
Carp04-4F	4F	63.5	3781.8	F	8	S	
Carp05-4F	4F	66.0	4422.5	F	9	S	
Carp01-5F	5F	60.6	3333.9	M	7	S	
Carp02-5F	5F	62.9	3433.1	M	4	S	
Carp03-5F	5F	65.4	4178.7	M	6	S	
Carp04-5F	5F	69.9	5397.7	F	8	S	
Carp05-5F	5F	NA	2954.0	F	4	S	4 th annulus beginning to form on margin
Carp01-7F	7F	67.0	4680.5	F	5	S	may be 6 y.o.
Carp02-7F	7F	73.2	5967.6	M	8	S	spawning marks overlapping
Carp03-7F	7F	65.3	4374.3	F	7	S	
Carp04-7F	7F	67.3	4847.8	F	5	S	might be 6 y.o.
Carp05-7F	7F	63.6	3770.5	F	9	S	
Carp01-9F	9F	60.5	3623.1	F	5	S	all regens
Carp02-9F	9F	66.7	4062.5	F	6	S	well defined annuli
Carp03-9F	9F	66.4	4672.0	F	8	S	outer spawning marks very close
Carp04-9F	9F	71.7	6015.8	F	10	S	distinct annuli
Carp05-9F	9F	68.0	5136.9	F	8	S	may be 9 y.o.

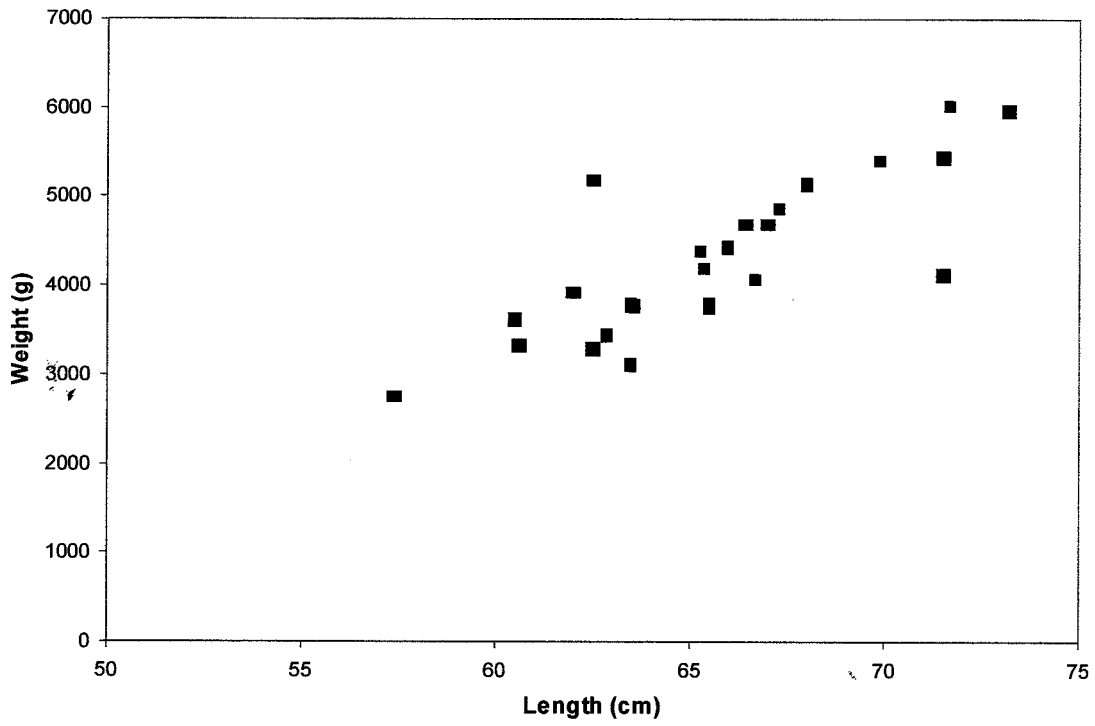


Figure 3. Length-weight relationship of common carp captured from all sites on the Charles River, November 1999.

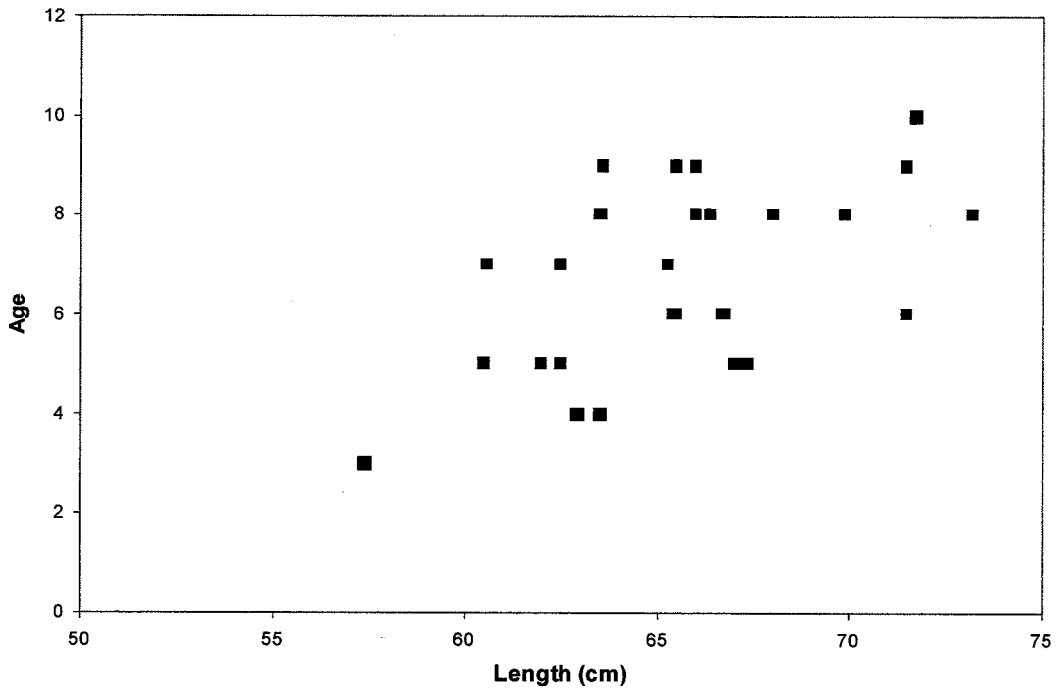


Figure 4. Length-age relationship of common carp captured from all sites on the Charles River, November 1999.

C. Discussion

The use of the scale method for aging younger, prespawn fish specimens is considered to be highly reliable by many researchers. However, in larger fish such as those aged in this study, the obscuring of the circuli forming the inner annuli, and overlapping or loss of outer annuli (spawning marks), frequently cause an understatement of the true age of the fish (Chilton and Beamish 1982). Also, since the inner annuli in older specimens were frequently obscured in scales observed in this study, it was decided to consider the first spawning mark as representing the third year annulus (representing the estimated two years of age prior to sexual maturity).

Common carp, show sexual dimorphism in the age of sexual maturation, with males typically spawning one year early than females. A limiting factor in determining the age of sexual maturity in common carp from this river system was the lack of younger specimens. Only two of the youngest fish had distinct inner annuli. One fish, a four year old male, first spawned at age two, and the second fish, a three year old female, first spawned at age three.

In summary, the ages given for common carp in this section should be considered as estimates, not true ages. As the determined age of a carp progresses above three to four years, the true age of the fish, if not equal to the determined age, is most likely greater than the determined age. The actual age of larger fish might be significantly higher than the determined age.

Two quality control methods were used to determine the precision of the age determination methods employed in this section. In the first method, scales from ten randomly selected specimens previously aged were aged a second time by the same primary reader. Due to the variability in aging scales from such large specimens, age determinations which differed by one year or less were considered to be in agreement. Using this method, the age determination from the second reading was within one year of the age determined in the initial reading for eight out of the ten specimens.

In the second method, a secondary experienced reader determined the age of the same ten randomly selected common carp scale samples used in the first quality control measure. Between readers, age determinations that differed by one year or less were considered to be in agreement. Using this method, the age determination made by the secondary reader was in agreement with the primary reader for nine out of the ten samples analyzed. The secondary readers age determination was within two years of the primary readers age determination for all ten samples.

Section 3. Yellow Perch and Black Crappie Age Determination

A. Methods

Between 11 and 27 fish were sampled from each of the six sampling sites. Scales were taken

from selected specimens representing the range of lengths in the composite sample or all specimens. Scales of the composite samples from each site were pooled together in one scale envelope. Scales from these envelopes were cleaned and mounted between microscopic slides as described in Section Two. Ages were determined from the three largest, and three smallest scales for each composite sample. Using this information, the range of ages of the fish for each composite sample was determined.

B. Data

Table 4: Range of lengths and ages of composite fish specimens captured in the Charles River, November 1999.

Composite Species	Site	Range of Lengths (cm)	Sex Distribution female/male	Range of Ages	Comments
Black Crappie	1F	15.2-24.8	9F/5M	2-6	Weak annuli formation
Yellow Perch	3F	18.7-27.6	8F/3M		No scales delivered
Yellow Perch	4F	19.1-25.1	14F/1M	2-6	Strong annuli formation
Yellow Perch	5F	18.9-24.1	19F/3M	2-5	Strong annuli formation
Yellow Perch	7F	17.1-24.1	11F/5M	4-5	1 st annulus unapparent, annuli moderately distinct
Yellow Perch	9F	18.7-26.7	12F/4M	2-5	

C. Discussion

The annuli from the scales of the black crappie in Site One were particularly difficult to discern. Overall, the inner circuli in these scales were obscured. Intercirculi spacing was very uniform in many scale samples, and there appeared to be a considerable number of false annuli. The ages determined for these specimens might be considerably different from their true ages.

One quality control method was used to determine the precision of the age determination methods in this section. In this method a secondary experienced reader determined the range of age of all five composite scale samples. Both readers had identical age determinations for the youngest age range in the composite for all sites.

The oldest ages for the ranges were identical in four out of five sites. The one difference being the high value for the age range of the black crappies in Site 1F, in which the primary reader determined the range of ages to be two to five years old, and the secondary reader determined the range of ages to be two to six years, a consensus age range of two to six was determined.

Appendix A. Comparison of the length-weight relationship and length-age relationship of largemouth bass captured from sites 1F, 3F, 4F, 5F, 7F, and 9F, and comparisons of individual sites to a composite of largemouth bass captured from all sites on the Charles River, November 1999.

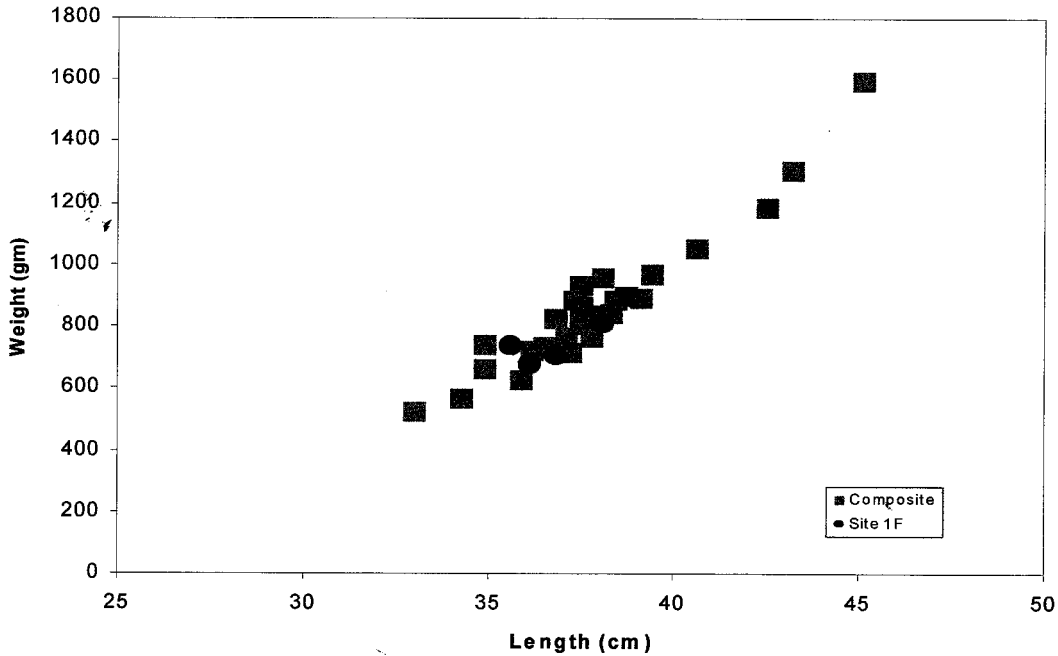


Figure A. Comparison of the length-weight relationship of largemouth bass captured from Site 1F to a composite of largemouth bass captured from all sites on the Charles River, November 1999.

