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WINTER 2005 VOL. 56, No. 3

VIRGINIA JOURNAL OF SCIENCE

OFFICIAL PUBLICATION OF THE VIRGINIA ACADEMY OF SCIENCE

THE VIRGINIA JOURNAL OF SCIENCE

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Virginia Journal of Science
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Prospects of Kenaf as an Alternative Field Crop in Virginia[1]

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ABSTRACT

Kenaf (*Hibiscus cannabinus* L.), a warm-season annual plant, has shown potential as an alternate source of fiber in the United States. Although preliminary research has indicated feasibility of kenaf production in Virginia, production details are lacking. Field experiments were conducted during 1995 and 1996 to determine optimal row spacing and fertilizer needs, and to compare available kenaf cultivars. Although results indicated that differences in dry matter yields from four row spacings (30, 60, 90, and 120 cm) and four rates each of N, P, and K fertilizers (50, 100, 150, and 200 $kg \cdot ha^{-1}$) were not statistically different, the yields were adequate ranging from 8.8 to 16.0 $t \cdot ha^{-1}$ with an average yield of 12.5 $t \cdot ha^{-1}$. Dry matter yields for narrow-leaf cultivars proved superior to broad-leaf, and the overall results demonstrate that kenaf can be easily produced in Virginia.

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.), a relative of cotton (*Gossypium hirsutum* L.) and okra (*Abelmoschus esculentus* L.), is a warm-season annual plant that originated in northern Africa and has been used as a cordage crop for many years in India, Russia, and China (Dempsey, 1975). Kenaf research in the USA began during World War II to supply cordage material for the war effort (Wilson et al, 1965). During the 1950s and early 1960s, it was determined that kenaf was an excellent cellulose fiber source for a large range of paper products (newsprint, bond paper, corrugated liner board, etc.). It was also determined that pulping kenaf required less energy and chemical inputs for processing than standard wood sources (Nelson et al., 1962). More recent research and development work indicates that kenaf is also suitable for use in building materials (particle boards of various densities, thicknesses, with fire and insect resistance), absorbents, textiles, livestock feed, and fibers in new and recycled plastics (Webber and Bledsoe, 1993).

These observations indicate that kenaf could be potentially grown in Virginia to diversify cropping systems, to provide alternative materials for paper mills, and to meet varied industrial needs. Virginia State University's New Crops Program, established in 1991, initiated a kenaf research project in 1992. The objectives of this project were

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[1] Contribution of Virginia State University, Agricultural Research Station Journal Article Series No. 247. The use of trade names or vendors does not imply approval to the exclusion of other products or vendors that may also be suitable.

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to conduct preliminary production research and to determine the feasibility of kenaf production in Virginia. Research conducted in Virginia during 1992-1994 indicated that kenaf has significant potential as an alternate crop in Virginia (Bhardwaj and Webber, 1994; Bhardwaj et al., 1995). However, information regarding desirable agronomic practices such as cultivar selection, fertility requirements, and plant densities, specifically for Virginia was not available. Therefore, experiments were conducted to identify: (1) high yielding varieties, (2) optimum levels of nitrogen, phosphorous, and potassium fertilizers, and (3) ideal row spacing.

MATERIALS AND METHODS

Three experiments were conducted during each of 1995 and 1996 at Randolph farm of Virginia State University, located in Ettrick, Virginia (37^0 14' N Latitude and 77^0 26' W Longitude) at an approximate elevation of 71 m. The soil type was an Abel sandy loam (fine loamy mixed thermic Aquatic Hapludult) soil that typically has a pH of 6.1 to 6.4.

In the first experiment, four inter-row spacings (30, 60, 90, and 120 cm) were evaluated with two kenaf cultivars: "Everglades 41" (A kenaf variety with broad leaves) and "Everglades-71" (A kenaf variety with narrow leaves). Three replications of a split-plot design with varieties in main plots and row spacings in sub-plots were planted on May 22, 1995, and May 20, 1996. Each plot consisted of three rows with a 60 cm spacing between sub-plots. These plots received 100 kg · ha^{-1} each of nitrogen (N), phosphorous (P), and potassium (K). In the second experiment, four rates (50, 100, 150, and 200 kg·ha^{-1}) each of N, P, and K, were evaluated with Everglades 41 variety in four replications of a split-plot design with N in main plots, P in sub-plots, and K in sub-sub-plots. Each plot consisted of three rows with inter-row spacing of 75 cm with one row left blank between the plots. These experiments were planted on May 23, 1995, and May 20, 1996. In the third experiment, 21 kenaf cultivars were planted on May 23, 1995, and May 21, 1996, in a randomized complete block design with three replications. Each plot consisted of three rows with inter-row spacing of 75 cm. These plots received 100 kg·ha^{-1} each of nitrogen (N), phosphorous (P), and potassium (K).

Approximately 100 seeds of each cultivar were planted in each 3 m long row. In each experiment, weeds were controlled with a pre-plant-incorporated application of 1.5 l· ha^{-1} of trifluralin herbicide. These experiments were not irrigated. Data were recorded for dry matter yield and plant height from samples harvested manually at the ground level after a hard freeze in early January had effectively killed the plants. During 1995, a 1-m sample was taken from the middle row of each plot in each experiment; and in 1996, a 2-m sample was harvested. After a two-month storage period, meant to stabilize the moisture content to a constant value and to dry the material, the harvested material was measured and the yield calculated in t·ha^{-1}. All data were analyzed using General Linear Models procedure of SAS (SAS, 1996).

RESULTS AND DISCUSSION

Row-Spacing: The differences in dry matter yield, averaged across two cultivars, for the four row spacings were not significant (Table 1). However, the closer spacing of 30 cm between rows showed a numerically higher yield of 11.1 t·ha^{-1}. The dry matter yields of Everglades 41 (8.2 t·ha^{-1}) and Everglades 71 (8.6 t·ha^{-1}) were also

TABLE 1. Effect of row-spacing on kenaf dry matter yield and plant height during 1995 and 1996 at Ettrick, Virginia.

Row Spacing	Dry Matter ($t{\cdot}ha^{-1}$)	Plant height (cm)
30 cm	11.1 a*	233.1 a*
60 cm	7.2 a	233.4 a
90 cm	7.2 a	231.3 a
120 cm	8.0 a	243.7 a
Mean	8.4	235.4

* Means across two cultivars (Everglades 41 and Everglades 71) and three replications each during two years. Means followed by similar letters are not different according to Least Significant Difference (5% level).

statistically similar (data not shown). The interactions between row spacing and cultivars for dry matter yield and plant height were not significant. The row spacing effects on plant height were also not significant. This research demonstrates kenaf's adaptability to varying plant densities. However, averaged across all row spacings, plants of Everglades 71 kenaf variety were taller (242 cm) than those of Everglades 41 kenaf variety (229 cm). No data were recorded on stalk diameter, however visual observations indicated that stalk diameter in closer row spacings was less than that of widely-spaced rows. Since a possibility of using kenaf as a forage crop exists, the closer row spacing may be desirable as it would reduce the woody component of kenaf harvested at green stage for feeding the livestock. However, the economics of kenaf seed would need to be considered. Kenaf, being a tropical plant, does not produce seed in the United States. Most kenaf seed is produced in Mexico or Carribean locations. Use of closer row spacing would entail more seed and would increase production costs.

Nutrient Needs: The dry matter yields and plant heights, following application of four rates (50, 100, 150, and 200 $kg{\cdot}ha^{-1}$) each of N, P, and K, are presented in Table 2. The kenaf dry matter yield and plant height did not differ significantly in response to fertilizer rates. However, the highest dry matter yield of 11.4 $t{\cdot}ha^{-1}$ was obtained upon application of 50 $kg{\cdot}ha^{-1}$ N. The residual N content in the experimental area was approximately 14 $kg{\cdot}ha^{-1}$, therefore, kenaf needs up to 64 $kg{\cdot}ha^{-1}$ N for optimal production. The response of kenaf to P and K applications was not significant. Previous observations (Rangappa et al., 2002) have indicated that soil at this experimental site, which is generally considered to be typical of most soils in the Southern Piedmont region in Virginia, contains approximately 54 to 77 $mg{\cdot}kg^{-1}$ P and 52 to 64 $mg{\cdot}kg^{-1}$ K. These levels, generally, provide adequate P and K for most crops, and positive responses to additional applications of these nutrients are not very common. These results indicate that the nutrient needs of kenaf are quite modest.

Varietal Evaluations: The mean dry matter yield from 21 kenaf varieties was 12.5 $t{\cdot}ha^{-1}$ (Table 3) which compares favorably with kenaf yields reported from other areas in the United States. The dry matter yields ranged from 8.8 to 16.0 $t{\cdot}ha^{-1}$, respectively for GR 2563 and 78-18RS-10 kenaf varieties. The plant height varied from 229.7 to 288.6 cm, respectively for Tainung #1 and 78-18-GS-3 kenaf varieties. A significant positive correlation (+0.28, P=0.001) indicated that taller plants resulted in higher dry matter yields.

TABLE 2. Dry matter yield and plant height of kenaf following four rates each of N, P, and K fertilizers during 1995 and 1996 at Ettrick, Virginia.

Fertilizer	Dry Matter Yield ($t \cdot ha^{-1}$)			Plant Height (cm)		
Rate	N	P	K	N	P	K
50 $kg \cdot ha^{-1}$	11.4 a*	10.6 a	10.8 a	277.6 a	274.7 a	270.7 a
100 $kg \cdot ha^{-1}$	10.9 a	11.3 a	11.8 a	273.8 a	277.2 a	282.6 a
150 $kg \cdot ha^{-1}$	10.6 a	10.6 a	10.3 a	277.2 a	276.2 a	277.9 a
200 $kg \cdot ha^{-1}$	10.6 a	11.0 a	10.5 a	278.8 a	279.1 a	276.2 a
Mean	10.9	10.9	10.9	276.8	276.8	276.8

*Means followed by similar letters are not different according to Least Significant Difference (5% level). The interactions between N, P, and K were non-significant. The means of individual nutrients were obtained from averaging over all rates of other two nutrients i.e. the mean of N is averaged over all rates of P and K, the mean of P is averaged over all rates of N and K, and the mean of K is averaged over all rates of N and P.

TABLE 3. Dry matter yield and plant height of 21 kenaf cultivars when grown during 1995 and 1996 at Ettrick, Virginia.

Variety	Dry Matter Yield($t \cdot ha^{-1}$)	Plant Height (cm)	Leaf Shape
78-18RS-10*	16.0**	270.0*	Narrow
Everglades 71	14.3	266.3	Narrow
45-9	14.3	257.7	Narrow
SF 192	14.3	265.2	Narrow
15-2	14.3	278.5	Narrow
KK 60	14.1	280.3	Narrow
78-18GS-3	13.7	288.6	Narrow
Gautemala 51	13.5	267.0	Narrow
SF 459	13.1	259.2	Narrow
Tainung #1	13.0	279.3	Narrow
Gautemala 45	12.4	229.7	Broad
C 2032	12.4	247.2	Broad
Everglades 41	12.0	239.2	Broad
7N	12.0	253.8	Broad
Tainung #2	11.9	284.3	Narrow
Guatemala 4	11.7	258.0	Broad
C-108	10.9	245.8	Broad
Indian	10.6	229.8	Narrow
Cubano	10.5	274.8	Broad
Guatemala 48	9.1	270.0	Broad
GR 2563	8.8	241.8	Broad
Mean	12.5	261.3	-
LSD(.05)	3.4	31.2	-

* These are the names of kenaf varieties that are assigned by developers of these varieties.
** Means across two years and three replications per year. The year x cultivar interaction was non-significant.

