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Interspecific food relationships of Anurans in northwestern Montana and fluoride accumulation in amphibians and reptiles in northwestern Montana

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INTERSPECIFIC FOOD RELATIONSHIPS OF ANURANS IN NORTHWESTERN MONTANA AND FLUORIDE ACCUMULATION IN AMPHIBIANS AND REPTILES IN NORTHWESTERN MONTANA

By

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Interspecific food relationships were studied between three species of Anura to define the nature of competition for the resource. Analysis of the contents of the stomachs from representatives of Rana pipiens, Rana pretiosa, and Bufo boreas was completed.

The taxonomic composition and number of each taxon were similar in the samples from the ranids. Primary dietary items were Coleoptera, Diptera, Hymenoptera, Odonata, and Arachnida. Competition for food in sympatric situations must be acute. Separation between the species was, at least partially, the result of differing abilities to tolerate water temperatures. R. pipiens tended to inhabit warmer water than R. pretiosa. Sympatric populations occurred in transitional areas between valleys and mountains.

Bufo boreas did not compete directly with the ranids for food. Separation was the result of different habitat utilization and different primary food items. Formicidae formed the major portion of the diet of the toads. Toads were more terrestrial than the ranids and, therefore, more independent of water temperature.

Bones from Rana pipiens, Rana pretiosa, Bufo boreas, Chrysemys picta belli, and two species of Thamnophis were analyzed for fluoride to evaluate the potential of amphibians and reptiles as indicators of fluoride pollution. Analyses were completed potentio-metrically.

All of the species accumulated fluoride in three general patterns. First, larger (older) specimens contained more fluoride than smaller (younger) specimens of the same species from the same site. Second, those specimens which were collected from sites which were closer to the aluminum plant or were located in the area which was covered by the emissions from the plant contained more fluoride than specimens of the same species and of the same size (age) from other areas. Third, fluoride was accumulated throughout the life of the organism. Interspecific differences were found in the rate of accumulation of fluoride.

Amphibians and reptiles may be used as indicators of fluoride pollution. However, because interspecific variation in the accumulation of fluoride may be great, analyses should be completed in corroboration with other species (both plant and animal).

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TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

CHAPTER I

INTRODUCTION

Studies of the effects of pollutants such as fluoride, heavy metals, and sulfur compounds, typically involve species of plants and animals which are directly important to man (Kilgore and Doutt, 1967). The study of the effects of pollutants on amphibians and reptiles has received little attention. Casual reference is occasionally made to a small sample in a report which concerns another animal (Rudd, 1964).

Pollutants may adversely effect a species directly or through slow accumulation (Hunt, 1966). The effects of sublethal concentrations of fluoride on amphibians and reptiles are not known. However, based upon studies of other animals (fish, birds, and mammals) two general categories of sublethal effects may be formed. These include accumulation which (1) may cause crippling or malformation and (2) may be passed to predators.

The susceptibility of individuals and of species is not well understood but exposure and genetic resistance are thought to alter the response (Boyd, Vinson, and Ferguson, 1963). The life cycle, physical structure, and physiology also play important roles in amphibian and reptilian susceptibility (Rudd, 1964; Cope, 1965; Boyd, Vinson and Ferguson, 1963), but exact roles have not been defined.

The literature which concerns fluoride is extensive. Discussions and reviews by Simons (1965), Stacy, Tatlow, and Sharpe (1961), National

Science Foundation (1971), Vostal (1971) and Hodge and Smith (1965) provide access to the chemistry, biological effects and distribution of fluoride in the environment. The major studies of pollution by various forms of fluoride have concentrated on the effects of fluoride on vegetation, domestic animals and man (Shupe et al., 1940; Saunders, 1961). Little investigation has centered on the effects of fluoride upon wildlife (Marier and Rose, 1971). Initially, investigation which concerned wildlife and their habitat was intended to establish lethal values (Spector, 1956).

Nonlethal levels of fluoride have received increasing attention in the last ten years. Nonlethal levels of fluoride have been studied in a variety of plants, insects, fish, birds and mammals (Gilmour, 1963; Dewey, 1973; Carlson, 1973; and Gordon, 1974).

The accumulation of fluoride by the flora and fauna which occur near the Anaconda Aluminum Reduction Plant at Columbia Falls, Montana, is well documented. Carlson (1972) analyzed vegetation for fluoride. The area over which 'abnormal' levels of fluoride were found included 179,200 acres which are situated within the 10 ppm isopol line. The U.S. Environmental Protection Agency (1973) demonstrated that the isopol values which define the average content of fluoride in the vegetation "clearly decreased as distance from the aluminum plant increases". The effects of the prevailing wind modified the pattern of distribution of fluoride to appear as an elongated oval. The work by Carlson and Dewey (1973) concentrated on establishing concentration of fluoride in terrestrial and aquatic insects in an attempt to show the transfer of fluoride levels. Carlson, et al. (1974) reported a "strong statistical association between airborne toxicants and outbreaks of forest insects". The association is facilitated by the reduction of the general health of the forest. Carlson and Hammer (1974) found significant impact by insects on the radical growth of lodgepole pine. Dewey (1973) and Carlson (1973) reported on the accumulation of fluoride by terrestrial insects. Higher concentrations of fluoride were found in the flora and fauna which were collected near the aluminum plant (E.P.A., 1974). An increase of "several orders of magnitude" were found to occur with successive steps in trophic levels (E.P.A., 1974).

Purpose

The primary purpose of the investigation was to determine whether amphibians and reptiles accumulated an abnormal amount of fluoride in the vicinity of a single emission source. Secondary and tertiary purposes included the investigation of interspecific food relationships between the resident Anura and further evaluation of the accumulation of fluoride, such as between different age groups and trophic levels.

Location of aluminum reduction plant

The Anaconda Aluminum Company reduction plant is located approximately 1.5 miles north of Columbia Falls, Montana, at the base of Tea Kettle Mountain which rises to a height of 5,936 feet to the east of the plant. The topography to the west and south of the plant is rather flat for approximately 15 miles. The topography to the northwest, north, northeast, and southeast is mountainous with deep valleys. The higher peaks in the general vicinity rise to a height of 8,000 to 9,000 feet, (Carlson and Dewey, 1971).

The process and pollutant emissions

Raw aluminum is produced through electrolytic dissociation of alumina (Al_2O_3). The process is accomplished in a molten slurry of cryolite (Na_3AlF_3) and fluorspar (C_aF_2). Gaseous and particulate fluorides as well as carbon monoxide, carbon dioxide and hydrocarbons are released during the electrolytic process (Hickey, 1968).

The amount of fluoride which has been released into the air has varied as new equipment has been brought into production and as control equipment has been added or modified. Fluoride emissions have varied from 1900 lb/day in 1955-1964 through 3600 lb/day in 1965-1967 to a high of 7500 lb/day in 1968-1970. In 1971 fluoride emissions were reduced to 2585 lb/day. Emissions were further reduced to 1235 lb/day (estimated) in 1975. The production of aluminum has increased from 200 tons per day during 1955 through 1964 to 300 tons per day during 1965 through 1967. In 1968 production of aluminum increased to 500 tons per day. Production has remained relatively constant from 1968 through 1975 (E.P.A., 1974).

Meteorological studies

The E.P.A. analyzed wind flow patterns in the area which surrounds the aluminum plant at Columbia Falls, Montana, and concluded that wind dispersal of airborne fluoride was affected by the topography of the area.

Daytime and nighttime flow patterns were reversed along a southwest to northeast gradient. Daytime wind flow was predominantly toward the northeast. Assessment of the rate of fluoridation at 40 stations indicated the prevailing winds carry more fluoride toward the northeast. Fluoridation rate as measured in 1970 by the E.P.A. decreased rapidly with increased distance along radians from the aluminum plant. Data generated by the United States Forest Service (Carlson, 1972) concurs.

Exception to this pattern occurred in the valleys which lay to the northeast of Tea Kettle Mountain. The height of the local mountains modified the wind flow patterns at specific localities. Shelter may be afforded by the canyon topography of the area. Mixing of surface and high level winds distributed the airborne fluoride more toward the northeast. The higher topography may force the prevailing surface wind over the smaller valleys (E.P.A., 1974).

CHAPTER II

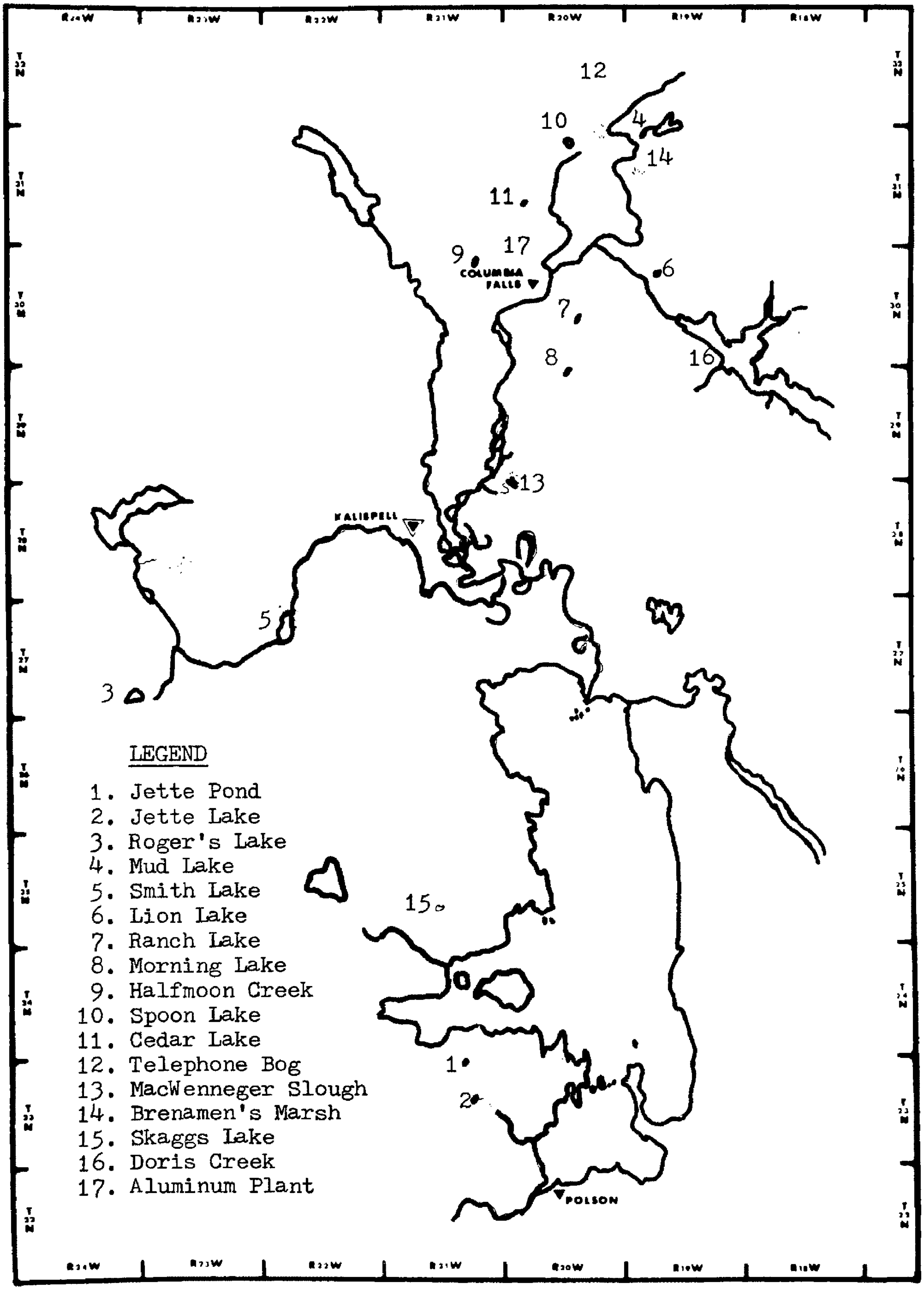
DESCRIPTION OF SITES OF COLLECTION

The herpetofauna of the northern Flathead Valley was sampled from sixteen sites. Although the major groups of amphibians and reptiles in the area were represented in the collection, no attempt was made to include all resident species. The selection of the sites was based upon (1) the location in relationship to the Anaconda Aluminum Plant at Columbia Falls, (2) records of previous collections which indicated the presence of the ranids and (3) the location in relationship to the valley and mountain topography of the area. Map 1 provides orientation to the northern Flathead Valley and to the location of the sites.

Jette Pond

Jette Pond is a small mountain pond which is located in Section 2 of Township 23 North, Range 21 West, Lake County. Until 1972, the pond was surrounded by a thick forest of ponderosa pine, lodgepole pine, a few fir and a variety of underbrush. Recently the area was selectively logged. A sloped area which was as large as the pond and which bordered the pond had been denuded of all vegetation. Soil erosion into the pond was noted. The pond was used frequently by a herd of 40-50 cattle which resulted in the brown color of the water.

A variety of plant and animal species resided in the vicinity. *Ricciocarpus* and *Potamogeton* were abundant in the pond. One clump of



Map 1. Orientation map showing Anaconda Aluminum Plant and the various sites of collection.

Typha occurred close to the western edge. Hyla regilla, Ambystoma macrodactylum, Bufo boreas and Rana pretiosa were observed in and around the pond throughout the period of collection. Two species of Thamnophis inhabited the area around the pond. Collections and observations were made at Jette Pond on June 28, July 4, 13, 17, 29 and August 4, 1974.

Jette Lake

Jette Lake is located to the southwest of Flathead Lake in Section 14 of Township 23 North, Range 21 West, Lake County. It is supplied primarily by springs and, to a lesser extent, by run off. The lake is drained via an intermittent stream into Flathead Lake. The lake and the surrounding acreage are privately owned. At the time of the study the perimeter of the 40 acre lake was undergoing subdivision. Selective logging was also in progress in the area which is drained by the lake. Cattle grazed in the vicinity and drank from the lake.

Ponderosa pine, scattered clumps of alder and willow, as well as cottonwood grew near the margin of the lake. One small and two larger grassy areas also bordered the lake. Both Polygonum and Potamogeton were abundant in the lake. Two species of Thamnophis inhabited the wooded riparian areas around the lake. Numerous Chrysemys picta belli inhabited the lake. The turtles were observed to be most active in the early evening during which time they fed along the shore. At least two species of Salmo were present in the lake. Rana pipiens inhabited the marginal areas around the lake. The frog was most abundant in the areas which were more open. Bufo boreas inhabited the area which surrounded the lake, including both the riparian and terrestrial habitats. The herpetofauna of Jette Lake was observed and sampled on June 27, 28, July 3, 12, 17 and August 4, 1974.