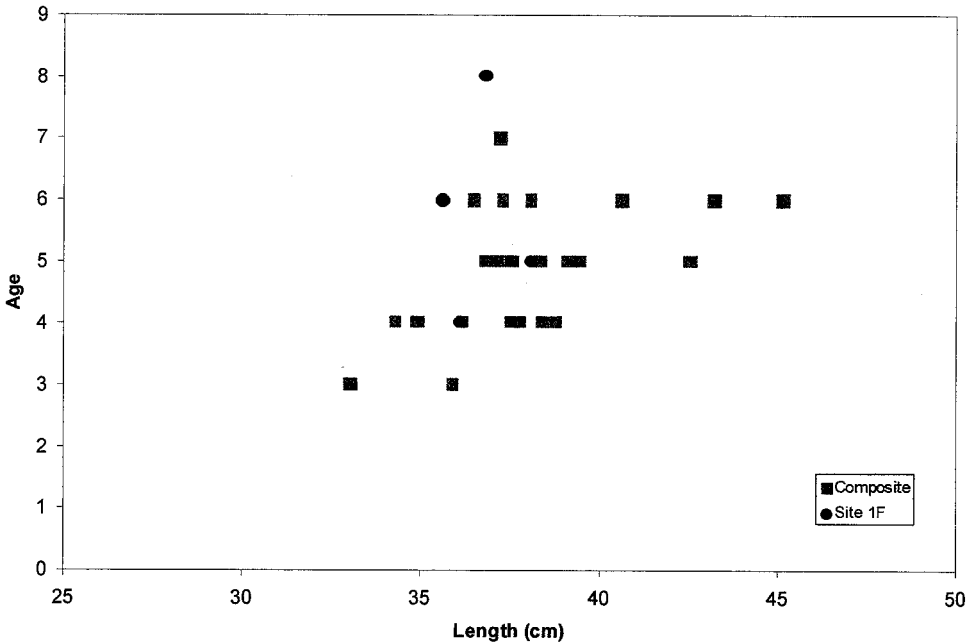


Figure B. Comparison of the length-age relationship of largemouth bass captured from Site 1F to a composite of largemouth bass captured from all sites on the Charles River, November 1999.

Appendix A. (Cont'd).

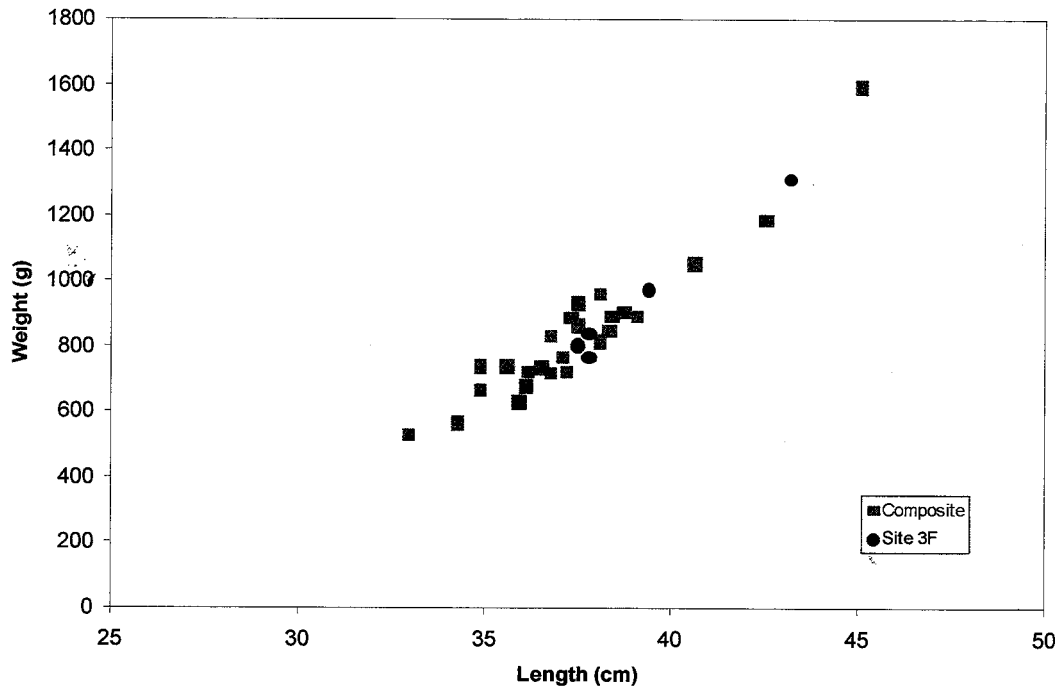


Figure C. Comparison of the length-weight relationship of largemouth bass captured from Site 3F to a composite of largemouth bass captured from all sites on the Charles River, November 1999.

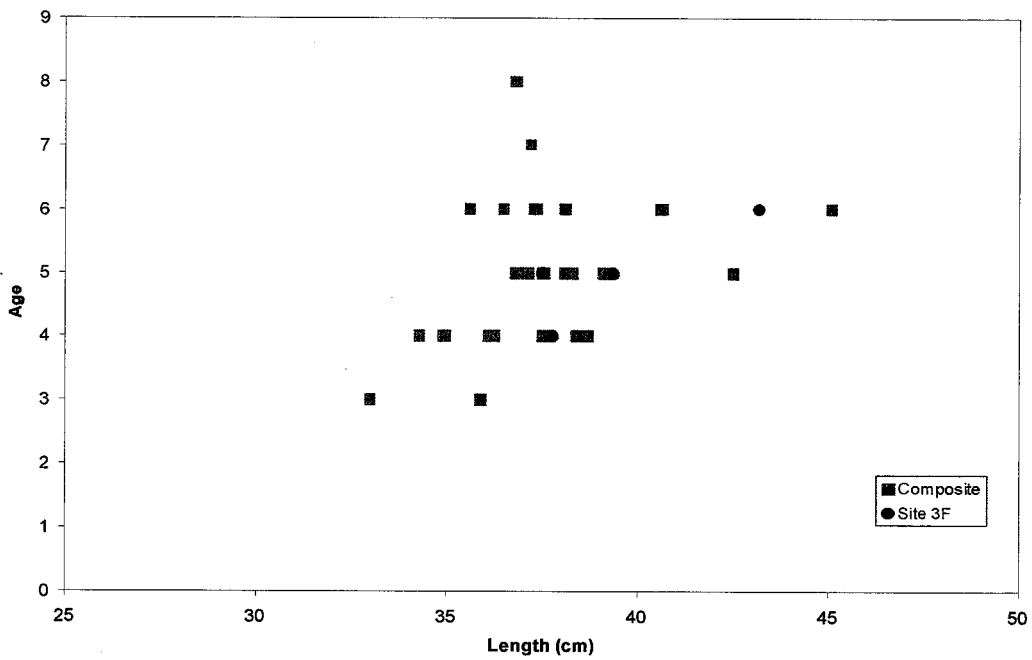


Figure D. Comparison of the length-age relationship of largemouth bass captured from Site 3F to a composite of largemouth bass captured from all sites on the Charles River, November 1999.

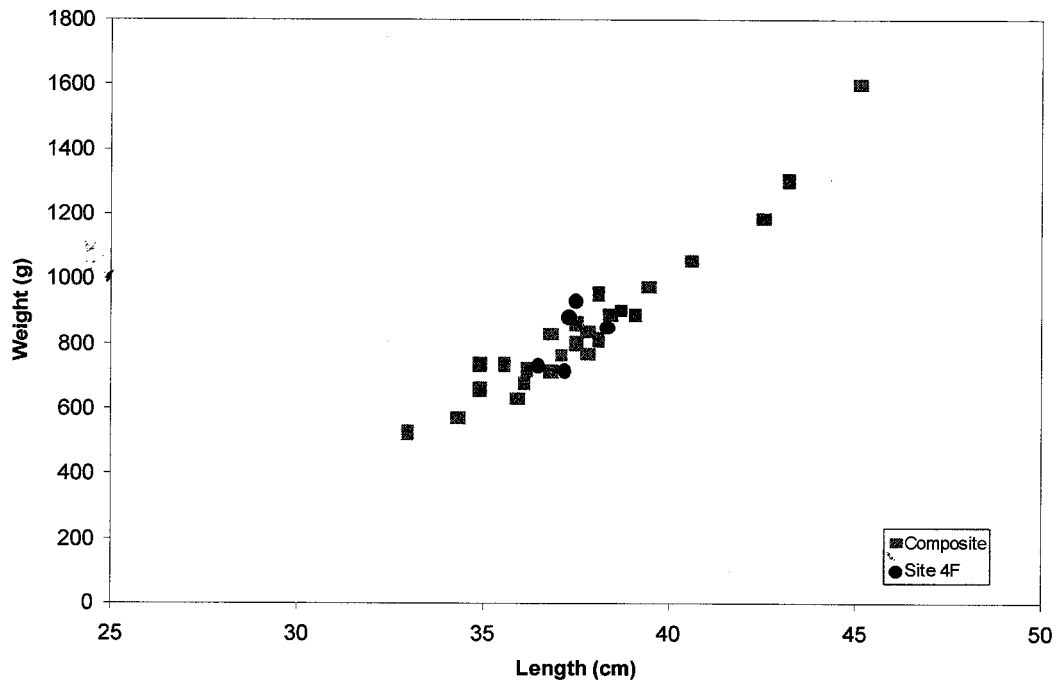


Figure E. Comparison of the length-weight relationship of largemouth bass captured from Site 4F to a composite of largemouth bass captured from all sites on the Charles River, November 1999.

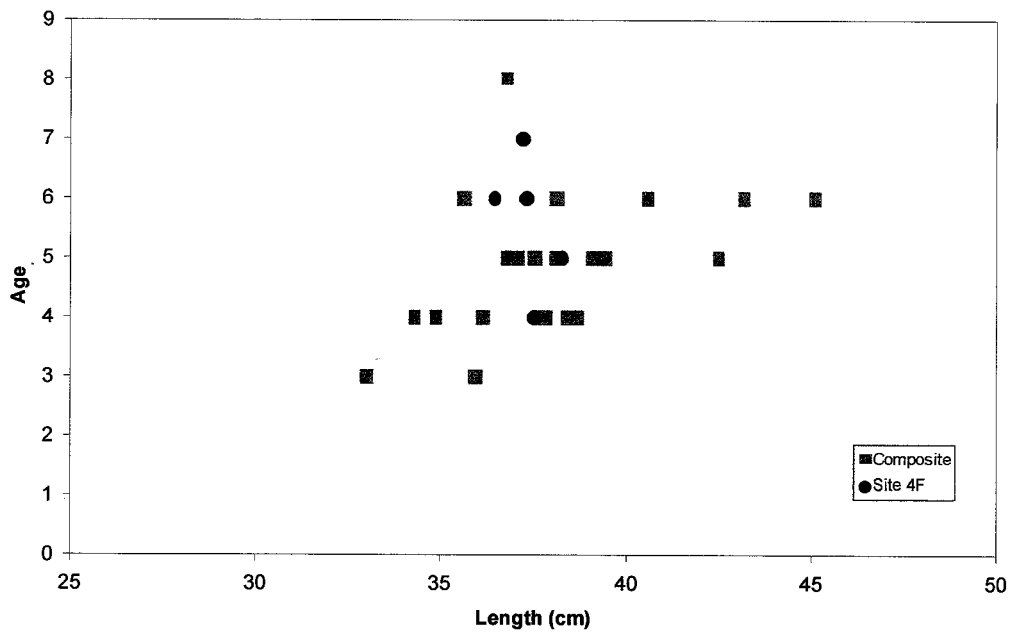


Figure F. Comparison of the length-age relationship of largemouth bass captured from Site 4F to a composite of largemouth bass captured from all sites on the Charles River, November 1999.

Appendix A. (Cont'd).

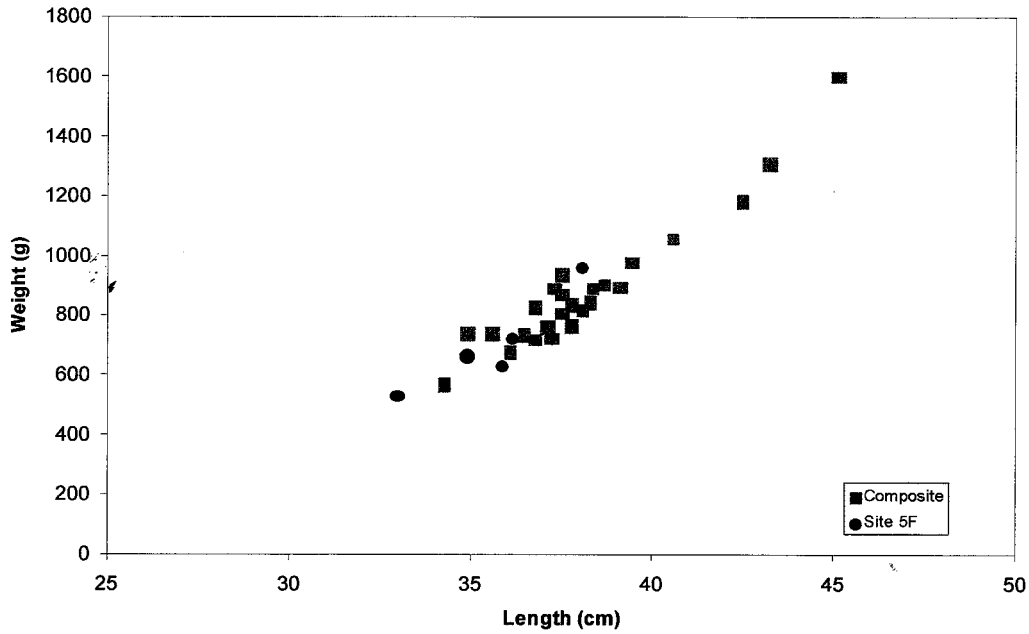


Figure G. Comparison of the length-weight relationship of largemouth bass captured from Site 5F to a composite of largemouth bass captured from all sites on the Charles River, November 1999.