Among the 21 kenaf varieties evaluated, 12 had narrow leaves and 9 had broad leaves (Table 3). A comparison of narrow-leaf shape group of kenaf varieties with broad-leaf shape group of kenaf varieties indicated that narrow-leaf group had a significantly (5% probability) higher yield and significantly taller plants (13.6 $t \cdot ha^{-1}$ and 268.8 cm, respectively) as compared to broad-leaf group (11.1 $t \cdot ha^{-1}$ and 251.1 cm, respectively). A problem with narrow leafed-cultivars is that the leaves superficially resemble those of marijuana (*Cannabis sativa* L.). There have been instances where narrow-leafed kenaf plants have been mistaken for marijuana plants. However, there are simple differences between kenaf and marijuana for identification purposes. The marijuana stalks are four-sided without thorns whereas kenaf stalks are generally round and have thorns. A marijuana leaf consists of seven or nine individual leaves joined at a common stem, whereas kenaf leaves are classified as compound leaves with seven lobes (Somers, 1991). We suggest that it may be desirable to grow broad-leafed cultivars at least until kenaf becomes a popular crop and potential confusion can be avoided.

CONCLUSIONS

The main goal of these research efforts was to evaluate the feasibility of kenaf production in Virginia. Results from two years of research indicate that under Virginia conditions, kenaf can be successfully planted at varying row spacings. The fertilizer needs of kenaf seem to be modest, approximately 64 $kg \cdot ha^{-1}$ of nitrogen may be adequate. The P and K content in most soils in Virginia is expected to be adequate for kenaf production. Our results also indicated that up to 16 t ha^{-1} dry matter yield can be obtained from kenaf grown as an annual crop. These results indicate that kenaf can be easily produced in Virginia.

ACKNOWLEDGMENT

The seeds for various experiments and details of kenaf cultivars were provided by C.L. Webber, III. The experiments were conducted by Harbans L. Bhardwaj. Both authors analyzed the data and prepared the manuscript.

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Virginia Journal of Science
Volume 56 Number 3
Fall 2005

Fish Consumption Patterns of Populations in Vicinities of Lake Kastoria and Lake Pamvotis, Greece

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ABSTRACT

Objectives are to establish fish consumption patterns of populations in vicinities of two lakes (Kastoria and Pamvotis) in Greece for use in the assessment of risks associated with consumption of fishes in these agri-chemically impaired lakes. Parameters measured were demographics (i.e., gender, age, weight, education level, occupation, residency), freshwater fish eating frequency, species and sizes of fishes consumed, and fish consumption habits [i.e., quantity, parts, and preparation method). All annual mean site-specific consumption rates of the four gender-age class sub-populations surveyed in vicinity of Lake Kastoria (avg. range= 0.103-0.29 kg/day) exceed those of Greece (0.066 kg/day), EU (0.068 kg/day), Spain (0.104 kg/day), Portugal (0.159 kg/day), and the USEPA default value (0.054 kg/day) with two exceptions. Female consumption rates (0.087-0.103 kg/day) of *Perca fluviatilis* were below annual consumption rates of Spain and Portugal. Similarly, annual mean site-specific consumption rates of *Anguilla anguilla* and *Cyprinus carpio* by male (0.199-0.210 kg/day) and female adults (0.096-0.157 kg/day) in vicinity of Lake Pamvotis exceeded those of Greece, EU, Spain, Portugal, and the USEPA default value. Survey results indicate better-educated Greeks to be higher consumers of fish; however, market availability appears to be a stronger determinant of food choice in comparison to health education. All populations in vicinities of both lakes preferred to eat fried fishes with one exception: grilled *C. carpio* from Lake Pamvotis was preferred by female and male adults.

Keywords: fish consumption, risk assessment, chronic daily intake, Greece

INTRODUCTION

Bioconcentration of environmental contaminants is receiving increased attention from Europe's general public, for-profit and non-profit institutions, regulatory agencies of countries in the European Community (EC), and the European Environmental Agency (EEA)(Hill, 1999; Millstone et al., 2000; Tait, 2001; Tait and Quin, 1998; WHO, 1989; UNEP, 1999). Increased concern stems from increased public and governmental awareness of their impacts on human health, tourism, and other economic issues that effect gross domestic markets (Braxton and Frewer, 1998; Hites et al., 2004; Miles and Frewer, 1998; World Bank, 1994). Public demand for healthful foods is causing considerable shifts in commodity markets (Millstone et al., 2000; Tait, 2001; Kafka and Alvensleben, 1997; World Bank, 1992), and demands for clean water has now become a dominant issue in the tourism industry (UNEP, 1999; World Bank, 1994).

In Greece, fish consumption has been increasing since 1995, concurrent with public awareness that seafood is healthy and can substitute for meat consumption (USDA. 2003). Per capita consumption of fisheries products in Greece is 24 kg (USDA. 2003). With the increased use and exploitation of localized fisheries comes an incremental increase in exposure to local pollution problems (Szucs and Grasselli, 2004; World Bank, 1992). The problem may be particularly acute in highly localized fisheries such as those in lakes, rivers, and natural water dependent aquaculture facilities as they tend to be located in, or impacted by, high yield agriculture areas (Stickney, 1979; Landau, 1992; Stevenson, 2004). Such is the case in Lake Kastoria, Lake Pamvotis, and Lake Vistonis in Greece. For example, we identified 10 pesticides (Atonik, Cobex, Efmathrin=Efmetren/Permethrin, Fusilade, Primextra, Ridomil, Sencor, Targa, Thimet, and Thiodan=Endosulfan)) that were routinely applied prophylatically to agricultural fields draining into Lake Vistonis in 1993, where acute toxicity had been reported to decimate the population of *Cyprinus carpio* in an aquaculture facility operated by monks (Alpanezos, pers. comm.). Consumption of freshwater fishes from Lake Pamvotis has diminished in recent years because of increased public awareness of the pollution state of the lake (Filos, pers. comm., 2002). Whereas studies in the variability of fish consumption within 10 European countries, nutrient content of foods in Greece, policy analysis on food safety in Europe, and the association of cooking methods to cancer have been conducted (Boulous et al., 1996; Millstone et al., 2000; Rohrmann et al., 2002; Welch et al., 2002), there appear to be no published data on human consumption patterns of freshwater fisheries products for use in calculating risks associated with consumption of freshwater fishes in Greece.

Our objective is to establish fish consumption patterns of populations in vicinities of two lakes (Kastoria and Pamvotis) in Greece for use in assessing risks associated with consumption of fishes in these two agri-chemically impaired lakes.

STUDY AREA

Lake Kastoria (~28 km^2; karst basin=304 km^2; avg. depth=4 m; max. depth=8.5 m), a eutrophic lake with large concentrations of phytoplankton and mats of submerged aquatic vegetation (Aliakmon River drainage), is located in Kastoria (Macedonia Prefecture) in northwestern Greece (Skoulikidis et al., 1998). The lake, which receives runoff from agricultural operations (e.g., corn, apple orchards, livestock) and a furrier industry, has dropped ~ 1.5 m in the last 5 years, primarily through water withdrawal for agricultural operations (Filos, pers. comm.). Mean physical and nutrient concentrations in the lake are pH (8.2), total phosphorus (39 μg/l), P-PO_4 (31.3 μg/l: max=62.5), N-NO_2 (5.8 μg/l; max=19.0), N (15.8 μg/l; max=1011); N-NO_3 (22.4 μg/l), and N-NH_4 (288 μg/l)(Hadjibiros et al., 1998). Mean heavy metal concentrations for Pb (31.1 ppb), Zn (32.8-81.2 ppb), Cu (6.6-19.4 ppb), Cd (0.7 ppb), Hg (0.25 ppb) and As (11.1 ppb) have been recorded from Lake Kastoria (Hadjibiros et al., 1998). Main sources of cadmium, copper, lead, and zinc are fertilizer and pesticide residues (Hadjibiros et al., 1998).

Lake Pamvotis (22 km^2; basin=330 km^2; avg. depth=5.5 m; max. depth=11 m) is an eutrophic lake beside the city of Ioannina (Epiros Prefecture) in western Greece (Skoulikidis et al., 1998). Mean physical and nutrient concentrations in the lake are pH (8.4), total phosphorus (38 μg/l), $P\text{-}PO_4$ (31.7 μg/l: max=91), $N\text{-}NO_2$ (2.2 μg/l; max=65.3), N (24.1 μg/l; max=926); $N\text{-}NO_3$ (27.3 μg/l), and $N\text{-}NH_4$ (62.8 μg/l) (Hadjibiros et al., 1998). Mean heavy metal concentrations for Zn (33.1 ppb), Cu (5.2 ppb), Cd (1.6 ppb), and As (1.9 ppb) have been reported from Lake Pamvotis (Hadjibiros et al., 1998). Eutrophication is primarily a result of domestic wastewater inputs (Hadjibiros et al., 1998).

MATERIALS AND METHODS

Fish consumption patterns were determined through the use of personal interviews with 90 people selected at random in Kastoria Greece for Lake Kastoria; and 135 people in Ioannina, Greece for Lake Pamvotis. Fish consumption survey interviews, modeled after guidelines and recommendations in USEPA (1998), were conducted in Greek for Lake Kastoria on June 5, 6, 10, and 17, 2002; and in Ioannina for Lake Pamvotis, Greece on June 6, 7, 8, 9, 11, 28, 29, 30 and July 1, 2002. Variables recorded included: demographics [i.e., gender, age, weight (kg), education (0=none, 1=elementary, 2=middle school, 3=1^{st}-2^{nd} years of high school, 4=3^{rd} and 4^{th} years of high school, 5=graduate school), occupation (0=unemployed/retired; 1=student; 2=housewife; 3=agricultural worker/fisherman; 4=blue collar; 5=professional), residency (1=yes, 2=no), domicile distance from lake (m)] and eating statistics [i.e., general marine and freshwater fish eating frequency (days/year); specific freshwater species and sizes (kg) eaten (*Perca fluviatilis*, *Rutilus rutilus* for Lake Kastoria, and *Anguilla anguilla*, *Cyprinus carpio*, *Silurus aristotelis* for Lake Pamvotis), specific eating frequency (days/year) per freshwater species from each lake, and fish consumption habits [i.e., quantity (kg), parts (frequency consumption of muscle, skin, bones, head, intestine), and preparation method (fry, grill, boil, head boil, soup frequency). Selection of fish species was based on freshwater fish eating preference, species availability per lake, and ecological feeding type: food chain position (predator, prey); feeding type (i.e., carnivore, herbivore, omnivore), and feeding position (e.g. bottom) as follows: herbivorous bottom feeder=*R. rutilus* and *C. carpio*; carnivorous bottom feeder= *S. aristotelis*; carnivorous pelagic feeder: *P. fluviatilis*; and carnivorous bottom feeder= *A. anguilla*.

Four gender-age class groups (male adult $\geq$ 18 yo; female adult $\geq$ 18 yo; male youth <18 yo; female youth <18 yo) conform to those specified in USEPA (1998). Percentages and other proportional measurements were converted into arcsin equivalents to normalize variance prior to statistical tests. Spearman's correlation analysis (SAS, 2002) was used to identify significant correlations among variables per lake and species. Option NOMISS was employed to eliminate observations with missing values as pairwise correlation matrices may not be nonnegative definite, and the pattern of missing values may bias results (SAS, 2002). Analysis of variance followed by Duncan's multiple range test (SAS, 2002) was used to test differences in each variable among gender-age class groups by lake and species in order to distinguish group specific consumption patterns from overall consumption patterns.

RESULTS

Lake Kastoria demographics- Mean ages and weights of male (47.3 y; 85.9 kg) and female (48.5 y; 63.5 kg) adults were significantly greater than those of female (12 yo; 37.4 kg) and male (10.4 y; 38.6 kg) youth (Table 1). Mean grade level (1.75) of male youth was significantly lower than those of male and female adults (range=2.7-2.8; Table 1). Occupation level of male adults (3.8) was significantly greater than those of female adults (2.8) and male and female children (Table 1). Mean eating days (126.2) of marine and freshwater fishes by male adults were significantly greater than those (range=76.9-105.8 days) of other interview groups, and negatively correlated with weight (coefficient=-0.3175; p=0.0336; Table 1).

