J. Environ Radioactivity **12** (1990) 131–141

Variation of ^{137}Cs Levels Between Sexes, Body Sizes and Collection Localities of Mosquitofish, *Gambusia holbrooki* (Girard 1859), Inhabiting a Reactor Cooling Reservoir

Michael C. Newman & I. Lehr Brisbin, Jr

Savannah River Ecology Laboratory, PO Drawer E, Aiken, South Carolina 29802, USA

(Received 12 June 1989; revised version received 17 October 1989; accepted 30 October 1989)

ABSTRACT

*Whole body concentrations of ^{137}Cs were determined for 190 mosquitofish (*Gambusia holbrooki*) collected in April 1987 from three locations in a former cooling reservoir which had been contaminated with ^{137}Cs from production reactor effluents between 1961 and 1964. Male fish collected near the point where the reactor effluent had entered the reservoir tended to have higher ^{137}Cs concentrations than those from the other locations. Females did not differ in ^{137}Cs concentrations between the three locations. Females at the site of contaminant entry tended to decrease in ^{137}Cs concentration as body size increased. These results suggest that radionuclide whole body concentrations may vary in unexpected ways between sex or size classes within a given species and that such differences may also vary within microgeographic scales.*

INTRODUCTION

Studies of the environmental distributions of radionuclides and other contaminants usually emphasize species of direct trophic consequence to humans (e.g. food crops, domestic livestock, fish and game). Smaller and less conspicuous species generally receive less attention and, when they do, factors which may affect the distribution of contaminants across microgeographic scales and between various size and sex cohorts within the population are seldom evaluated. Nevertheless, such species can be important links in food webs which ultimately lead to humans and thus

J. Environ. Radioactivity 0265-931X/90/$03·50

they may, to some degree, play a role in determining human exposure to released contaminants.

In most freshwater systems in the southeastern United States, the mosquitofish (*Gambusia holbrooki*; formerly *G. affinis* var. *holbrooki*) is an abundant and widespread prey species for many forms of larger fish and wildlife. In addition, the high tolerance of this species for a variety of extreme environmental conditions, its consequential ubiquitous distribution and relatively high density in many of the habitats where it is found, make it an ideal candidate for consideration as an indicator species (Newman & Mitz, 1988; Newman & Doubet, 1989). It is therefore an appropriate subject with which to study contaminant distribution on a microgeographic scale and the distribution of contaminants between different sex/size classes within the population. The latter information is necessary to understand how contaminant distribution within food webs may be altered by changes in the population characteristics of important prey species.

The Pond B reservoir of the US Department of Energy's Savannah River Site (SRS) near Aiken, South Carolina, USA, offers a unique opportunity to study the uptake and cycling of radionuclides within aquatic food webs. This system has been contaminated since accidental releases from a nuclear production reactor were introduced into the reservoir over 20 years ago. Over the ensuing years, ^{137}Cs has been incorporated into the biota of this reservoir, including the abundant mosquitofish. Previous studies (Alberts *et al.*, 1979; Brisbin, 1989; Whicker *et al.*, 1989) provide a background to understand better the current distribution patterns of this nuclide within both biotic and abiotic components of this reservoir.

The study reported here describes the distribution of ^{137}Cs in the resident Pond B mosquitofish population with emphasis on variations in whole body burdens of fish from three locations in the reservoir. Differences in ^{137}Cs body burdens due to sex and size of fish within the population are also evaluated.

MATERIALS AND METHODS

Site description and sampling

The SRS is a 750 km^2 site near Aiken, South Carolina that has been closed to public access since 1952. Several reactors on the site produce nuclear materials for national defense. Langley & Marter (1973) have described the site's geology, hydrology, climate and major biota. Bennett &