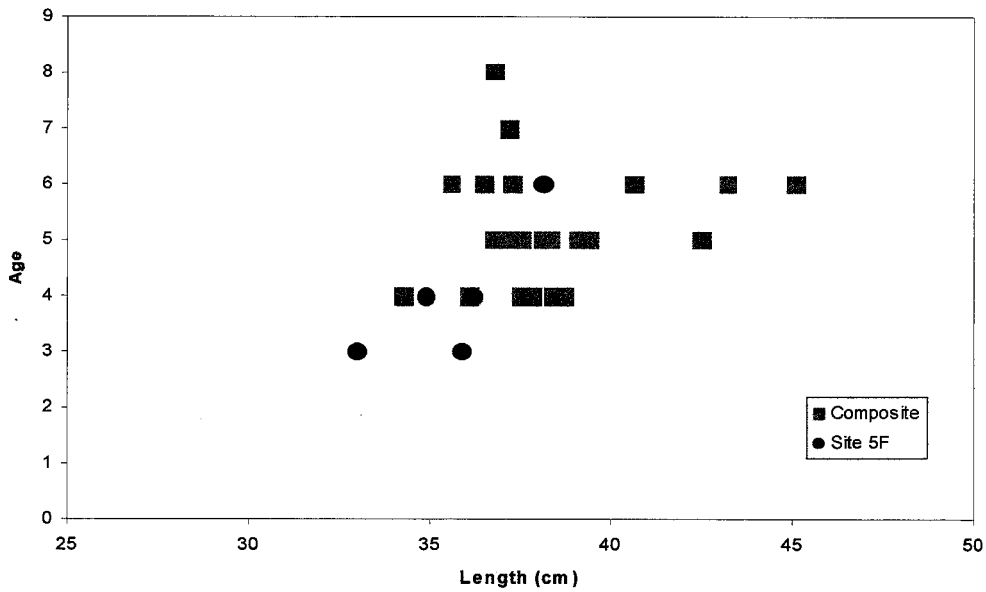


Figure H. Comparison of the length-age relationship of largemouth bass captured from Site 5F to a composite of largemouth bass captured from all sites on the Charles River, November 1999.

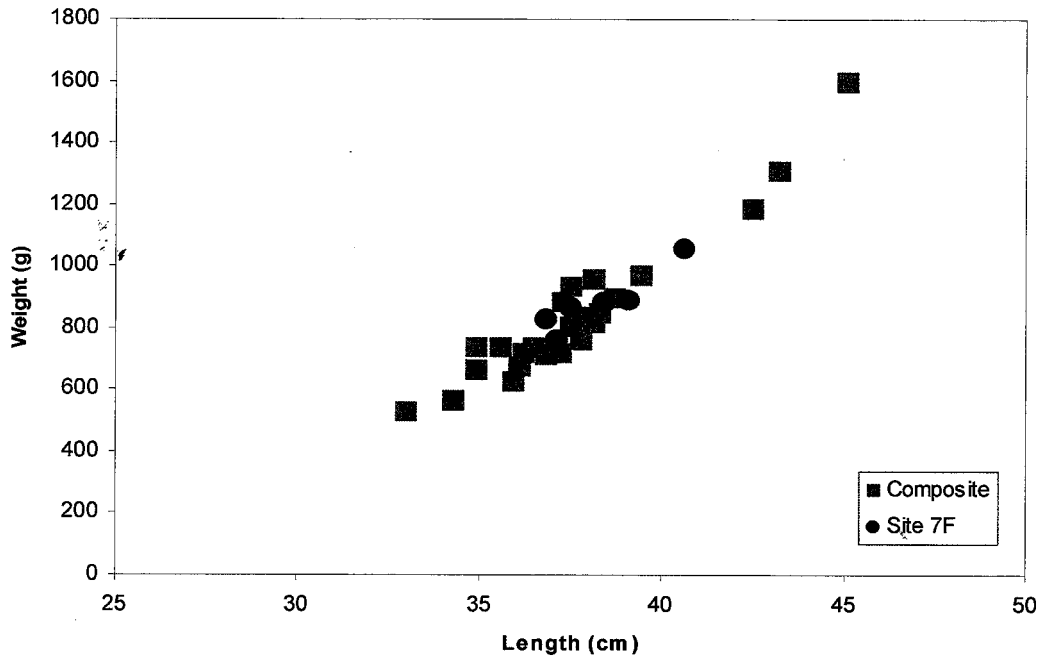


Figure I. Comparison of the length-weight relationship of largemouth bass captured from Site 7F to a composite of largemouth bass captured from all sites on the Charles River, November 1999.

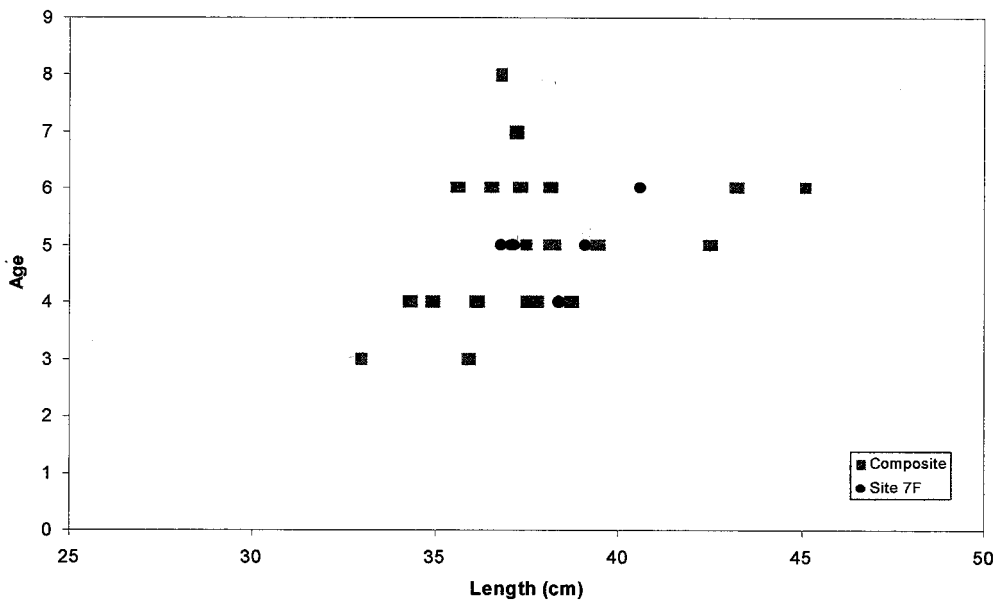


Figure J. Comparison of the length-age relationship of largemouth bass captured from Site 7F to a composite of largemouth bass captured from all sites on the Charles River, November 1999.

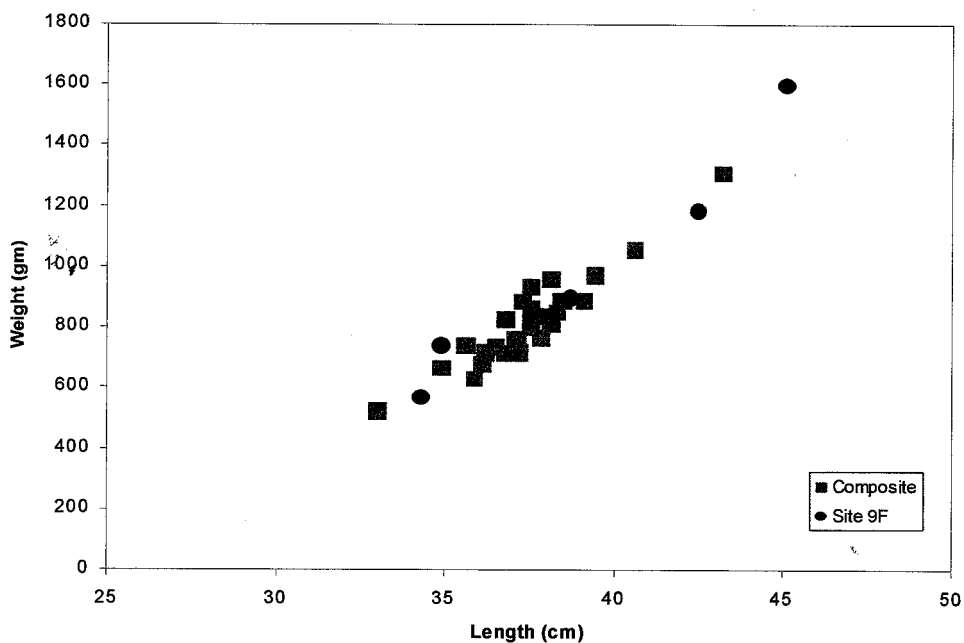


Figure K. Comparison of the length-weight relationship of largemouth bass captured from Site 9F to a composite of largemouth bass captured from all sites on the Charles River, November 1999.

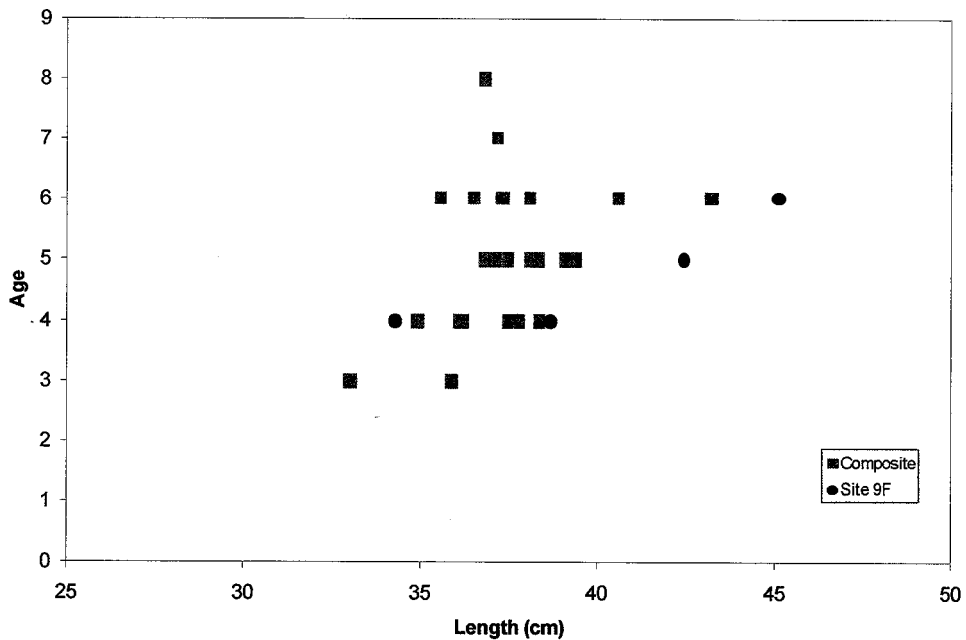


Figure L. Comparison of the length-age relationship of largemouth bass captured from Site 9F to a composite of largemouth bass captured from all sites on the Charles River, November 1999.

Appendix B. Comparison of the length-weight relationship and length-age relationship of common carp captured from sites 1F, 3F, 4F, 5F, 7F, and 9F, and comparisons of individual sites to a composite of common carp captured from all sites on the Charles River, November 1999.

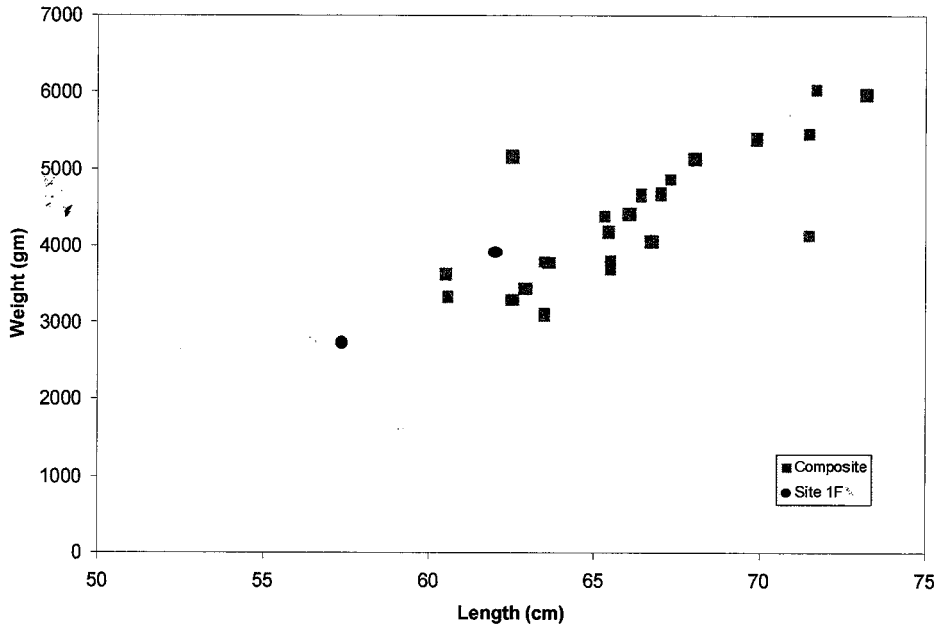


Figure M. Comparison of the length-weight relationship of common carp from Site 1F to a composite of common carp captured from all sites on the Charles River, November 1999.