Lake Kastoria Consumption Patterns by Species Consumed:

Perca fluviatilis: Annual mean consumption of *P. fluviatilis* ranged from 0.087 kg/day in female adults to 0.273 kg/day in male adults and (Table 2), and did not vary significantly among gender-age groups (F=1.70; p=0.1889). Mean fish size (range=0.32-0.50 kg) and number of days (range= 57.5-73.9) of eating *P. fluviatilis* did not vary significantly among all gender-age groups (Table 3). However, quantity ($\bar{x}$=0.93 kg) of *P. fluviatilis* consumed per meal by male adults was significantly greater than those (range $\bar{x}$=0.34-0.45 kg) consumed by other gender-age groups (Table 3).

Interviewee weight was significantly positively correlated with age (p<0.0001), education (p=0.0037), occupation (p<0.0001) quantity of *P. fluviatilis* consumed (p=0.004), and eating skin (p=0.0072) and bones (p=0.0218) of the species. Frequency of eating skin was correlated with quantity of *P. fluviatilis* consumed (p=0.0204). *Perca fluviatilis* consumption days were correlated positively with frequency of eating skin (p=0.0163) and negatively with eating heads (p=0.0099). Boiling heads of *P. fluviatilis* was correlated with frequency of eating bones (p=0.0136) and soup (p<0.0001).Fish size and frequency of eating fried *P. fluviatilis* were negatively correlated (coefficient=-0.4906), whereas fish size and frequency of eating grilled *P. fluviatilis* were positively correlated (coefficient=0.3580; p=0.0376) indicating that smaller fish were fried and larger ones grilled. Mean percents (65-67) of consumption of fried *P. fluviatilis* were significantly greater than those of grilled ($\bar{x}$ range=27-35) and boiled ($\bar{x}$ range=0-3) in female and male adults (Table 4).

Mean skin eating frequencies (range=82-90 %) in female and male adults were significantly greater than those ($\bar{x}$ range=0-33 %) in female and male youth (Table 3). Mean frequencies of eating heads (60 %) and bones (40 %) by male adults were significantly greater than those ($\bar{x}$ range=0-1 %) in female and male youth (Table 3). Mean percents (range 33-55) of consumption of fried *P. fluviatilis* were significantly greater than those of other preparation methods except by female youth (Table 3). All *P. fluviatilis* were gutted prior to consumption, regardless of preparation method.

Rutilus rutilus: Annual mean consumption of *R. rutilus* ranged from 0.185 kg/day in female adults to 0.297 kg/day in male children (Table 2), and did not vary significantly among gender-age groups (F=1.68; p=0.1970). Mean quantity ($\bar{x}$=0.93 kg) of *R. rutilus* consumed per meal by male adults was significantly greater than those ($\bar{x}$ range=030-0.48 kg) consumed by other gender-age groups (Table 5). Mean fish size (range=0.10-0.12 kg) and number of days (range= 77.0-96.7) of eating *R. rutilus* did not vary significantly among all gender-age groups (Table 5).

Interviewee weight (correlation=0.5340; p=0.0028) and occupation (correlation=0.4178; p=0.0269) were significantly correlated with quantity of *R. rutilus* consumed. Age was significantly correlated with eating skin (correlation=0.36984; p=0.0483) and bones (correlation=0.4550; p=0.0131) of *R. rutilus*. Eating skin was significantly correlated with occupation (correlation=0.3908; p=0.0398), and eating heads (correlation=0.4761, p=0.0090) and bones (correlation=0.3864; p=0.0384), negatively correlated with grilling *R. rutilus* (correlation=-0.3864; p=0.0384), and number of days per year consuming *R. rutilus* (correlation=-0.5811; p=0.0009).

Frequencies of eating skin, heads, and bones (fried or grilled) did not vary significantly among gender-age groups (Table 5). Percent frequencies ($\bar{x}$ range=67-80 %) of eating fried *R. rutilus* by all gender-age groups was significantly greater than eating the species either grilled ($\bar{x}$ range=20-33 %) or boiled (0 %)(Table 5). All *R. rutilus* were gutted prior to consumption, regardless of preparation method, and none was boiled to make soup.

Lake Pamvotis demographics (based on 135 interviews): Mean ages and weights of male (43.2 y; 81.9 kg) and female (44.6 y; 68.6 kg) adults were significantly greater than those of female (10.1 y; 40.6 kg) and male (11.3 y; 44.4 kg) youth (Table 1). Mean educational levels of female (3.8) and male (3.5) adults were significantly greater than those of male (1.4) and female (1.2) youth (Table 1). Occupation levels of female (3.7) and male (3.0) adults were significantly greater than those of female (1.1) and male youth (0.96)(Table 1). Mean distance (1270.8 m) between homes and lakes of male youth were significantly greater than those of adult females (640.2) and males (640.0; F=2.81; p<0.042). Mean eating days ($\bar{x}$ range=40.5-60.9) of marine and freshwater fishes did not vary significantly among all gender-age groups (F=7.86; p<0.0001).

Anguilla anguilla: Annual mean consumption of *A. anguilla* ranged from 0.010 kg/day in female children to 0.199 kg/day in male adults (Table 6), and did not vary significantly among gender-age groups (F=1.31; p=0.3193). Mean number of consumption days (55) for *A. anguilla* by male adults was significantly greater than those ($\bar{x}$ range=1-6.5) for female and male youth (Table 6).

Age (correlation=0.5360; p=0.0001), weight (correlation=0.3828; p=0.0087), and frequency of eating fried fish (correlation=0.5647; p<0.0001) were correlated with frequency of eating *A. anguilla*. Frequency of eating soup made from *A. anguilla* was significantly correlated with boiling heads (correlation=1.0; p<0.0001) eating bones (correlation=1.0; p<0.0001), eating heads (correlation=1.0; p<0.0001).

Quantity, fish size, frequency of eating skin, heads, bones by any preparation method (i.e., fried, grilled, or boiled) of *A. anguilla* did not vary significantly among gender-age groups (Table 6). Consumption of fried *A. anguilla* (63 %) was significantly greater than other preparation methods (grilled, 37 %; boiled, 0 %) in female adults (Table 7). Correspondingly, male adults ate fried (48 %) and grilled (48 %) *A. anguilla* significantly more than boiled (4 %) *A. anguilla* (Table 7). All *A. anguilla* were gutted prior to consumption, regardless of preparation method.

Cyprinus carpio: Annual mean consumption of *C. carpio* ranged from 0.056 kg/day in female children to 0.210 kg/day in male adults (Table 1), and did not vary significantly among gender-age groups (F=1.17; p=0.33427). Mean quantity (0.86 kg) of *C. carpio* consumed per meal by male adults was significantly greater than those ($\bar{x}$ range=0.21-0.25 kg) consumed by female and male youth (Table 8).

Interviewee weight was significantly correlated with age (correlation=0.4768, p=0.0011), education level (correlation=0.4768, p=0.0011), occupation (correlation=0.0.5868, 0.0011), and quantity of *C. carpio* consumed (correlation=0.7959, p<0.0001). Occupation was correlated with eating grilled *C. carpio* (correlation=0.4041; p=0.0406) and negatively correlated with eating fried *C. carpio* (correlation=-6645; p=0.0002). Interviewees consuming a greater quantity of *C. carpio* were more likely to boil *C. carpio* (correlation=0.6040; p=0.0009), heads of *C. carpio* (correlation=0.6040; p=0.0009) and eat soup (correlation=0.6018; p=0.0009).

Eating skins of *C. carpio* was correlated with frequencies of eating heads (correlation=0.9117; p<0.0001), boiling *C. carpio* (correlation=0.5449; p=0.0033), boiling heads (correlation=0.5449; p= 0.0033), and eating soup made from boiled flesh and heads of *C. carpio* (correlation=0.5328; p=0.0042). Eating heads of *C. carpio* was correlated with boiling flesh of *C. carpio* (correlation=0.5976; p=0.0010), heads of *C. carpio* (correlation=0.5976; p=0.0010), and eating soup (correlation=0.5844; p=0.0014). Likewise, eating bones of *C. carpio* was correlated with boiling flesh (correlation=0.5547; p=0.0027), boiling heads (correlation=0.5547; p=0.0027); and frequency of eating soup made from *C. carpio* (correlation=0.7167; p<0.0001). Grilling *C. carpio* was inversely correlated with frying the species (correlation=-0.5437; p=0.0034). Grilling was correlated with number of consumption days for *C. carpio* (correlation=0.4710; p=0.0132).

Percentages of eating grilled *C. carpio* in female adults (78) and male adults (63) were significantly greater than those for fried (18-28 %) and boiled (4-9 %) in female and male adults (Table 7). Fried *C. carpio* (70 %) consumed by female youth was significantly greater than that consumed boiled (0 %)(Table 7).

Silurus aristotelis: Annual mean consumption of *S. aristotelis* ranged from 0.02 kg/day in male adults to 0.042 kg/day in female adults (Table 1), and did not vary significantly among gender-age groups (F=0.30; p=0.8217).

Interviewee age was significantly correlated with quantity of *S. aristotelis* consumed (correlation=0.5546; p=0.0258)(Table 9). Eating skin was significantly correlated with eating heads (correlation=0.5092; p=0.0440), grilling (correlation=0.8783;p<0.0001), and number of consumption days (correlation=0.7398; p<0.0001) for *S. aristotelis*. Consumers living closer to Lake Pamvotis ate *S. aristotelis* on fewer days than those living further away (correlation=-0.3357; p=0.0278).

Mean numbers of consumption days (7.5-11.9 days), quantity (0.27-0.50 kg) and fish size (0.61-1.1 kg) of *S. aristotelis*, parts eaten [skin (38-100 %), head (0-40 %), bones (0-12 %)], and preparation methods [fry (88-100 %), grill (50-100 %)] did not vary significantly among gender-age groups (Table 9). Consumption of fried ($\bar{x}$ range=69-70 %) *S. aristotelis* by female and male adults was significantly greater than that for grilled ($\bar{x}$ range=30-31 %) or boiled (0) *S. aristotelis* (Table 7). There was no difference in the percent of fried (50) and grilled (50) *S. aristotelis* consumed by female or male youth (Table 7). All *S. aristotelis* were gutted prior to consumption, regardless of preparation method, and none was boiled to make soup.

DISCUSSION

This is the first published study to generate site-specific consumption rates of freshwater fishes in Greece that can be applied as chronic daily intake values (CDI) in determining carcinogenic and non-carcinogenic human health risks associated with the consumption of fish tissues. All annual mean site-specific consumption rates of gender-age populations in vicinity of Lake Kastoria (avg. range= 0.103-0.29 kg/day) exceed those of Greece (0.066 kg/day; USDA, 2003 and Welch et al., 2002), EU (0.068 kg/day; Leatherhead Food RA, 2001), Spain (0.104 kg/day; Szucs and Grasselli, 2004), Portugal (0.159 kg/day; Szucs and Grasselli, 2004), and the USEPA default value (0.054 kg/day; USEPA, 1991) with two exceptions. Female consumption rates (0.087-0.103 kg/day) of *P. fluviatilis* were below annual consumption rates of Spain and Portugal (Table 2). Similarly, annual mean site-specific consumption rates of *A. anguilla* and *C. carpio* by male (0.199-0.210 kg/day) and female adults (0.096-0.157 kg/day) in vicinity of Lake Pamvotis exceeded those Greece, EU, Spain, Portugal, and the USEPA default value (USEPA, 1991) (Table 2). Many of our site-specific consumption rates for the two lakes rival those of Native Americans in Alaska whose annual mean consumption rate is 0.324 kg/day, six times greater the USEPA default value (TERA, 1999). As there are such high consumption rates for these two different inland populations, and as exposure varies considerably under different circumstances, we concur with the WHO (1999) which strongly encourages responsible authorities in countries to characterize risk on the basis of local measured or predicted site-specific exposure scenarios and not default values such as those available for USEPA models. To date, no default values have been issued by the EEA. Application of site-specific consumption rates as the CDI in risk assessment investigations is warranted as current consumption rates (0.0.66 and 0.068 kg/day) listed for Greece (USDA, 2003 and Welch et al., 2002), and the EU (Leatherhead Food RA, 2001) would underestimate effects of chronic pesticide and other contaminant exposures to local populations utilizing freshwater fishes as a protein source. For example, the EEA (2004) indicates that pesticides are the most common cause of acute and sub-chronic poisonings because of the amounts of pesticides used in comparison with other chemicals, their high toxicity, and inappropriate storage. EEA (2004) did not comment on long-term exposures to pesticides, but indicated that scientific evidence and information concerning actual exposures to chemical substances and their possible health effects is lacking in most European countries, and that the lack of data for health impact assessment poses a big problem. Further, no association between exceedances of EU standards or (WHO) guidelines for pesticides and the incidence of morbidity or mortality has been established, possibly because of the safety margin built into EU standards/WHO guidelines is considerable, and because of the scarcity of appropriate studies (EEA, 2004).