Roger's Lake

Roger's Lake is located in Section 30 of Township 27 North and Range 27 West, Flathead County. Most of the perimeter of the lake is privately owned and access is limited. Several springs emerge along the edge of the marsh. The lake was rehabilitated (poisoned and restocked) in 1967 by the Montana Fish & Game.

During the period of study, the Scirpus, Chara and Sterinomena were abundant along the shore of the western end. Large balls of Nostoc were common. A long section of grass separated the margin of the water from the second growth ponderosa pine and poplar forest. Rana pipiens, Rana pretiosa and Bufo boreas inhabited the margin of the lake but were not abundant. Chrysemys picta belli and two species of Thamnophis occurred in and around the lake. The herpetofauna of Roger's Lake was observed and sampled on June 29, 30, July 2, 3, 14, 15, 17, 30, 31 and August 3, 1974.

Mud Lake

Mud Lake is located in Section 9 of Township 31 North, Range 19 West, Flathead County. The lake is a small drop-shaped body of water connected to the outflow of Lake Five. Lake Five receives a great recreational use. However, Mud Lake receives only occasional recreational use. The lake was rehabilitated in 1968 by the Montana Fish & Game.

At the time of study the lake margin was overgrown with alders and willows. Just inside the margin of the lake was a large mat of Scirpus which varied in width between a few feet and several yards. Scattered clumps of Nuphar occurred in the shallow areas near the Scirpus mat. Typha also occurred around the margin of Mud Lake. Polygonum and Potamogeton occurred in restricted localities in the lake. Rana pretiosa, Rana pipiens and Bufo boreas inhabited the shores of Mud Lake and Lake Five. R. pretiosa was more abundant in Mud Lake than Rana pipiens. Collections and observations were made on June 30, July 15, 16, and August 2, 1974.

Smith Lake

Smith Lake is located in Sections 4 and 9 of Township 27 North, Range 22 West, Flathead County. Smith Lake is a wide, shallow lake on Ashley Creek. The water level fluctuates seasonally.

During the period of study, tall grasses lined most of the margin of the lake. Cattle grazed some of the grass areas; other areas were cut for hay and still other areas were neither cut nor grazed. Myriophyllum outlined the cooler water of the channel. Typha, Scirpus, Alisma, Nuphar, Carex, Potamogeton and Glyceria inhabited parts of the lake and the marginal area. Rana pipiens were abundant and all age groups were represented. Bufo boreas was also present. Chrysemys picta belli occurred in the lake. Two species of Thamnophis inhabited the grassy margin of the lake. The herpetofauna of Smith Lake was observed and sampled on June 29, July 4, 13, 14, 31 and August 3, 1974.

Lion Lake

Lion Lake is located in Sections 9 and 16 of Township 30 North, Range 19 West, Flathead County.

On the day of observation Lion Lake was cold and contained few plants. The forest ended abruptly at the edge of the water which occasionally shaded the shore. Marginal vegetation included willows, alders, and pine trees. *Rana pretiosa* and *Bufo boreas* occurred around the lake. Two species of *Thamnophis* also inhabited the area. The herpetofauna of Lion Lake was observed and sampled on July 30, 1974.

Ranch Slough

Ranch Slough is located in Section 21 of Township 31 North, Range 19 West, Flathead County. The slough is surrounded by private land which is used primarily as pasture for cattle.

At the time of study the range grass of the pasture was inundated around the margin of the slough. No shrubs, brush, or trees grew near the water. *Rana pipiens* was not abundant but was established. However, only one age group is evident along the shore. Newly metamorphosed *Bufo boreas* inhabited the margin of the lake. The darker areas of ground between clumps of grass were crowded with toadlets. Two species of *Thamnophis* were present around the slough. Observation and sampling of the herpetofauna of the Ranch Slough were conducted on August 1, 1974.

Morning Slough

Morning Slough is located in Section 3 of Township 29 North, Range 20 North, Flathead County. Morning Slough is surrounded by private land and access is restricted.

When observations were made, the marsh area had no definite border. The grass and surrounding areas were inundated. The gradation between the grass and the truly aquatic plants was subtle. The center of the marsh consisted of dense Typha mats and intervening waterways. Scirpus grew among Potamogeton and Polygonum. Areas of each type were indistinct. However, some areas of broad leaf Potamogeton did exist. A few willows stood around the edge of the marsh. The surrounding meadows were grazed by cattle. Several Rana pipiens were found among the aquatic vegetation at the edge of the dense Typha area. The frogs were sitting on the broadleaf Potamogeton which floated in approximately $1\frac{1}{2}$ feet of water. Observations and sampling were made at Morning Slough on August 1, 1974.

Halfmoon Creek

Halfmoon Creek flows through Section 2 of Township 30 North, Range 21 West, Flathead County.

The site of collection on Halfmoon Creek was 3/4 mile north of the community of Halfmoon. The creek meandered through intermittently dense forest of fir and pine. The covered areas were cool and dark. Other areas were sunlit and warm wherever the sun penetrated the canopy. The area from which the collection was made along the creek was overgrown by fir and pine trees. A few alders also grew in the area. Rana pretiosa were collected from the bank of the creek. Sampling and observations were made at Halfmoon on August 1, 1974.

Spoon Lake

Spoon Lake is located in Section 3 of Township 31 North, Range 20 West, Flathead County.

At the time of study, Spoon Lake was not heavily vegetated. The stones of the bottom near the shore were covered with brown, green and tan colored algae. Numerous logs blocked the outflow and fallen logs lined the edge of the lake. The west end of the lake was narrow. Large *Scirpus* mats lined the sides. *Rana pretiosa* was abundant. *Bufo boreas* and *Thamnophis spp*. occurred along the forested banks and on the *Scirpus* mats. Collections and observations of the herpetofauna were made on June 21 and July 1, 1974.

MacWenneger Slough

MacWenneger Slough is located in Section 31 of Township 29 North, Range 20 West and Section 1 of Township 28 North, Range 20 West, Flathead County. MacWenneger Slough is a large marsh ox-bow in which a variety of vegetation abounds.

At the time of study, *Typha*, *Nuphar*, *Nymphia*, *Nytella*, *Elodea*, *Ceratophyllum* and *Scirpus* were common throughout the marsh. The water of the marsh was relatively warm and slightly acid. *Rana pipiens* was abundant and *Chrysemys picta belli* was present. Although no snakes were collected, *Thamnophis spp*. was probably present. Collection and observation of the herpetofauna of MacWenneger Slough were made on August 1, 1974.

Telephone Bog

The area which was designated as Telephone Bog is located in Section 2 of Township 31 North, Range 20 West, Flathead County. The site was called 'Telephone Bog' because the Forest Service Map indicates a telephone near the junction of the North Fork Road and Blankenship Road. The 'bog' is the result of a cold spring seepage along the Forest Service Road.

On the day of collection, a variety of algae grew in the water along with Skunk Cabbage and terrestrial plants which had been inundated. Cedar and ponderosa pine grew in the vicinity. A few *Rana pretiosa* inhabited the margin of the pool. Observations and collections were made at Telephone Bog on June 30, and August 1, 1974.

Cedar Lake

Cedar Lake is located in Sections 20 and 29 of Township 31 North, Range 20 West, Flathead County. The lake is approximately 2 acres in size.

During the period of observation, the marginal area was bog-like. Both *Polygonum* and *Potamogeton* were present in the lake. The forest around the lake contained a few cedar trees, but consisted mostly of a mixture of species of pine and fir. Both *R. pipiens* and *R. pretiosa* were present in numbers which favored the spotted frog. No toads or snakes were seen, however, they probably inhabit the area. Observation and collection were made on July 17, 1974.

Doris Creek

Doris Creek is located in Section 1 of Township 29 North, Range 19 West, Flathead County. The origin of the creek is in the Swan Mountain Range and it drains into Hungry Horse Reservoir. The creek is permanent, rocky, cold and fast flowing.

The collection of *R. pretiosa* was made in the shallows of the creek. The vegetation in the vicinity included ponderosa pine, alder and terrestrial grasses. Because the shallow water area was the result of run off, no aquatic vegetation was observed. The terrestrial grasses were inundated. Collection and observations were made on July 1, 1974.

Brenamen's Marsh

Brenamen's Marsh is located in Section 21 of Township 31 West, Range 19 North, Flathead County. The marsh is situated in a depression near Highway U.S. 2.

At the time of study the margin of the marsh was Scirpus mat which was occasionally interrupted with clumps of alder. No frogs were noted among the vegetation. One Thamnophis and one Chrysemys picta belli were collected on July 30, 1974.

Skaggs Lake

Skaggs Lake is located in Sections 22 and 27 of Township 31 West, Range 25 North of Lake County. The lake is situated near the top of the ridge of mountains north of the town of Proctor.

On the day of observation, the area which surrounded the lake was thickly forested. A narrow strip of rocky shore separated the woods from the water. The beach area was strewn with logs and branches. Aquatic vegetation was sparse, primarily algae. Several size classes of Rana pipiens were present. Chrysemys picta belli were abundant. Collection and observations were made on July 3, 1974.

CHAPTER III

METHODS

General methods

Specimens of *Rana pretiosa*, *Rana pipiens* and *Bufo boreas*, together with two species of *Thamnophis* and *Chrysemys picta belli* were collected from sixteen localities which were located throughout the northern part of the Flathead Valley. The name and locus of each site as determined from a U.S. Dept. of Agriculture, Forest Service map, Flathead National Forest (north half) are presented in Columns A & B of Table 1. Table 1 also presents the species which were present (Column C), number of each species which were retained (Column D), and dates of collections (Column E).

General activity of the anurans was observed from a short distance with the aid of binoculars. The general habits of feeding, movement, basking, and predator avoidance were noted for each species when observed. Observations were made concerning the habitats which were frequented as well as those which were avoided. Observations were made over several 24 hour periods to establish specific patterns. Time of activity and the temperature of ambient air and water and substrate were recorded for the periods of activity and nonactivity. With the exception of small specimens, cloacal temperatures were recorded for all specimens which were captured. Each specimen was assigned a number and was measured. Total length, snout-urostyle length and width of the head (measured just

TABLE 1

LIST OF SITES, LOCI, RESIDENT SPECIES, NUMBER OF RETAINED SPECIMENS, AND DATES OF COLLECTION

A	B	C	D	E
1. Jette Pond	T23N, R21W, S2	R. pretiosa Bufo boreas	4 1	6-26; 7-4, 13, 17, 29, 8-4
2. Jette Lake	T23N, R21W, S14	R. pipiens Bufo boreas	7 3	6-27, 28; 7-3 12, 17; 8-4
3. Roger's Lake	T27N, R27W, S30	R. pretiosa R. pipiens Bufo boreas	6 7 1	6-29, 30; 7-2, 3, 14, 15, 17, 30; 8-3
4. Mud Lake	T31N, R19W, S9	R. pretiosa R. pipiens Bufo boreas	6 3 1	6-30; 7-15, 16; 8-2
5. Smith Lake	T27N, R22W, S4&9	R. pipiens Bufo boreas	20 1	6-29; 7-4, 13, 14, 31; 8-3
6. Lion Lake	T30N, R19W, S9&16	R. pretiosa Bufo boreas	2 1	7-30
7. Ranch Slough	T31N, R19W, S21	R. pipiens	8	8-1
8. Morning Slough	T29N, R20W, S3	R. pipiens	3	8-1
9. Halfmoon Creek	T30N, R21W, S2	R. pretiosa	4	8-1
10. Spoon Lake	T31N, R20W, S3	R. pretiosa Bufo boreas	7 2	6-21; 7-1
11. Cedar Lake	T31N, R20W, S20&29	R. pipiens R. pretiosa	2 3	7-17
12. Telephone Bog	T31N, R20W, S2	R. pretiosa	2	8-1
13. MacWenneger Slough	T29N, R20W, S31	R. pipiens	6	8-1
14. Brenamen's Marsh	T31N, R19W, S21	(no Anura)		7-30
15. Skaggs Lake	T31N, R25W, S22&27	R. pipiens	4	7-3
16. Doris Creek	T29N, R19W, S1	R. pretiosa	2	&-1

behind the eyes) were also noted for all specimens.

All specimens which were captured but not retained were marked by clipping toes of the hind feet. Care was exercised to insure the survival of the specimen. (Only the terminal phalanx was removed). Each specimen was clipped in a unique pattern to facilitate identification of the individual upon recapture.

Food analyses

A check was made of each specimen, by squeezing the gut, to determine whether the stomach was full. Only those specimens which contained material in their stomachs were sacrificed. Each retained specimen was injected with 70% ETOH, was tagged with an individual number and was stored in 70% ETOH.

The stomach was removed from each specimen which had been retained. Each stomach was stored separately until the contents were analyzed. The number of members of each taxon which were contained in the stomach was recorded.

Fluoride analysis

In the laboratory, the entire left hind leg was removed from each specimen. More bone was removed from smaller specimens to ensure sufficient weight of dry defatted bone for analysis.

The leg bones of each specimen were boiled, separately, in Alconox until clean. Any clinging flesh was removed by brushing the bones with a toothbrush. The bones were reboiled in Alconox and then dried in a forced air oven for at least 12 hours. When dry, the bones were broken and boiled in at least six changes of petroleum ether to remove fat.

The bones were then redried in the forced air oven.

The clean, defatted, dry bones were weighed into nickel crucibles, and ashed at 600°C for 12 hours. When cool, the ash was dissolved with 2ml of 20% hydrochloric acid ($HClO_4$) and the solution was diluted to a volume of 100ml with 50% T.I.S.A.B. in distilled water.

An orion specific ion electrode was used to determine the fluoride activity potentio-metrically of each sample.

One member of each of twenty pairs of femur bones was examined with the aid of a variable power dissecting microscope for evidence of fluorosis, pitting, nodules or other aberrations which might indicate acute poisoning by fluoride.

Thirty-three anuran stomach samples were selected and grouped for analysis of fluoride content. Selection and grouping were based upon three criteria: (1) animals which were collected at the same site were selected, (2) animals which were collected on the same date were grouped, and (3) at least one specimen of a different species was included in each group (with the exception of one group). The contents of each stomach were dried separately in a forced air oven until dry. The samples were broken into small pieces and thoroughly mixed, then were weighed into nickel crucibles and 0.05 grams of flux (CaO) were added. A homogenous mixture was prepared by stirring with a glass rod. The samples were moistened with distilled water and charred for thirty minutes. The samples were then ashed at 600°C for 12 hours. When cool, the ash was dissolved in 20% $HClO_4$ and the solution was diluted to 100ml with 50% T.I.S.A.B. in distilled water.