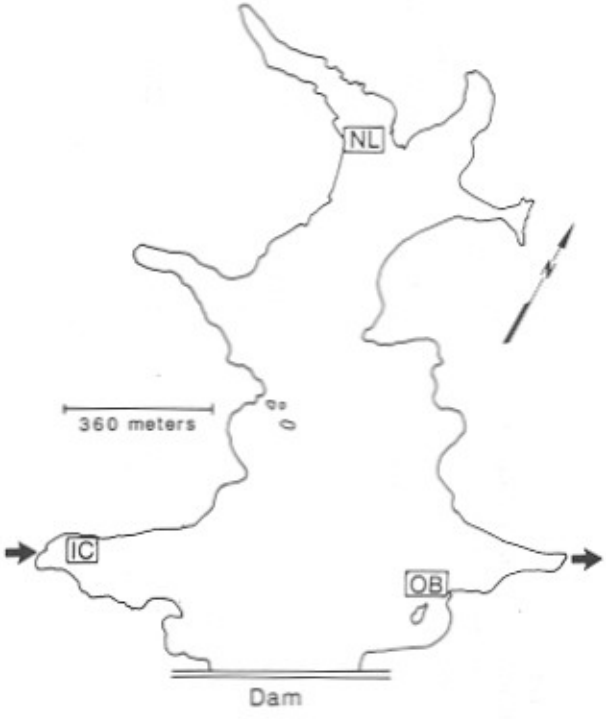

Fig. 1. Locations of mosquitofish collection in April 1987, from the Pond B reservoir of the US Department of Energy's Savannah River Site near Aiken, South Carolina: IC = Inlet Canal, NL = North Lake and OB = Outlet Bay. Arrows represent locations where heated, and later contaminated, nuclear production reactor effluents entered and then left the reservoir between 1961 and 1964.

McFarlane (1983) have provided more detailed information on the fish of the SRS.

Pond B is an 87-ha impoundment constructed in 1961 to receive heated effluent from an operating production reactor. The reactor was shut down and discharge to Pond B ceased in June 1964 after an inadvertent release of radionuclides into the effluent from a damaged fuel element (Alberts *et al.*, 1979). Approximately 5·6 TBq (150 Ci) of ^{137}Cs, along with other fission products, were released to the canal which leads from the reactor to Pond B (Ashley & Zeigler, 1980; Fig. 1). A fraction of the radionuclides released to Pond B subsequently passed out of the reservoir and entered a larger reservoir further to the south (Par Pond). Redistribution and cycling of ^{137}Cs and other radionuclides have occurred in these areas over the ensuing 23 years. The easily measurable levels of ^{137}Cs in several ecosystem components have been quantified by Whicker *et al.* (1989) and

TABLE 1
Sex, Wet Weight, Wet:Dry Weight Quotient and ^{137}Cs Concentrations in Mosquitofish

			Wet wt (g)		*Dry:wet ratio*		*^{137}Cs (Bq/g wet wt)*		*Concentration factor[a] (Bq/g dry wt)/ (Bq/liter)*
Site	*Sex*	*N*	*Median*	*Range*	*Median*	*Range*	*Median*	*Range*	
NL	F	28	0·26	0·12–1·20	0·28	0·25–0·35	1·68	0·80–3·37	7 895
NL	M	28	0·16	0·10–0·23	0·26	0·26–0·33	1·73	0·74–2·94	8 755
OB	F	55	0·31	0·15–0·96	0·25	0·21–0·37	1·61	0·90–2·98	8 474
OB	M	29	0·17	0·10–0·28	0·22	0·21–0·24	1·72	0·71–2·76	10 287
IC	F	31	0·28	0·13–0·54	0·27	0·24–0·28	1·51	0·84–3·66	7 359
IC	M	19	0·16	0·10–0·26	0·26	0·23–0·30	2·23	0·69–5·02	11 285

[a]Assuming 0·76 Bq/liter (Whicker *et al.*, 1989).

Brisbin (1989). Cesium-137 is the only cesium radionuclide present in significant amounts in the reservoir today. Although some ^{134}Cs was initially released into the area, it is unlikely that this isotope provided a significant contribution to the observed counts. The ratio of ^{137}Cs to ^{134}Cs was shown to be approximately 20:1 in biota from the Par Pond and Steel Creek areas of the SRS (Marter, 1970). Other gamma-emitting nuclides were also present in relatively small quantities in these areas (Marter, 1970).

Mosquitofish ($N = 190$) were collected with dipnets from three shoreline locations in the Pond B reservoir (Fig. 1) in April 1987. During this sampling, these live-bearing fish were actively reproducing and most females were gravid. Sample sizes, reported by sex, are given for each of the collection localities in Table 1.