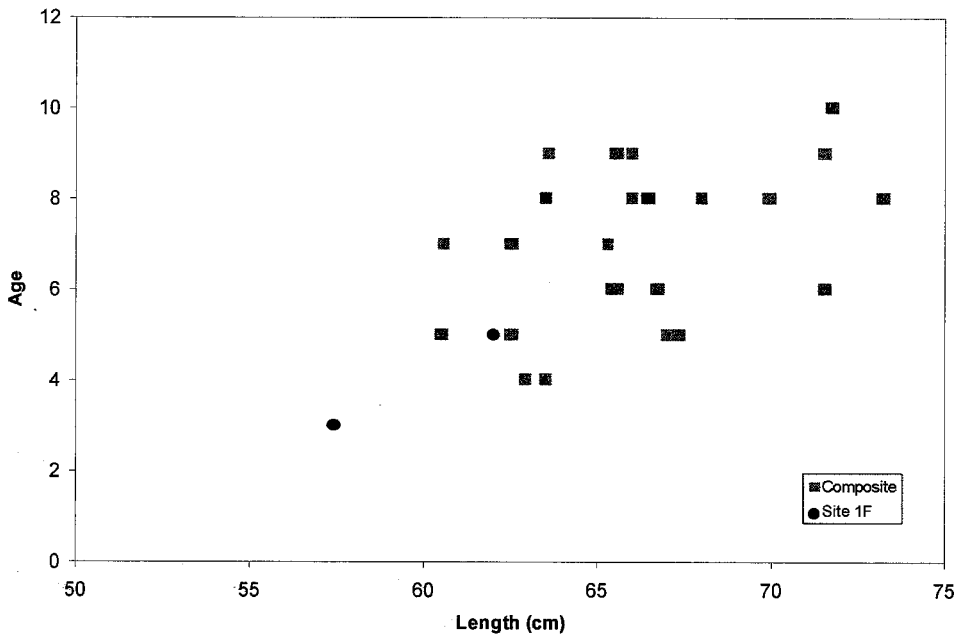


Figure N. Comparison of the length-age relationship of common carp captured from Site 1F to a composite of common carp captured from all sites on the Charles River, November 1999.

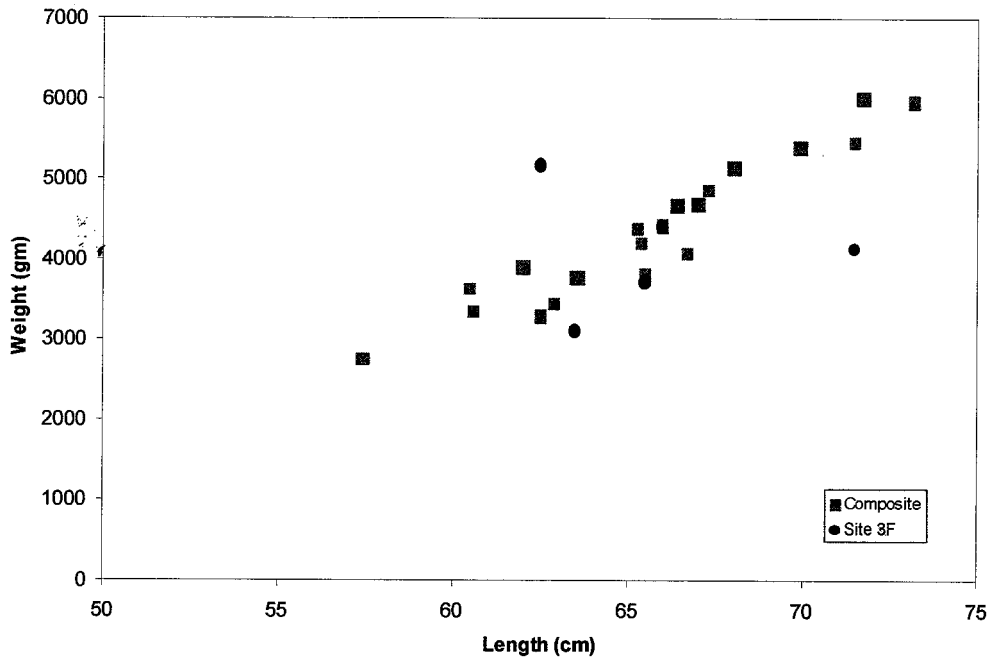


Figure O. Comparison of the length-weight relationship of common carp from Site 3F to a composite of common carp captured from all sites on the Charles River, November 1999.

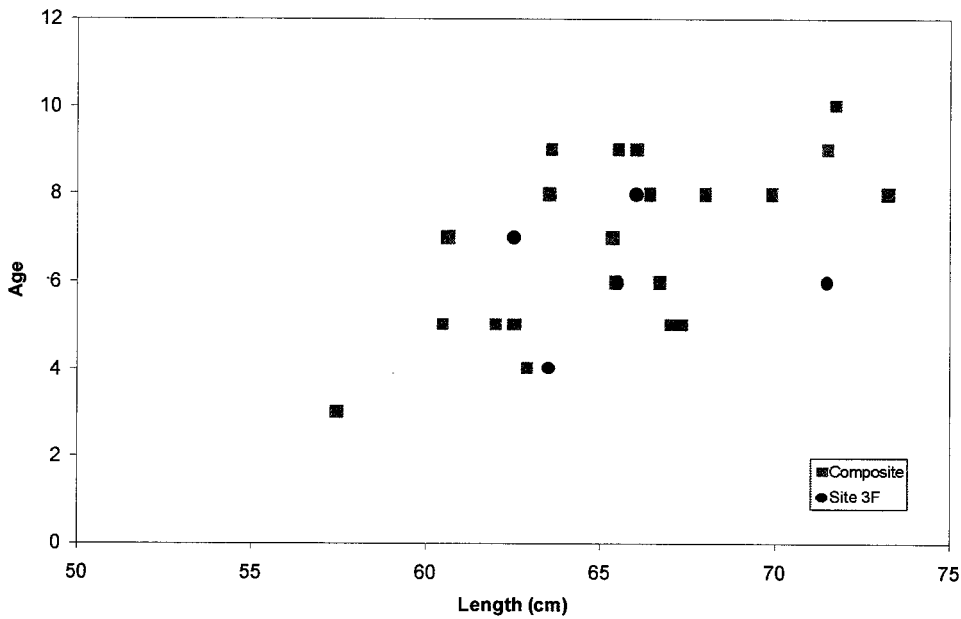


Figure P. Comparison of the length-age relationship of common carp captured from Site 3F to a composite of common carp captured from all sites on the Charles River, November 1999.

Appendix B. (Cont'd).

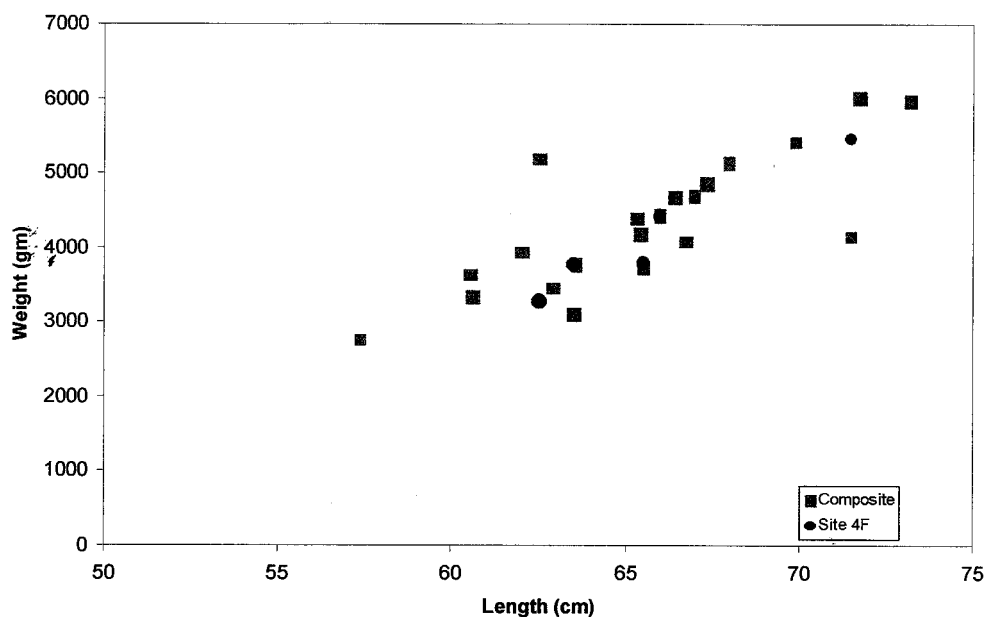


Figure Q. Comparison of the length-weight relationship of common carp from Site 4F to a composite of common carp captured from all sites on the Charles River, November 1999.

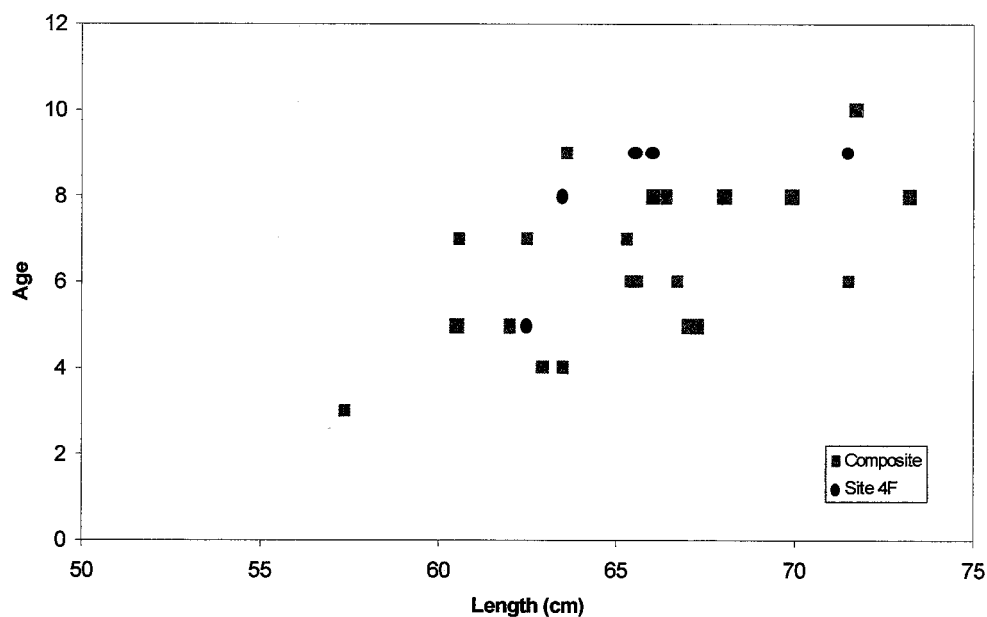


Figure R. Comparison of the length-age relationship of common carp captured from Site 4F to a composite of common carp captured from all sites on the Charles River, November 1999.

Appendix B. (Cont'd).

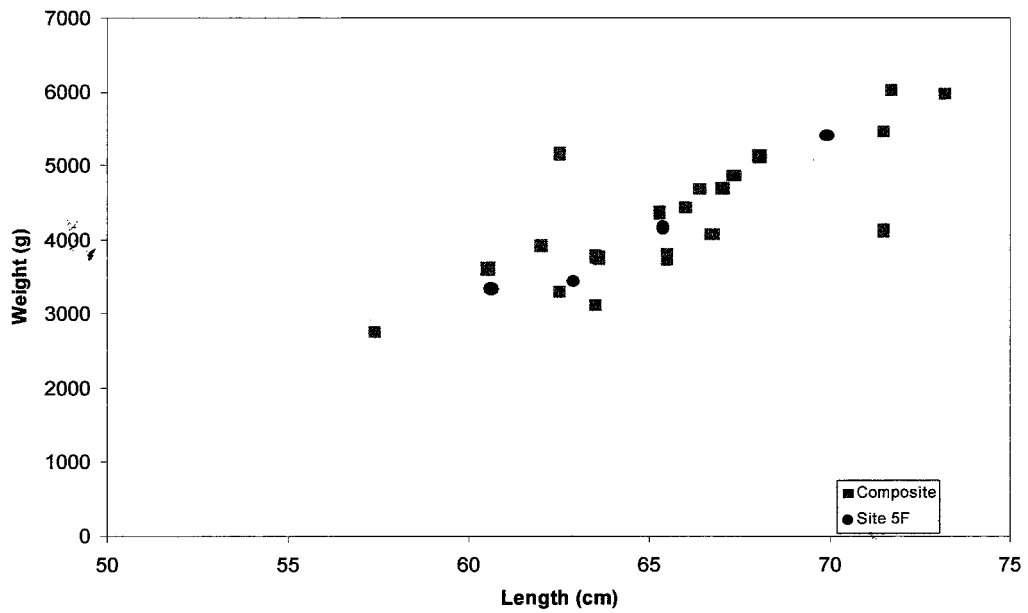


Figure S. Comparison of the length-weight relationship of common carp from Site 5F to a composite of common carp captured from all sites on the Charles River, November 1999.