As for the bones, the fluoride activity in millivolts was determined with an orion specific ion electrode.

The specific ion electrode was calibrated before the analyses with solutions of standard fluoride concentration. The calibration was checked twice during and once following the analyses of samples. The millivolt value of the standard concentration and the weight of each sample were typed into a preprogramed computer which determined the fluoride activity in parts per million (ppm) on a dry weight basis.

Reliability of method

Carlson and Hammer (1974) reported on the comparability of the specific ion electrode and the semi-automated colormetric methods of analysis for fluoride. They concluded that the specific ion electrode method provides results which are "accurate and comparable to those obtained by the semi-automated method or other specific ion methods".

It should be noted that some variation is to be expected between the results of separate analysis of aloquats of the same sample. The variation reflects the nonhomologus distribution of fluoride which exists throughout the sample, both before and after preparation. The variation also reflects differences in temperature and electrode sensitivity.

CHAPTER IV

RESULTS

Rana pipiens

Description of Rana pipiens

Rana pipiens Shreber (1782) is a medium sized frog. The dorsal ground color may be green, grey or brown. Two irregular rows of rounded dark spots are situated between raised lateral folds which may be bronze, yellow or whitish in color. Two irregular rows of spots mark each side. The spots on the legs appear to be bands when the legs are folded in a sitting position. One or more spots are usually on the eyelids. All spots are usually outlined with a lighter color. The venter is colored whitish to yellowish. The skin is slippery and typically smooth, but may be slightly tubercular. A prominent light line extends from the nostril to the arm. The line is bordered by a dark strip. The body is slender. The legs are long. The webs of the hind feet are deeply indented. Prominent tubercles are located beneath the joints of the toes, the inner sole tubercle is small. There is no outer tubercle (Wright and Wright, 1949; Stebbins, 1966).

Associated temperatures of Rana pipiens

The range of the temperature of the air in which *Rana pipiens* was captured was between 86° and 58°F. The range of the temperature of the water was between 85° and 56°F. The range of the temperature of the body was between 84°F and 60°F. Rana pipiens maintained a body temperature which approximated the ambient air and water temperatures (Figure 1). Within this temperature regime, the frogs were actively seeking food or sitting quietly among the vegetation. The difficulty of capture in addition to the lack of frogs at other air and water temperatures suggests that these data approach the limits of temperature which are required for optimum activity. Leopard frogs may occasionally be found in cooler water, and in warmer water.

Food of Rana pipiens

The contents of the stomachs of fifty-two leopard frogs (Figure 2) consisted of 35.3% $\pm$ Coleoptera, which were primarily Tenebrionidae and Carabidae. Odonata constituted 13.8% $\pm$ of the diet, most of which were Coenagrionidae. Arachnida made up 11.6% $\pm$ and Diptera contributed 11.3% $\pm$ to the total sample. The remainder of the contents (27% $\pm$) were members of eleven other groups of organisms, including 1.1% $\pm$ of toadlets. The largest contribution by a minor group (Hymenoptera) was 9.7% $\pm$ (Table 2).

The presence of such a variety of organisms indicates that leopard frogs are opportunistic feeders. The taxonomic composition of the samples suggests that leopard frogs feed in a variety of habitats, but primarily feed in association with the riparian sector. The occurrence in the samples of members of the Tenebrionidae and Carabidae, both of which are frond dwelling forms, and the occurrence of members of the Chrysomelidae and Curculionidae, both of which feed upon foliage, indicates the leopard frogs feed from the ground as well as from the foliage. The presence of

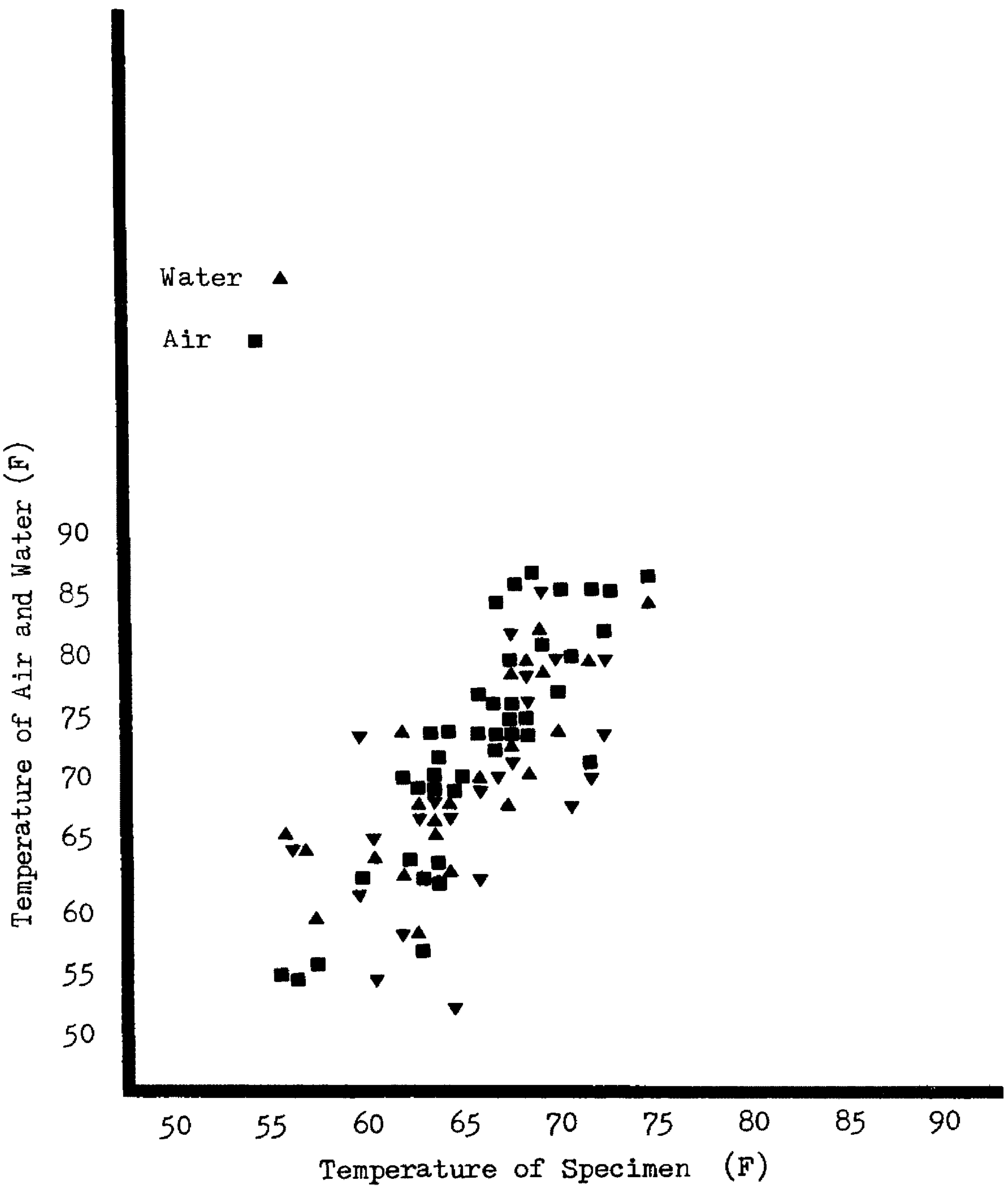


Figure 1. Relationship between the body temperature of Rana pipiens and the temperature of the air and water.

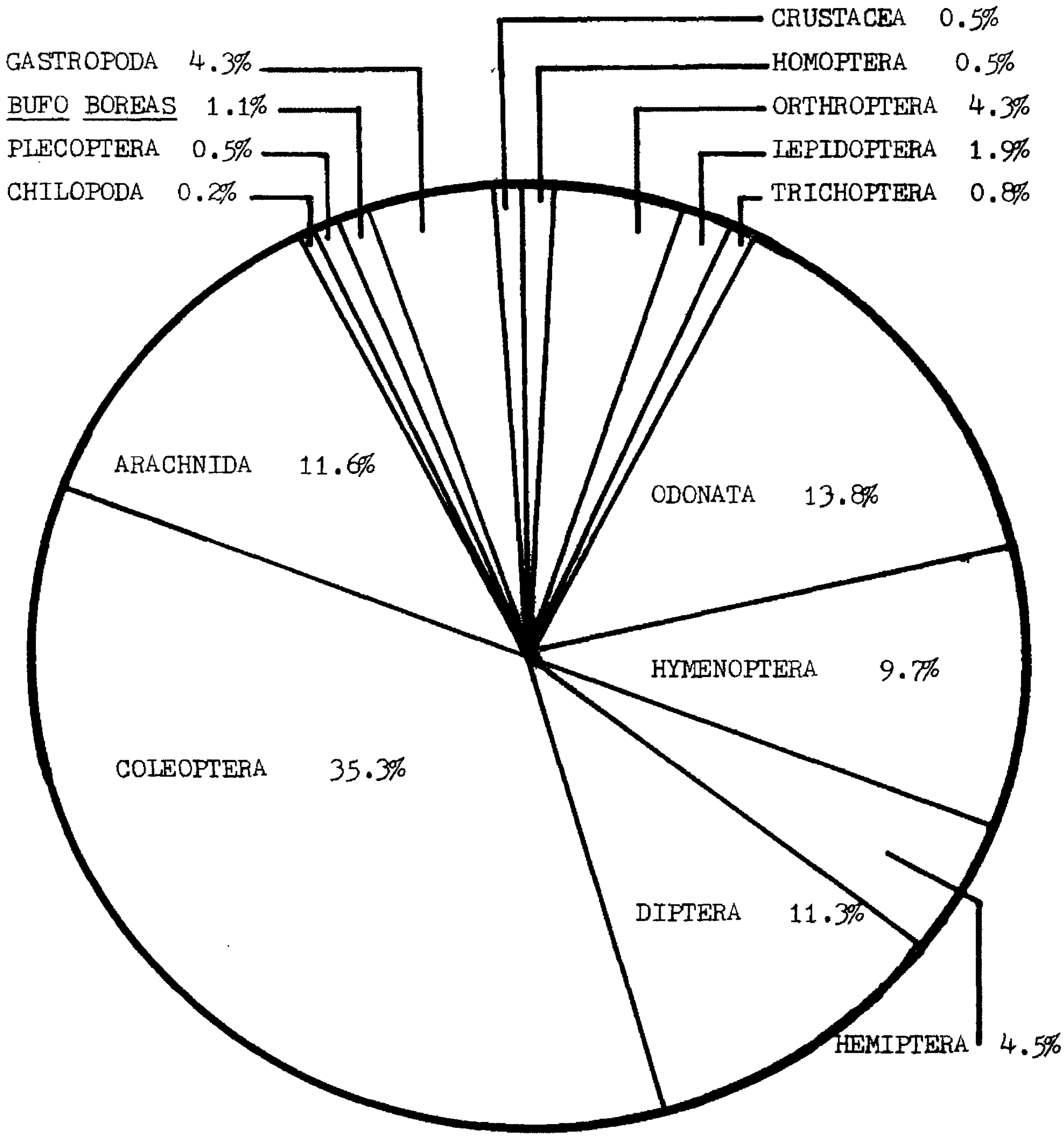


Figure 2. Food items which were consumed by Rana pipiens.

TABLE 2

SUMMARY OF FOOD ITEMS WHICH WERE EATEN BY RANA PIPIENS FROM SELECTED SITES IN NORTHWESTERN MONTANA

FOOD ITEM			NUMBER
INSECTA			
	Coleoptera		5
		Cantharidae	3
		Carabidae	39
		Chrysomelidae	31
		Cicendlidae	1
		Coccinellidae	2
		Curculionidae	6
		Dytiscidae	1
		Elateridae	2
		Scarabaeidae	7
		Silphidae	7
		Staphylinidae	11
		Tenebrionidae	16
	Diptera		2
		Anthomyiidae	2
		Chironomidae	18
		Dolichopidae	6
		Muscidae	3
		Simulidae	2
		Syrphidae	1
		Tipulidae	8
	Hemiptera		
		Gerridae	7
		Miridae	2
		Pentatomidae	1
		Saldidae	5
	Homoptera		
		Cercopidae	1
		Membracidae	1
	Hymenoptera		
		Apidae	2
		Braeonidae	1
		Formicidae	23
		Ichneumonidae	1
		Megachilidae	1
		Sphecidae	5
		Vespidae	3
	Lepidoptera		
		Larvae	7
	Odonata		
		Aeschnidae	4
		Coenagrionidae	40
		Libellulidae	7

FOOD ITEM	NUMBER
Orthroptera	
Acrididae	11
Tetrigidae	5
Plecoptera	2
Trichoptera	3
AMPHIBIA	
Salientia	
Bufonidae	4
GASTROPODA	16
ARTHROPODA	
Arachnida	
Acarina	1
Araneae	42
Opiliones	2
CHILOPODA	1
DIPLOPODA	
Julida	2
	373 Total

adult Odonata and other flying insects, denotes that the frogs capture these forms either as the insects rest on vegetation or as they fly. Both patterns occur, but repeated observations indicate that the insects are more often at rest on some support rather then flying when captured by the frogs. Because leopard frogs have relatively large mouths and tend to feed rather aggressively, the size of the prey seldom limits its capture. Large odonates such as Aeschnidae and Libellulidae were consumed by the frogs. The difference between the numbers of each family of Odonata reflects the habits of the dragonflies rather than the frog and the frequency with which the dragonflies rest near the frogs. The occurrence of aquatic snails in the stomach samples illustrates the habit of the frogs to feed near the water. The relatively small number of insects which were completely aquatic, such as Dytiscidae, compared to those forms which live on the surface of the water or on the shore further supports the habits of the frogs to feed in the riparian area. The single most unique item of food was newly metamorphosed toadlets. The occurrence of toadlets illustrates that leopard frogs willingly feed upon any organism which is of appropriate size and movement.