Average consumption of fishes has increased from 0.039 kg/day (Trichopoulou and Lagiou, 1998) to 0.066 kg/day in Greece from 1980-2003 (USDA, 2003 and Welch et al., 2002). The Greek Ministry of Health and Welfare (2003) states that fish and seafood can physiologically substitute meat and eggs, but culinary, practical, and economic constraints dictate a recommendation of about one serving of fish (0.060 kg) per day. Trichopoulou and Lagiou (1998) indicate that fish and seafood availability in Greece decreases with proximity to urban areas, and can be attributed to the immediate availability of sea products in rural costal areas and islands. Our high consumption rates of fishes from both of these inland lakes, where fishes are plentiful and readily available, are consistent with the statement by Trichopoulou and Lagiou (1998) who reported that one would expect better educated Greeks to be higher consumers of fish; however, market availability appears to be a stronger determinant of food choice in comparison to health education in this case: better educated Greeks are usually residing in the urban and not the rural areas.

Consumption frequencies of fish from Pamvotis Lake were lower than those from Kastoria Lake. This is consistent with the report of Filos (pers. comm., 2002) who indicated consumption of freshwater fishes from Pamvotis Lake had decreased in recent years as public awareness of the lake's pollution had increased. The significantly lower educational levels of adults in Kastoria coupled with fewer pollution reports for Lake Kastoria (Filos, pers. comm., 2002) are probably related to higher consumption rates at this lake.

A study of consumer perceptions of food-related hazards (including pesticide residue health risks) in 16 European countries and the USA conducted by Kafka and

Alvensleben (1997) indicated the index of concern (112) for Greece was greater than the European mean (100), USA (109), and all other European countries (range=63 in Spain-103 in Norway) except Germany (143) and Austria (136). This may reflect the recent and increased frequency of reports about pesticides and environmental and human health concerns available in Greece (Braxton and Frewer, 1998; Miles and Frewer, 1998; EEA, 2003). For example, pesticides have been identified as important freshwater pollutants in Greece (Albanis, 1992; Albanis et al., 1994). Greece authorized the use of nine of the 12 hazardous pesticides on the EEA's Water Framework Directive Priority List, but does not monitor any of them (EEA, 2003), despite documented exceedances (0.2-0.6 mg/kg; limit of detection=0.05 mg/kg) of maximum residue limit (MRL) in grapes tested from Greece (Brown, 2004); presence of organochlorine pesticide residues in human breast milk associated with food consumption patterns of mothers (Schinas et al., 2000); higher than normal concentrations of pesticides in mousaka, bean soup, infant food, and feta cheese (Boulous et al., 1996); and mammogram abnormalities and malignant changes in tumors of female greenhouse workers in Crete (Dolapsakis et al., 2001).

Data on food cooking methods in the EEA and Greece are scarce, even though there is epidemiologic evidence that consumption of fried, grilled or barbecued meat and fish that are well-done or browned may be associated with an increased cancer risk (Rohrmann et al., 2002). All populations in vicinities of both lakes preferred to eat fried fishes with one exception: grilled *C. carpio* from Lake Pamvotis was preferred by female and male adults (Tables 4 and 7). Preference for fried fish may reflect the transition from traditional Mediterranean and Cretan diets (Simopoulos, 2001) to a more western style diet in Greece, a phenomenon described for younger dwellers in urban areas of the country by Costacou et al. (2003). This is in contrast to the report by Rohrmann et al. (2002) who found that frying was more often noted in northern Europe, and roasting and stir-frying were more often used in the south.

ACKNOWLEDGEMENTS

Funded in part by the Thomas F. and Kate Miller Jeffress Memorial Trust, Science Museum of Virginia, and University of Richmond. Both authors collected and analyzed data, and prepared the manuscript. We thank D. Bobori, Ichthyology Department, Aristotle University, Greece, and P. Filos, for assisting in conducting interviews.

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TABLE 1. Comparison of demographic characteristics of adult and youth males and females surveyed in vicinities of Lake Kastoria and Lake Pamvotis, Greece from June-July, 2002.

Age (yr) F=54.1; p<0.0001	Pamvotis ♀ Child 10.1	Kastoria ♂ Child 10.4	Pamvotis ♂ Child 11.3	Kastoria ♀ Child 12.0	Pamvotis ♂ Adult 43.2	Pamvotis ♀ Adult 44.6	Kastoria ♂ Adult 47.3	Kastoria ♀ Adult 48.5
Weight (kg) F=51.1; p<0.0001	Kastoria ♀ Child 37.4	Kastoria ♂ Child 38.6	Pamvotis ♀ Child 40.6	Pamvotis ♂ Child 44.4	Kastoria ♀ Adult 63.5	Pamvotis ♀ Adult 68.6	Pamvotis ♂ Adult 81.9	Kastoria ♂ Adult 85.9
Education[a] F=17.5; p<0.0001	Pamvotis ♀ Child 1.2	Pamvotis ♂ Child 1.4	Kastoria ♂ Child 1.8	Kastoria ♀ Child 2.0	Kastoria ♀ Adult 2.7	Kastoria ♂ Adult 2.8	Pamvotis ♂ Adult 3.5	Pamvotis ♀ Adult 3.8
Occupation[b] F=29.8; p<0.0001	Pamvotis ♂ Child 1.0	Kastoria ♀ Child 1.0	Kastoria ♂ Child 1.0	Pamvotis ♀ Child 1.1	Kastoria ♀ Adult 2.8	Pamvotis ♂ Adult 3.0	Pamvotis ♀ Adult 3.7	Kastoria ♂ Adult 3.8
Quantity consumed (kg/meal) F=8.45; p<0.0001	Pamvotis ♀ Child 0.24	Pamvotis ♂ Child 0.27	Kastoria ♀ Child 0.32	Kastoria ♂ Child 0.43	Kastoria ♀ Adult 0.45	Pamvotis ♀ Adult 0.46	Pamvotis ♂ Adult 0.72	Kastoria ♂ Adult 0.93
Days of eating fish F=7.86; p<0.0001	Pamvotis ♂ Adult 40.5	Pamvotis ♀ Child 43.4	Pamvotis ♂ Child 47.2	Pamvotis ♀ Adult 60.9	Kastoria ♀ Adult 76.9	Kastoria ♂ Adult 80	Kastoria ♀ Child 105.9	Kastoria ♂ Child 126.2

TABLE 2. Consumption rates (kg) of *Perca fluviatilis*, *Rutilus rutilus*, *Anguilla anguilla*, *Cyprinus carpio*, and *Silurus aristotelis* by populations in vicinity of Lake Kastoria and Lake Pamvotis, Greece in 2002, and fish consumption rates of other selected populations.

Lake Kastoria		kg/day		
	♂ adult	♀ adult	♂ child	♀ child
P. fluviatilis	0.273	0.087	0.172	0.103
R. rutilus	0.244	0.185	0.297	0.219
Lake Pamvotis				
A. anguilla	0.199	0.096	0.027	0.010
C. carpio	0.210	0.157	0.079	0.056
S. aristotelis	0.023	0.042	0.035	0.035
		kg/day		
USEPA default[1]		0.054		
EU[2]		0.068		
Greece[3]			0.066	
Portugal[4]		0.159		
Spain[4]			0.104	
USA Subsistence fishermen		0.086		
USA MN Native Americans		0.390		

1 – USEPA, 1991a
2 – Leatherhead Food RA, 2001
3 – USDA, 2003
4 – Szucs and Grasselli, 2004

TABLE 3. Results of Duncan's Multiple Range Test of population consumption parameters for *Perca fluviatilis*. Underscored means do not differ (p=0.05).

Species eating frequency	Female youth	Male youth	Male adult	Female adult
Mean %	50	67	83	84
F=1.32; p=0.2814				
No. days eating species	Female youth	Female adult	Male adult	Male youth
Mean days	57.5	62	71.8	73.9
F=0.14; p=0.9382				
Quantity consumed/meal	Female youth	Male youth	Female adult	Male adult
Mean (kg)	0.34	0.41	0.45	0.93
F=4.92; p=0.0067				
Fish size	Male youth	Female adult	Female youth	Male adult
Mean (kg)	0.32	0.43	0.5	0.5
F=0.59; p=0.6265				
% skin consumption	Female youth	Male youth	Female adult	Male adult
Mean %	0	33	82	90
F=7.7; p=0.0006				
Eating heads	Female youth	Male youth	Female adult	Male adult
Mean %	0	10	36	60
F=2.75; p=0.0598				
Eating bones	Male youth	Female youth	Female adult	Male adult
Mean %	0	0	10	40
F=2.89; p=0.0519				
Eating fried fish	Female youth	Male youth	Male adult	Female adult
Mean %	50	78	90	91
F=1.32; p=2859				
Eating grilled fish	Female adult	Male youth	Male adult	Female youth
Mean %	55	56	60	75
F=0.17; p=0.9173				
Eating boiled fish	Female youth	Male youth	Male adult	Female adult
Mean %	0	0	0	10
F=0.68; p=0.5733				
Soup eating frequency	Female youth	Male youth	Male adult	Female adult
Mean %	0	0	0	20
F=0.68; p=0.5733				

TABLE 4. Percent fried, grilled, and boiled *Perca fluviatilis* and *Rutilus rutilus* consumed by female and male adults and youth in vicinity of Lake Kastoria, Greece, June, 2002. Underscored means do not differ significantly (p=0.05).

Perca fluviatilis			
Female adult	Boil	Grill	Fry
Mean	3.0	30.0	67.0
F=13.91; p=<0.0001			
Female youth	Boil	Fry	Grill
Mean	0	37	63
F=2.60; p=0.1288			
Male adult	Boil	Grill	Fry
Mean	0	35	65
F=13.00; p<0.0001			
Male youth	Boil	Grill	Fry
Mean	0	33	67
F=7.62; p=0.0027			
Rutilus rutilus			
Female adult	Boil	Grill	Fry
Mean	0	20	80
F=39.07; p<0.0001			
Female youth	Boil	Grill	Fry
Mean	0	25	75
F=10.53; p=0.0044			
Male adult	Boil	Grill	Fry
Mean	0	33	67
F=12.04; p=0.0002			
Male youth	Boil	Grill	Fry
Mean	0	33	67
F=15.07; p=0.0003			

TABLE 5. Results of Duncan's Multiple Range Test of population consumption parameters for *Rutilus rutilus*. Underscored means do not differ (p=0.05).

Species eating frequency	Male youth	Female adult	Female youth	Male adult
Mean %	67	91	100	100
F=2.01; p=0.1339				
No. days eating species	Female adult	Male adult	Male youth	Female youth
Mean days	77	81.1	88	96.7
F=0.10; p=0.9594				
Quantity consumed/meal	Female youth	Female adult	Male youth	Male adult
Mean (kg)	0.3	0.45	0.48	0.93
F=6.15; p=0.0028				
Fish size	Male youth	Female adult	Female youth	Male adult
Mean (kg)	0.1	0.1	0.1	0.12
F=1.64; p=0.2050				
% skin consumption	Male youth	Female youth	Male adult	Female adult
Mean %	67	75	89	100
F=1.33; p=0.2855				
Eating heads	Female youth	Male youth	Male adult	Female adult
Mean %	25	50	67	70
F=0.90; p=0.4559				
Eating bones	Male youth	Female youth	Female adult	Male adult
Mean %	17	25	60	67
F=1.75; p=0.1821				
Eating fried fish	Female youth	Male youth	Male adult	Female adult
Mean %	89	100	100	100
F=0.72; p=0.5504				
Eating grilled fish	Female adult	Female youth	Male adult	Male youth
Mean %	40	50	56	67
F=0.34; p=0.7948				

TABLE 6. Results of Duncan's Multiple Range Test of population consumption parameters for *Anguilla anguilla*. Underscored means do not differ (p=0.05).