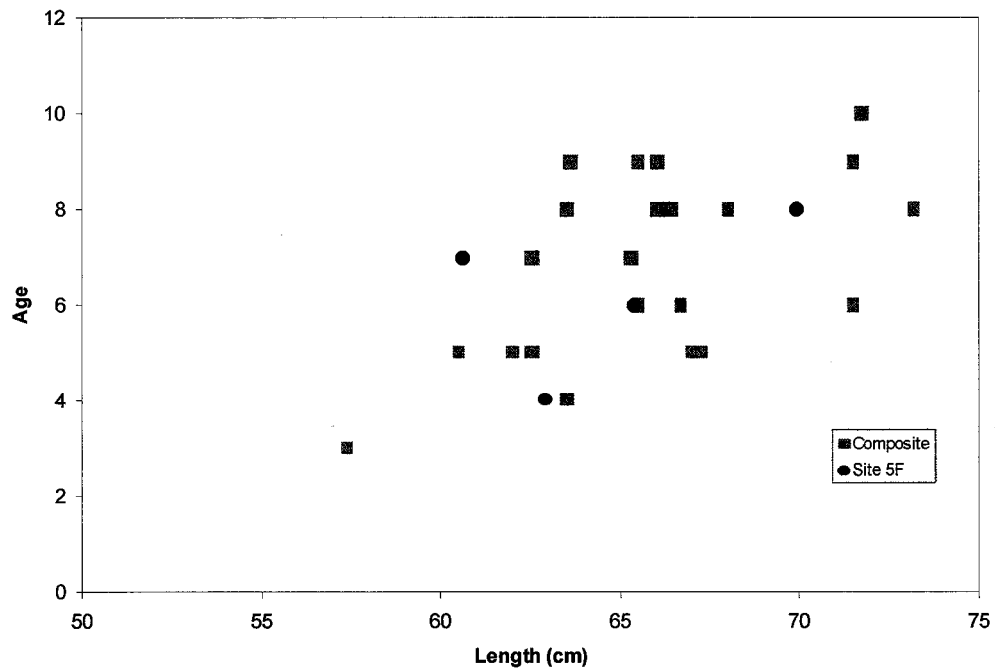


Figure T. Comparison of the length-age relationship of common carp captured from Site 5F to a composite of common carp captured from all sites on the Charles River, November 1999.

Appendix B. (Cont'd).

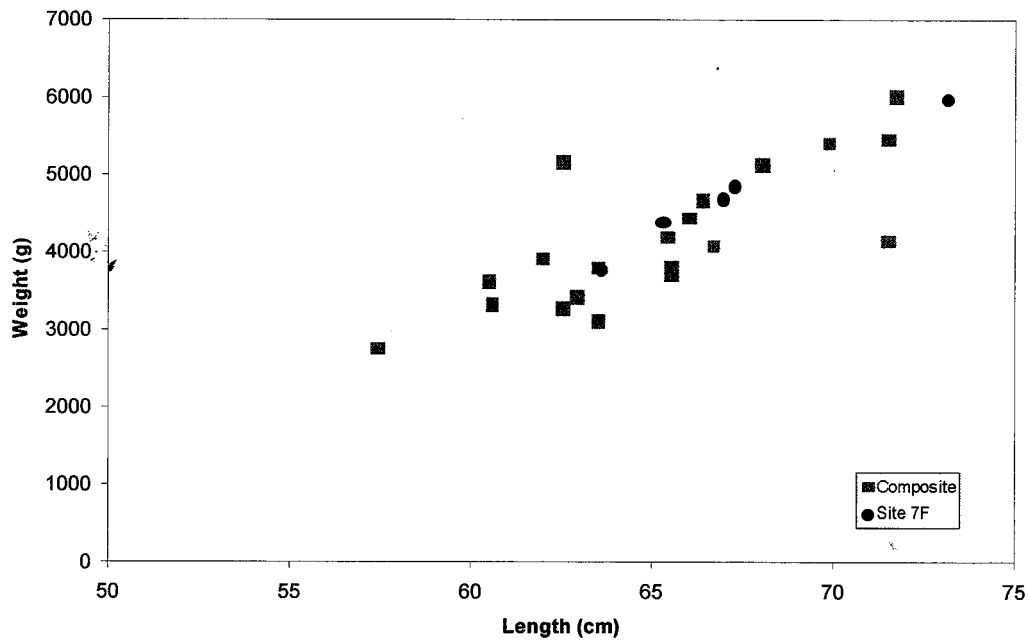


Figure U. Comparison of the length-weight relationship of common carp from Site 7F to a composite of common carp captured from all sites on the Charles River, November 1999.

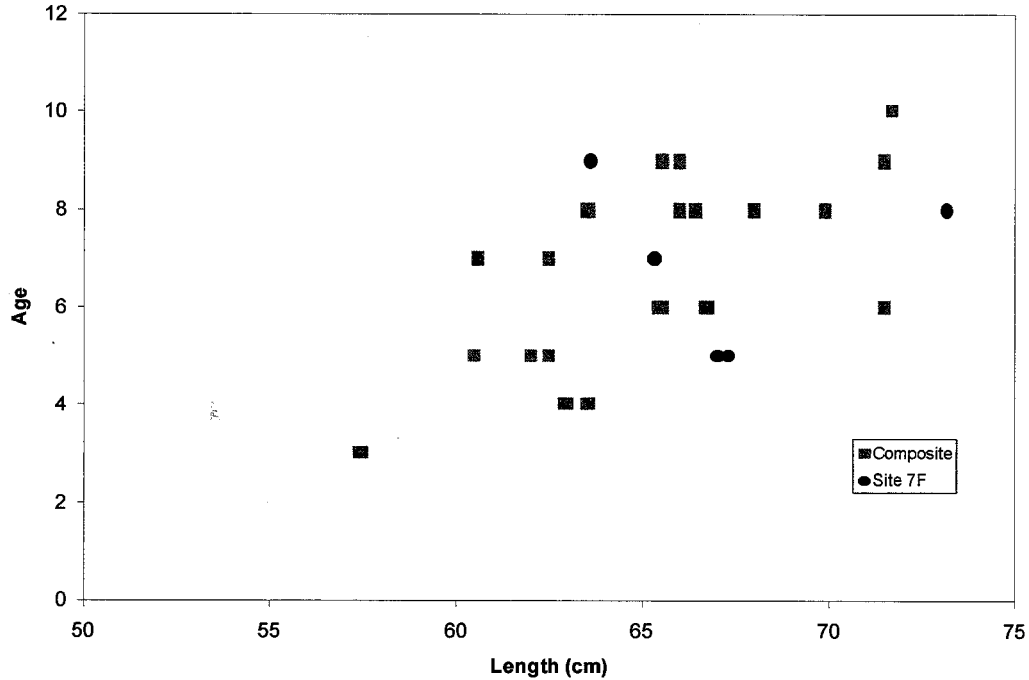


Figure V. Comparison of the length-age relationship of common carp captured from Site 7F to a composite of common carp captured from all sites on the Charles River, November 1999.

Appendix B. (Cont'd).

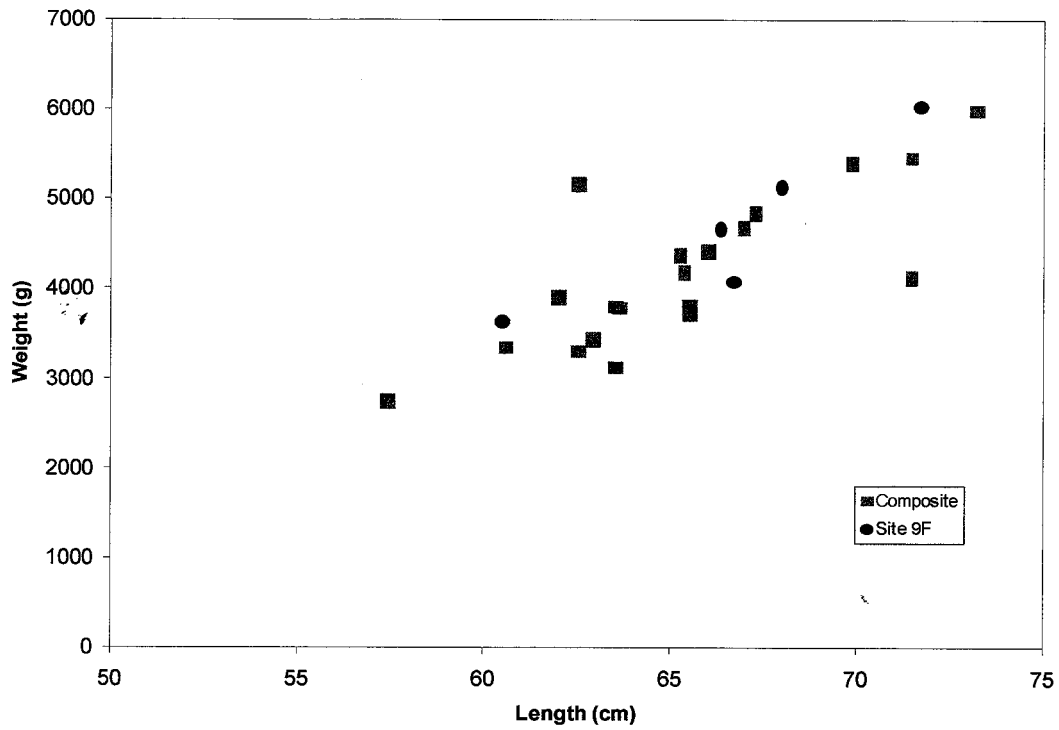


Figure W. Comparison of the length-weight relationship of common carp from Site 9F to a composite of common carp captured from all sites on the Charles River, November 1999.