Cesium-137 quantification

Captured mosquitofish were allowed 24 h without food (at 22°C) to clear their guts prior to processing. Each fish was placed into a tared, polyethylene scintillation vial, weighed and lyophilized to a constant weight (12 h). Wet and dry weights were then measured for each fish.

Cesium-137 activity was estimated for each sample using a Beckman Gamma 8000 gamma counter. The 662 keV gamma photon from ^{137}Ba was quantified using a window of 550–760 keV. Empty vials that had been lyophilized and handled similarly to the sample vials were used for estimation of background counts. Instrument counting efficiency was estimated daily using a Beckman 0·1 μCi (3·7 kBq) ^{137}Cs phantom. Each sample was counted for three 30-min periods and an average (30 min) count was calculated for each sample. All but three of the 190 sample counts were above the instrument detection limit (0·123 Bq or 3·3 pCi). The three that were below the detection limit (0·106, 0·117 and 0·119 Bq) were not censored (replaced with the designation, 'below DL'), but were included in these analyses as recommended by Gilbert & Kinnison (1981) and Newman *et al.* (1989).

RESULTS

Radiocesium whole body concentrations of the 190 fish sampled are summarized in Table 1 and Fig. 2. Preliminary univariate analysis of both whole body ^{137}Cs concentrations and log-transformed ^{137}Cs concentrations indicated that a two-parameter, lognormal distribution provided the best fit for these data. For this reason, all concentrations were log-

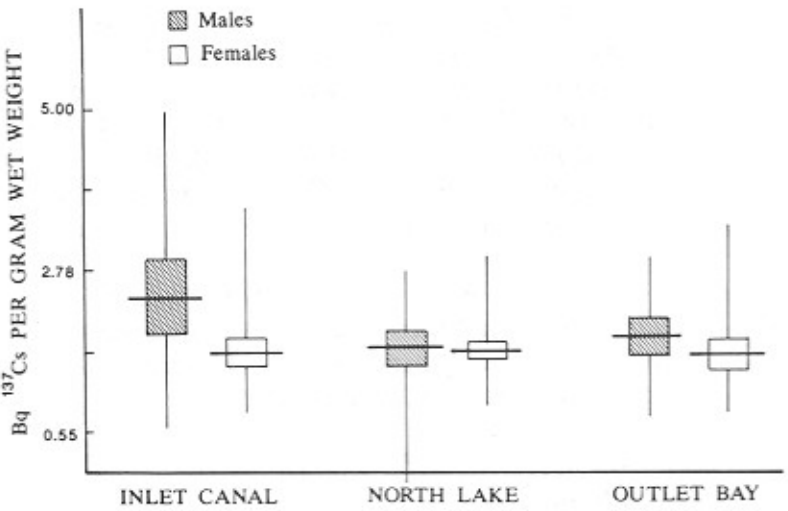

Fig. 2. Radiocesium whole body burdens of male and female mosquitofish collected from three locations (Fig. 1) in the Pond B reservoir of the US Department of Energy's Savannah River Site. Horizontal lines represent means, rectangles represent ± two standard errors and vertical lines represent the range of samples of 19/31, 29/55, and 28/28 males/females for the Inlet Canal, North Lake and Outlet Bay sites, respectively.

transformed prior to conducting statistical tests. Analyses of variance showed that the whole body concentrations in males differed between the three collection localities at a level closely approaching statistical significance ($F = 2{\cdot}91$; $df = 2, 73$; $P = 0{\cdot}061$, $\alpha = 0{\cdot}05$), while there were clearly no significant differences in the whole body concentrations of females from the three locations ($F = 0{\cdot}32$; $df = 2, 111$; $P = 0{\cdot}73$). The greater significance of the difference in concentrations between locations as shown by males as opposed to females was due to the higher concentrations of radiocesium found in males collected at the site nearest the former point of reactor effluent entry (Fig. 2). When the test for differences between locations was re-run for all males, after deleting the data for those from this locality, all suggestion of significant differences disappeared ($F = 0{\cdot}14$; $df = 1, 56$; $P = 0{\cdot}709$).