Fluoride in the food of Rana pipiens

The contents of the stomachs of twelve leopard frogs which had been captured in 1974, plus the contents of stomachs of frogs which had been captured in 1958 and a composite sample of the contents of six frogs which were captured prior to 1952 were analyzed for fluoride (Table 3). The fluoride content of the three stomach samples from frogs which had been captured at Roger's Lake during July, 1974, compares favorably to the amount of fluoride which was contained in stomachs of two frogs which had

TABLE 3

FLUORIDE CONTENT OF THE FOOD OF *RANA PIPIENS* FROM SELECTED SITES IN NORTHWESTERN MONTANA

LOCALE	PPM F^-	AVERAGE	DATE
Jette Lake	18.3		6-19
	43.9	29.1	6-28
	25.1		6-28
Roger's Lake	10.9		7-14
	12.1	23.3	7-31
	47.1		7-31
Cedar Lake	46.6		8-16
	64.9	50.9	8-16
Smith Lake	41.6		7-31
	28.8	35.2	7-31
Roger's Lake	18.7		7-28-58
	47.7	32.2	7-28-58
Control (composite of 6)		43.3	pre-1952

been captured at Roger's Lake during July, 1958. These data indicate that the exposure to fluoride via food has been relatively constant at Roger's Lake. Of the recent collection, the stomachs of the specimens from Cedar Lake averaged more fluoride than samples from the other sites. The highest concentration of fluoride was contained in one of the samples from Cedar Lake, 64.9 ppm F^-. Although the size of the sample was small, these data suggest that exposure to fluoride via food is greater closer to the aluminum plant.

Fluoride in the bone of *R. pipiens*

The left femurs from 60 *Rana pipiens* from nine different locations were analyzed for fluoride. Five control specimens which had been collected prior to 1952 were also analyzed (Figure 3). The accumulation of fluoride by leopard frogs must be considered in terms of the size of the animals. Because differences in the amount of ambient fluoride exist between sites, only those animals which are of similar size (close in approximate age) may be compared (Table 4). For example, frogs from Ranch Slough and Morning Slough are approximately the same size. Yet, frogs from Ranch Slough contained less fluoride than those from Morning Slough. The difference is the result of exposure and ambient concentration. The difference in the concentration of fluoride between larger specimens from Smith Lake and Roger's Lake further illustrates the importance of size (age) of the frogs in the determination of a polluted area. The largest frog from Roger's Lake is more than two centimeters larger than the largest individual from Smith Lake. This difference represents 2 or more years of growth and the concentration of fluoride by

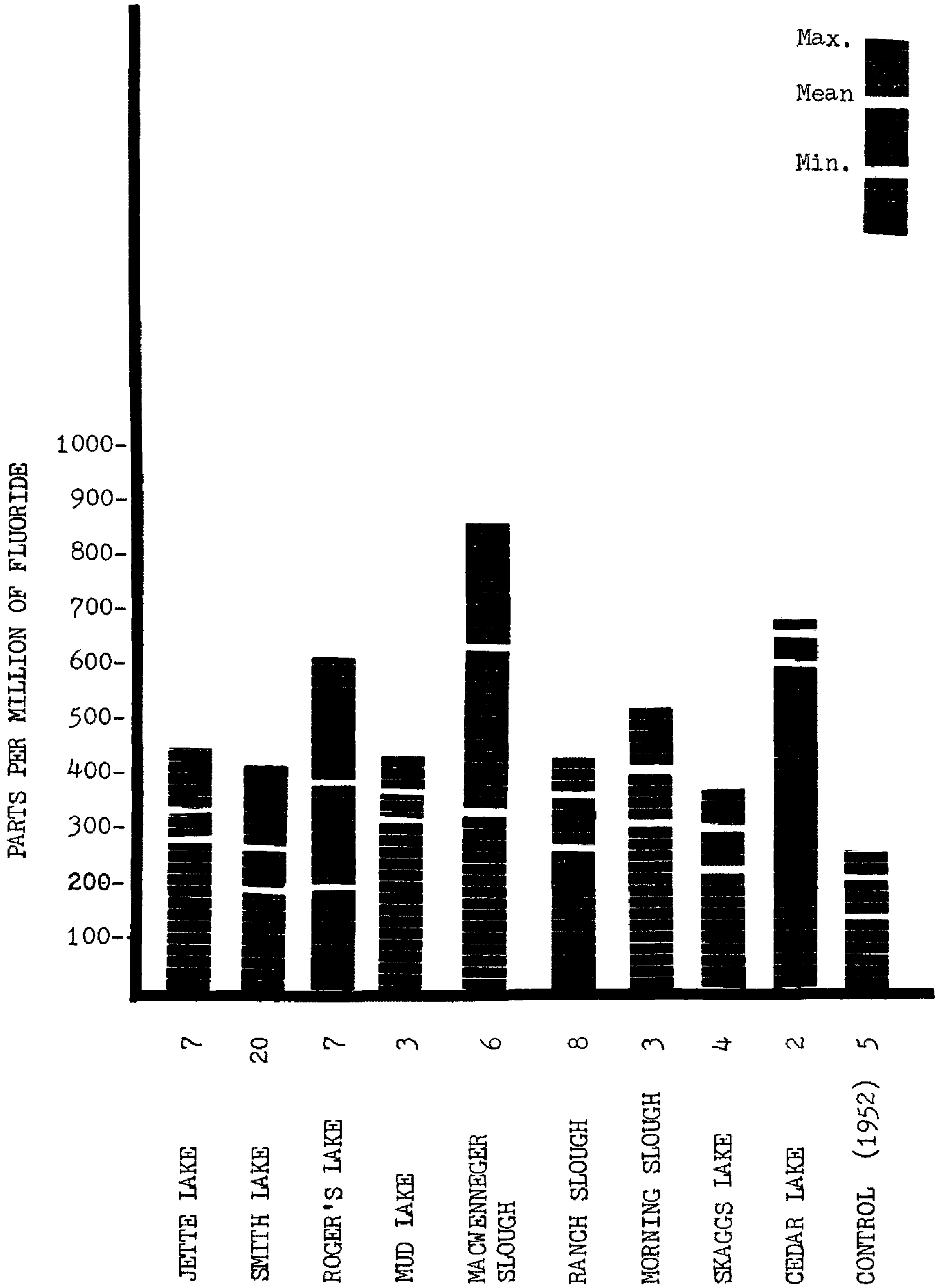


Figure 3. Fluoride content in the bone of Rana pipiens.

TABLE 4

FLUORIDE CONTENT IN THE BONE OF *RANA PIPIENS* FROM SELECTED SITES IN NORTHWESTERN MONTANA

LOCALE	N	TOTAL LENGTH (cm)			PPM F^-		
		max.	mean	min.	max.	mean	min.
Jette Lake	7	19.8	17.0	13.6	388.0	295.7	233.4
Smith Lake	20	19.2	13.8	11.2	345.0	213.1	145.4
Roger's Lake	7	21.6	13.3	7.4	537.7	303.1	154.3
Mud Lake	3	16.2	11.2	7.7	359.3	309.7	264.9
MacWenneger Slough	6	19.5	13.3	9.6	791.4	631.4	278.4
Ranch Slough	8	11.1	10.6	10.3	370.6	310.4	215.0
Morning Slough	3	11.5	10.9	10.6	452.3	347.6	251.0
Skaggs Lake	4	17.7	17.2	16.6	324.0	292.1	243.6
Cedar Lake	2	18.0	17.7	17.4	584.7	547.6	510.7
Control (pre-1952)	5	19.5	16.8	15.5	225.7	184.5	102.4

the two frogs reflects the difference noted in the size (age) 537.7 ppm and 345.0 ppm F^- respectively. The comparison of size and contained fluoride of specimens from Cedar Lake and Skaggs Lake shows that although the difference in size is small, the difference in contained fluoride is great. This is primarily the result of the ambient concentration at the localities. Because of the similarity of the habitats, exposure (sheltering and activity) is of little importance. Cedar Lake is closer to the aluminum plant and the frogs which were captured near the lake contained more fluoride than those from the area of Skaggs Lake.

The accumulation of fluoride by different size (age) groups of *R. pipiens* is illustrated by specimens from MacWenneger Slough (Table 5). Clearly, smaller (younger) frogs contain less fluoride than larger (older) frogs from the same site.

TABLE - 5

FLUORIDE CONTENT IN DIFFERENT AGE GROUPS OF *RANA PIPIENS*

LOCALE	N	TOTAL LENGTH (CM)			PPM F^-		
		max.	mean	min.	max.	mean	min.
MacWenneger Slough	4	11.9	10.5	9.6	480.2	429.2	278.4
MacWenneger Slough	2	19.5	18.5	17.5	791.4	732.5	673.7

Rana pretiosa

Description of *Rana pretiosa*

Rana pretiosa Baird & Girard (1853) is a medium sized frog. The dorsal ground color may be yellowish, greenish or reddish. Zero to many irregular roundish black spots may occur between the lateral folds. The spots are light centered. The lateral folds may be lighter than, or the same as, the ground color. The lower sides of the body are light yellowish, cream or grayish. The venter is lighter yellowish cream than the sides. The throat and chest areas are marbled with gray. Dorsal surface of the legs may be barred or spotted when folded. The venter surfaces of the legs are salmon red in adults. The color may form a thick 'U' on the belly. The completeness of the shape varies. No salmon red is present in newly metamorphosed individuals. The light glandular stripe which extends from under the eye to the arm is interrupted at the end of the jaw. The dorsum is typically rough. The lateral folds are raised. The tympanum is smaller than the eye. The eyes are small and are set obliquely, slightly elevated. The webs of the hind feet extend to the tips of all toes except the 4th, the terminal phalanx of which is free (Wright and Wright, 1949; Stebbins, 1966).

Associated temperatures of *Rana pretiosa*

The range of temperature of the air in which *Rana pretiosa* was captured was between 85°F and 59°F. The range of temperatures of the water was between 80°F and 41°F. The range of the temperature of the body was between 83°F and 54°F. *Rana pretiosa* consistently maintained its temperature slightly below that of the ambient air and slightly above that of the ambient water (Figure 4). The spotted frog is able to inhabit a rather cool riparian environment. This is reflected in the temperature regime of the habitat which is utilized by the frog.

Food of *Rana pretiosa*

The contents of the stomachs of fifty spotted frogs (Figure 5) consisted of 34.8%± Coleoptera, slightly less than one-half of which were

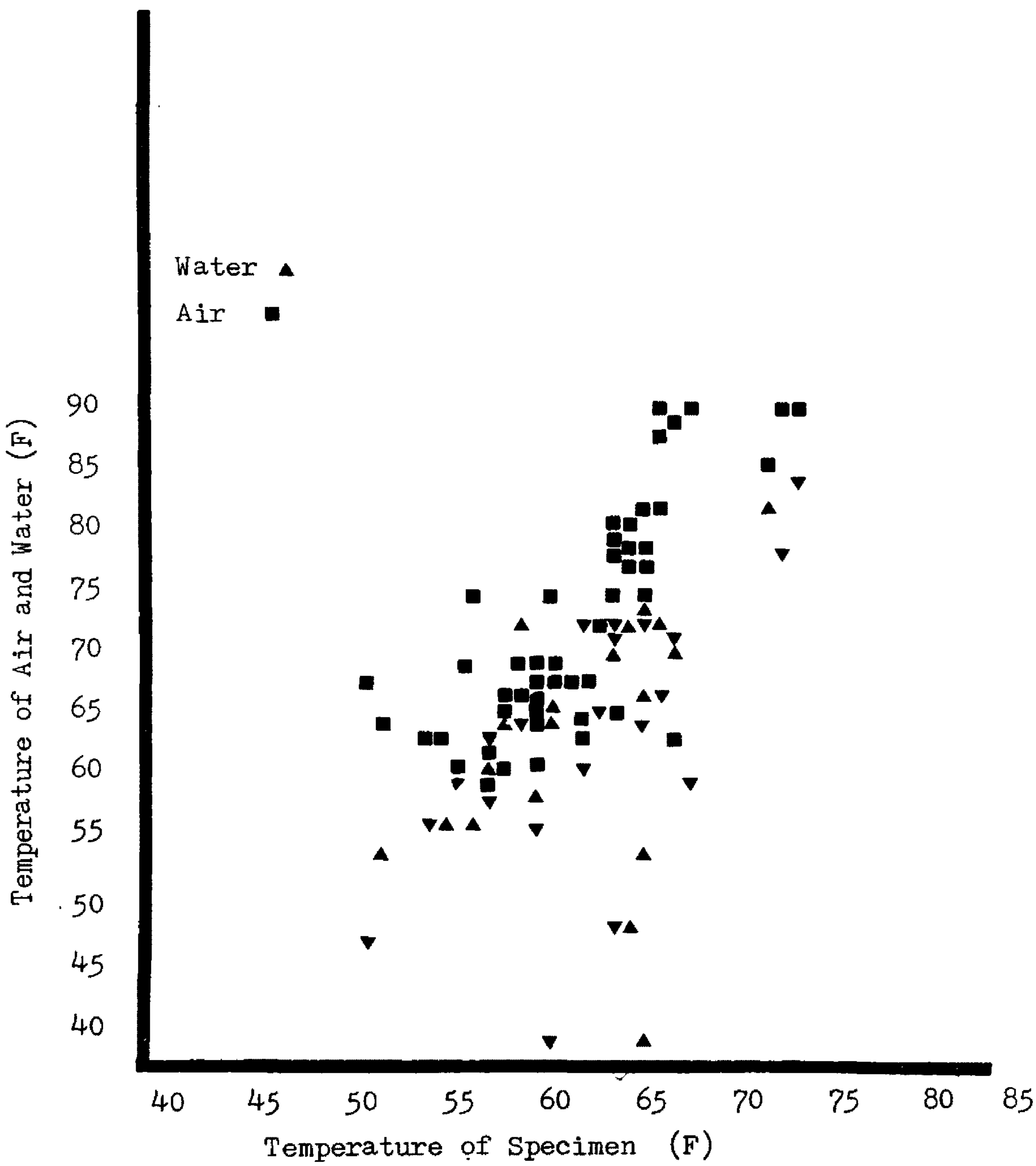


Figure 4. Relationship between the body temperature of Rana pretiosa and the temperature of the air and water.