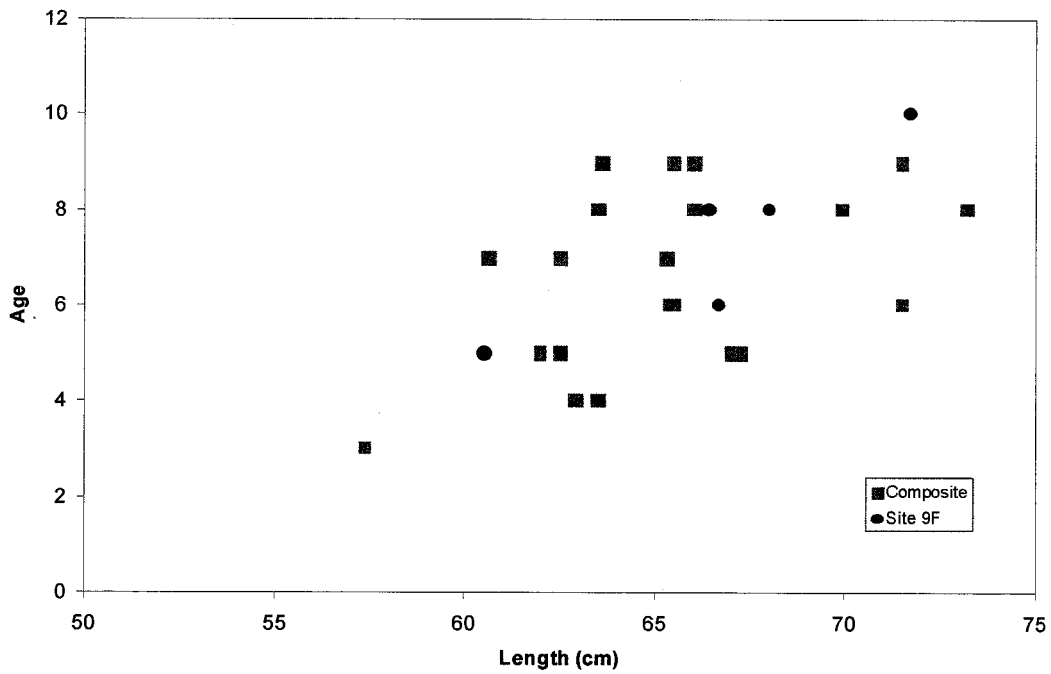


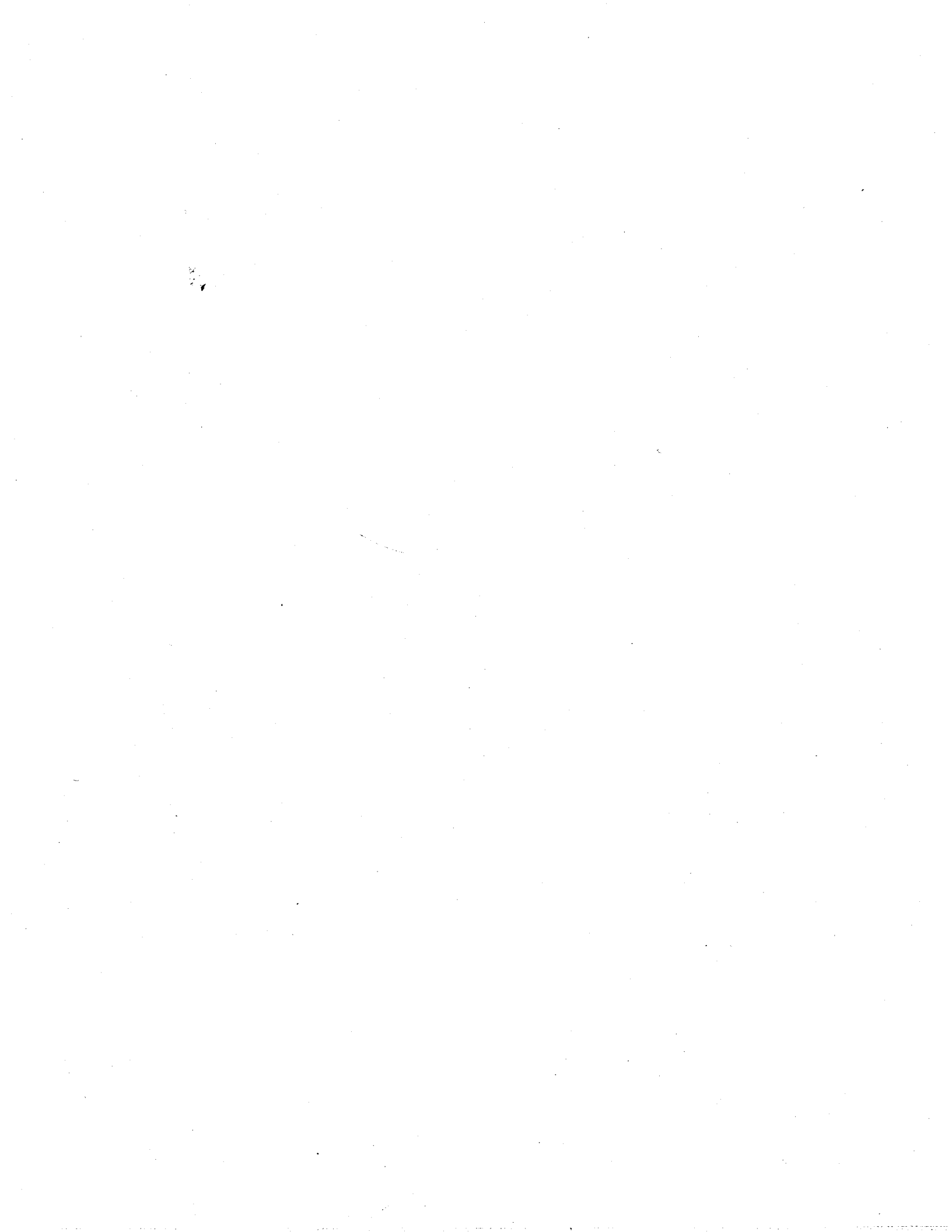
Figure X. Comparison of the length-age relationship of common carp captured from Site 9F to a composite of common carp captured from all sites on the Charles River, November 1999.

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APPENDIX II
ANALYTE TABLES



METALS CONCENTRATIONS IN CHARLES RIVER FISH

mg/kg wet weight (ppm)

Station	Sample Type	Cd	Cr	Cu	Ni	Pb	Se	Zn	Fe
1F	calico bass fillet	ND	ND	0.2	ND	ND	0.4	5.3	7.4
1F	carp fillet	ND	ND	0.5	ND	ND	ND	7.6	8.7
3F	carp fillet	ND	ND	0.3	ND	ND	ND	6.6	8.7
4F	carp fillet	ND	ND	0.2	ND	ND	ND	9.5	10.3
9F	carp fillet	ND	ND	0.4	ND	ND	0.5	11.0	9.4
1F	LMB fillet	ND	0.2	0.2	ND	ND	ND	3.8	2.4
3F	LMB fillet	ND	0.2	0.2	ND	ND	ND	3.8	3.4
4F	LMB fillet	ND	6.1	1.1	3.5	ND	0.6	3.3	28.6
5F	LMB fillet	ND	0.3	0.3	ND	ND	ND	4.2	3.1
9F	LMB fillet	ND	ND	0.2	ND	ND	0.4	3.8	2.8
3F	yellow perch fillet	ND	ND	0.9	ND	ND	ND	5.4	3.5
4F	yellow perch fillet	ND	ND	0.3	ND	ND	ND	5.5	5.7
5F	yellow perch fillet	ND	3.3	1.8	1.9	ND	ND	5.8	22.8
9F	yellow perch fillet	ND	0.1	0.3	ND	ND	0.3	5.0	3.2
1F	calico bass offal	ND	1.3	0.5	ND	0.2	ND	34.8	18.2
1F	carp offal	ND	3.5	1.8	1.7	0.5	0.7	78.1	30.4
3F	carp offal	ND	0.5	1.6	ND	0.9	ND	117.6	26.9
4F	carp offal	ND	0.9	2.4	ND	1.3	1.1	131.0	41.1
9F	carp offal	ND	1.2	2.5	0.4	2.0	0.5	169.6	47.0
1F	LMB offal	ND	0.9	0.6	ND	ND	ND	20.3	15.0
3F	LMB offal	ND	0.7	0.6	ND	ND	ND	16.5	15.7
4F	LMB offal	ND	1.1	1.0	ND	ND	ND	24.6	18.5
5F	LMB offal	ND	15.1	3.6	8.1	ND	ND	25.7	77.8
9F	LMB offal	ND	0.6	0.7	ND	0.2	0.8	17.2	24.6
3F	yellow perch offal	ND	0.6	0.4	ND	0.6	0.5	18.8	15.9
4F	yellow perch offal	ND	0.9	0.8	ND	1.3	ND	31.9	28.6
5F	yellow perch offal	ND	1.18	0.74	0.24	1.49	ND	30.72	24.24
9F	yellow perch offal	ND	1.2	1.7	0.2	1.6	ND	31.5	31.2

Total Mercury Concentrations in Charles River Fish

mg/kg wet weight (ppm)

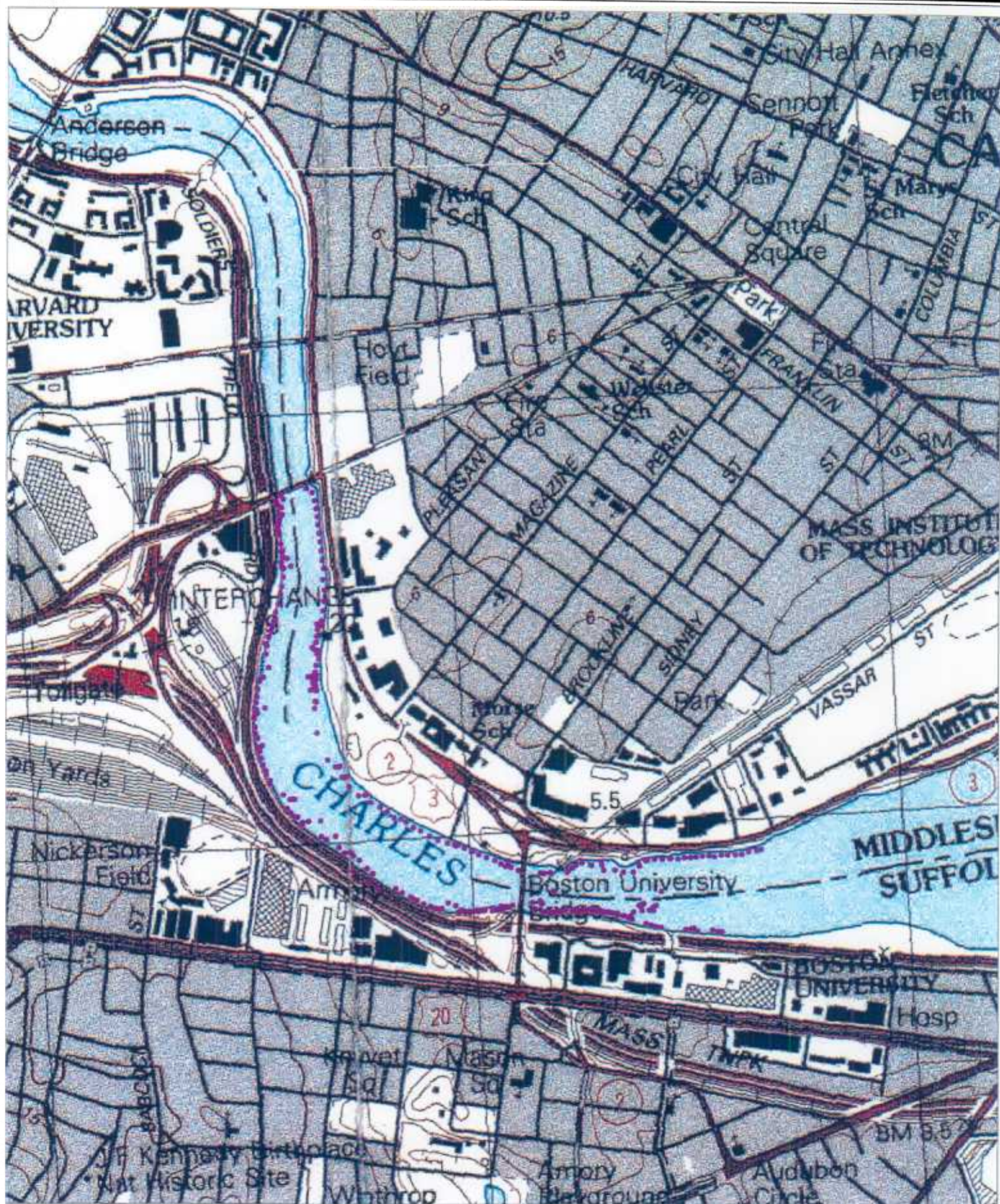
Station Location	Sample Type & Station	Hg (ug/g) ppm wet weight	Hg (ug/g) ppm dry weight	R.L. (ppm) dry weight	% solids
1F	calico bass fillet 1F	0.26	1.30	0.15	20
1F	carp fillet 1F	0.11	0.41	0.14	27
3F	carp fillet 3F	0.07	0.30	0.05	24
4F	carp fillet 4F	0.07	0.30	0.05	23
5F	carp fillet 5F	0.09	0.41	0.17	23
7F	carp fillet 7F	0.12	0.52	0.16	24
9F	carp fillet 9F	0.19	0.70	0.14	27
1F	LMB fillet 1F	0.48	2.30	0.18	21
3F	LMB fillet 3F	0.20	0.93	0.93	22
4F	LMB fillet 4F	0.24	1.10	0.17	22
5F	LMB fillet 5F	0.21	0.93	0.15	23
7F	LMB fillet 7F	0.22	1.00	0.16	22
9F	LMB fillet 9F	0.26	1.20	0.16	22
3F	yellow perch fillet 3F	0.10	0.49	0.49	20
4F	yellow perch fillet 4F	0.09	0.44	0.12	20
5F	yellow perch fillet 5F	0.12	0.58	0.16	20
7F	yellow perch fillet 7F	0.18	0.86	0.17	21
9F	yellow perch fillet 9F	0.15	0.76	0.15	20
1F	calico bass offal 1F	0.12	0.41	0.15	30
1F	carp offal 1F	0.05	0.13	0.10	37
3F	carp offal 3F	0.03	0.08	0.05	34
4F	carp offal 4F	0.03	0.08	0.05	37
5F	carp offal 5F	0.03	0.09	0.08	33
7F	carp offal 7F	0.05	0.16	0.16	34
9F	carp offal 9F	0.07	0.21	0.10	32
1F	LMB offal 1F	0.26	0.95	0.15	27
3F	LMB offal 3F	0.09	0.28	0.05	31
4F	LMB offal 4F	0.11	0.33	0.16	32
5F	LMB offal 5F	0.12	0.36	0.15	32
7F	LMB offal 7F	0.08	0.28	0.18	29
9F	LMB offal 9F	0.15	0.49	0.15	30
3F	yellow perch offal 3F	0.07	0.21	0.05	31
4F	yellow perch offal 4F	0.03	0.12	0.05	29

PCB's & Pesticides in Charles River Fish

mg/kg wet weight (ppm)