Species eating frequency	Female youth	Male youth	Female adult	Male adult
Mean %	8	25	31	62
F=3.13; p=0.0356				
No. days eating species	Female youth	Male youth	Female adult	Male adult
Total days	0.9	6.4	35.4	55
F=2.88; p=0.0473				
Quantity consumed/meal	Female adult	Male youth	Female youth	Male adult
Mean (kg)	0.28	0.3	0.35	0.78
F=3.02; p=0.0754				
Fish size	Female youth	Female adult	Male youth	Male adult
Mean (kg)	0.3	1.1	1.3	1.43
F=0.40; p=0.7562				
% skin consumption	Male youth	Female youth	Male adult	Female adult
Mean %	0	0	50	50
F=0.73; p=0.5535				
Eating heads	Female youth	Male youth	Female adult	Male adult
Mean %	0	0	25	38
F=0.43; p=0.7352				
Eating bones	Male youth	Female youth	Female adult	Male adult
Mean %	0	0	0	17
F=0.24; p=0.8636				
Eating fried fish	Female youth	Male youth	Male adult	Female adult
Mean %	0	50	88	100
F=2.73; p=0.0944				
Eating grilled fish	Male youth	Female adult	Male adult	Female youth
Mean %	50	75	88	100
F=0.47; p=0.7063				
Eating boiled fish	Female youth	Male youth	Female adult	Male adult
Mean %	0	0	0	12
F=0.24; p=0.8636				
Soup eating frequency	Female youth	Male youth	Female adult	Male adult
Mean %	0	0	0	38
F=0.24; p=0.8636				

TABLE 7. Percent fried, grilled, and boiled *Anguilla anguilla*, *Cyprinus carpio*, and *Silurus aristotelis* consumed by female and male adults and youth in vicinity of Lake Pamvotis, Greece, June, 2002. Underscored means do not differ significantly (p=0.05).

Anguilla anguilla				
Female adult	Boil	Grill	Fry	
Mean	0	37	63	
F=9.65; p=0.0059				
Male Adult	Boil	Grill	Fry	
Mean	4	48	48	
F=9.41; p=0.0012				
Cyprinus carpio				
Female adult	Boil	Fry	Grill	
Mean	4	18	78	
F=9.56; p=0.0059				
Female youth	Boil	Grill	Fry	
Mean	0	30	70	
F=4.63; p=0.0323				
Male adult	Boil	Fry	Grill	
Mean	9	28	63	
F=6.07; p=0.0067				
Male youth	Boil	Grill	Fry	
Mean	0	50	50	
F=1.50; p=0.2953				
Silurus aristotelis				
Female adult	Boil	Grill	Fry	
Mean	0	30	70	
F=12.38; p=0.0012				
Female youth	Boil	Grill	Fry	
Mean	0	50	50	
F=99.99; p<0.0001				
Male adult		Boil	Grill	Fry
Mean	0	31	69	
F=10.30; p=0.0008				
Male youth	Boil	Grill	Fry	
Mean	0	50	50	
F=99.99; p<0.0001				

TABLE 8. Results of Duncan's Multiple Range Test of population consumption parameters for *Cyprinus carpio*. Underscored means do not differ (p=0.05).

Species eating frequency	Male youth	Female youth	Male adult	Female adult
Mean %	38	42	69	77
F=1.82; p=0.1580				
No. days eating species	Male youth	Female youth	Male adult	Female adult
Total days	47	47.4	50.3	84
F=0.62; p=0.6035				
Quantity consumed/meal	Female youth	Male youth	Female adult	Male adult
Mean (kg)	0.21	0.25	0.52	0.86
F=7.17; p=0.0014				
Fish size	Male youth	Female adult	Female youth	Male adult
Mean (kg)	4	5.6	5.9	7.6
F=1.32; p=0.2929				
% skin consumption	Female youth	Female adult	Male youth	Male adult
Mean %	20	20	33	44
F=0.49; p=0.6900				
Eating heads	Male youth	Female adult	Female youth	Male adult
Mean %	0	20	20	44
F=0.93; p=0.4403				
Eating bones	Male youth	Female youth	Female adult	Male adult
Mean %	0	0	0	10
F=0.64; p=0.5977				
Eating fried fish	Female adult	Male adult	Female youth	Male youth
Mean %	30	33	40	67
F=0.42; p=0.7379				
Eating grilled fish	Male youth	Female youth	Male adult	Female adult
Mean %	67	80	89	90
F=0.36; p=0.7841				
Eating boiled fish	Female youth	Male youth	Female adult	Male adult
Mean %	0	0	10	22
F=0.66; p=0.5855				
Soup eating frequency	Female youth	Male youth	Female adult	Male adult
Mean %	0	0	20	56
F=0.76; p=0.5302				

TABLE 9. Results of Duncan's Multiple Range Test of population consumption parameters for *Silurus aristotelis*. Underscored means do not differ (p=0.05).

Species eating frequency	Female youth	Male youth	Female adult	Male adult
Mean %	17	25	42	47
F=0.77; p=0.5183				
No. days eating species	Male adult	Female youth	Female adult	Male youth
Total days	7.5	7.9	10.3	11.9
F=0.15; p=0.9285				
Quantity consumed/meal	Female youth	Male youth	Male adult	Female adult
Mean (kg)	0.27	0.27	0.5	0.5
F=0.53; p=0.6693				
Fish size	Male adult	Female adult	Male youth	Female youth
Mean (kg)	0.61	0.62	1.1	1.1
F=1.62; p=0.2374				
% skin consumption	Male adult	Female adult	Male youth	Female youth
Mean %	38	60	100	100
F=1.12; p=0.3789				
Eating heads	Male youth	Female youth	Male adult	Female adult
Mean %	0	0	25	40
F=0.44; p=0.7256				
Eating bones	Male youth	Female adult	Female youth	Male adult
Mean %	0	0	0	12
F=0.29; p=0.8348				
Eating fried fish	Male adult	Male youth	Female youth	Female adult
Mean %	88	100	100	100
F=0.29; p=0.8348				
Eating grilled fish	Male adult	Female adult	Male youth	Female youth
Mean %	50	60	100	100
F=0.69; p=0.5768				

Virginia Journal of Science
Volume 56 number3
Fall 2005

Assessment of Human Health Risks from Chemically Contaminated Lake Fishes In Greece

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ABSTRACT

Objectives were to conduct screening level surveys of locally consumed fish tissues in vicinities of two lakes (Kastoria and Pamvotis) in Greece to determine the presence of halogenated organic compounds and determine carcinogenic and non-carcinogenic human health risks associated with the consumption of sampled fish tissues. Results estimate the Incremental Lifetime Cancer Risks (ILCR) and Hazard Index (HI) values for the two local populations using site-specific population data. These results were compared to analyses conducted using U.S. Environmental Protection Agency default values in an effort to determine the applicability of USEPA default values to assessments of risks in non U.S. populations. Using site specific data, 87 % of the mean ILCRs calculated for total populations and sub-populations (i.e. female adult, female youth, male adult and male youth) consuming fishes from the two lakes we studied were above USEPA's acceptable cancer risk of $1.0E^{-06}$; 53 % of the mean HIs were greater than 1.0. The USEPA default value (0.054 kg/d) for ingestion rate (IR) is considerably lower than the mean site specific IRs derived from populations in vicinity of Lake Kastoria (0.20; min.=0.09; max.=0.29 kg/d) and Lake Pamvotis (0.10; min.=0.01; max.=0.21 kg/d). These differences point to the need for the development of default values specific to the regions and population consumption patterns within Greece.

Keywords: human health risk assessment, PCB, pesticides, Greece, European Union

INTRODUCTION

The hazards of pesticides are receiving increased attention in European Union (EU) countries. For example, the European Environmental Agency (EEA) of the EU removed a large number of pesticides from the market in July 2003 (Pesticide News, 2003). Pesticide use in Greece during 1989 was 7,811 tons, and has likely increased significantly since that time due to changing dynamics in Greece's agribusiness ((Dassenakis, 2000; Lekakis, 1998).

Published studies of human health risks associated with pesticide exposure in Greece are scant. Dolapsakis et al. (2001) have reported on occupational exposure to pesticides currently used in Crete, finding that female greenhouse workers had elevated incidencies of mammogram abnormalities, including tumors. Additionally, Schinas et al. (2000) have reported that organochlorine pesticide residues in human breast milk from southwest Greece are correlated with dietary intake of pesticides.

Some investigators in Greece have also reported on the concentrations of pesticides in water and fish. For example, Georgakopoulos-Gregoriades and Vassilopoupou (2004) studied organochlorine levels in muscle of *Lepidorhombus boscii* (four-spotted megrim, an edible flatfish) in the Aegean Sea and found concentrations of DDT ranging from 12.5-32.3 ng/g, and those of PCBs between 4.5-12.1 ng/g. Charizopoulos and Papadopoulou-Mourkidou (1999) found one or more pesticides in 90 % of 205 rainwater samples. Atrazine was measurable in 30 % of the 205 samples. In Greece's freshwater Lake Kastoria, all four pesticides sampled for were found at all locations sampled. Concentrations of atrazine (avg.=0.7 mg/l; range=0.5-0.9 mg/l)and endosulfan (avg.=0.51 mg/l; range=0.2-1.0 mg/l) were higher than those of fenathion (avg.=0.053 mg/l), chronomeb (avg.=0.056 mg/l)(Bobori, 1998).

However, no comprehensive data base of contamination in groundwater, surface water, or dietary commodities could be located, suggesting that these potential contaminant inputs are not routinely monitored for risk management purposes. Additionally, a thorough review of the literature indicates no formal human health risk assessments of pesticides in fish tissues in Greece have been published (pers. comm. Joachim Scholderer, MAPP Centre at the Aarhus School of Business, Denmark, 2004; Joyce Tait, Univ. Edinburgh, 2004; George Chryssochoidis, Agribusiness Laboratory, Agricultural University of Athens, 2004).

Despite this apparent lack of monitoring and assessment of the fate and transport of pesticides and other contaminants in the environment, the Greek government has recently authorized the use of nine organic pesticides with known human health impact potentials: endosulfan, anthracene, simazine, trifluralin, alachlor, atrazine, chlorpyrifos, diuron, isoproturon, chlorfenvinphos, and naphthalene (EEA, 2000).

Our objectives were to conduct screening level surveys of locally consumed fish tissues in vicinities of two lakes (Kastoria and Pamvotis) in Greece to determine the presence of halogenated organic compounds and determine carcinogenic and non-carcinogenic human health risks associated with the consumption of sampled fish tissues.

METHODS AND MATERIALS

Fish Collection and Processing: Species collected were those consumed by local populations (Maurakis et al., 2005). Lake Kastoria fish were obtained from local fishermen immediately after they finished fishing their gill net (58 mm bar mesh) on 19 June 2002. For Lake Pamvotis, live fish maintained in lake water pumped once through tanks, were purchased from a fish tavern on the Island of Ioannina on 21 June 2002.

Collections were placed in plastic bags, labeled with collection site, species, and date of collection, placed on ice and transported to University of Ioannina within 24 hours where they were stored at –20 C until processed.