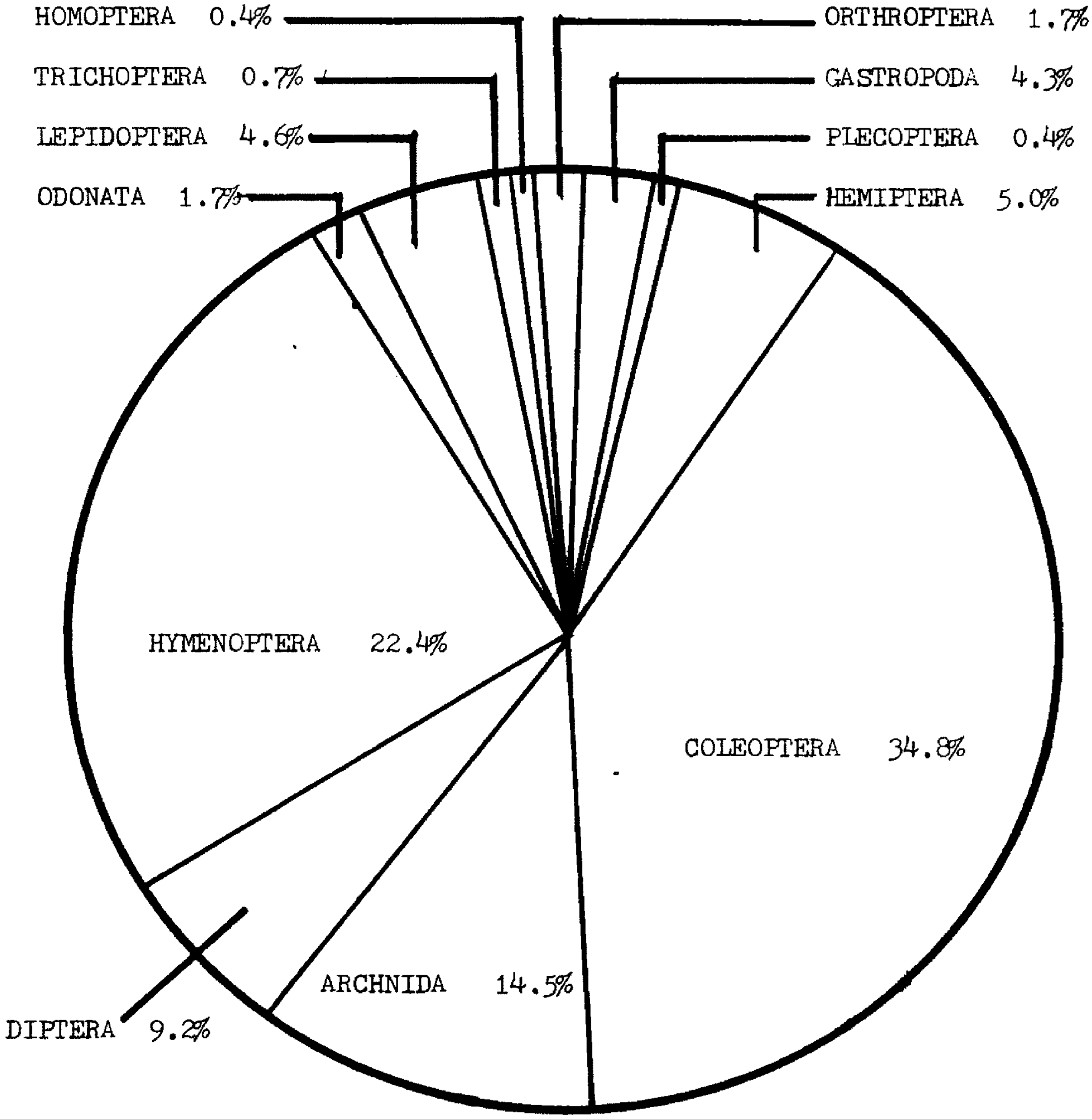


Figure 5. Food items which were consumed by Rana pretiosa.

Carabidae. Hymenoptera made up 22.4%± of the diet. The majority of Hymenoptera in the samples was Formicidae. The Arachnida contributed 14.5%±, mostly Araneae. Diptera constituted 9.2%± of the contents of the stomachs. Tipulidae and Calliphoridae made up the majority of the composite sample of Diptera. Eight other groups of food items made up 24%± of the sample. The largest contribution by a minor group (Hemiptera) was 5.0%± (Table 6).

The occurrence of such a large variety of food items in the stomachs of spotted frogs indicates that the frogs feed opportunisticly. The taxonomic composition of the samples suggests that spotted frogs feed primarily in the riparian habitat but may occasionally venture into meadows or woods. The presence of ground dwelling insects, foliage insects, and flying insects illustrates the versatility of the feeding pattern of _R. pretiosa_. The occurrence of numbers of the Gerridae in the samples denotes that the frogs obtain food from the water as well as the land. The numerous snails which were found in the sample, support the finding. Spotted frogs fed upon a variety of flying insects which were probably at rest when eaten. The lack of the larger Odonata (Aeschnidae and Libellulidae) reflects the habits of the dragonflies to rest on taller objects and the relatively nonagressive feeding behavior of the frog. The size of the dragonflies in relation to the size of the frog may exert limited influence on the nonselection by the frog. The representation in the samples of Hymenoptera again reflects the habits of the insects. Those Hymenoptera which frequent muddy areas were eaten more often than those which do not.

TABLE 6

SUMMARY OF FOOD ITEMS WHICH WERE EATEN BY *RANA PRETIOSA* FROM SELECTED SITES IN NORTHWESTERN MONTANA

FOOD ITEM	NUMBER
INSECTA	
Coleoptera	13
Cantharidae	7
Carabidae	76
Chrysomelidae	14
Cicendelidae	10
Coccinellidae	3
Curculionidae	18
Dytiscidae	3
Elateridae	4
Haliplidae	1
Histeridae	1
Meloidae	1
Scarabaeidae	2
Staphylinidae	4
Tenebrionidae	20
Diptera	4
Calliphoridae	11
Chironomidae	5
Muscidae	4
Simulidae	5
Syrohidae	2
Tipulidae	15
Hemiptera	
Gerridae	18
Miridae	3
Nabidae	1
Pentatomidae	1
Reduviidae	2
Saldidae	1
Homoptera	
Cercopidae	1
Cicadellidae	1
Hymenoptera	1
Apidae	4
Bombidae	1
Formicidae	100
Megachilidae	1
Sphecidae	2
Vespidae	7

TABLE 6 Continued

FOOD ITEM	NUMBER
Lepidoptera	
Adult	2
Larvae	22
Odonata	
Coenagrionidae	9
Orthroptera	
Acrididae	1
Gryllacridae	1
Tetrigidae	7
Plecoptera	2
Trichoptera	4
GASTROPODA	22
ARTHROPODA	
Arachnida	
Acarina	2
Araneae	68
Opiliones	4
	451 Total

Fluoride in the food of *R. pretiosa*

The contents of thirteen stomachs of *R. pretiosa* plus a composite sample from prior to 1952 were analyzed for fluoride (Table 7). The average quantity of fluoride from the several sites, with the exception of Cedar Lake, either approximated the pre-1952 level or was less than the pre-1952 level. Samples from Cedar Lake, which was the closest site to the aluminum plant, contained more fluoride than the other sites. Although the number and composition of the samples restrict the interpretation of these data, it is strongly suggested that those frogs which inhabited areas closer to the plant were exposed to greater concentrations of fluoride via their food than those which inhabited areas further away.

Fluoride in the bone of *R. pretiosa*

The left femurs from 42 *Rana pretiosa* from nine different localities were analyzed for fluoride. Six control specimens which had been collected prior to 1952 were also analyzed (Figure 6). The accumulation of fluoride in those specimens which are approximately the same size may be compared to indicate the extent of fluoride pollution in an area (Table 8). For example, specimens from Jette Pond and Telephone Bog were approximately of similar size yet the largest amount of fluoride from a frog from Jette Pond was less than the smallest amount of fluoride in a frog from Telephone Bog. The difference may be attributed to the drift of the airborne fluoride from the aluminum plant. The difference which is noted between specimens from Halfmoon Creek and Doris Creek results from the pattern of dispersal of the airborne fluoride as well as the sheltering effect of Columbia Mountain. Specimens from Lion Lake which was close to Doris Creek, but within the typical dispersal pattern

TABLE 7

FLUORIDE CONTENT OF STOMACH SAMPLES FROM *RANA PRETIOSA* FROM SELECTED SITES IN NORTHWESTERN MONTANA

LOCALE	PPM F^-	AVERAGE	DATE
Spoon Lake	34.6		6-21
	15.3	21.8	7-1
	15.5		7-1
Jette Pond	31.8		7-29
	52.6	34.2	7-29
	18.3		7-29
Mud Lake	23.9		6-30
	16.5	20.2	6-30
Roger's Lake	10.2		7-15
	11.9	11.0	7-31
Cedar Lake	41.0		7-16
	45.5	43.8	7-16
	45.0		7-16
Control (composite of 6)		33.1	Pre-1952

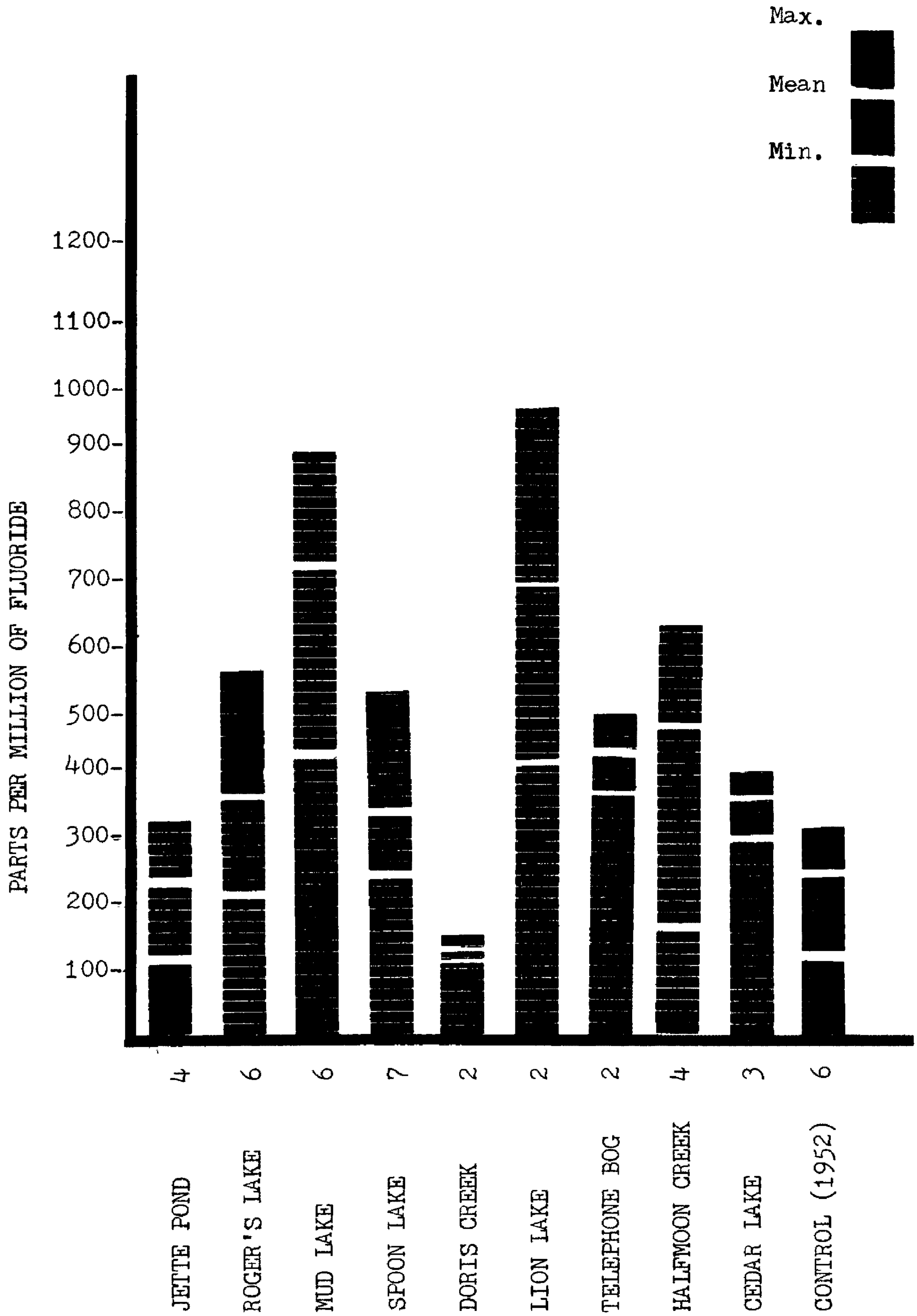


Figure 6. Fluoride content in the bone of <u>Rana pretiosa</u>.

TABLE 8

FLUORIDE CONTENT OF BONE SAMPLES FROM *RANA PRETIOSA* FROM SELECTED SITES IN NORTHWESTERN MONTANA

LOCALE	N	TOTAL LENGTH (cm)			PPM F^-		
		max.	mean	min.	max.	mean	min.
Jette Pond	4	20.7	14.2	9.2	332.7	229.1	103.3
Roger's Lake	6	17.1	10.1	7.2	580.6	365.9	210.4
Mud Lake	6	20.0	16.5	11.4	903.2	730.0	438.8
Spoon Lake	7	18.2	10.8	8.6	539.9	358.6	257.1
Doris Creek	2	19.4	18.2	17.1	148.7	140.4	132.1
Lion Lake	2				995.8	714.9	434.0
Telephone Bog	2	20.9	16.5	12.2	520.5	457.6	394.7
Halfmoon Creek	4	19.3	16.0	13.2	669.3	485.0	165.4
Cedar Lake	3	16.7	16.5	16.3	439.7	380.8	309.9
Control (pre-1952)	6	19.7	15.6	13.3	335.6	248.3	122.3

of the airborne fluoride, contain more fluoride than those from Doris Creek. Reference to the map and to Table 8 illustrates that the concentration of fluoride in the bone of spotted frogs is greater when the specimens were collected near the aluminum plant or from within the area which is covered by the emission from the plant. Other concentrations of fluoride, such as the 580^{+} ppm F^{-} in the large specimens from Roger's Lake may be attributed to the age of the individual.

The accumulation of fluoride by different size (age) groups of R. pretiosa is illustrated by specimens from Mud Lake (Table 9). Clearly, the smaller frogs contained less fluoride than large frogs. It should be further noted that several years of growth separate the two groups, yet the mean concentration of fluoride are separated by only 200 ppm F^{-}. It may be inferred from these data that spotted frogs accumulate about one-half of their total fluoride by the second or third year of life.

TABLE - 9

FLUORIDE CONTENT IN BONE FROM DIFFERENT AGE GROUPS OF RANA PRETIOSA FROM SELECTED SITES IN NORTHWESTERN MONTANA

LOCALE	N	TOTAL LENGTH (CM)			PPM F^{-}		
		max.	mean	min.	max.	mean	min.
Mud Lake	2	11.4	10.7	10.1	702.5	570.6	438.8
Mud Lake	3	19.9	19.2	18.7	841.7	785.6	639.0

Bufo boreas

Description of Bufo boreas

Bufo boreas Baird and Girard (1852) is a large toad with a flat and broad body. The ground color may be brown, gray or green. The color is pale vinaceous buff or pale smokey gray. The belly is occasionally mottled with pale black. The dorsal skin is granulous with numerous irregularly spaced warts. No cranial crests are present. The paratoid glands are oval. The tibia exhibits a rounded gland. A well developed tarsal fold is present (Wright and Wright, 1949; Stebbins, 1966).

