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Predicting Fish Species Diversity in Lotic Freshwaters of Greece

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ABSTRACT

Objectives were to test the hypothesis that stream order and stream width alone account for species diversity in drainages of Greece, and to create a mathematical model that predicts fish diversity in small and medium sized freshwater streams in the southern Balkan Peninsula in accord with the stream classification system proposed by the European Environmental Agency (EEA). Thirty-seven species of fishes in 12 families (Petromyzontidae, Anguillidae, Cyprinidae, Cobitidae, Balitoridae, Mugilidae, Salmonidae, Peociliidae, Gasterosteidae, Moronidae, Centrarchidae, and Blenniidae) were collected in five stream orders (1-5) from 19 river drainages in Greece in 1993 and from 2000-2002. Numbers of species were significantly correlated with stream order (+), width (+), and depth (+), and elevation (-). Results of stepwise regression indicated that stream order, elevation, stream depth, and river km were significant factors associated with ichthyofaunal diversity, and were used to create a regression model to predict species diversity (up to 5th order streams). We conclude that geo-specific factors (i.e., small, isolated drainages with limited water budgets, geological history, dry climate, and low annual rainfall) should be included in the EEA monitoring design for lotic waters in harsh environments of southern Mediterranean countries as these features differ from those of central, eastern, and northern European countries with larger watersheds.

INTRODUCTION

Gretes and Maurakis (2001) examined relationships between fish species diversity and physiochemical features of freshwater streams, and hypothesized that stream order and stream width alone were the significant factors accounting for species diversity based on collections from four drainages (Strymon-Aggitis, Axios, Aliakmon, and Aoos) in Greece. Their study is significant as it is the first attempt to systematically examine fish diversity relative to the physiochemical environment of lotic waters in small, isolated drainages in Greece. Lotic freshwater systems in Greece are relatively harsh environments with limited water budgets as a result of the country's complicated geological history, and dry climate with low annual rainfall (Hadjibiros et al., 1998; Gretes and Maurakis, 2001). Studies by Gretes and Maurakis (2001) and Economou et al. (1999), who investigated fish species diversity in streams of western Greece, may have significant implications for European Environmental Agency (EEA) stream biodiversity policies, monitoring guidelines, and management programs. For example, EEA recently formulated a freshwater stream monitoring program and a biodiversity initiative to inventory, identify and describe aquatic and terrestrial species in European Union (EU) countries (Nixon et al., 1996). Data from Gretes and Maurakis (2001) and Economou et al. (1999) can be used to create the requisite baseline for future biodiversity inventories, water quality assessments, environmental impact statements, and a host of other ecological studies. However, EEA guidelines for collecting these baseline data are applied to all countries of the EU without consideration to unique geo-specific factors (e.g. semi-arid conditions and small drainage areas) that occur in southern European countries such as Greece. In Greece, there is a paucity of ecological and distributional data for fishes in lotic freshwater streams, many of which annually experience significant natural and anthropogenic perturbations (e.g. desiccated stream beds in summer, water extraction for irrigation, and pollution)(Bobori, et al., 2001, Hadjibiros et al., 1998). Natural perturbations (e.g. droughts) and anthropogenic influences (i.e., water extraction, canalization, channelization, damming, introductions of exotic species, and agricultural, industrial, and municipal waste inputs) on freshwater resources have become more pervasive over the past 40 years, resulting in elimination and degradation of surface water resources and aquatic habitats in Greece (Bobori 1996; Bobori et al., 2001; Hadjibiros, et al., 1998; Lekakis, 1998; Economidis et al., 2000; Economou et al., 1999; OECD, 2000). These perturbations have fragmented and otherwise altered freshwater fish habitats and communities and led to extirpation of some native species in both lotic and lentic environs (Economidis, 1995; Economou et al., 1999; Hadjibiros, 1998; Lekakis, 1998;). Currently, Greece is without a national action plan to monitor and manage aquatic resources (i.e., aquatic habitats, threatened fishes, and inland fisheries)(Bobori et al., 2001; Economidis, 1995; Economou et al., 1999; Hadjibiros, 1998; Lekakis, 1998; OECD, 2000).

Our first objective is to test the hypothesis of Gretes and Maurakis (2001) that stream order and stream width alone account for species diversity with a more robust set of data that includes collections from previously sampled drainages (i.e., Aliakmon, Aoos, Axios, and Strymon-Aggitis) and from 15 additional drainages (Piros-Tethreas, Pinios, Alphios, Pamisos, and Evrotas of Peloponnesos; Kalamas, Aheron, Louros, Arachthos of Epiros; Acheloos, Evinos, Sperchios and Kifissos of Sterea Hellas; and Gallikos and Loudias of Macedonia)(Fig. 1). Our second objective is to present a mathematical model that predicts fish diversity in small and medium sized freshwater streams in the southern Balkan Peninsula in accord with the stream classification system proposed by the EEA.

MATERIALS AND METHODS

Fishes were collected with a 1.2 x 3 m seine (stretch mesh=0.64 cm), a 12 Volt Smith-Root Model VII DC backpack electroshocker or a 24 Volt Smith-Root Model backpack electroshocker in June and July, 1993, and from June-July in 2000, 2001, and 2002 along with physiochemical stream data (Table 1). Fishes were preserved in 10 % formalin except for six collections preserved in 70 % ethanol and were deposited in the Ichthyology Laboratory of Aristotle University (Greece) and University of Richmond (USA). Collection data from 2000 are presented in Gretes and Maurakis (2001) and those from 1993 and 2001-2002 in Appendix 1. Methods for identifying stream order, gradient (m/km), elevation (m), stream width (m), stream depth (m), pH, water temperature (^{o}C), river kilometer (km), and Jaccard Coefficient follow those in Gretes and Maurakis (2001).