Sample processing followed the *Guidance For Assessing Chemical Contaminant Data For Use In Fish Advisories* (USEPA 1993) *Guidance for Conducting Fish and Wildlife Consumption Surveys* (USEPA 1998) and the good laboratory practices of EU Directive 93/99EEC outlined in Hill (1999). Stainless steel equipment was used in processing samples for organics analysis. Prior to preparing each sample, equipment was washed with a detergent solution, rinsed with tap water, wiped with isopropanol and rinsed with deionized water. Work surfaces were wiped down with isopropanol, allowed to dry and covered with heavy-duty aluminum foil, which was changed after each sample was processed.

Total length (mm) and weight (g) were recorded for each specimen to determine sample variability within species. Species processed as whole body samples (*Rutilus rutilus*) were scaled, gutted and rinsed with deionized water. Species processed as skin-on fillets (*Perca fluviatilis*) were scaled prior to filleting (belly flap discarded) and rinsed with deionized water. Species processed as skin-off fillets (*Cyprinus carpio, Anguilla anguilla, Silurius arestotilis*) were partially scaled as needed to facilitate filleting and then skinned after filleting. Carp roe was resected for sampling as consumption survey data (Maurakis et al., 2005) indicated carp roe was also consumed. Tissue samples were weighed on a digital scale covered with heavy-duty aluminum foil which was replaced after each weighing. If fillets from an individual fish had a total weight of less than 200 grams, fillets were composited with one or more fillets from the same species to generate a tissue sample of at least 200 grams (USEPA 1993). Fillet samples were wrapped in heavy duty aluminum foil with a sample identification tag labeled for: sample location, species, sample type (individual or composite), total length(s) and weight, field sample number, and sample date. Carp roe samples were placed in plastic jars with Teflon lined lids.

Sample Analysis: All samples were stored at –20 C until they were packed in ice and shipped by overnight courier from Thesoloniki, Greece to Richmond, Virginia. In Richmond, samples were stored at –5 C until they were packed in ice and transported to the Department of Environmental and Aquatic Animal Health at the Virginia Institute of Marine Science (VIMS), College of William and Mary, Gloucester Point, Virginia, USA. Final sample preparation and chemical analyses were conducted using VIMS Analytical Protocol for Hazardous Organic Chemicals in Environmental Samples (Hale, 1994) with modest modifications to improve extraction results and sample throughput.

A total of 27 samples were analyzed for polychlorinated Biphenyls (PCBs) by congener analysis and organochlorine pesticide burdens. Samples were homogenized and lyophilized, then sub-sampled and subjected to enhanced solvent extraction (Schantz, 1997) using methylene chloride as the solvent. Multiple surrogate standards (PCBs: PCB-30, PCB-65, PCB-121 and PCB-204) were added prior to sample extraction to span the molecular weight range of the targeted analytes. Size exclusion chromatography (SEC) purification of extracts was accomplished using an HPLC column using methylene chloride as the solvent at a flow rate of 5 ml/min. Polarity separation of post-SEC extracts was conducted on 2000 mg silica gel solid phase extraction (SPE) columns. Extracts were separated into two silica-derived fractions. The first (eluted with 5 ml of 100% hexane) contained aliphatics and was not further processed. The second silica gel fraction (eluted with 6 ml of 60:40 hexane/methylene chloride) contained the PCBs and organochlorine pesticides. Solvent volumes were reduced under purified nitrogen.

The second SPE fractions were then spiked with an internal standard, pentachlorobenzene, for quantitation purposes. Separation and detection of contaminants were accomplished by high-resolution gas chromatography (GC) with electrolytic conductivity detection (ELCD), operated in the halogen selective mode. The ELCD selectively responds to halogenated analytes, i.e. those containing Cl, Br, F or I. A 1 to 2 ul injection of each purified extract was made onto a 60 m DB-5 column (0.32 mm ID and 0.25 um film thickness), using splitless injection and helium carrier gas. Organochlorine compound identifications were made via halogen retention indices (HRIs) and an existing VIMS analyte library. Extracts derived from VIMS quality control samples #001 and 005 were also subjected to full scan GC/MS to confirm compound identities indicated by the retention indices. Data were corrected for recovery of the PCB-204 surrogate.

Quality control measures also included the coincident processing of three blanks, consisting of pre-ignited sodium sulfate, with each set of samples lyophilized. Each sample was also spiked with the four PCB surrogate standards to determine analyte recovery rates. A certified reference material (CRM) fish (Carp-1, US National Institute of Standards and Technology (NIST)), recently analyzed for PCBs and selected pesticides to ascertain accuracy of the VIMS methodology (Schantz, 1997) was also analyzed with the Greek samples, as were four samples of *C. carpio* from the New River, Virginia, USA, which were also recently analyzed by VIMS.

Risk Analysis: Analyses of health risks associated with consumption of fish containing the identified compounds were performed using US Environmental Protection Agency (USEPA) Risk Assessment Guidance for Superfund (RAGS) (USEPA 1989; 1991a). In general, analyses for determining carcinogenic health risks:

1. determine the Intake factor (IF) term by inputing either site specific or default population exposure information;
2. determine an Exposure Point Concentration (EPC) for an identified compound;
3. determine a Chronic Daily Intake (CDI) value for each compound by multiplying the EPC by the IF; and
4. determine an Incremental Lifetime Cancer Risk (ILCR) by applying a compound's cancer potency factor (CSF) from EPA's Integrated Risk Information System (IRIS, 2002) to the CDI. Individual ILCRs are then analyzed to determine the range of risk as well as the mean risk for the population.

Analyses for determining non-carcinogenic health effect risks follow much the same general procedure but use an Oral Reference Dose (RfD) in place of the CSF and generate a final value termed a Hazard Index (HI). Individual HIs are analyzed to determine the range of non-carcinogenic risk as well as a mean non-carcinogenic risk.

Compounds were included in the risk analyses if there was sufficient toxicological data in IRIS to support the algorithms or if they represented breakdown products of parent compounds that could be readily characterized in IRIS. Breakdown products for chlordane, DDT, DDE and DDD were grouped with their parent compounds.

The algorithm for calculating the IF was:

$$IF = IR*FI*ED*EF/(BW*AT)$$

Where:	IR	=	ingestion rate (kg/d or kg/meal)
	FI	=	fraction ingested from contaminated source
	ED	=	exposure duration (yrs)
	EF	=	exposure frequency (d/yr or meals/yr)
	BW	=	body weight (kg)
	AT	=	averaging time (d)

Determinations of IF used both site-specific exposure data from Maurakis and Grimes (2005), and RAGS default exposure inputs for IR, ED, EF and BW in USEPA (1991a) to determine the applicability of USEPA default values to assessments of human health risks in populations outside the United States. The value of FI was set at 1.0 as data from Maurakis and Grimes (2005) indicated local populations obtained their fish primarily from the respective lake sampled in the region. Values for AT were set at 70 years for analyses of carcinogenic risks and 30 years for non-carcinogenic risks (USEPA 1991a). Default values used were: IR= 0.054 kg/d, FI= 1, AT-cancer= 25,550 d (70 yr x 365 d/yr), AT-non-cancer= 10,950 d (30 yr x 365 d/yr), ED= 30 years, EF= 350 d/yr, and BW= 70 kg -adult, (IRIS, 2002; USEPA 1991a).

Due to the relatively small size of most of the data sets, (i.e. < six samples), many EPCs were set at the maximum detected compound concentrations. Data sets with greater than six samples used the 95% upper confidence limit (UCL) of the compound concentrations as the EPC (EPA 1989).

The algorithm for calculating chronic daily intake (CDI) of a target compound was:

$$CDI\ (mg/kg\text{-}d) = EPC*IF$$

Where:	EPC	=	concentration of identified compound in tissue (mg/kg)
	IF	=	Intake factor

The CDI for each compound was multiplied by the CSF to calculate the ILCR estimate for that compound. Estimated ILCRs were summed for all compounds identified in a species collected from the respective lakes to determine an individual's total ILCR estimate for exposure to all target compounds identified in the species. Individual total ILCRs were analyzed to determine minimum, maximum and mean risk estimates for the general fish consuming population, and four sub-populations: adult men, adult women, male children (age < 18 years) and female children (age < 18 years). Tests for significant differences in sub-population ILCR estimates were made using the SAS GLM procedure followed by Duncan's Multiple Range Test (SAS, 2002).

Estimated HIs were summed for all compounds identified in a species collected from the respective lakes to determine an individual's HI estimate for exposure to all target compounds identified in the species. Individual HIs were analyzed to determine minimum, maximum and mean HI estimates for the general fish consuming population, and the adult men, adult women, male children (age<18 years) and female children (age<18 years) sub-populations. Tests for significant differences in sub-population HI estimates were made using the SAS GLM procedure followed by Duncan's Multiple Range Test (SAS, 2002). The RfD for Aroclor 1254 was used to assess non-cancer risks for total PCBs as this PCB mixture is reflective of the PCB congeners that bioaccumulate in fish (IRIS 2002).

Calculation of ILCRs and HI values for *S. aristotelis* from Lake Pamvotis were made separately from the calculations for *C. carpio* and *A. anguilla* because surveys for consumption of *S. aristotelis* were conducted on different dates and thus sampled a different population than that surveyed for consumption habits of *C. carpio* and *A. anguilla*.

RESULTS

Fish Collection and Processing: A total of 27 fish from two species groups (*P. fluviatilis*, 17 and *R. rutilus*, 10) were collected from Lake Kastoria, and a total of 37 fish from three species (*C. carpio*, 7; *A. anguilla*, 7, and *S. aristotelis*, 23) were collected from Lake Pamvotis. Lake Kastoria collections yielded sufficient amounts of tissue samples for four fillet samples of the predator *P. fluviatilis* and two fillet samples of the prey species *R. rutilus*. Lake Pamovits collections resulted in five fillet samples and five roe samples from the bottom feeder *C. carpio*, seven fillet samples from the bottom feeder *A. anguilla*, and four fillet samples from the predator *S. aristotelis*. Percent difference in total length of samples composited for analysis ranged from 1.9 to 16.7 percent, well within USEPA recommended $\leq$ 25 percent range (USEPA 1993).

Sample Analysis:

Quality control measures: Contamination detectable in the sodium sulfate blanks was typically less than 1 ug/kg (dry weight basis) per component. Recovery of PCB-204 in surrogate samples ranged from 110 to 133%. These recovery values were used to correct results from the extraction process as the majority of chlorinated analytes eluted between PCB-121 and PCB-204 and had intermediate volatilities, leaning towards that of PCB-204.

Total PCB values were in excellent agreement with the total PCB values for the 25 peaks quantitated by NIST for the CRM fish. Values for 4,4-DDT and 4,4-DDE for the CRM fish were also in excellent agreement with the NIST values. Results for 4,4-DDD were low, about 45% of expected. As this compound was consistently found in all samples, the low recoveries may underestimate the concentrations of this compound in fish tissues. The percent differences between PCB extraction results and those of the earlier extraction results for the New River fish were 2-4%.

Samples showed some signs of decomposition upon receipt by the VIMS laboratory due to thawing during transport. Although lipid composition was likely altered by sample decomposition, the halogenated analytes targeted for analysis are generally resistant to environmental degradation and their concentrations would not be expected to be significantly altered by the levels of decomposition associated with the samples. This assumption is supported by the pattern of increasing halogenated organics concentrations with increasing percent lipid in the samples.

Sample results: The dominant halogenated organics detected within parameters of the analytical protocol were degradation products of DDT, principally 4,4'-DDE, and PCBs (congeners 153/132, 138 and 180). Other halogenated organics identified in most samples included: hexachlorobenzene and associated degradation products; Chlordane and associated degradation products; and several un-identified halogenated organics. Concentrations of the 16 un-identified halogenated organics in Lake Pamvotis samples ranged from 0.1–18.1 ppb. Concentrations of the 19 un-identified halogenated organics in Lake Kastoria samples ranged from 0.1–2.7 ppb (Table 1).