Station	Sample type	% solids	% Lipids	Alpha Chlordane	Gamma Chlordane	Total Chlordane	DDD	DDE	DDT	Dieldrin	Endrin	Aroclor 1254	Aroclor 1260
1F	Calico Bass Fillet Composite	20	0.6	0.0039	0.0011	0.0050	0.0066	0.0239	ND	0.0013	ND	0.0817	0.0235
1F	Carp Fillet Composite	27	5.5	0.0457	0.0237	0.0694	0.0715	0.1234	ND	0.0177	ND	0.3869	0.1262
3F	Carp Fillet Composite	24	5.9	0.0075	0.0237	0.0312	0.2141	0.2163	ND	0.0127	ND	1.4315	0.7407
4F	Carp Fillet Composite	23	3.9	0.0138	0.0297	0.0435	0.1598	0.1457	ND	0.0122	ND	1.1150	0.4959
5F	Carp Fillet Composite	23	3	0.0132	0.0229	0.0361	0.1197	0.1340	ND	0.0103	ND	1.2178	0.8492
7F	Carp Fillet Composite	24	5	0.0162	0.0220	0.0382	0.1071	0.1067	ND	0.0187	ND	1.0798	0.0361
9F	Carp Fillet Composite	27	7.3	0.0845	0.0431	0.1276	0.2241	0.2573	ND	0.0335	0.0274	2.4649	1.1738
1F	LMB Fillet Composite	21	0.3	0.0019	0.0006	0.0025	0.0048	0.0226	ND	0.0012	ND	0.0752	0.0544
3F	LMB Fillet Composite	22	0.9	0.0030	0.0026	0.0056	0.0187	0.0470	ND	0.0028	ND	0.2134	0.1314
4F	LMB Fillet Composite	22	1.1	0.0033	0.0041	0.0074	0.0388	0.0875	ND	0.0037	ND	0.4812	0.2721
5F	LMB Fillet Composite	23	0.7	ND	0.0007	0.0007	0.0138	0.0435	ND	0.0027	ND	0.2488	0.1225
7F	LMB Fillet Composite	22	0.3	0.0030	0.0022	0.0052	0.0122	0.0283	ND	0.0027	ND	0.1277	0.0775
9F	LMB Fillet Composite	22	1	0.0013	0.0021	0.0034	0.0228	0.0440	ND	0.0036	ND	0.3354	0.1511
3F	YP Fillet Composite	20	0.4	0.0016	0.0023	0.0041	0.0102	0.0175	ND	0.0019	ND	0.1213	0.0533
4F	YP Fillet Composite	20	0.5	0.0011	0.0027	0.0038	0.0123	0.0194	ND	0.0021	ND	0.1451	0.0497
5F	YP Fillet Composite	20	0.3	0.0074	0.0014	0.0088	0.0057	0.0134	ND	0.0017	ND	0.1125	0.0483
7F	YP Fillet Composite	21	0.5	0.0023	0.0017	0.0040	0.0076	0.0146	ND	0.0027	ND	0.1543	0.0558
9F	YP Fillet Composite	20	0.4	0.0029	0.0014	0.0043	0.0079	0.0124	ND	0.0017	ND	0.1369	0.0475
1F	Calico Bass Offal Composite	30	2	0.0161	0.0045	0.0206	0.0330	0.0822	ND	0.0059	ND	0.2892	0.1383
1F	Carp Offal Composite	37	12.5	0.0877	0.0443	0.1320	0.1473	0.2739	ND	0.0130	ND	0.9007	0.3752
3F	Carp Offal Composite	34	12.8	0.1704	0.0893	0.2597	0.4893	0.3917	ND	0.0611	ND	2.7212	1.7158
4F	Carp Offal Composite	37	5	ND	0.0360	0.0360	0.2200	0.2400	ND	0.0162	ND	2.0000	0.8800
5F	Carp Offal Composite	33	11.7	0.0433	0.0841	0.1274	0.4334	0.6094	ND	0.0439	ND	5.0195	2.8377
7F	Carp Offal Composite	34	12	0.0168	0.0380	0.0548	0.2641	0.2905	ND	0.0370	ND	3.0667	0.9818
9F	Carp Offal Composite	32	12.8	0.0258	0.0844	0.1102	0.6075	0.4375	ND	0.0476	ND	4.9054	1.9753
1F	LMB Offal Composite	27	3.1	0.0219	0.0057	0.0276	0.0698	0.2972	0.0047	0.0080	ND	0.8463	0.5539
3F	LMB Offal Composite	31	5.5	0.0091	0.0169	0.0260	0.1604	0.3135	ND	0.0183	ND	1.4727	0.9130
4F	LMB Offal Composite	32	4.7	0.0094	0.0135	0.0229	0.1998	0.5589	ND	0.0162	ND	3.0028	1.8169
5F	LMB Offal Composite	32	4.9	0.0200	0.0210	0.0410	0.1522	0.3368	ND	0.0218	ND	1.5960	0.9114
7F	LMB Offal Composite	29	4.8	0.0321	0.0198	0.0519	0.1521	0.2604	ND	0.0242	ND	1.5635	0.9705
9F	LMB Offal Composite	30	5.6	0.0510	0.0159	0.0669	0.1869	0.2498	ND	0.0406	ND	2.1924	1.1775
3F	YP Offal Composite	31	3.7	0.0116	0.0286	0.0402	0.1800	0.1843	ND	0.0198	ND	1.1561	0.5377
4F	YP Offal Composite	29	4.3	0.0231	0.0250	0.0481	0.1644	0.1892	ND	0.0236	ND	1.3472	0.5134
5F	YP Offal Composite	24	3.2	ND	0.0038	0.0038	0.0799	0.1389	ND	0.0137	ND	1.1451	0.7476
7F	YP Offal Composite	30	3.6	0.0285	0.0186	0.0471	0.1202	0.1568	ND	0.0352	ND	0.1539	0.4925
9F	YP Offal Composite	30	3.5	0.0088	0.0178	0.0266	0.1656	0.1837	ND	0.0246	ND	1.8542	0.6888

APPENDIX III
SEGMENT MAPS AND GPS SHOCK PATHS



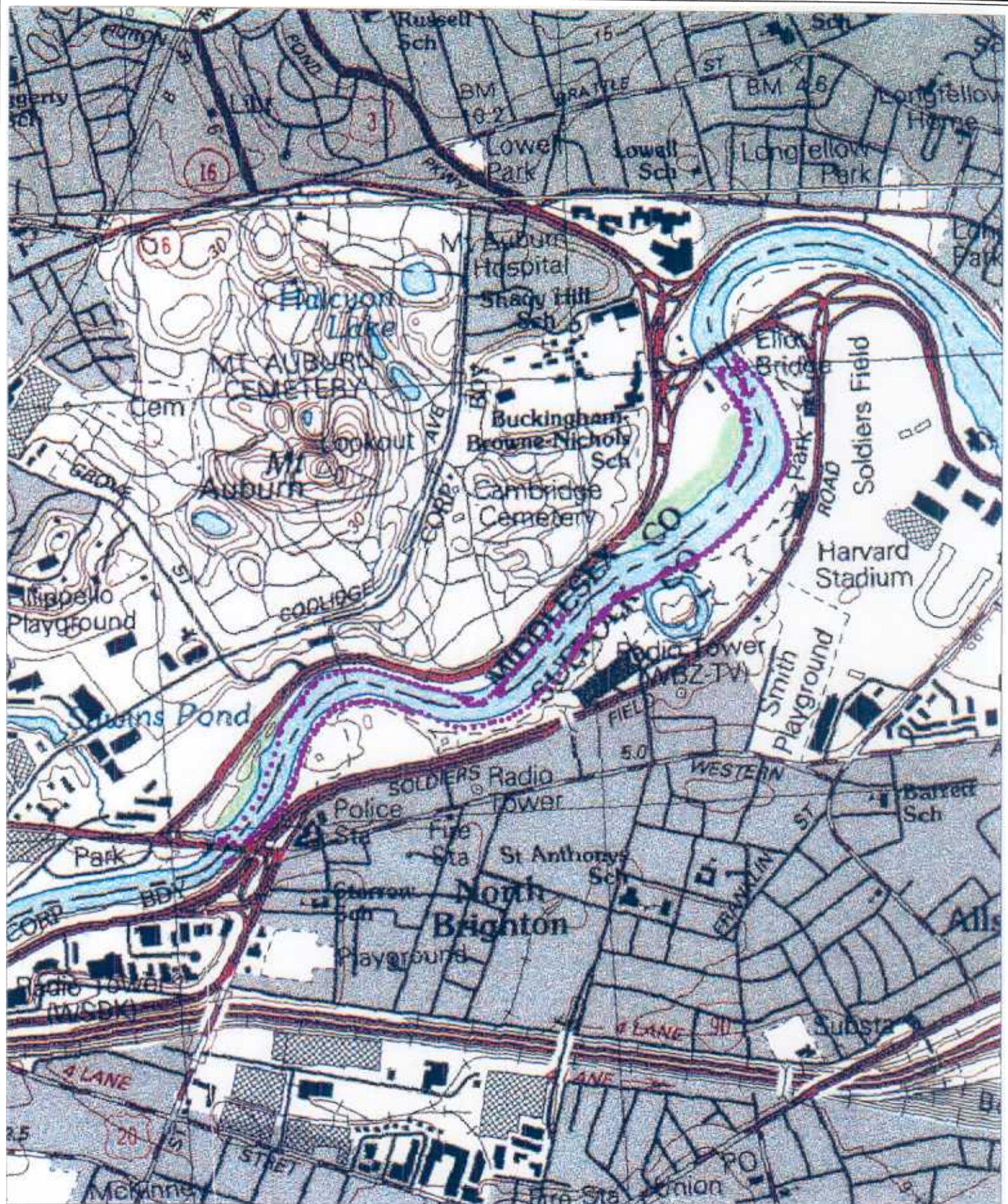
• Survey Boat Route

0.1 0 0.1 0.2 Miles

Charles River Fish Survey Segment 5F Shock Path

Data Sources: Quadrangle from USGS at 1:24,000. Fish survey from EPA at 1:500.





**Charles River Fish Survey
Segment 4F Shock Path**

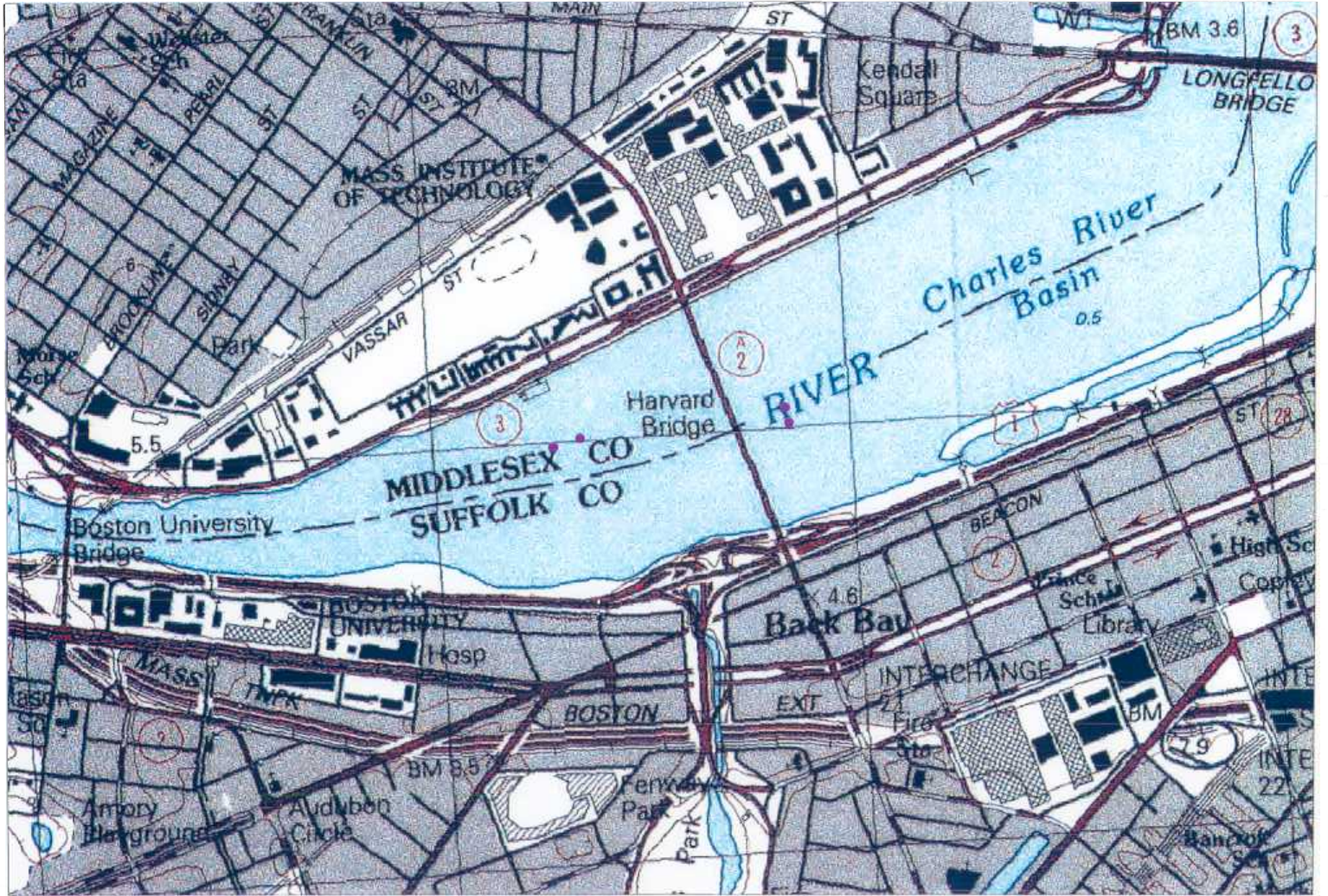
Data Sources: Quadrangle from USGS at 1:24,000. Fish survey from EPA at 1:500.