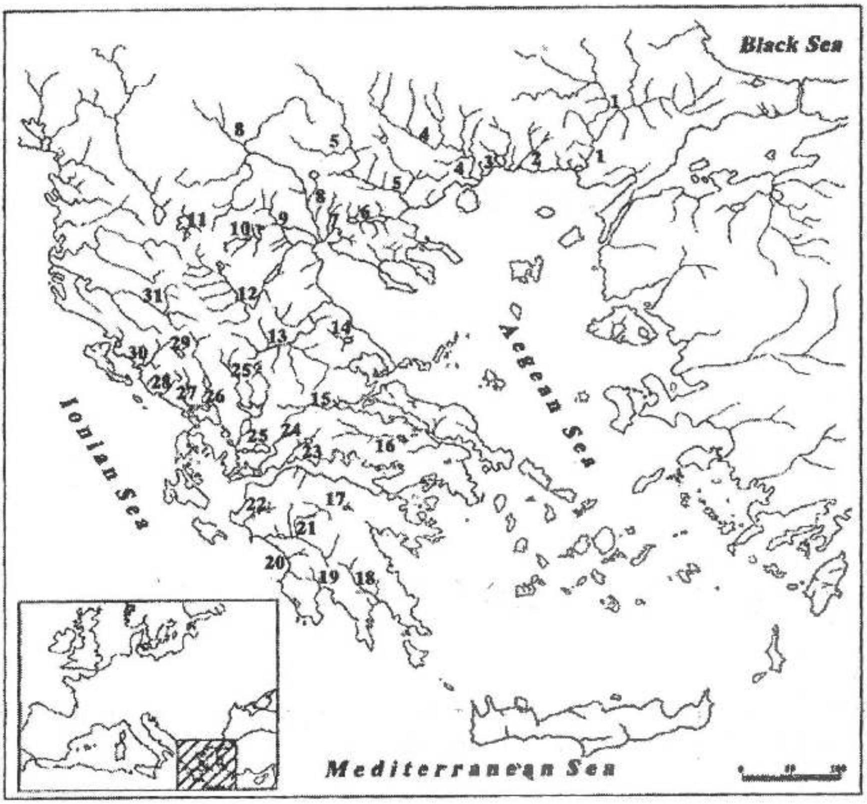

TABLE 1. Drainage, collection number, stream order, elevation (m), stream width (m), stream depth (m), gradient (m/km), water temperature (^{o}C), and river kilometers from mouth to collection location in the Acheloos, Aheron, Aliakmon, Aoos, Arachthos, Axios, Evinos, Gallikos, Kalamas, Kifissos, Loudias, Louros, Sperchios, and Strymon-Aggitis drainages on mainland Greece, and the Alphios, Evrotas, Pamisos, Pinios, and Piros drainages in Peloponnesos (P), Greece in 1993 and from 2000-2002.

Correlation analyses (SAS, 2002) were performed to determine significant relationships among numbers of species, stream order, elevation, stream width, stream depth, gradient, pH, water temperature and river kilometer in individual and combined drainages. A General Linear Model followed by Duncan's Multiple Range Test (SAS, 2002) was used to determine significant differences among average numbers of species and Jaccard Coefficients per stream order. Multiple stepwise regression (forward entry at p=0.15 and backward elimination, SAS, 2002) was used to determine factors accounting for significant variation in species diversity. Backward stepwise regression began with all stream factors in the model and removed the least significant ones until all remaining terms were statistically significant. The backward elimination method drops factors whose deletions causes the smallest decrease in R^2.

RESULTS

A total of 37 species was collected in five stream orders (1, 2, 3, 4, and 5) from 19 river drainages in Greece in 1993 and from 2000-2002 (Tables 2a-c; Gretes and Maurakis, 2001). Twenty-six species (10 families: Petromyzontidae, Anguillidae, Cyprinidae, Cobitidae, Mugilidae, Salmonidae, Moronidae, Peociliidae, Gasterostei-

TABLE 1. Drainage, collection number, stream order, elevation (m), stream width (m), stream depth (m), gradient (m/km), water temperature (^{o}C), and river kilometers from mouth to collection location in the Acheloos, Aheron, Aliakmon, Aoos, Arachthos, Axios, Evinos, Gallikos, Kalamas, Kifissos, Loudias, Louros, Sperchios, and Strymon-Aggitis drainages on mainland Greece, and the Alphios, Evrotas, Pamisos, Pinios, and Piros drainages in Peloponnesos (P), Greece in 1993 and from 2000-2002.

Drainage	Collection Number	Order	Elevation (m)	Width (m)	Depth (m)	pH	Gradient (m/km)	Temp (^{o}C)	River km (km)
Arachthos	485	1	559.6	9.0	0.25	.	5.9	20.0	95.5
Arachthos	504	1	144.8	1.5	0.02	8.0	3.5	26.1	41.4
Arachthos	484	2	517.3	7.0	0.50	.	5.7	17.0	90.2
Arachthos	486	2	555.1	8.0	0.25	.	5.8	22.0	94.9
Arachthos	487	3	473.1	17.0	0.35	.	5.6	15.0	84.3
Arachthos	503	3	130.8	8.0	0.21	8.0	3.4	27.2	39.0
Kalamas	495	1	137.2	5.0	0.05	8.1	1.9	11.1	71.9
Kalamas	499	1	28.7	2.0	0.20	7.6	1.4	20.6	20.5
Kalamas	493	2	422.8	9.0	0.30	8.5	4.6	6.7	91.8
Kalamas	494	2	152.5	8.0	0.08	8.6	2.0	11.7	78.1
Kalamas	496	2	114.0	5.0	0.09	8.0	2.3	19.4	50.2
Kalamas	525	2	386.8	12.0	0.15	8.0	7.2	15.6	54.0
Kalamas	497	3	81.1	15.0	0.30	8.2	1.6	20.0	51.5
Kalamas	498	3	34.1	30.0	0.28	8.4	1.0	20.6	32.9
Sperchios	518	2	10.1	4.0	0.20	6.4	0.0	31.0	12.0
Sperchios	521	2	347.2	5.0	0.20	8.0	5.4	22.7	63.9
Sperchios	522	3	347.2	12.0	0.30	8.0	5.4	20.6	64.5
Sperchios	520	4	150.6	7.5	0.25	8.0	2.9	22.2	52.1
Sperchios	519	5	42.7	11.5	0.04	8.0	1.3	19.4	32.9
Aheron	500	1	11.3	3.0	0.11	7.8	1.3	18.3	8.4
Louros	501	3	144.8	10.0	0.03	7.8	3.5	22.2	42.0
Louros	502	3	109.4	12.0	0.22	7.6	2.9	16.7	37.8
Louros	5011	3	198.1	15.0	0.32	8.2	4.2	15.6	46.8