Risk Analysis:

Descriptive statistics and resultant EPC values: The ten compound groups used in

TABLE 1. Exposure point concentrations (EPC) by chemical and species (*Perca fluviatilis*, *Rutilus rutilus*, *Anguilla anguilla*, *Cyprinus carpio*, and *Silurus aritotelis*) used in analyses of human health risks for populations living in vicinities of Lake Kastoria and Lake Pamvotis, Greece; and concentration ranges for unidentified halogenated organic compounds found in tissue samples.

	Lake Kastoria		Lake Pamvotis			
(ppb)	*P. fluviatilis*[1]	*R. rutilus*[1]	*A. anguilla*[1,2]	*C. carpio*[1]	*C. carpio* eggs[1]	*S. aristotelis*[1]
Chemical						
alpha-BHC		0.04	0.23 [2]			
beta-BHC			0.05 [1]			
gamma-BHC		0.05	0.64 [2]	0.05		
hexachlorobenzene	0.03	0.13	0.78 [2]	0.15	0.19	0.47
heptachlor epoxide		0.04	0.41 [2]	0.11		0.28
chlordane	0.18	1.17	2.53 [2]	1.87	1.15	3.65
DDD	0.15	1.83	10.34 [2]	3.38	2.21	12.16
DDE	4.7	45	94.5 [2]	34.54	23.61	86.97
DDT	0.08	2.34	6.85 [2]	2.2	1.11	3.47
Total PCBs	1.61	7.0	36.8 [2]	13.7	10.4	30.9
Unidentified (range)[3]	0.1-0.5	0.1-2.7	0.1-4.4	0.2-18.1	- [4]	0.1-5.7

[1]=maximum concentration used as EPC due to sample size <6.
[2]=95% UCL used as EPC when sample size >6.
[3]=No risk assessments were performed for unidentified halogenated organic compounds.
[4]=Unidentified halogenated organic compounds were not quantified in *C. carpio* egg samples.

the risk analyses were: Total PCB (includes all identified PCB congeners); Alpha Benzene Hexa Chlorine (BHC); Beta BHC; Gamma BHC; Hexachlorobenzene; Chlordane (includes cis-chlordane, trans-chlordane, and chlordane); DDE (includes 2,4 DDE and 4,4 DDE); DDT (includes DDT, 4,4 DDT, and DDT related); DDD (includes 4,4 DDD, and DDD-olefin); and Heptachlor epoxide. Exposure Point Concentrations were selected from maximum detected compound concentrations in all species except *A. anguilla*, which had a sufficiently robust data set to use the 95% UCL as the EPC for most compounds (Table 1).

Determination of IF:

Not all individuals surveyed in Maurakis et al. (2005) consumed all species of fish collected from a particular lake. No IF values were calculated for 11 individuals surveyed from the Lake Kastoria population as they did not consume either *P. fluviatilis* or *R. rutilus*. Therefore, these individuals were not included in estimations of risks using site specific population data. Five individuals reported consuming *P. fluviatilis* but not *R. rutilus*, while two individuals reported consuming *R. rutilus* but not *P. fluviatilis*. As a result of these differing consumption patterns, IF values were calculated for 32 and 29 individuals that consumed *P. fluviatilis* and *R. rutilus*, respectively. These data combinations resulted in the calculation of 34 IF values for the Lake Kastoria risk assessment.

No IF values were calculated for 16 individuals surveyed from the Lake Pamvotis population as they did not consume either *C. carpio* or *A. anguilla*, and therefore these individuals were not included in estimations of risks using site specific population data. Fifteen individuals reported consuming *C. carpio* but not *A. anguilla*; three individuals reported consuming *A. anguilla* but not *C. carpio*. As a result of these differing consumption patterns, IF values were calculated for 27 and 15 individuals that consumed *C. carpio* and *A. anguilla*, respectively. These data combinations resulted in the calculation of 30 IF values for the Lake Pamvotis risk assessment for these two species. No IF values were calculated for 27 individuals surveyed from the Lake Pamvotis population as they did not consume *S. aristotelis*; therefore these individuals were not included in estimations of risks using site specific population data. Deletion of these 27 individuals resulted in the calculation of 16 IF values used to calculate risk for consumption of *S. aristotelis*.

USEPA Default IF:

The USEPA default IF value was calculated from the default inputs presented above. Determinations of ILCRs and HIs using the default IF do not consider the effects of site specific age, sex, body weight or other exposure factor variability.

Determination of ILCR and HI:

Lake Kastoria – P. fluviatilis and *R. rutilus consuming population*:

The mean site specific ILCR for the general population was $3.80E^{-5}$ (range=$3.10E^{-7}$ – $2.00E^{-4}$). The ILCR from the USEPA default model was $1.17E^{-5}$. Mean site specific ILCRs in the sub-populations ranged from $1.3E^{-5}$ in female children to $6.9E^{-5}$ in male adults. The mean ILCR in adult men was significantly greater than mean ILCRs in other Lake Kastoria sub-populations (Table 2).

The mean site specific HI for the general population was 1.2 (range= 0.1-4.2) compared to 0.32 derived from the USEPA default model. Mean HI values in the sub-populations ranged from 0.9 in adult women and female children to 1.9 in male children. Mean HIs were not significantly different between sub-populations (Table 2).

Lake Pamvotis - C. carpio and *A. anguilla consuming population*:

The mean site specific ILCR for the general population was $1.20E^{-4}$ (range=$2.2E^{-7}$–$4.5E^{-4}$). The ILCR from the USEPA default model was $9.31E^{-5}$. Mean site specific ILCRs in the sub-populations ranged from $1.0E^{-5}$ in female children to $2.4E^{-4}$ in male adults. The mean ILCR in adult men was significantly greater than mean ILCRs in other Lake Pamvotis sub-populations (Table 2).

The mean site specific HI in the general population was 2.9 (range = 0.1 - 9.3), compared to 3.49 derived from the USEPA default model. Mean HI values in the sub-populations ranged from 1.1 in female children to 5.0 in adult men. The mean HI in male adults was significantly greater than the mean HIs in other Lake Pamvotis sub-populations (Table 2).

Lake Pamvotis - S. arestotelis consuming population:

The mean site specific ILCR for the general population was $1.8E^{-5}$ (range=$6.4E^{-7}$ – $1.1 E^{-4}$). The ILCR from the USEPA default model was $9.3E^{-5}$. Mean site specific ILCRs in the sub-populations ranged from $6.2E^{-6}$ in male children to $3.0E^{-5}$ in adult women. Mean ILCRs were not significantly different between sub-populations (Table 2).

The mean site specific HI for the general population was 0.5 (range = 0.0-2.3) compared to 3.49 derived from the USEPA default model. Mean HI values in the sub-populations ranged from 0.3 in adult men to 0.7 in adult women and female children. Mean HIs were not significantly different between sub-populations (Table 2).

DISCUSSION

Our analyses are based on guidelines, risk models, and default values of the USEPA and not those of the EEA as the latter has not published human health risk models and default values for contaminants in fish products. However, in 2003 the European

TABLE 2. Site specific mean, minimum, and maximum Incremental Lifetime Cancer Risks (ILCR) and Hazard Indexs (HI) for the general population and subpopulations (adult male, adult female, male youth (age<18 years), and female youth (age<18 years) consuming *Perca fluviatilis* and *Rutilus rutilus* in Lake Kastoria, and *Anguilla anguilla*, *Cyprinus carpio*, and *Silurus aristotelis* in Lake Pamvotis, Ioannina, Greece in 2002.

	Lake Kastoria			Pamvotis (*A. anguilla* & *C. carpio*)			Pamvotis (*S. aristotelis*)		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Total population									
ILCR:	3.10E-07	2.00E-04	3.80E-05	2.20E-07	4.50E-04	1.20E-04	6.40E-07	1.10E-04	1.80E-05
HI:	0.1	4.2	1.2	0.1	9.3	2.9	0.0	2.3	0.5
Adult men									
ILCR	6.90E-06	2.00E-04	6.9E-05 (*)	3.70E-05	4.50E-04	2.4E-04 (*)	6.40E-07	5.30E-05	1.40E-05
HI	0.1	3.9	1.3	0.9	9.3	5 (*)	0.0	0.8	0.3
Adult women									
ILCR	2.20E-06	6.90E-05	3.40E-05	3.20E-06	3.10E-04	9.70E-05	8.60E-07	1.10E-04	3.00E-05
HI	0.1	2.2	0.9	0.1	6.9	2.2	0.0	2.3	0.7
Male children									
ILCR	3.10E-07	4.70E-05	1.90E-05	4.49E-06	1.90E-05	1.10E-05	5.70E-06	6.70E-06	6.20E-06
HI	0.1	4.2	1.9	0.4	3.2	1.5	0.5	0.7	0.6
Female children									
ILCR	1.80E-06	2.50E-05	1.30E-05	2.20E-07	1.80E-05	1.00E-05	7.00E-06	7.00E-06	7.00E-06
HI	0.2	2.2	0.9	0.1	1.8	1.1	0.7	0.7	0.7

* Group's estimated risk is significantly greater than estimated risks for other sub-groups from the same lake.

Commission did list cancer risks from environmental, diet, and genetic factors as a priority (EEA, 2004). USEPA uses an ILCR risk range of $1x10^{-6}$ to $1x10^{-4}$, and an HI < 1.0 (USEPA 1989, USEPA 1991(a)). The guidance is widely interpreted as setting an ILCR of $1E^{-06}$ as the rate of occurrence of cancer that could be expected in the absence of xeno-biotic compounds (i.e. the point of departure). Increasing departures from this level of risk suggests an increasing need for actions to manage risks to human health, as would HIs elevated to 1.0 or greater. Once ILCRs approach or exceed $1E^{-04}$, and HIs approach or exceed 1.0, EPA will often require remedial action to be taken to reduce risk factors to levels conducive to management through institutional or othercontrols.

Using site specific data, 87 % of the mean ILCRs calculated for total populations and sub-populations (i.e. female adult, female youth, male adult and male youth) consuming fishes from the two lakes we studied were above USEPA's acceptable cancer risk of $1.0E^{-06}$; 53 % of the mean HIs were greater than 1.0. For example, the mean site specific ILCR ($1.2E^{-04}$) and HI values (2.9) for the general population consuming *A. anguilla* and *C. carpio* from Lake Pamvotis exceed the levels where the USEPA would often require remedial action. The sub-population estimated to be atthegreatest risk for carcinogenic health risks is adult males (mean ILCR=$2.4E^{-04}$; mean HI=5.0).

In Lake Kastoria, mean ILCR ($3.8E^{-05}$; max.=$2.0E^{-04}$) for the general population consuming *P. fluviatilis* and *R. rutilus* also exceed USEPA's point of departure for cancer risk but is below the level where USEPA would likely require remedial action beyond some form of institutional control such as risk communication and / or a consumption advisory. However, the mean HI value for the general population (1.2, max.=9.3) is above this level where the USEPA would likely require remedial action beyond the use of institutional controls. As in Lake Pamvotis, adult men are the sub-population at greatest risk. For *S. aristotelis*, the mean site specific ILCR for the general population ($1.80E^{-05}$, max.=$1.1E^{-04}$) and adult sub-populations (range=$1.40E^{-05}$- $3.0E^{-05}$) exceed the USEPA's point of departure for cancer risk; however, the mean ILCRs are below the level where the USEPA would likely require remedial action beyond some form of institutional control such as risk communication and / or consumption advisory. However, maximum ILCR values exceed this level.

The USEPA default value (0.054 kg/d) for ingestion rate (IR) is considerably lower than the mean site specific IRs derived from populations in vicinity of Lake Kastoria (0.20; min.=0.09; max.=0.29 kg/d) and Lake Pamvotis (0.10; min.=0.01; max.=0.21 kg/d). These differences point to the need for the development of default values specific to the regions and population consumption patterns within Greece.

In both lakes, primary drivers for risk were consumption values (particularly those of men), and concentrations of PCBs and DDT related compounds present in fish tissues (low recoveries of DDD may underestimate risks from DDT and associated breakdown products). While the concentrations of halogenated compounds in the fishes we studied in Greece were lower than those reported for some U.S. sites (USEPA, 1991b; Grimes, 1994 and 1995), the substantially higher consumption rates of the local populations studied in Greece make health risks associated with these contaminant levels meet or exceed those at sites in the US where risk management actions have been implemented to protect human health.