• Survey Boat Route

0.1 0 0.1 0.2 Miles





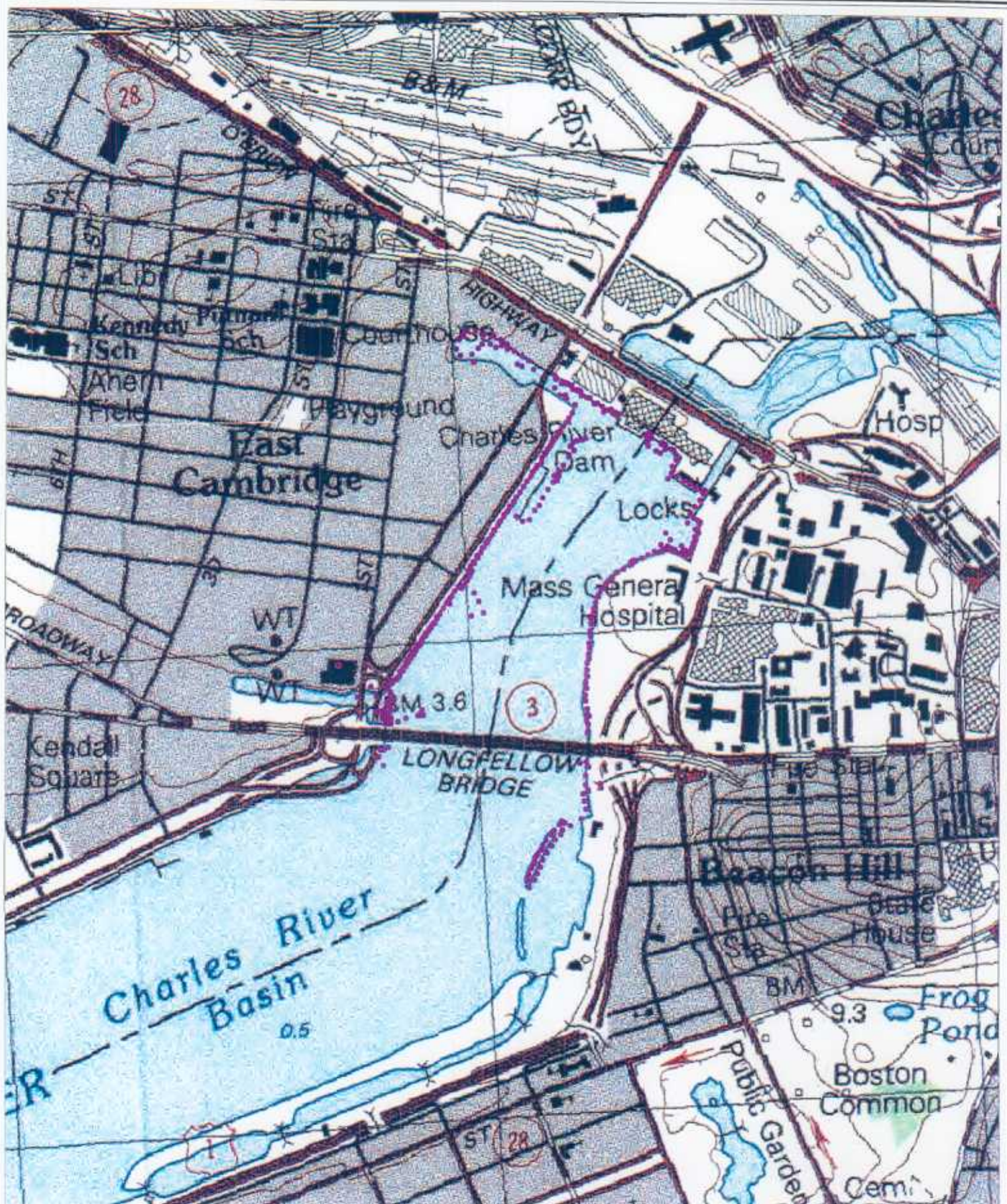
**Charles River Fish Survey
Segment 7F Shock Path**

Data Sources: Quadrangle from USGS at 1:24,000. Fish survey from EPA at 1:500.



• Survey Boat Route





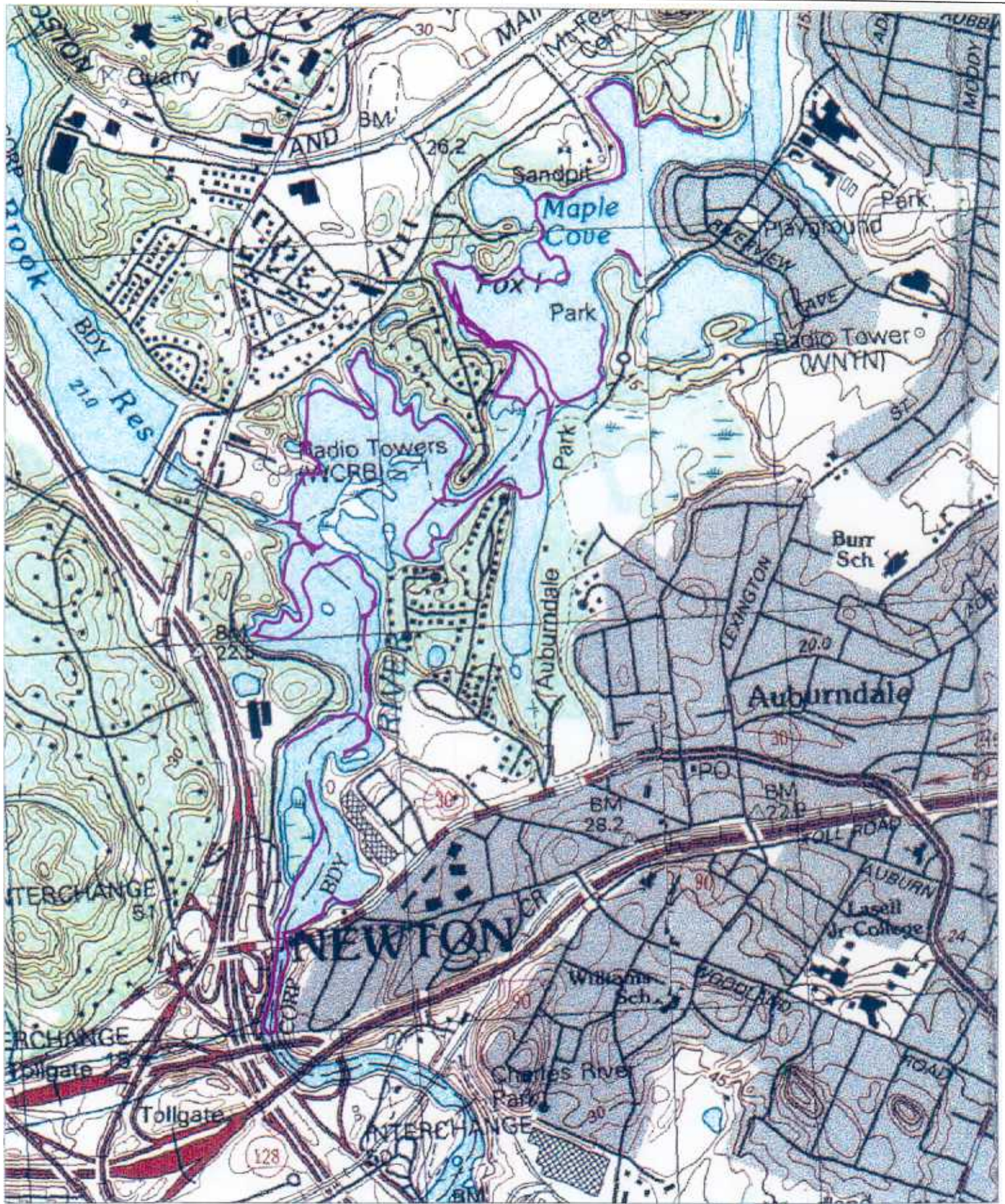
• Survey Boat Route



Charles River Fish Survey
Segment 9F Shock Path

Data Sources: Quadrangle from USGS at 1:24,000. Fish survey from EPA at 1:500.





**Charles River Fish Survey
Segment 1F Shock Path**

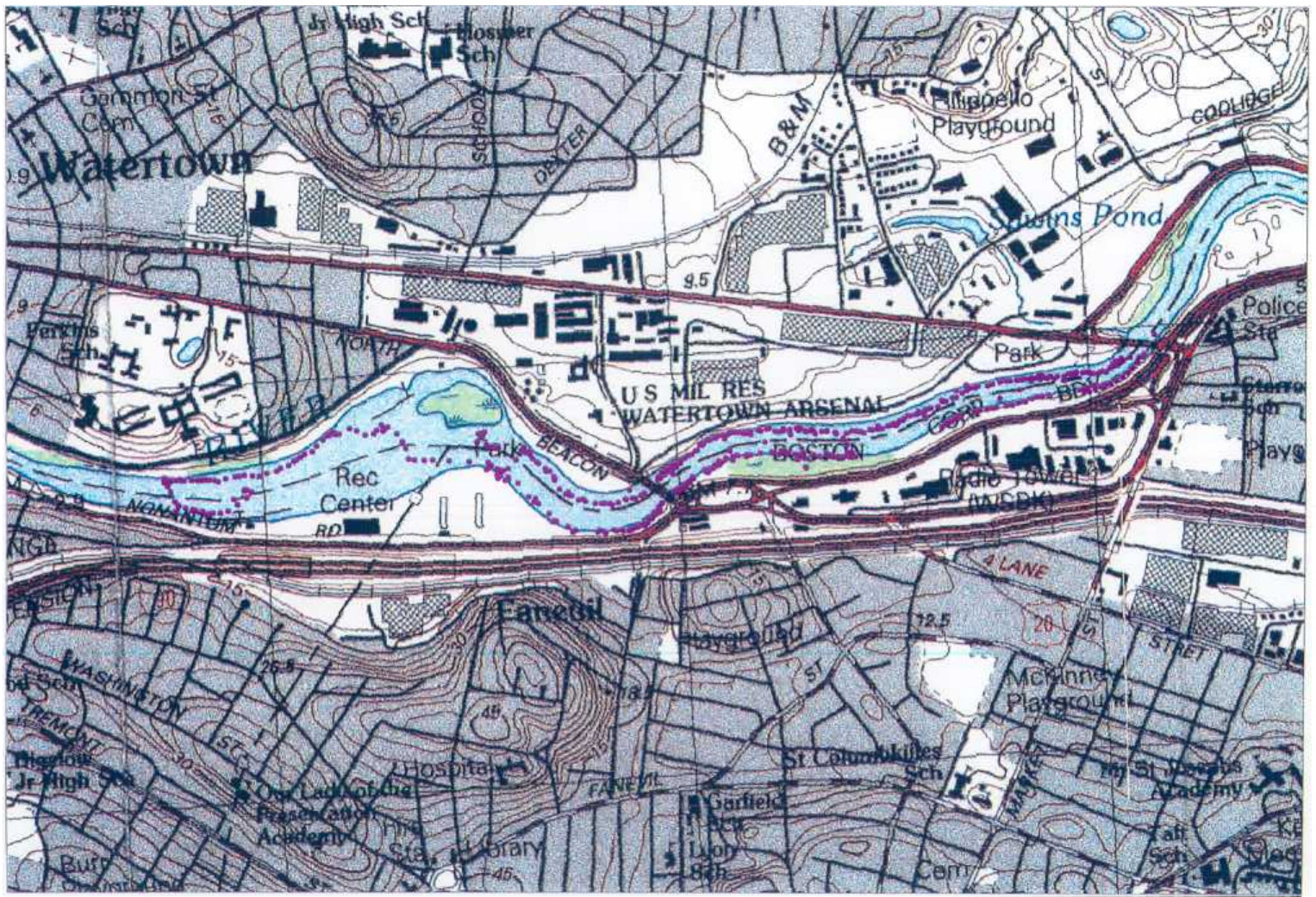
Data Sources: Quadrangle from USGS at 1:24,000. Fish survey from EPA at 1:500.



— Survey Boat Route

0.1 0 0.1 0.2 Miles





Charles River Fish Survey
Segment 3F Shock Path

Data Sources: Quadrangle from USGS at
1:24,000. Fish survey from EPA at 1:500.