continued

TABLE 1. *continued*

Drainage	Collection Number	Order	Elevation (m)	Width (m)	Depth (m)	pH	Gradient (m/km)	Temp (°C)	River km (km)
Evinos	507	1	120.0	.	.	.	5.6	.	21.6
Evinos	506	4	8.0	.	.	.	0.0	.	9.0
Evinos	508	4	84.4	15.0	0.15	8.4	3.4	24.0	24.6
Piros	512	1	175.6	3.0	0.06	7.8	7.4	18.3	23.7
Piros	513	1	246.6	3.0	0.04	8.0	9.6	22.2	25.7
Piros	510	2	67.1	4.0	0.20	8.2	3.6	26.0	18.6
Piros	511	2	91.4	4.5	0.03	8.3	4.9	27.2	18.7
Pinios (P)	514	1	129.2	3.0	0.02	8.0	2.3	20.0	55.4
Pinios (P)	515	2	213.7	6.0	0.04	8.0	3.5	21.1	61.8
Kifissos	516	3	83.8	5.0	0.42	7.7	3.9	25.6	21.6
Kifissos	517	3	117.3	12.0	0.50	7.8	2.1	15.0	57.0
Alphios	530	1	279.2	1.5	0.20	8.2	10.7	16.5	26.1
Alphios	531	1	159.4	0.5	0.10	8.0	6.3	28.2	25.2
Alphios	532	1	59.1	1.5	1.00	7.4	2.5	19.6	23.4
Alphios	535	1	78.3	3.5	.	.	1.8	.	44.2
Alphios	539	1	703.2	1.5	.	.	6.4	.	109.5
Alphios	540	1	665.1	0.5	0.30	7.1	6.2	13.1	107.4
Alphios	541	1	651.4	.	.	.	6.3	.	105.3
Alphios	542	1	649.8	1.3	0.15	7.6	6.1	12.9	105.9
Alphios	543	1	646.8	1.0	0.35	7.7	6.2	14.3	104.7
Alphios	544	1	635.2	10.0	0.25	7.1	6.3	16.1	100.2
Alphios	545	1	387.4	4.0	0.27	7.8	4.1	18.2	94.2
Alphios	529	2	25.6	3.0	0.20	8.0	1.6	26.8	15.6
Alphios	533	2	34.1	3.5	0.17	7.7	2.2	19.6	15.6
Alphios	538	2	397.4	2.5	0.05	7.5	5.0	23.1	79.2
Alphios	528	3	23.5	5.5	0.35	8.0	1.9	22.9	12.6

continued

TABLE 1. *continued*

Drainage	Collection Number	Order	Elevation (m)	Width (m)	Depth (m)	pH	Gradient (m/km)	Temp (^oC)	River km (km)
Alphios	536	3	75.0	22.0	1.10	8.0	1.7	17.0	43.8
Alphios	537	3	324.0	11.0	0.25	8.0	4.7	22.8	69.0
Alphios	527	4	0.0	99.9	0.90	8.0	0.0	21.5	4.2
Alphios	534	4	57.9	.	.	7.9	1.6	18.2	37.0
Alphios	9906	4	0.0	99.9	1.00	8.1	0.0	25.1	4.2
Pamisos	546	1	2.0	.	.		.		.
Pamisos	549	1	207.0	3.0	0.15	7.4	5.8	19.1	36.0
Pamisos	550	1	130.8	3.0	0.13	7.6	4.1	19.6	32.2
Pamisos	547	2	65.2	6.0	0.40	7.4	2.4	23.4	26.7
Pamisos	548	2	84.1	12.0	0.30	.	2.9	.	28.2
Pamisos	551	2	138.4	1.5	0.23	7.1	4.3	16.4	32.1
Pamisos	9923	2	100.0	.	.	8.0	3.8	20.3	26.4
Pamisos	552	3	53.9	8.0	0.27	7.8	2.2	21.7	24.3
Pamisos	553	3	30.5	11.5	0.47	7.6	1.9	24.8	15.9
Pamisos	554	3	11.9	8.0	.	.	0.0	.	14.4
Pamisos	555	3	7.0	40.0	.	.	0.0	.	9.3
Pamisos	556	3	0.6	50.0	0.30	7.9	0.0	22.2	3.6
Evrotas	557	1	269.1	.	.	.	4.3	.	63.0
Evrotas	558	1	485.9	2.2	0.13	8.1	7.6	13.1	63.6
Evrotas	563	3	108.8	6.0	.	.	3.2	.	33.6
Evrotas	654	3	92.0	4.0	0.35	8.0	3.7	22.9	25.2
Acheloos	523	1	902.2	2.5	0.06	8.0	6.7	13.9	135.6
Acheloos	524	1	896.1	6.0	0.20	8.0	6.8	15.6	132.5
Gallikos	459	4	62.5	6.0	0.15	.	3.2	19.5	19.8
Gallikos	460	4	49.4	11.0	0.45	.	2.2	21.1	22.2
Loudias	463	4	27.1	12.8	0.50	.	0.0	17.0	61.2

TABLE 2a. Presence (1) and absence (blank) of species by drainage and stream order collected in Aheron, Arachthos, Evinos, Kalamas, Louros, Kiffisos, and Sperchios drainages of Greece, 1993 and 2000-2002.