The boreal toad frequents a variety of habitats including forests, meadows and the shore of ponds, streams and lakes.

Associated temperatures of Bufo boreas

The range of air temperatures in which *Bufo boreas* was captured was between 84°F and 55°F. The range of the temperature of the water was between 79°F and 60°F. The range of the temperature of the body was between 78°F and 58°F. *Bufo boreas* maintained a body temperature which reflected that of the water more than the ambient air (Figure 7). The tolerance of the boreal toad of lower temperatures than reported here has been observed at other localities in Western Montana (R.B. Brunson, personal communication). Toads were active during the day and evening early in the summer, however, by August when temperatures during the day were very high, the toads were active only during the late evening and night.

Food of Bufo boreas

The contents of the stomachs of seven boreal toads (Figure 8) consisted of 75%± Hymenoptera, the majority of which was Formicidae. Coleoptera comprised 23%± of the sample. The majority of Coleoptera which had been eaten was Tenebrionidae and Carabidae. Arachnida composed 3%± of the total number of food items. Four other groups of food items made up a total of 2%± of the samples. Table 10 presents a summary of the food

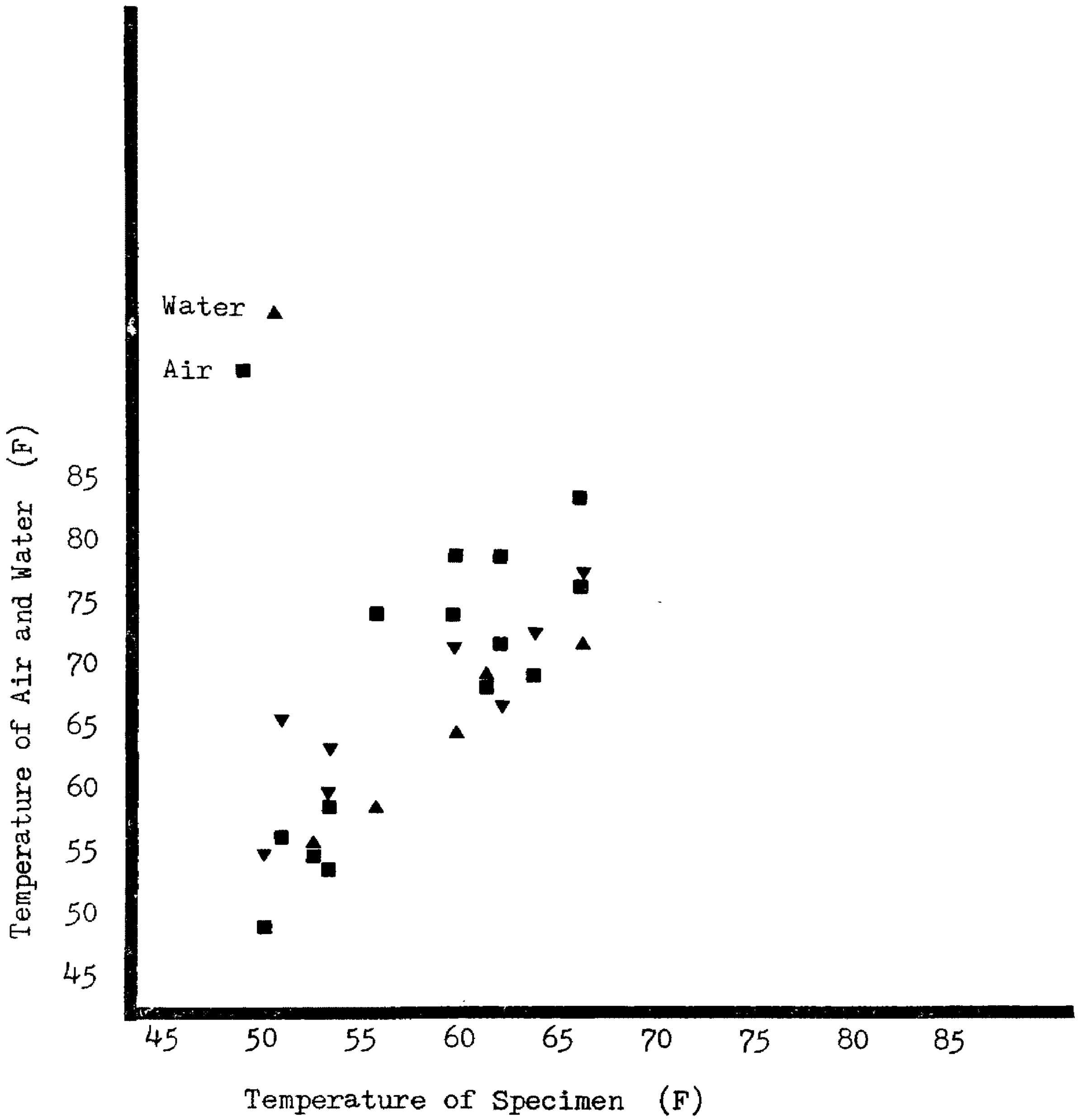


Figure 7. Relationship between *Bufo boreas* and the temperature of the Air and Water.

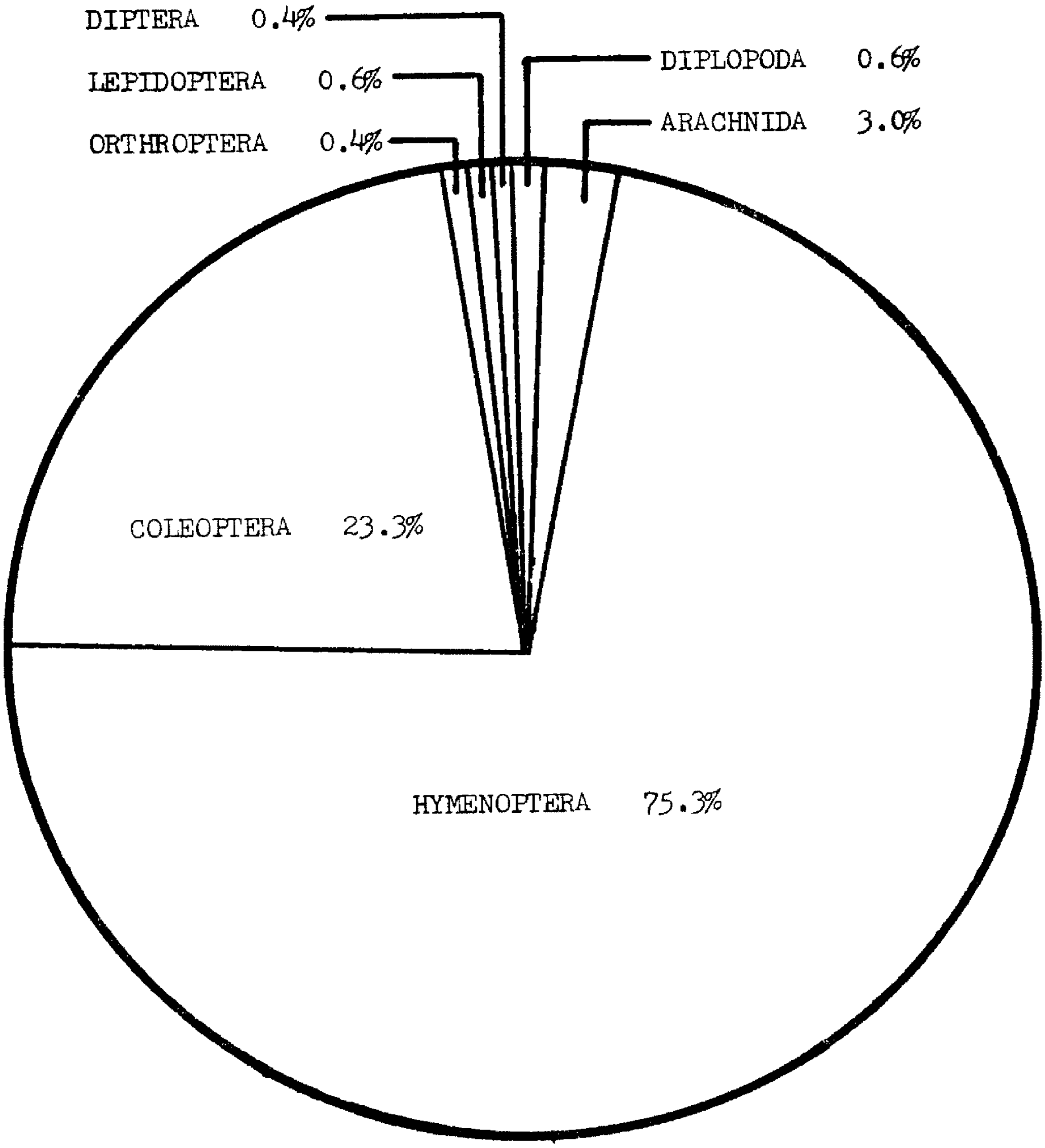


Figure 8. Food items which were consumed by *Bufo boreas*.

TABLE 10

SUMMARY OF FOOD ITEMS WHICH WERE EATEN BY *BUFO BOREAS* FROM SELECTED SITES IN NORTHWESTERN MONTANA

FOOD ITEM			NUMBER
INSECTA			
	Coleoptera		
		Carabidae	66
		Chrysomelidae	2
		Cicendelidae	1
		Curculionidae	18
		Dytiscidae	1
		Elateridae	4
		Silphidae	1
		Staphylinidae	1
		Tenebrionidae	21
	Diptera		
		Muscidae	1
		Tipulidae	1
	Hymenoptera		
		Formicidae	372
		Sphecidae	1
	Lepidoptera		
		Larvae	3
	Orthroptera		
		Acrididae	2
ARTHROPODA			
	Arachnida		
		Araneae	13
		Opiliones	2
DIPLOPODA			
	Julida		3
			513 Total

items which were found in the stomachs of the toads. The numbers of Formicidae which were contained in the stomach samples from the boreal toads illustrate three aspects of the feeding behavior. First, toads are primarily ground feeders. The occurrence of Formicidae, Carabidae, Arachnida and Diplopoda, which are principally ground dwelling forms, confirms the habit. The high incidence of debris such as leaves, grass, pine needles and dirt in the samples add support to the idea. Second, toads are opportunistic feeders. When a toad has the opportunity to feed upon numbers of one kind of insect, it will do so. Third, the recovery of Formicidae from all the samples indicates that ants are a major source of food for the toads throughout the period of activity.

The lack of flying insects such as Diptera, Homoptera, Odonata, Lepidoptera, and flying Hymenoptera corroborates the habit of the toad to feed from the ground. The absence of aquatic snails among the food items suggests that toads primarily feed away from the riparian sector. The presence of pine needles supports the suggestion of temporal separation from the ranids. Toads regularly contained pine needles, whereas frogs only occasionally contained pine needles. Spatial separation is further supported by the small size of the sample. Because collections were made only in the riparian area, the lack of numerous toads indicates that toads do not inhabit the shore areas as much as do the ranids.

Fluoride in the food of B. boreas

The contents of the stomachs of nine boreal toads were analyzed for fluoride (Table 11). All samples contained some fluoride (range from 8.0 ppm to 31.0 ppm F^-).

TABLE 11

FLUORIDE CONTENT IN THE FOOD OF *BUFO BOREAS* FROM SELECTED SITES IN NORTHWESTERN MONTANA

LOCALE	PPM F^-	AVERAGE	DATE
Jette Lake	8.0		6-19
	30.6	23.5	6-27
	31.9		6-28
Jette Pond	18.3		7-29
Smith Lake	24.1		7-31
Mud Lake	19.5		6-30
Spoon Lake	20.6		6-21
	31.0	25.8	7-1
Roger's Lake	30.0		7-15
Control (composite of 6)		35.1	Pre-1952

At specific localities those samples which were obtained from early in the summer contained less fluoride than those which were obtained later in the summer. For example, two samples from Spoon Lake which were collected on June 21 and July 1 contained 20.6 ppm and 31.0 ppm F^-, respectively. The composition of the samples was approximately the same. A series of three samples which were obtained on June 19, June 27 and June 28 from Jette Lake contained 8.0 ppm, 30.6 ppm and 30.9 ppm F^-, respectively. The composition of these samples varied but the samples illustrate the tendency of the insects to accumulate fluoride as the period of activity progresses.

A sample which was composed of the contents of the stomachs of six *Bufo boreas* which had been collected prior to 1952 in the northern Flathead Valley contained 35.1 ppm F^-. The samples consisted of stomach contents from toads which had been collected over most of the summer months.

Fluoride in the bone of *B. boreas*

The left femur of ten experimental toads and six control toads was analyzed for fluoride (Table 12). The boreal toads showed a substantial accumulation of fluoride by the time they had obtained approximately 20 centimeters in total length.

TABLE - 12

ACCUMULATION OF FLUORIDE BY *B. BOREAS* OF DIFFERENT SIZES (AGES)

Total length	ppm F^-	Locale
18.3	373.3	Jette Lake
18.7	453.8	Jette Lake
19.5	598.1	Jette Pond
22.9	591.7	Jette Lake

The fluoride content of the specimen from Jette Pond more closely approximated that of the control group. However, the individual from Jette Pond was smaller, and contained more fluoride, than the largest control specimen. The fluoride content of the smaller specimens from Spoon Lake and Jette Lake also approached that of the control group (Figure 9).

Specimens from other sites, although not much different in size, exhibited wide variation in their concentration of fluoride. This may be the result of accumulation with increased age or may result from the concentration of available fluoride in their environment or in their food. Two general patterns of accumulation of fluoride are evident from these data. First, as illustrated by specimens from Jette Lake and Jette Pond, smaller specimens tend to contain less fluoride than larger ones (Table 13). Second, as illustrated by specimens from Mud Lake and Spoon Lake, toads which are exposed to greater concentrations of fluoride tend to accumulate more fluoride (Table 14).