Analyses for differences in concentrations between the sexes were conducted for each location separately and indicated that weight-specific radiocesium levels in males were significantly higher than those of female fish at the inlet canal (IC; Fig. 1) site ($F = 8{\cdot}54$; $df = 1, 48$; $P = 0{\cdot}0053$), but concentrations did not differ significantly between the sexes at either of the other two locations ($F = 0{\cdot}66$; $df = 1, 82$; $P = 0{\cdot}42$, and $F = 1{\cdot}64$;

TABLE 2
Size Effects on ^{137}Cs Body Burdens of Mosquitofish (log (Bq/fish) = b (log g wet wt) + log a)

Site	*Sex*	*Slope (SE)*	*Intercept (SE)*	r^2	*F*	*PR > F*	*N*
NL	F	0·96 (0·12)	0·39 (0·15)	0·73	69·20	0·0001	28
NL	M	0·76 (0·36)	0·10 (0·67)	0·15	4·54	0·0427	28
OB	F	0·88 (0·08)	0·35 (0·09)	0·72	134·73	0·0001	55
OB	M	1·23 (0·28)	0·92 (0·50)	0·42	19·45	0·0001	29
IC	F	0·43 (0·11)	−0·29 (0·14)	0·36	16·24	0·0004	31
IC	M	−0·22 (0·31)	−1·49 (0·58)	0·03	0·50	0·4876	19

df = 1, 48; P = 0·2057 for the OB and NL sites, respectively; Figs 1 and 2).

The relationship between body burdens of contaminants and animal size was best described by the power equation:

$$Y = aX^b \tag{1}$$

where Y = body burden (Bq/individual), X = body weight (g), and a and b are parameter estimates derived from least squares linear regression of the log-transformed data (log Y versus log X) (Boyden, 1974, 1977). In these analyses, total body burdens are used instead of weight-specific concentrations (Bq/g) so as to avoid the presence of animal weight in the independent and dependent variables. As recommended in Newman & Doubet (1989), the bias associated with the regression of log-transformed data was corrected using the methods of Beauchamp & Olson (1973). The regression results of body burdens of male and female fish from the three sampling sites are presented in Table 2. Except for the regression for males from the IC site, all regression lines had slopes significantly different from 0 (α = 0·05). The wide scatter in the data for males from the IC site precluded reliable development of a regression model for size-dependent body burden. Females from the IC site displayed a b-value significantly less than 1. The b-values for fish from the OB and NL sites ($b \approx 1$) suggest that, assuming a constant exposure for all individuals within the population, the amount of ^{137}Cs in the fish is determined by the number of available tissue binding sites (pCi/g tissue = constant) (Boyden, 1974, 1977). However, b-values significantly less than 1 for both female and male fish from the IC site suggest that additional factors such a surface:volume quotient or size-dependent feeding behavior determine the size-dependence of ^{137}Cs body burden at this site.

DISCUSSION

High concentration factors for ^{137}Cs ($^{137}Cs_{fish}/^{137}Cs_{water}$) in Pond B fish have been attributed to the relatively low concentrations of potassium in Pond B waters (0·7 mg liter^{-1}; Tilly, 1975). Concentration factors for mosquitofish ranged from 7359 to 11 285 with males having generally higher values (Table 1). These were generally within the range reported by Whicker *et al.* (1989) for concentration factors for muscle tissue of piscivorous (8500) and planktivorous (11 000) fish from this reservoir.