	Aheron	Arachthos			Evinos	Kalamas			Louros	Kiffisos	Sperchios		
Species Order	1	1	2	3	4	1	2	3	3	3	2	4	5
Anguilla anguilla	1												
Alburnoides bipunctatus											1	1	1
Carassius gibelio							1			1			
Barbus albanicus		1	1	1									
Barbus cyclolepis											1	1	1
Barbus graecus										1			1
Barbus peloponnesius	1	1	1	1	1	1	1	1	1				
Leuciscus cephalus		1	1	1	1	1	1	1	1				1
Leuciscus cf. *svallize*		1	1										
Phoxinellus pleurobipunctatus	1	1	1	1	1	1	1	1	1				
Pseudophoxinus boeticus										1			
Pseudophoxinus stymphalicus							1		1	1		1	1
Rutilus ylikiensis										1	1		
Scardinius graecus										1			
Tinca tinca								1					
Cobitis arachthosensis						1							
Mugil cephalus								1					
Salmo trutta				1				1	1				
Gambusia holbrooki										1			
Salaria fluviatilis	1												
Gasterosteus aculeatus									1				
TOTAL	4	5	4	6	3	4	5	6	6	7	3	3	5

TABLE 2b. Presence (1) and absence (blank) of species by drainage and stream order collected in Piros, Pinios, Alphios, Pamisos, and Evrotas drainages of Greece, 1993 and 2000-2002.

	Piros		Pinios	Alphios				Pamisos			Evrotas		
Species Order	1	2	2	1	2	3	4	1	2	3	1	2	3
Petromyzon marinus							1						
Anguilla anguilla					1	1	1			1			1
Carassius gibelio							1						
Cyprinus carpio						1							
Barbus albanicus	1		1										
Barbus peloponnesius	1	1	1	1	1	1	1	1	1	1			
Leuciscus cephalus	1	1	1	1	1	1	1	1	1	1			
Leuciscus keadicus												1	1
Leuciscus cf. *svallize*						1							
Phoxinellus pleurobipunctatus		1		1	1	1	1						
Pseudophoxinus stymphalicus				1	1	1	1	1	1	1	1	1	1
Tropidophoxinellus spartiaticus								1	1	1	1	1	1
Mugil cephalus							1						
Salmo trutta						1							
Dicentrarchus labrax							1						
Gambusia holbrooki							1		1	1			
Salaria fluviatilis	1						1			1			
TOTAL	4	3	3	4	5	8	11	4	5	7	2	3	4

TABLE 2c. Presence (1) and absence (blank) of species by drainage and stream order collected in Gallikos, Loudias, and Acheloos drainages of Greece from 2000-2002.

		Gallikos		Loudias	Acheloos
Species	**Order**	4	5	4	1
Anguilla anguilla		1	1	1	
Alburnoides bipunctatus				1	
Carassius gibelio				1	
Cyprinus carpio			1	1	
Barbus peloponnesius		1	1	1	
Gobio gobio		1	1	1	
Pseudophoxinus stymphalicus					
Pachychilon macedonicum				1	
Gambusia holbrooki		1	1	1	
Rhodeus serricus			1	1	
Phoxinus phoxinus				1	
TOTAL		4	6	10	0

dae, and Blenniidae) were collected in the present study from the following drainages: Piros-Tethreas, Pinios, Alphios, Pamisos, and Evrotas of Peloponnesos; Kalamas, Aheron, Louros, Arachthos of Epiros; Acheloos, Evinos, Sperchios and Kifissos of Sterea Hellas; and Gallikos and Loudias of Macedonia; and 11 species (six families: Anguillidae, Cyprinidae, Cobitidae, Balitoridae, Salmonidae, and Centrarchidae) were collected in Strymon-Aggitis, Axios, Aliakmon, and Aoos drainages in Gretes and Maurakis (2001). Species diversity was significantly correlated with stream order (+), width (+), and depth (+), and elevation (-) (Table 3). Stream order was significantly correlated with stream width (+), stream depth (+), pH (+), elevation (-), and stream gradient (-) (Table 3). Average number of species (6.5) in stream order 5 was significantly greater than those (range 1.84-4.4) in stream orders 1, 2 and 3 (Table 4). Average number (4.8) of species in stream order 4 was significantly greater than that (1.84) in stream order 1 (Table 4). Jaccard index values between stream order pairs (1 & 2, 1 & 3, 1 & 4, 1 & 5; 2 & 3, 2 & 4, 2 & 5; and 3 & 4, 3 & 5) decreased progressively with increased distance downstream (Table 5). Percent (66.7) of shared species in stream order pair 1 & 1 was significantly greater than those (range=25.0-27.2) in stream order pairs 1 & 5 and 1& 4 (Table 6). Similarly percent (69.3) of shared species in stream order pair 2 & 2 was significantly greater than those (range=29.2-43.6) in stream order pairs 2 & 5 and 2 & 4 (Table 6). Percent (64.5) of shared species in stream order pair 3 & 3 was significantly greater than that (22.3) in stream order pair 3 & 5 (Table 6). Results of stepwise regression indicated that stream order, elevation, stream depth, and stream length (river km) were significant factors accounting for species diversity (numbers of species) (Table 7). These four factors and the intercept (1.43217) were used to create a model to predict species diversity where (Table 7):

$$\text{Diversity} == (1.43217*\text{slope}) + (0.87356*\text{order}) + (-0.00273*\text{elevation}) + (1.76413*\text{depth}) + (0.00525*\text{river km})$$

Number of species predicted by the model compare well with average number of species calculated from collections in stream orders 1-5 (Table 8) with one exception.