TABLE - 14

ACCUMULATION OF FLUORIDE BY B. BOREAS OF SIMILAR SIZE FROM DIFFERENT SITES

TOTAL LENGTH (CM)	PPM F^-	LOCALE
22.6	910.8	Spoon Lake
22.2	1810.6	Mud Lake

The difference between the concentration of fluoride by toads at the two sites may be attributed to the location in relationship to the pattern of dispersal of airborne fluoride from the Anaconda Aluminum Plant. Isopols which were based on analyses of 1970 and 1971 vegetation samples

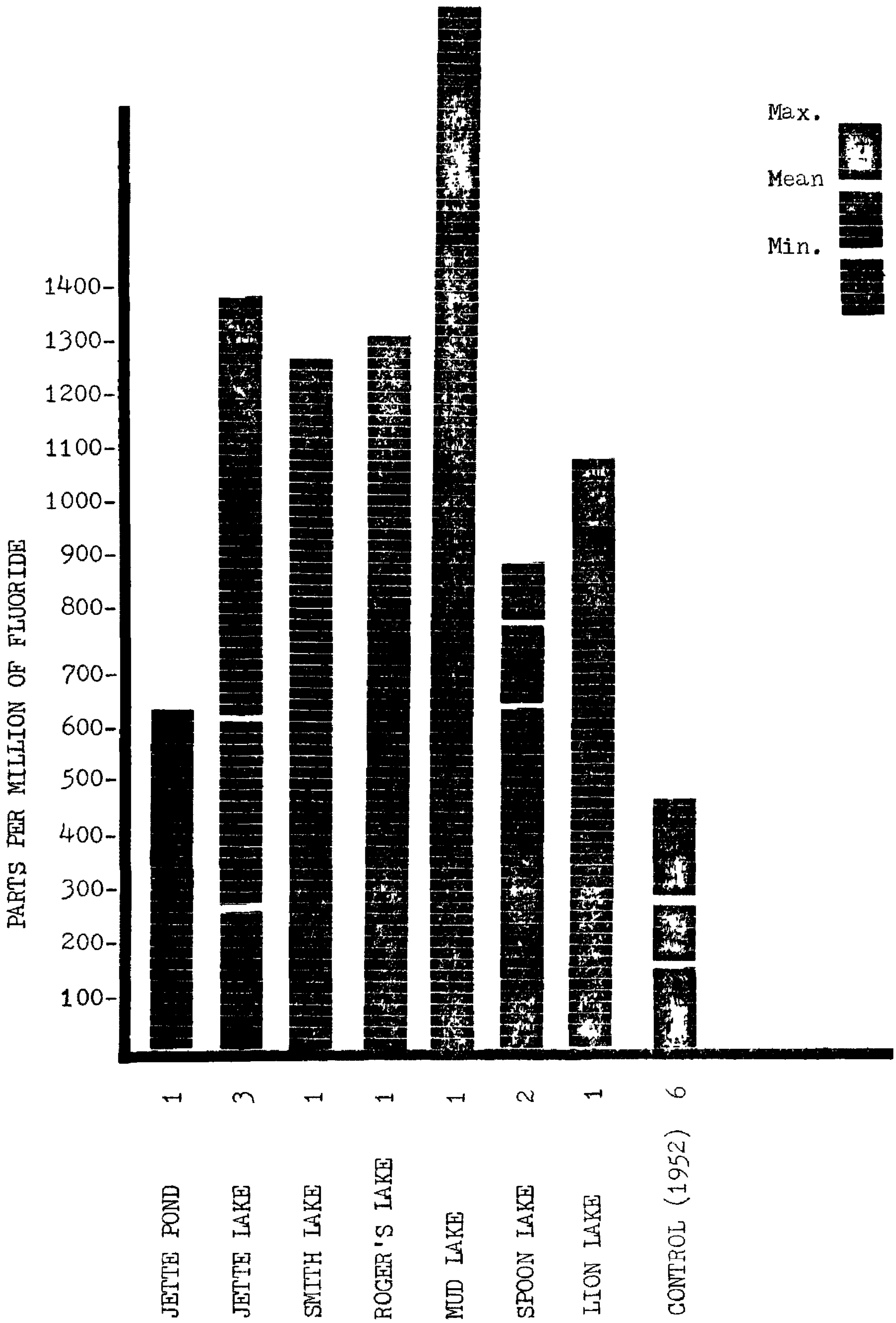


Figure 9. Fluoride content in the bone of Bufo boreas.

TABLE 13

FLUORIDE CONTENT IN THE BONE OF BUFO BOREAS FROM SELECTED SITES IN NORTHWESTERN MONTANA

LOCALE	N	TOTAL LENGTH (cm)			PPM F^-		
		max.	mean	min.	max.	mean	min.
Jette Pond	1	19.5			598.1		
Jette Lake	3	23.2	20.2	18.3	1409.0	611.2	228.3
Smith Lake	1	19.5			1284.1		
Roger's Lake	1	19.7			1312.5		
Mud Lake	1	22.2			1816.6		
Spoon Lake	2	22.6	20.8	19.1	910.8	798.1	673.3
Lion Lake	1	22.3			102.2		
Control (pre-1952)	6	22.7	18.7	12.3	456.3	241.3	121.8

establish that the Spoon Lake area receives a lower concentration than Mud Lake (E.P.A., 1974).

The accumulation of fluoride by a toad occurs throughout the life of the animal (Table 15). Eggs, tadpoles and toadlets contained less than 65 ppm F^-. Because toadlets were analyzed whole and because the concentration of fluoride is expressed on the basis of dry weight, the actual concentration in the bones of the toadlets may be slightly higher than reported. Some variation should be expected as a result of the differences in ambient concentration of fluoride at the sites from which these samples were obtained. Because toadlets have metamorphosed (ended the larval stage), and contain relatively little fluoride, the concentration of fluoride as illustrated by adults indicates that toads accumulate more fluoride as subadults and adults than as larvae and tadpoles.

Thamnophis spp.

Description and food of Thamnophis

Garter snakes, Genus Thamnophis, are slender snakes with keeled dorsal scales and single anal scutes, typically a pale vertebral stripe and a pale lateral stripe low on each side. Garter snakes may be found in and around ponds, streams, lakes, woods and meadows. Thamnophis spp. are carnivorous throughout their life.

The red-sided garter snake, Thamnophis sirtalis parietalis Say (1823) exhibits dark spotting on the dorsum, red bars on the sides and red lateral stripes which are often broad and dull. Colors may be bright (Stebbins 1966; Wright and Wright, 1957). This species frequents environments near water. Food has been reported to include fish, toads, frogs, tadpoles, salamanders, birds, small mammals, earthworms, slugs and leeches

TABLE 15

FL ORIDE CONTENT OF SELECTED STAGES IN
THE LIFE CYCLE OF BUFO BOREAS

STAGE	PPM F^-	TOTAL LENGTH (cm)
EGG	32.7	
TADPOLE	29.6, 61.0	
TOADLETS	42.0, 43.3	1.0-2.0
SUBADULT	236.0	12.3
ADULT	453.8	18.7
ADULT	591.7	22.9

(Stebbins, 1966).

Fluoride in stomach samples of *Thamnophis* spp.

The contents of 21 stomachs were analyzed for fluoride content (Table 16). These were selected to reflect the range of food which had been eaten by the two species of *Thamnophis*. The contents of stomachs selected from Smith Lake, Jette Lake, Roger's Lake, Spoon Lake, Mud Lake, Jette Pond and along Cedar Lake were analyzed. Leeches, *Hemophis* and *Erpobdella*, were removed from snakes which had been captured at Jette Lake, and Roger's Lake. These contained 10.3 ppm F^- and 7.7 ppm F^- and 1.1. The average fluoride content was 6.3 ppm F^-. Toadlets, which were substituted for specimens from snake stomachs were collected from Smith Lake (2 samples), Spoon Lake, and Roger's Lake. These samples contained 42.0, 43.3, 29.6 and 61.4 ppm F^-, respectively. *Rana pretiosa* were removed from one snake which had been captured at Roger's Lake and one snake which had been captured at Jette Pond. These samples contained 29.8 and 18.3 ppm of fluoride. *Rana pipiens* were removed from the stomachs of two snakes which had been captured at Roger's Lake. One specimen, a tadpole, contained 32.5 ppm F^-. The other, a newly metamorphosed animal, contained 61.3 ppm F^-. A group of 28 slugs, *Prophysaon*, which had been removed from snakes from Mud Lake was determined to contain 19.7 ppm fluoride. Two *Hyla regilla* which were recovered from a snake which had been collected from Jette Pond contained 36.3 ppm fluoride. Six *Ambystoma macrodactylum* which had been collected to represent snake food were analyzed for fluoride content. The salamanders contained 195.5 ppm fluoride. Fish, *Salmo* sp., were recovered from the stomachs of snakes which had been collected from Mud Lake (1 sample) and Jette Lake (2 samples). These

TABLE 16

FLUORIDE CONTENT OF FOOD ITEMS OF THAMNOPHIS SPP. FROM SELECTED SITES IN NORTHWESTERN MONTANA

FOOD ITEM	PPM F^-	AVERAGE
Leaches	7.9	
	10.3	6.3
	1.1	
Toadlets	42.0	
	43.3	
	29.6	44.0
	61.4	
Rana pretiosa	29.8	
	18.3	24.5
Rana pipiens	32.5	
	61.3	46.9
Slugs	19.7	
Hyla regilla	36.3	
Ambystoma macrodactylum	195.5	
Birds	68.1	
	61.2	45.5
	17.4	
Fish	25.1	
	69.1	37.5
	18.3	

contained 25.1, 69.1 and 18.3 ppm fluoride, respectively. The latter two were large and small sized fish. Two birds, a sparrow and a meadow lark, were recovered from snakes which had been collected from Roger's Lake. The parts of the sparrow contained 17.4 ppm fluoride. The composition of the sample was primarily bones and feathers. The two parts of the meadow lark contained 68.1 and 61.2 ppm fluoride. These samples were primarily composed of bone and flesh.

Those food organisms of snakes which contain bone rather than other sustentative tissue contained more fluoride. This does not necessarily mean that organisms with higher concentrations of fluoride contribute more to the transfer of fluoride to the predator. The strength of the chemical bond which holds the fluoride in the bone structure decreases the likelihood of direct transfer from the bone to the predator. The fluoride which has not been bound into calcified structures is readily available for utilization by the system of the predator. The rate of feeding and associated completeness of digestion effect the transfer of fluoride as much as does the selection of food items. The accumulation of fluoride in the bones of garter snakes corroborates the conveyance of fluoride between trophic levels.

Fluoride in snakes

A total of twenty-three *Thamnophis spp*. were analyzed for fluoride (Table 17). Two species, *T*. *elegans vagrans* and *T*. *sirtalis peritalis*, were collected from six locations in the northern Flathead Valley (Figure 10). No differences existed between the species or sexes in the tendency to accumulate fluoride. Generally, the larger snakes contained more fluoride than smaller snakes from the same site (Table 18). Because

TABLE 17

FLUORIDE CONTENT IN THE BONE OF THAMNOPHIS SPP. FROM SELECTED SITES IN NORTHWESTERN MONTANA

Thamnophis elegans vagrans

LOCALE	N	TOTAL LENGTH (cm)			PPM F^-		
		max.	mean	min.	max.	mean	min.
Jette Pond	2	74.0	68.3	62.7	551.7	550.0	548.3
Jette Lake	2	73.0	64.9	56.8	403.7	338.9	174.1
Roger's Lake	1	56.2			385.3		
Mud Lake	3	65.4	53.5	36.0	1347.2	874.1	277.0
Brenamen's Marsh	1	76.0			1267.9		
Cedar Lake	1				612.5		

Thamnophis sirtalis parietalis

LOCALE	N	TOTAL LENGTH (cm)			PPM F^-		
		max.	mean	min.	max.	mean	min.
Jette Pond	1	69.2			1102.7		
Jette Lake	3	60.0	53.1	41.5	278.2	233.1	210.5
Smith Lake	2	66.6	60.3	55.1	491.4	418.1	299.4
Spoon Lake	1				1249.4		
MacWenneger Slough	1				297.7		
Cedar Lake	1				1366.9		
Control	6	70.0	55.6	50.0	249.5	195.5	141.6

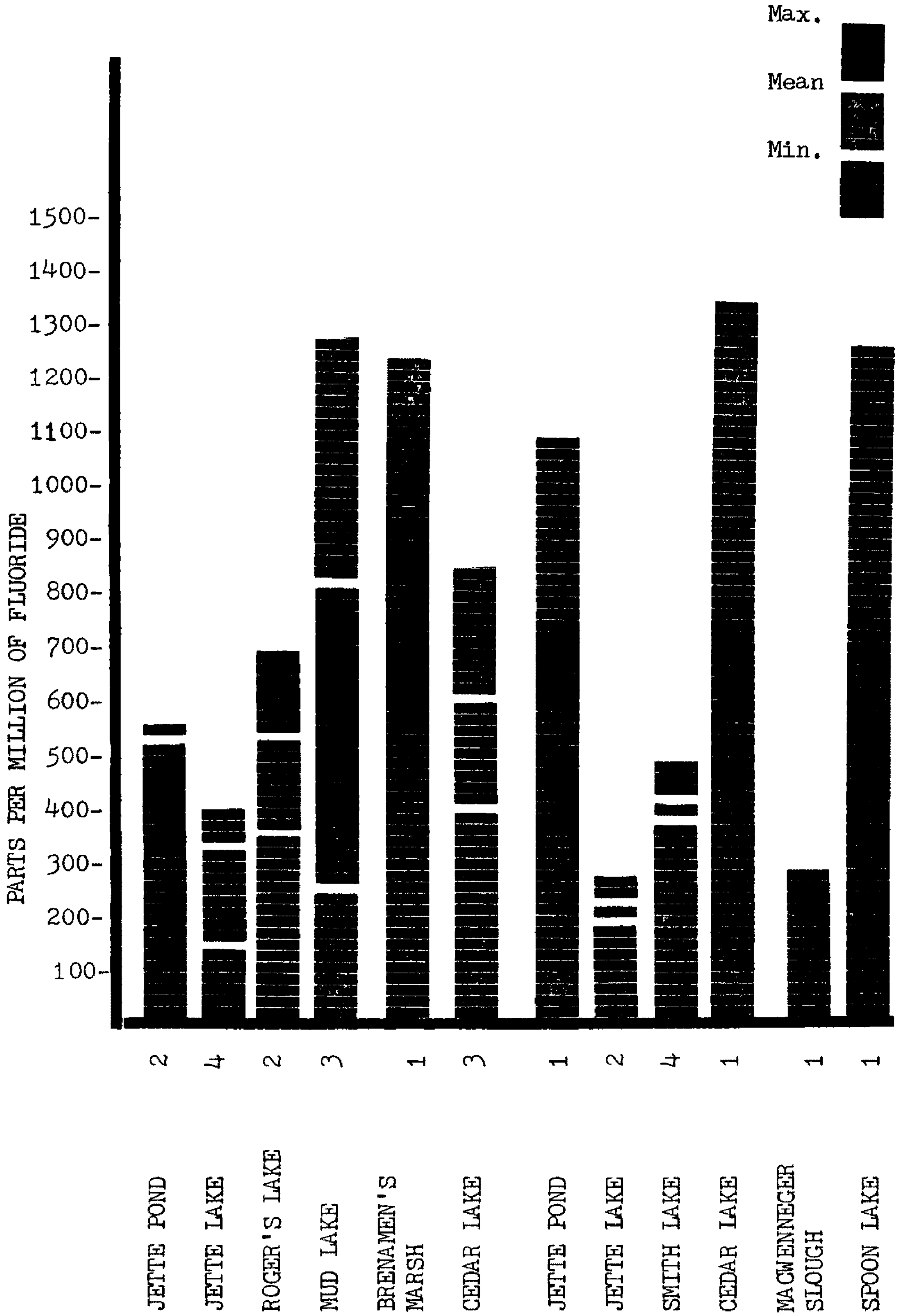


Figure 10. Fluoride content in the bone of *Thamnophis* spp.