Although an accurate assessment of the numbers of *Gambusia* resident in Pond B is not available, nor perhaps even feasible to obtain, the data obtained in this study allow an order-of-magnitude estimate of the minimal amount of radiocesium found in the total *Gambusia* standing crop of the reservoir. This minimal-estimate inventory may then be compared with similar estimates of the total amounts of radiocesium present in other biotic and abiotic components of this same ecosystem, as presented elsewhere by Brisbin (1989). Using the minimal estimate of fish density (19 individuals per meter of shoreline) obtained by Feder *et al.* (1984) for Par Pond, a neighboring reactor cooling reservoir on the SRS, the 9000 m of Pond B shoreline has an estimated minimum total population of 171 000 individuals. The average wet weight per individual fish in this study (males and females combined) was 0·269 g, yielding a minimum estimate of 46 kg live-weight biomass standing crop. At an average of 1·77 Bq (47·69 pCi) radiocesium per gram live weight (again calculated for males and females combined), a minimum total estimate of 81 420 Bq is obtained for the entire 87-ha lake, or 936 Bq/ha. This value was of the same range as estimates of the total radiocesium inventory in other Pond B biota such as benthic gastropods (1783 Bq/ha), waterfowl (1828 Bq/ha) or alligators (903 Bq/ha), as estimated by Brisbin (1989). This minimum inventory estimate of radiocesium for *Gambusia* represents less than 0·003% of the total estimate of 35 148 890 Bq/ha for all biota of the reservoir combined. Over 99% of biotic radiocesium is found in macrophyte biomass and all biota contain less than 0·5% of the total radiocesium for the reservoir as a whole. More than 99% of the ^{137}Cs is found in the lake sediments (Brisbin, 1989).

At the present time, we cannot identify a specific mechanism responsible for the sexual difference in ^{137}Cs concentrations or *b*-values less than 1 for size-dependent body burdens in female *Gambusia* from the site of former effluent entry (IC) but not for those from the other two locations sampled in Pond B. However, these data and background information suggest that the dispersal behavior of this species combined with microgeographic discontinuities in ^{137}Cs distribution in the pond may contribute to the observed differences.

Adult male mosquitofish show a higher tendency to disperse than adult females (Brown, 1985). At sites with a relatively uniform spatial distribution of ^{137}Cs in abiotic and biotic components (sites NL and OB), this effect of migration on size- or sex-dependent body burdens would be minimal since migrants would come from nearby areas of similar background ^{137}Cs contamination. However, there is considerable microgeographical discontinuity in ^{137}Cs contamination near the IC site. Cesium-137 activity increases rapidly in sediments and plants with distance into the input canal. Although mosquitofish were netted from an area immediately outside of the canal, it is quite likely that the fish sampled contained recent migrants from within the canal proper. If the sex and size effects on migration described above are applicable to Pond B mosquitofish, then it would provide an explanation for the greater variability of the IC site male data. Individuals from areas with more variable levels of ^{137}Cs contamination would be expected from sampling this location. This is also consistent with the higher concentrations of ^{137}Cs in males from the IC site (2·23 Bq/g wet wt) versus females from this site (1·51 Bq/g wet wt) or males of the other sites (NL = 1·73; OB = 1·72 Bq/g wet wt; Table 1).

Another factor which could influence the higher amount of unexplained variability of the data from males relative to females from all three sites is the sexual difference in mosquitofish growth kinetics. Growth of female mosquitofish is more uniform throughout their lives than that of male mosquitofish. Growth of male mosquitofish reaches a plateau much earlier than that of females (Brown, 1985). Consequently, there would be a poorer correlation between male fish size and duration of exposure (age) than between female fish size and duration of exposure.

Regardless of what factors are responsible for the patterns of radiocesium distribution seen in Pond B *Gambusia*, the sex- and location-specific nature of these patterns have important implications for the design of radiocesium monitoring or assessment programs in which this species, because of its broad distribution, abundance and importance in various aquatic food webs, may be targeted as an indicator species. Thus, for example, any monitoring program using this species as an indicator of changes in the levels of biotic radiocesium contamination should take particular care to identify and separate the sexes. Furthermore, sampling locations should be precisely defined, marked and resampled carefully on a microgeographic scale—preferably with several such sites being sampled in each time period. The variance structure within a sampling location should be defined as well as that between locations with the most appropriate statistical methods being those associated with a nested sampling design. To the extent that patterns similar to those found in this study may also exist in other species, radiocesium monitoring programs which sample other small fish or aquatic vertebrates should carefully

consider possible sex-, weight- and location-specific differences in body burdens in both the design of such studies and the interpretation of the data that they produce.

ACKNOWLEDGEMENTS

This research was supported by contract DE-AC09-76SROO-819 between the US Department of Energy and the University of Georgia's Savannah River Ecology Laboratory. S. G. McDowell, H. Zippler and S. Presnell provided assistance with both field collections and laboratory analyses. P. Dixon provided helpful discussion concerning counting statistics and critical readings of the manuscript were provided by P. Leberg, G. Meffe, A. Towns, and F. W. Whicker.