TABLE 3. Results of correlation analysis (correlation coefficient, probability, and n) of diversity (total number of species), drainage, stream order, elevation, stream width, stream depth, pH, stream gradient, water temperature, and river km for collections in the Acheloos, Aheron, Aliakmon, Aoos, Arachthos, Axios, Evinos, Gallikos, Kalamas, Kifissos, Loudias, Louros, Sperchios, Strymon-Aggitis on mainland Greece, and the Alphios, Evrotas, Pamisos, Pinios, and Piros in Peloponnesos, Greece in 1993 and from 2000-2002. $p<0.05$.

	Diversity	Order	Elevation	Width	Depth	pH	Gradient	Temp.	River km
Diversity	1.00000 98	0.59721 <.0001 98	-0.26327 0.0088 98	0.47906 <.0001 92	0.50355 <.0001 91	0.25060 0.0535 60	-0.07438 0.4667 98	0.14206 0.1744 93	-0.02331 0.8197 98
Order		1.00000 108	-0.39883 <.0001 107	0.46467 <.000 98	0.40608 1<.0001 91	0.26238 0.0411 61	-0.23085 0.0167 107	0.18469 0.0747 94	-0.16869 0.0824 107
Elevation			1.00000 107	-0.20957 0.0394 97	-0.1049 0.3223 91	1-0.13272 0.3079 61	0.61803 <.0001 107	-0.40297 <.0001 94	0.82724 <.0001 107
Width				1.00000 98	0.54559 <.0001 91	0.19455 0.1470 57	-0.13536 0.1862 97	0.07260 0.4965 90	-0.12520 0.2218 97
Depth					1.00000 91	-0.07328 0.5880 57	-0.03782 0.7219 91	-0.07973 0.4551 90	0.02487 0.8150 91
pH						1.00000 61	-0.03418 0.7937 61	-0.06461 0.6208 61	-0.03192 0.8070 61
Gradient							1.00000 107	-0.11845 0.2555 94	0.77557 <.0001 107
Temp.								1.00000 94	-0.27829 0.0066 94
River km									1.00000 107

TABLE 4. Results of Duncan's multiple range test (SAS, 2002) for average number of species per stream order in combined river drainages (Acheloos, Aheron, Aliakmon, Aoos, Arachthos, Axios, Evinos, Gallikos, Kalamas, Kifissos, Loudias, Louros, Sperchios, Strymon-Aggitis on mainland Greece, and the Alphios, Evrotas, Pamisos, Pinios, and Piros in Peloponnesos, Greece, 1993 and 2000-2002.

Stream Order	1	2	3	4	5
Average # Species	1.84	3.07	4.4	4.8	6.5

F=13.57
p>F=0.0001
df=97

TABLE 5. Results of Jaccard Index tests by stream order pair for all combined drainages (Acheloos, Aheron, Aliakmon, Aoos, Arachthos, Axios, Evinos, Gallikos, Kalamas, Kifissos, Loudias, Louros, Sperchios, Strymon-Aggitis) on mainland Greece, and the Alphios, Evrotas, Pamisos, Pinios, and Piros in Peloponnesos, Greece, 1993 and 2000-2002.

Order pair	n	Average Jaccard	Std.Dev.	Min	Max	Range
1-1	3	66.67	28.87	50.00	100.00	50.00
1-2	20	49.38	20.52	20.00	80.00	60.00
1-3	21	44.98	15.66	20.00	75.00	55.00
1-4	5	27.20	9.39	17.00	36.00	19.00
1-5	2	25.00	1.41	24.00	26.00	2.00
2-2	7	70.14	16.54	50.00	100.00	50.00
2-3	23	62.70	18.52	30.00	100.00	70.00
2-4	7	43.63	3.63	38.00	50.00	12.00
2-5	2	29.15	5.87	25.00	33.30	8.30
3-3	6	60.17	24.26	33.00	100.00	67.00
3-4	5	50.50	9.07	38.50	58.00	19.50
3-5	3	22.33	2.52	20.00	25.00	5.00
4-5	1	60.00		60.00	60.00	0

The absolute difference between predicted (2.02) and actual (1.84) numbers of species in stream order 1 was significant ($t=2.69$; $p=0.0116$).

DISCUSSION

We do not agree with Gretes and Maurakis (2001) that stream order and stream width alone account for fish species diversity in freshwater streams in Greece. Rather, our stepwise multiple regression analyses of a significantly more robust data set (four times greater than theirs) indicate that four factors (stream order, elevation, stream depth, and river km) account for fish species diversity in freshwater systems we studied in Greece (Table 7). As in Gretes and Maurakis (2001), stream order remains the most significant factor, but their other factor, stream width, is not a significant variable in the regression analysis that accounts for species diversity. Instead, elevation, stream depth, and river km (distance from collecting site to mouth of receiving river) in

TABLE 6. Results of Duncan's multiple range test (SAS, 2002) for average Jaccard value by stream order pair comparison in combined river drainages (Acheloos, Aheron, Aliakmon, Aoos, Arachthos, Axios, Evinos, Gallikos, Kalamas, Kifissos, Loudias, Louros, Sperchios, Strymon-Aggitis) on mainland Greece, and the Alphios, Evrotas, Pamisos, Pinios, and Piros in Peloponnesos, Greece, 1993 and 2000-2002. Underscored averages do not differ at p=0.05.

Order pair	1-5	1-4	1-3	1-2	1-1
Average	25.0	27.2	45.2	47.9	66.7
F=2.99 p>F=0.0289 df=47					
Order pair	2-5	2-4	2-3	2-2	
Average	29.2	43.6	63.9	69.3	
F=5.61 p>F=0.0033 df=35					
Order pair	3-5	3-4	4-5	3-3	
Average	22.3	50.5	60.0	64.5	
F=3.32 p>F=0.0705 df=12					

TABLE 7. Results of stepwise regression (forward entry, backward elimination, SAS, 2002) for modeling numbers of freshwater fish species in river drainages of Greece. Significance indicated by an asterisk at p≤0.15.