As there is a void of published information on fish consumption data and human health risks associated with the consumption of fish tissues contaminated with organochlorine pesticides in Greece, and published reports for the same in EU countries, our investigation is the first such study for Greece, and second for an EU country. Although there are studies indicating chemical loads for waters and sediments in some areas of the Mediterranean, no published reports have linked these chemical loads to human health effects. There also appears to be few studies surveying local fish consumption habits for the purpose of developing inputs to risk assessment models.

Pesticide concentrations in samples of fishes from EU members have not been reported as of the pesticide residue committee meeting in May, 2004 (Brown, 2004). Likewise, there are no adequate EU toxicity data for about 75 % of 3,000 substances in use; and no adequate EU ecotoxicity data for 50-75 % of the 3,000 priority High Production Volume Chemicals (HPVC) reviewed by the EU (EEA and UNEP, 1999). EEA (2004) and EEA and UNEP (1999) indicated there were significant gaps in toxicity and exposure data, and announced a major lack of human health and exposure data for these priority chemicals. To date, approximately 400 risks assessments have been conducted by EU member states; however, none are made available to the public as they are listed as confidential data (EEA and UNEP, 1999).

This study shows that chemicals from industrial and agricultural use over the past 50 – 60 years have come to rest in the rivers, lakes and other water bodies of Greece. Albanis (1992) found 2,4-D was found in Greek rivers and streams at every location tested between May and August, and that 17 % of applied 2,4-D ended up in water. Due to their environmental persistence and lipophilic nature, these compounds are bioaccumulating in the regional food chains and place the health of humans, as well as other (e.g. wildlife) top consumers on those food chains at potentially significant risk. This scenario not only opens the door to human health risk assessment issues, but also to environmental risk assessment for the aquatic and terrestrial biodiversity in the country. In a report of the OECD Project on pesticide aquatic risk indicators, OECD (2002) reported that the projects first focus on aquatic risks was a logical starting point as it would be simpler and a reasonable first step toward a comprehensive suite of risk indicators for assessment of terrestrial and human health risk.

The size and nature of populations at risk from contaminated fishes in Greece are unknown. Since exposure varies considerably under different circumstances, and the risk assessment process contains inherent uncertainties (USEPA 1991a), we concur with WHO (1999) which strongly encourages responsible authorities in countries to characterize risk using site-specific exposure scenarios and not default values. Use of USEPA default values would have underestimated the risk to Greek populations based on IR alone. Despite the use of maximum chemical concentrations in most cases, risk values in our study should be considered conservative as they focused only on identifiable halogenated organic compounds and did not look at risk contributions from non-halogenated organics, unidentifiable halogenated organics or metals. Accordingly, the rivers, lakes, seas sediments in and around Greece should be sampled and analyzed for organic and inorganic contaminants and the results inventoried in a GIS based data warehouse to guide and focus future risk assessment efforts.

Risk management decisions, such as recommending consumption limits, are beyond the scope of this study and will require a fuller characterization of both exposure and consumption issues if cost effective actions are to be correctly identified. If consumption patterns in other southern European Union countries are comparable to those of Greece, the EU should consider conducting similar studies in those countries. The need for such studies will continue to grow as the issue becomes more evident in the light of globalization and the associated growth of international environmentalism such as that reflected in the compliance provisions of HACCP (USFDA, 2001), Codex Maximum Residue Limits for Pesticides and Extraneous Maximum Residue Limits (Codex Alimentarius Commission, 1997) and guidelines of World Health Organization (WHO, 1999).

ACKNOWLEDGEMENTS

Funded in part by the Thomas F. and Kate Miller Jeffress Memorial Trust, Science Museum of Virginia, Aristotle University, and University of Richmond. E. G. Maurakis, D. V. Grimes, and D. Bobori made collections, analyzed data, and prepared the manuscript; R. Hale of Virginia Institute of Marine Science performed qualitative and quantitative analyses of fish tissue samples; and J. Jones, VA Dept. of Environmental Quality, calculated risk indices.

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EDUCATION SECTION ABSTRACTS FROM THE 83rd ANNUAL MEETING MAY 19-20 JAMES MADISON UNIVERSITY HARRISONBURG, VA

DEVELOPMENT OF NEW ASTRONOMY EXHIBITS AT THE SCIENCE MUSEUM OF VIRGINIA. K. D. Wilson, Science Museum of Virginia, Richmond VA 23220. Astronomy has been a part of the Science Museum of Virginia's program since its earliest days. However it has never had any permanent astronomy exhibits other than its planetarium. A temporary, traveling exhibit, "Night Visions" opened in 1987 but left the exhibit floor several years ago. In recent years few, if any, permanent museum exhibits were devoted to this vast and interesting topic. In 2004 the museum began fast track development of Cosmic Visions, a new 3,000 square foot gallery of eight astronomy exhibits, scheduled to open in the fall of 2005. Topics of these exhibits will include: phases of the moon, reasons for seasons, meteorites, star pattern perspectives, the current night sky, current astronomical discoveries, the celestial sphere, and astronomical telescopes. These exhibits were designed to be highly interactive and support many Virginia Standards of Learning (SOLs).

SOL ENRICHMENT: RAISING SCORES IN ELEMENTARY SCIENCE. Patricia D. Fishback and David B. Hagan, Center for Science Education, Science Museum of Virginia, Richmond, VA 23220. Since 1999, the Science Museum of Virginia has collaborated with educators in Richmond Public Schools to design a program that would help solve the problem of low scores on students' 3rd and 5th grade science SOL tests. Although a direct statistical correlation to the program is not possible, the students' scores improved dramatically and the program has been renewed every year. Gains made by students who are in the program are about twice what they are for students who are not in the program. Teachers as well as students benefit from the in-class series of hands-on science lessons. The program continues to grow, and has been conducted in Petersburg and Chesterfield. A description of the program and its effects, such as changes in scores and increased teacher competency and comfort regarding science, will be presented.

LAB DEVELOPMENT FOR INTRODUCTORY BIOLOGY. Brittany P. Trant & Lynn O. Lewis, Dept. of Biological Sciences, University of Mary Washington, Fredericksburg VA 22401. According to the TIMSS study and Project 2061, Americans are falling behind other leading countries in science and math achievement. Also by valuing breadth over depth in the curriculum, we are weakening the ability of our citizens to question and make informed decisions regarding their lives. In present classrooms, science is focused on lecture and highly structured labs with little room for independent analysis or methods. Because of this students never truly learn what scientific inquiry is until they choose to enter a science field. Therefore, the goal for this study was to implement "real world" labs that can be used in conjunction with traditional teaching methods. Thus allowing students to develop critical thinking skills, learn how to apply knowledge, and still meet the standards outlined by state and federal law. Labs were designed to integrate a variety of biological topics (scientific method and procedures, photosynthesis and respiration, protein function, data interpretation, disease, evolution, ecology, human physiology) that encouraged students to explore alternate solutions to current problems while learning how to question and support an argument.

MATHEMATICS: REASONS TO VALUE IT. Michael Gentry[1] & James McCrory[2], [1]Department of Mathematics & [2]Department of Education, Mary Baldwin College, Staunton, Virginia, 24401. Although mathematics is valuable as a tool of science to describe or model the physical world, and scientists like Stephen Hawking, Lucasian professor of mathematics at Cambridge University, live life by the numbers, for the vast majority of American citizens, mathematics is important for other reasons. It is argued that mathematics is relevant to everyday concerns at work, home, and school because it teaches important life lessons.

RAT BASKETBALL: A SLAM DUNK FOR EDUCATION AT THE SCIENCE MUSEUM OF VIRGINIA. Leeanna T. Pletcher, Science Museum of VA, 2500 W. Broad St., Richmond VA 23220. Norway rats, *Rattus norvegicus*, were trained using operant and classical conditioning to perform a complex task. A skinner box 17.5" wide, 27" wide and around 15 inches tall was constructed. Training began March 31st, 2004 in the Science Museum of Virginia's Biolab 1, training sessions have been open to museum visitors. Feeding pellets were used as positive reinforcement for each target behavior. Training took between 4 and 7 months and consisted of 15- 30 minute sessions where one of 13 target behaviors must be performed 50 repetitions. Two rats successfully completed training and first performed the Rat Basketball Demonstration on August 17th, 2004. During this 20 minute demonstration, museum guests are introduced to the topics of operant conditioning and basic theories of learning by watching two rats play a game of basketball. Information about the natural history of rats is also shared. The Rat Basketball Demonstration has proven popular with museum visitors and is now running twice daily.

TEACHING AND LEARNING SCIENCE IN A WIRELESS CLASSROOM ENVIRONMENT USING TABLET PC'S. Mark Cline, Ed Oakes, Neil Sigmon, P. Niels Christensen, Marissa Smith, Kristy Crigger & Joe King, Radford University. The Tablet PC would seem to be a perfect fit for the classroom with wireless connectivity. When integrated into the wireless teaching and learning environment that includes appropriate instructional technologies, software applications and technical support, one could expect new problems as well as advantages. Beginning the Fall of 2004 Radford University instituted a wireless campus environment and distributed tablet pcs to many faculty. A multidisciplinary "Wireless Classroom Project" was conducted through its Honors Academy during the fall and spring semesters of the past academic year, in which students as well as faculty had access to the wireless campus environment during class time. Our panel of faculty, students and director of academic computing discuss the advantages and limitations discovered through various implementations of this collaborative faculty and student project.

VIRGINIA'S BIODIVERSITY: A SERIES OF HANDS-ON ACTIVITIES FOR ELEMENTARY STUDENTS. Emily Betts[1,2] & Eugene G. Maurakis[2,3], [1]Virginia Commonwealth University, [2]Science Museum of Virginia, [3]University of Richmond.
The Science Museum of Virginia's mission calls for the education of Virginia citizens in the concepts of science and in the study of Virginia's natural resources. Elementary teachers, especially those who have not specialized in science, need resources to improve science understanding and exposure among their students. The Virginia Biodiversity Activities were conceived to satisfy this need for affordable hands-on activities that encourage student-led learning. The objectives of the Virginia Biodiversity program are to educate students and the public about biodiversity, give needed attention to invertebrate groups and allow students to make observations, ask questions and make comparisons. The Virginia Biodiversity program consists of five activities about butterflies, insects, invertebrates from the Chesapeake Bay, fossils and rocks and minerals. Evaluations by pre-service and VAST teachers, and 3rd grade students indicate that activities meet needs for hands-on activities in elementary classrooms with appropriate group size and specimen care. Funded in part by Junior League of Richmond and Virginia Museum of Natural History.

CREATION OF A NATURE CENTER TO SUPPLEMENT ELEMENTARY SCHOOL SCIENCE INSTRUCTION. J. Orion Rogers[1] & James T. Togers[2], [1]Radford University, Radford, VA 24142, [2]Radford High School, Radford, VA 24141.
Literature surveys reveal that schoolyard habitats, such as organic gardens, wetlands and wildflower meadows, provide students a context for learning science and promote opportunities for interdisciplinary teaching. This project involved creating a nature center at Belle Heth Elementary School in Radford, Virginia during May and June of 2002. A 720 square feet plot of neglected land adjacent to the school grounds was cleared, and three study areas were planned to enhance teaching and learning of selected science SOLs. The project required over 266 hours of planning and labor as well as over $632 in donations of money and materials. Objectives included providing students opportunities to conduct field identification of rocks, plants, birds and butterflies as well as experiments on plant growth and predation and bird dietary preferences. Study areas were designed for botany with 27 different plant specimens, for geology with nine different rock specimens and for birds with a birdbath, robin shelf, bluebird house and bird feeders. Data revealed that experiential learning enhanced science instruction and Virginia Science SOL Test passing scores increased from 80.6% in 2001-02, the year before the nature center was established, to 90.6% in 2003-04.

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