REFERENCES

Alberts, J. J., Tilly, L. J. & Vigerstad, T. J. (1979). Seasonal cycling of caesium in a reservoir. *Science*, **203**, 649–51.

Ashley, C. & Zeigler, C. C. (1980). Releases of radioactivity at the Savannah River Plant 1954 through 1978. Report DPSPU. 75-25-1, E. I. DuPont de Nemours and Co., Aiken, South Carolina.

Beauchamp, J. J. & Olson, J. S. (1973). Corrections for bias in regression estimates after logarithmic transformation. *Ecology*, **54**, 1403–7.

Bennett, D. H. & McFarlane, D. H. (1983). The fishes of the Savannah River Plant: National Environmental Research Park. SRO-NERP-12 Savannah River Ecology Laboratory, Aiken, South Carolina.

Boyden, C. R. (1974). Trace element content and body size in molluscs. *Nature (London)*, **251**, 311–14.

Boyden, C. R. (1977). Effect of size upon metal content of shellfish. *J. Mar. Biol. Assoc. UK*, **57**, 675–714.

Brisbin, I. L., Jr (1989). Radiocaesium levels in a population of American alligators: a model for the study of environmental contaminants in free-living crocodilians. *Proceedings of the Eighth Working Meeting of the IUCN/SSC Crocodile Specialist Group*, Gland, Switzerland, Published by International Union of Conservation, Nature and Nature Reserves, ed. F. W. King, pp. 60–73.

Brown, K. (1985). Demographic and genetic characteristics of dispersal in the mosquitofish, *Gambusia affinis* (Pisces: Poeciliidae). *Copeia*, **1985**(3), 597–612.

Feder, J. L., Smith, M. H., Chesser, R. K., Godt, M. J. W. & Asbury, K. (1984). Biochemical genetics of mosquitofish. II. Demographic differentiation of populations in a thermally altered reservoir. *Copeia*, **1984**, 108–19.

Gilbert, R. O. & Kinnison, R. R. (1981). Statistical methods for estimating the mean and variance from radionuclide data sets containing negative, unreported or less-than values. *Health Phys.*, **40**, 377–90.

Langley, T. M. & Marter, W. L. (1973). The Savannah River Plant Site. AEC Research and Development Report DP-1323, Savannah River Laboratory, Aiken, South Carolina.

Marter, W. L. (1970). Radioactivity in the environs of Steel Creek. Publ. DPST-70-435 of Savannah River Laboratory, Aiken, South Carolina.

Newman, M. C., Dixon, P. M., Looney, B. B. & Pinder, J. E. III (1989). Estimating mean and variance for environmental samples with below detection limit observations. *Water Res. Bull.* **25**, 905–16.

Newman, M. C. & Doubet, D. K. (1989). Size dependence of mercury (II) accumulation kinetics in the mosquitofish, *Gambusia affinis* (Baird & Girard). *Arch. Environ. Contam. Toxicol.*, **19**, 819–25.

Newman, M. C. & Mitz, S. V. (1988). Size dependence of zinc elimination and uptake from water by mosquitofish, *Gambusia affinis* (Baird & Girard). *Aquat. Toxicol.*, **12**, 17–32.

Tilly, L. J. (1975). Changes in water chemistry and primary productivity of a reactor cooling reservoir (Par Pond). In *Mineral Cycling in Southeastern Ecosystems* (Proceedings of a Symposium held at Augusta, GA), ed. F. G. Howell, J. B. Gentry & M. H. Smith. Technical Information Center, US Energy Research and Development Administration.

Whicker, F. W., Pinder, J. E. III, Bowling, J. W., Alberts, J. J. & Brisbin, I. L. Jr (1989). Distribution of ^{137}Cs, ^{90}Sr, ^{238}Pu, ^{239}Pu, ^{241}Am and ^{244}Cm in Pond B, Savannah River Plant. Publication SREL-35, UC-66e of the Savannah River Ecology Laboratory, Aiken, South Carolina.