Source	DF	Sum of Squares	Mean Square	F Value	Prob >F
Model	7	53.30305	7.61472	12.768	0.0001
Error	18	10.73541	0.59641		

Parameter Estimates

Variable	Parameter Estimate	Standard Error	T for Ho: Parameter=0	Prob > |T|
Intercept	1.43217	0.84265	1.70	0.093*
Order	0.87356	0.16311	5.36	<0.001*
Elevation	-0.00273	0.00101	-2.69	0.009*
Depth	1.76314	0.75525	2.34	0.022*
Length	0.00525	0.00358	1.47	0.146*
Width	0.01564	0.01103	1.42	0.160
Gradient	0.00388	0.00540	0.72	0.474
Temp. (^{o}C)	-0.01551	0.03366	-0.46	0.646

Diversity = (1.43217*slope) + (0.87356*order) + (-0.00273*elevation) + (1.76413*depth) + (0.00525*river km)

TABLE 8. Comparison of actual average diversity and predicted diversity of fishes (derived from model) per stream order based on samples collected in drainages in Greece.

Stream order	Predicted # species	Avg. Actual # species
1	2.02	1.84
2	3.26	3.10
3	4.56	4.40
4	5.82	4.80
5	6.59	6.50

addition to stream order are significantly more important than stream width in accounting for species diversity. Stream width cannot be discounted as unimportant, as it was correlated with species diversity, stream order, elevation, and stream depth. However, the backward stepwise regression analysis removed stream width based on its low p-value as it did not improve the explanation provided by the other variables.

Our mathematical model derived from stepwise multiple regression can be used to predict fish species diversity in all altitude classes (>800 m, 500-800 m, 200-500 m, <100-200 m) and the relative sizes of monitoring sites (small sized rivers of stream order 1-3; and medium sized rivers of stream order 4-5) of the stream monitoring system proposed in the European Freshwater Monitoring Network Design (Nixon et al., 1996). Our model, however, may not be an appropriate tool to predict fish species diversity for the EEA's large river monitoring sites (6^{th} order and above). We did not experience 6^{th} or larger stream orders in Greece, and as such, our model does not reflect data from large rivers. Absence of 6^{th} order rivers in Greece is related to the isolated and small river drainages (maximum length =~320 km for Aliakmon River in Greece) whose water budgets are limited by geological history, dry climate, and low annual rainfall of the country (Hadjibiros et al., 1998). We recommend these factors (i.e., geological history, dry climate, and low annual rainfall) should be considered in a monitoring design for Mediterranean countries of southern European whose climate, geology, and hydrology differ from those of central, eastern, and northern European countries with larger watersheds.

Fish species diversity predicted from our model compare well with the average fish species diversity per stream order (Table 8). Differences between predicted and actual numbers of species collected at each collecting station are not significant with one exception. The absolute differences between actual and predicted fish species diversity were significant only for stream order 1 ($p=0.0116$). Significant variability in number of species in 1^{st} order streams was due to several of these streams being devoid of fishes (e.g. high elevations of 1^{st} order snow-melt streams in Acheloos drainage).

Values for the intercept, stream order, elevation, stream depth, and river km from the multiple regression model can be manipulated easily in a spreadsheet and used to calculate species diversity with factors (stream order, elevation, and river km) taken from maps and measurement of stream depth for a particular location. The predictive model of species diversity can be a useful evaluation tool in environmental impact studies, pollution impact assessment, and other stream monitoring programs. For example, the actual species diversity was much lower than predicted in 3^{rd} order sections of the Louros River, suggesting that this portion had been impacted by channelization. Collecting locations three kilometers upstream and five km downstream of this 3^{rd} order reach exhibited values consistent with those predicted for a healthy system. Likewise, the absence of species in the 3rd order reaches of the milky colored Evrotas River still contaminated with sewage effluent eight and 13 km downstream from Sparta, Peloponessos in Greece were significantly lower than those (4 species each) at points 18 km downstream of Sparti where water clarity and presumably water quality had improved, and in the control station in the Evrotas River, 0.5 km upstream of the municipal sewage discharge of Sparti.

The pattern of decreasing homogeneity of species with increased distance downstream among stream order pairs (e.g. 1 and 2, 1 and 3, 1 and 4, etc.; Tables 5 and 6) compare well with the results reported by other researchers (Gretes and Maurakis, 2001; Hutchinson, 1993; Hynes, 1970; Maurakis et al., 1987; Rahel and Hubert, 1991; and Paller, 1994) in streams they studied in Australia, North America, and Europe. We concur with Gretes and Maurakis (2001) that stream order can be used to emulate an ecological unit, and thus account for species diversity along a river continuum except where physical factors change rapidly within the same stream order.

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APPENDIX 1

Fish collections by river drainage, collection number (EGM=Eugene G. Maurakis), region/prefecture, locality, and date in rivers of Greece in 1993, and 2000-2002.

Piros-Tethreas: EGM-Greece-510. Achaia. Tethreas River (2nd order), 0.25 km east of Hai Kali, about 30 km southwest of Patras, Peloponnesos,18 June 2001. EGM-Greece-511. Achaia. Piros River (2nd order), 1.5 km NE of Isoma and about 15 km SSW of Patras, 18 June 2001. EGM-Greece-512. Achaia. Unnamed tributary (1st order) of Piros River, 1 km southeast of Prevedos about 18 air km south of Patras, Peloponnesos, 18 June 2001. EGM-Greece-513. Achaia. Tethreas River (1st order) at Kiparissi near M. Aghios, 12 km SE of Kritharakia and about 22 km S of Patras, 18 June 2001.

Pinios (Peloponnesos): EGM-Greece-514. Ilia. Unnamed tributary (1st order) of Pinios River, 3.5 km W of Simopoulo and 15 km NE of Amaliada, 19 June 2001. EGM-Greece-515. Ilia. Pinios River (2nd order), 4 km southwest of Karpeta, about 40 air km east of Amalidia, Peloponnesos,19 June 2001.

Alphios. EGM-Greece-327. Arkodis creek at Ladonas, 20 km NW of Panayitsa, about 35 km NW of Tripoli, 5 July 1993. EGM-Greece-329, unnamed tributary of Alphios River between Vlahena and Kalivia, 53 km NW of Tripoli, 5 July 1993. EGM-Greece-527. Ilias. Alphios River, 4th order, 1.7 km SE of Pirgos, 23 June 2002. EGM-Greece-528. Ilias. Enipeas River (3rd order), 2.5 km E of Varvasena, about 8 km

E of Pirgos. 23 June 2002. EGM-Greece-529. Ilias. Unnamed tributary (2nd order), 1.5 km N of Smila, about 13 km ENE of Pirgos, 23 June 1002. EGM-Greece-530. Ilias. Unnamed tributary (1st order), 1 km S of Neraida, about 22 km NE of Pirgos. EGM-Greece-531. Ilias. Unnamed tributary (1st order), 3 km W of Neraida, about 19 km ENE of Pirgos, 23 June 2002. EGM-Greece-532. Ilias. Unnamed tributary (1st order), 2 km E of Sopi, about 12 km NNE of Pirgos, 23 June 2002. EGM-Greece-533. Ilias. Unnamed tributary (2nd order), 2 km Nne of Paleo Varvasena, about 10 km E of Pirgos, 23 June 2002. EGM-Greece-534. Ilias. Alphios River (4th order) 1.5 km N of Issova, about 30 km ESE of Pirgos, 24 June 2002. EGM-Greece-535. Ilias. Unnamed tributary of Alphios River, 0.5 km W of Dafnoula, about 48 km SE of Pirgos, 24 June 2002. EGM-Greece-536. Ilias. Alphios River (3rd order), 2 km NE of Dafnoula, about 50 km ESE of Pirgos, 24 June 2002.EGM-Greece-537. Arkadia. Alphios River (3rd order), 1 km NE of Kiparissia, about 8 km NW of Megalopoli, 24 June 2002. EGM-Greece-538. Arkadia. Unnamed tributary (2nd order) of Alphios River near ancient theater, 1 km N of Megalopoli, 24 June 2002. EGM-Greece-539. Arkadia. Unnamed tributary of Alphios River, 3 km NE of Kasea, about 12 km E of Megalopolis and 12 km W of Tripoli, 25 June 2002. EGM-Greece-540. Arkadia. Spring of Alphios River (1st order), 1.5 km ENE of K. Asea, 11 km E of Megalopoli and 13 km W of Tripoli, 24 June 2002. EGM-Greece-541. Arkadia. Unnamed tributary of Alphios River aside train water tank in K. Asea, 25 June 2002. EGM-Greece-542. Arkadia. Unnamed tributary (1st order) of Alphios River, 0.75 km ESE of K. Asea, about 10 km E of Megalopoli, 25 June 2002. EGM-Greece-543. Arkadia. Unnamed tributary (1st order) of Alphios River, 1 km S of K. Asea, about 3 km N of Dafni,10 km E of Megalopoli, and 13 km W of Tripoli, 25 June 2002. EGM-Greece-544. Arkadia. Unnamed tributary (1st order), 2.5 km N of Mamaria, about 5 km W of K. Asea, and 8 km E of Megalopoli, 25 June 2002. EGM-Greece-545. Arkadia. Unnamed tributary (1st order), 1.5 km NW of Routsi, about 9 km SE of Megalopoli, 25 June 2002.

Pamisos. EGM-Greece-546. Messenias. Unnamed (1st order) tributary of Pamisos River, 25 June 2002. EGM-Greece-547. Messenias. Unnamed tributary (2nd order) of Pamisos River, 0.5 km E of Zevgolatio, about 26 km E of Kiparissia, 25 June 2002. EGM-Greece-548. Messenias. unnamed tributary (2nd order) of Pamisos River, 1 km E of Malta, about 26 km E of Kiparissia, 25 June 2002. EGM-Greece-549. Messenias. Unnamed tributary (1st order) of Pamisos River, 4 km S of Koklas, 1.5 km NE of Malthi and 15 km E of Kiparissia, 25 June 2002. EGM-Greece-550. Messenias. Unnamed tributary (1st order), 0.5 km N of Koklas and 18.5 km E of Kiparissia, 25 June 2002. EGM-Greece-551. Messenias. Unnamed tributary (2nd order) of Pamisos River, 0.5 km E of Koklas and 18 km E of Kiparissia, 26 June 2002. EGM-Greece-552. Messenias. Pamisos River (3rd order), 1 km S of Neochori and about 25 km N of Kalamata, 26 June 2002. EGM-Greece-553. Messenias. Pamisos River (3rd order) at Valira, about 16 km N of Kalamata, 26 June 2002. EGM-Greece-556. Kalamata. Pamisos River (3rd order) at bridge in Kalamata, 26 June 2002.

Evrotas. EGM-Greece-557. Lakonias. Unnamed (1st order) tributary of Evrotas River, about 15 km NW of Sparti, 27 June 2002. EGM-Greece-558. Lakonias. Unnamed tributary (1st order) of Evrotas River, 0.5 km S of Kastorio and 20 km NW of Sparti, 27 June 2002. EGM-Greece-559. Lakonias. Unnamed tributary (1st order) of Evrotas River, 1 km SE of Verdonia and about 10 km NW of Sparti, 27 June 2002. EGM-Greece-560. Lakonias. Unnamed tributary (2nd order) at bridge in Kokkinorachi, 2.5 km N of Sparti, 27 June 2002. EGM-Greece-561. Lakonias. Evrotas River (3rd order), 0.5 km N of Sparti, 27 June 2002. EGM-Greece-562. Lakonias. Evrotas River (3rd order), 1 km E of Riza and 8 km SE of Sparti, 28 June 2002. EGM-Greece-563.

Lakonias. Unnaled tributary (2^{nd} order) of Evrotas River, about 1.5 km E of Lefkochoma and about 13 km SE of Sparti, 28 June 2002. EGM-Greece-564. Lakonias. Evrotas River (3^{rd} order), about 8 km E of Lefkohoma and about 20 km SE of Sparti, 28 June 2002.

Kalamas. EGM-Greece-493. Ioannina. Kalamas River (2^{nd} order), 2.5 km W of Doliana and 16 km E of Kitismata, 12 June 2001. EGM-Greece-494. Ioannina. Unnamed tributary (2^{nd} order) of Kalamas River, 8.5 km NW of Lefkothea and 25 km WNW of Ioannina, 12 June 2001. EGM-Greece-495. Ioannina. Unnamed tributary (1^{st} order) of Kalamas River, 1 km SSW of Soulopoulo and about 22 km W of Ioannina, 13 June 2001. EGM-Greece-496. Ioannina. Unnamed tributary (2^{nd} order) of Kalamas River, 4.5 km E of Vrosina and 24 km W of Ioannina, 13 June 2001.EGM-Greece-497. Ioannina. Kalamas River (3^{rd} order), 0.25 km W of Vrosina and about 24 km W of Ioannina, 13 June 2001.EGM-Greece-498.Despotia. Kalamas River (3^{rd} order), 0.5 km NW of Neraida and 16 km E of Igoumenitsa, 13 June 2001.EGM-Greece-499. Despotia. Unnamed tributary (1^{st} order) of Kalamas River, 0.5 km N of Pigadoulia and 12 km NE of Igoumenitsa, 13 June 2001. EGM-Greece-525. Ioannina. Kalamas River (2^{nd} order), about 30 km NW of Ioannina, 24 June 2001.

Aheron. EGM-Greece-501. Thesprotia. Unnamed tributary (1^{st} order) of Kokitos River, 1.5 km NW of Themelo and about 10 km SE of Parga, 14 June 2001.

Louros. EGM-Greece-500. Ioannina.Louros River (3^{rd} order), 0.5 km E of Panagia and 16 km N of Filipiada, 14 June 2001. EGM-Greece-5011. Ioannina. Louros River (3^{rd} order), 5 km NW of Panagia and 21 km NW of Filipiada, 14 June 2001. EGM-Greece-502. Ioannina. Louros River (3^{rd} order), 3 km N of Aghios Georgios, about 8 km N of Filipiada and 10 km NW of Arta, 14 June 2001.

Arachthos. EGM-Greece-484. Unnamed tributary (2^{nd} order) of Arachthos River, 1 km SW of Potamia, about 20 km NE of Metsovo, 24 June 2000. EGM-Greece-485, Unnamed tributary (1^{st} order) of Arachthos River, 2 km NW of Ambelos, about 20 km SW of Metsovo, 24 June 2000. EGM-Greece-486. Unnamed tributary (2^{nd} order) of Arachthos River, 4.5 km N of Potamia, 0.5 km N of Ambelos, and 19 km W of Metsovo, 24 June 2000. EGM-Greece-487. Unnamed tributary (3^{rd} order) of Arachthos River, 3 km E of Baltouma, 16 km SW of Metsovo, 24 June 2000. EGM-Greece-503. Arta. Unnamed tributary (3^{rd} order) of Lake Pournarion, 0.1 km N of Melates and 15 km NE of Arta, 15 June 2001. EGM-Greece-504. Arta. Unnamed tributary (1^{st} order), 1.6 km ENE of Melates and 17 km NE of Arta, 15 June 2001.

Evinos. EGM-Greece-506. Sterea Ellada. Evinos River (4^{th} order), 4 km W of Perithori and 20 km W of Nafpaktos, 17 June 2001. EGM-Greece-507. Sterea Ellada. Unnamed tributary (1^{st} order) of Evinos River, 1 km E of Velvina and about 6 km NW of Nafpaktos, 17 June 2001. EGM-Greece-508. Sterea Ellada. Evinos River (4^{th} order) at Miavia, about 10 km NW of Nafpaktos, 17 June 2001.

Kiffisos. EGM-Greece-516. Viotia. Kiffisos River (3^{rd} order), about 0.25 km N of Lake Yliki and 1.5 km SE of Akrefnio, and about 67 km NW of Athens, 20 June 2001. EGM-Greece-517. Viotia. Kiffisos River (3^{rd} order), 3 km NE of Aeronia and about 8 km N of Livadia, 20 June 2001.

Sperchios. EGM-Greece-518. Fthiotida. Unnamed tributary (1^{st} order) of Sperchios River, about 0.75 km NE of Damasta and 13 km SE of Lamia, 21 June 2001. EGM-Greece-519. Fthiotida. Sperchios River (5^{th} order), 1 km S of Lianokladi and 14 km W of Lamia, 22 June 2001. EGM-Greece-520. Fthiotida. Sperchios River (4^{th} order), 7.5 km W of Makrakomi and about 36 km W of Lamia, 22 June 2001. EGM-Greece-521. Fthiotida. Sperchios River (2^{nd} order), 1 km NW of Aghios Georgios and 50 km WNW of Lamia, 22 June 2001. EGM-Greece-522. Fthiotida. Sperchios

River (3rd order), 1 km NW of Aghios Georgios and about 50km WNW of Lamia, 22 June 2001.

Acheloos. EGM-Greece-523. Evritania. Unnamed tributary (1st order) of Acheloos River, about 1 km W of Miriki and about 5 km E of Karpenessi, 22 June 2001. EGM-Greece-524. Evritania. Unnamed tributary (1st order) of Acheloos River, about 3 km W of Miriki and 3 km E of Karpenessi, 22 June 2001.

Gallikos. EGM-Greece-459. Gallikos River, 1.6 km SW of Petroto, about 25 km S of Kilkis, 16 June 2000. EGM-Greece-460. Gallikos River drainage, Gallikos River, 1 km W of Meseo, about 25 km S of Kilkis, 16 June 2000.

Loudias. EGM-Greece-463. Loudias River, 0.5 km W of Poliplantanos, about 9 km S of Skidra, 18 June 2000.