TABLE 18

FLUORIDE CONTENT IN *THAMNOPHIS SPP.* OF DIFFERENT SIZES (AGES)

LOCALE	TOTAL LENGTH (cm)	SNOUT-VENT LENGTH (cm)	PPM F^-
Smith Lake	43.8	33.6	410.7
	66.6	59.3	491.4
	72.6	55.3	470.6
Mud Lake	36.0	25.7	277.0
	59.2	45.9	998.2
	65.4	48.9	1347.2
Roger's Lake	56.2	43.4	385.3
	62.8	47.5	1036.4

growth slows with increased age, a snake which was a few centimeters longer than another may have been several years older and therefore, potentially, contained more fluoride. For example, two snakes from Mud Lake were 65.4 cm and 59.2 cm in total length and contained 1347.2 ppm F^- and 958.2 ppm F^-, respectively. The larger snakes exhibited greater variation in the amount of fluoride which was contained in the bone than do smaller snakes.

Species of Thamnophis are carnivorous throughout their lives; young are born alive. Other than by absorption through the skin and lungs, the fluoride which was present in the bone must have come from the diet. The diet of the garter snakes encompasses a variety of organisms, all of which contained fluoride (Table 16). Both herbivorous and carnivorous forms of prey were represented.

Chrysemys picta belli

Description and food of Chrysemys picta belli

The western painted turtle, Chrysemys picta belli Gray (1844) is an aquatic turtle which may be found in ponds, marshes and lakes in the northern Flathead Valley. It is often seen sunning on logs or around the shore. The turtles actively feed along the shore during the early evening.

The carapace is low, smooth and unkeeled. The color of the dorsal surface is dark olive with yellow striping coloration on the marginals. The plastron is usually a yellow-orange with a centralized, mottled, dark figure. The neck, head and limbs are striped with yellow (Stebbins, 1966; Carr, 1952).

The primary foods of the turtle include aquatic plants, insects, spiders, earthworms, mollusks, crayfish, fish, frogs and tadpoles

(Stebbins, 1966). Stomach contents were not analyzed for fluoride.

Chrysemys picta belli and fluoride

Ten western painted turtles were collected and analyzed for the presence of fluoride in the femurs (Table 19 and Figure 11). A group of five turtles, 2 juveniles and 3 adults, were collected from Jette Lake. The juvenile specimens contained less fluoride than the adults (232.3, 275.9, 319.9, 504.2, 3595.6 ppm F^-, respectively).

The largest specimen which was recovered from Jette Pond contained over 3000.0 ppm fluoride. Inference may be made from this individual and from the general tendency of increased concentration of fluoride with increased size (age) that the specimen was much older than the other turtles and that turtles continue to accumulate fluoride after their growth rate has slowed substantially. This process involves the translocation of fluoride into internal sections of the bone.

Comparison of one juvenile female specimen which had been captured from Brenamen's Marsh to specimens of the same general size from Jette Lake revealed that the specimen from Brenamen's Marsh contained approximately twice as much fluoride (466.2 ppm F^- and 232.3 ppm F^-). This rapid accumulation may be attributed to the proximity of Brenamen's Marsh to the aluminum plant at Columbia Falls.

Three juvenile specimens were captured at Smith Lake. The specimens ranged between 10.8 cm and 11.5 cm in total length. The amount of fluoride which was contained in the specimens ranged between 979.5 to 1306.8 ppm. The smallest specimen contained the least amount of fluoride and the largest specimen contained the most.

Turtles tend to accumulate fluoride throughout their lives from

TABLE 19

FLUORIDE CONTENT OF BONE IN *CHRYSEMYS PICTA BELLI* FROM SELECTED SITES IN NORTHWESTERN MONTANA

LOCALE	CARAPACE LENGTH (cm)	PLASTRON LENGTH (cm)	PPM F^-
Jette Lake	10.3	9.7	232.3
	10.9	9.8	275.9
	14.7	13.4	504.2
	14.8	13.8	319.9
	16.3	14.7	3227.2
Smith Lake	10.8	10.3	693.3
	11.4	10.3	1000.0
	11.5	10.5	1306.8
Brenamen's Marsh	10.5	9.8	466.2
MacWenneger Slough	17.4	16.1	931.9
Skaggs Lake	(parts of shell)		4807.7
	(parts of shell)		5099.7

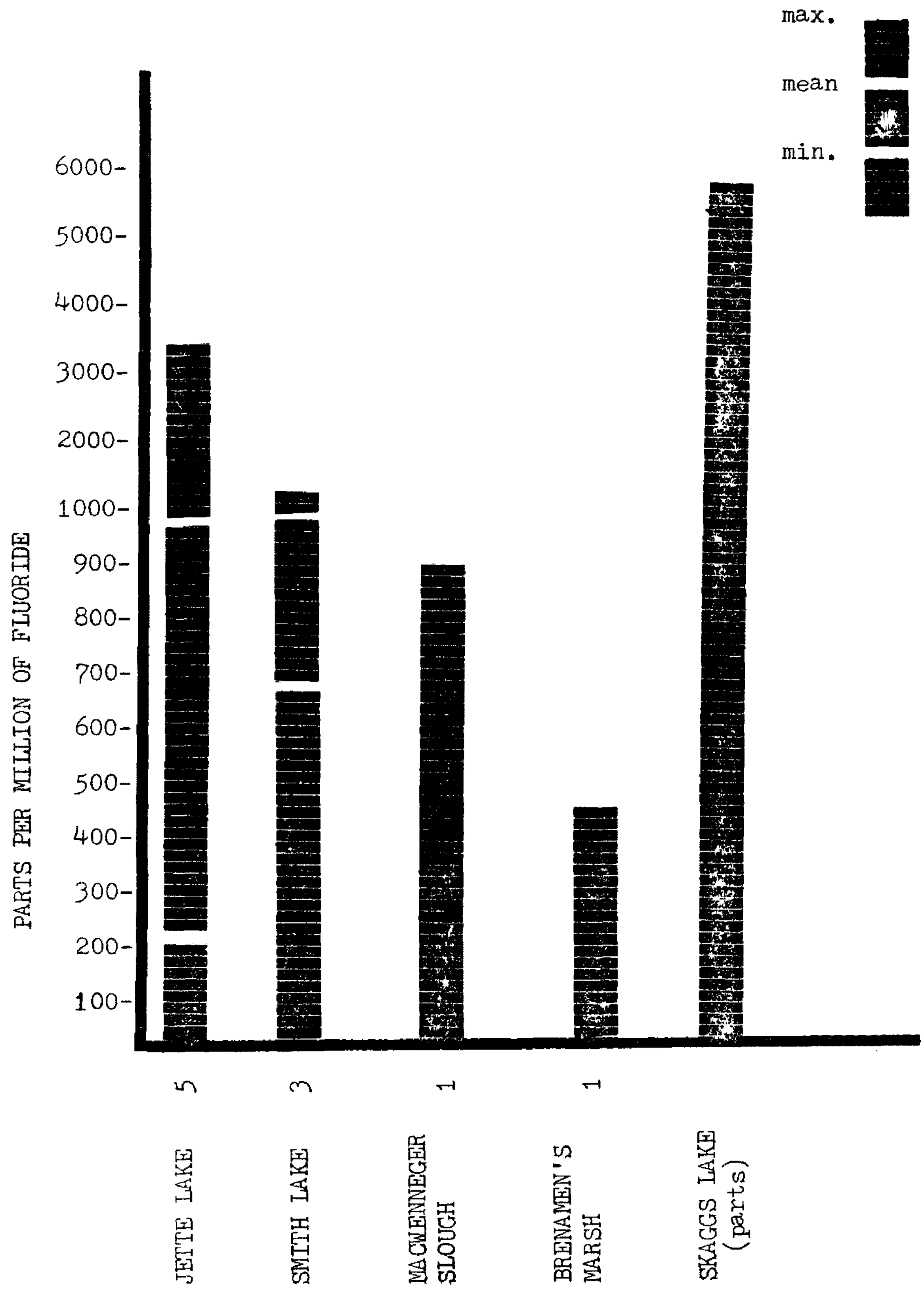


Figure 11. Fluoride content in the bone of *Chrysemys picta belli*.

their environment. When exposed to greater amounts of ambient fluoride, the turtles accumulate more rapidly. A direct correlation does not exist between the size of the organisms and the amount of fluoride which is contained in the bone, because no direct correlation exists between the size and the age of the animal.

CHAPTER V

DISCUSSION

Temperature relationships of Anura

A large region of overlap exists in the range of air and water temperatures which are tolerated by the two ranids. This allows the species to co-exist in specific areas. However, some separation in habitat utilization is achieved as a result of the ability of R. pretiosa to tolerate lower water temperatures in both the larval and adult stages. Further separation is achieved as a result of the ability of R. pipiens to tolerate higher water temperature in both the larval and adult stages (Figures 1, 4 & 7).

The ability to tolerate different water temperatures influences the distributions of the ranids in the northwest (Dumas, 1964). R. pretiosa populates the mountain ponds and streams where R. pipiens cannot. However, R. pipiens inhabits the warm sloughs of the valleys, R. pretiosa cannot. Both species inhabit a variety of locations in the valleys and foot hills in which the temperature range of the water is within the limits of each.

Bufo boreas tolerates slightly lower air temperatures than the ranids. Although not enough data are available, the relationship between environmental temperatures and the boreal toad resemble that of both ranids. The boreal toad inhabits areas in which the ranids are separated, such as

Spoon Lake (only *R. pretiosa*) and Smith Lake (only R. pipiens).

The crepuscular and nocturnal activity of the boreal toad suggests that it prefers cooler temperatures. However, the occasional diurnal activity further suggests that the range of temperature which is tolerated is wide. The periods of typical activity tend to temporarily separate *Bufo boreas* from the ranids, both of which are active throughout the day but tend to cease activity before sunset. *Bufo boreas* is active during the day in the early part of June. However, as daytime temperatures rise as the summer progresses, the bufonid tends to become more crepuscular until its activity is restricted to the late afternoon and evening hours.

Food relationships of Anura

Five principles govern the diet of the Anura. First, the diet is determined by the availability and abundance of the prey (Whitaker, 1961; Turner, 1959). Second, the diet reflects the habits (life cycle) and habitat of both the prey and predator (Linzey, 1967; Whitaker, 1961). Third, one or more families of insects may be stable in the diet but the importance may fluctuate as a result of the abundance and availability of other prey (Linzey, 1967). Fourth, the actual prey eaten by a species varies "from place to place, from time to time and from individual to individual" (Oliver, 1955). Fifth, vegetable matter is ingested incidently (Kilby, 1945; Turner, 1959). These principles are supported by the present study.

Analyses of samples from the stomachs of *R. pipiens* and *R. pretiosa* indicate that the two frogs feed primarily upon the same food items (Table 20). However, some differences are noted. These differences probably result from differences in the availability of food items from

TABLE 20

COMPARISON OF INGESTED MATTER EXCLUDING INORGANIC AND VEGETABLE MATTER BY SELECTED SPECIES OF ANURA FROM NORTHWESTERN MONTANA

FOOD ITEM	RANA PRETIOSA		RANA PIPIENS		BUFO BOREAS	
	#	%	#	%	#	%
Insecta						
Coleoptera	180	34.8	131	35.3	115	23.3
Diptera	48	9.2	42	11.3	2	0.4
Hemiptera	26	5.0	15	4.5	-	-
Homoptera	2	0.4	2	0.5	-	-
Hymenoptera	116	22.4	36	9.7	373	75.3
Lepidoptera	24	4.6	7	1.9	3	0.6
Odonata	9	1.7	51	13.8	-	-
Orthroptera	9	1.7	16	4.3	2	0.4
Plecoptera	2	0.4	2	0.5	-	-
Trichoptera	4	0.7	3	0.8	-	-
Amphibia						
Bufo boreas	-	-	4	1.1	-	-
Gastropoda	22	4.2	16	4.3	-	-
Arthropoda						
Arachnida	75	14.5	43	11.6	15	3.0
Chilopoda	-	-	1	0.2	-	-
Diplopoda	-	-	2	0.5	3	0.6

site to site, and the size of the items in relation to the size of the frog as well as the feeding behavior of the frogs.

The similarity of diet of the ranids indicates that in allopatric situations the adults do compete for the food resource which is available. The taxonomic composition of the samples and the numbers of prey suggest that in specific cases competition may be acute. The simultaneous occurrence and feeding in the riparian habitat support the hypothesis.

Interspecific competition is reduced by a difference in the sizes of prey which are acceptable to the ranids. R. pipiens can and does eat larger items than R. pretiosa. Turner (1959) found that for R. pretiosa the smaller frogs fed only upon small prey but that the larger frogs ingest items which range considerably in size. An analogous situation exists interspecifically and intraspecifically between the ranids in the present study.

The size of the frog in relation to the size of the prey influences the selection of food. Leopard frogs are larger at metamorphosis and tend to be larger than spotted frogs, therefore leopard frogs are able to feed upon larger prey. For example, Odonata were consumed five times more often by leopard frogs than by spotted frogs. This reflects the relative abundance of odonates in the habitats which are frequented by R. pipiens. More odonates were eaten in July than in June which coincides with the emergence of the adult forms of dragonflies. R. pipiens fed upon members of Coenagrionidae, Aeschnidae and Libellulidae. The size of the Coenagrionidae was smaller than the latter two families. The size of the prey did not retard feeding. Only members of Coenagrionidae were eaten by R. pretiosa even though Aeschnidae and Libellulidae were present at many of the same localities as the spotted frog. This reflects the preference for habitat by the odonates as well as the relatively non-aggressive feeding habits of the spotted frog. Because R. pretiosa did not consume another food item which was as large as members of the Aeschnidae and Libellulidae, slight selection of prey size may be inferred.

The consumption of toadlets (10-15 mm) by R. pipiens and not by R. pretiosa substantiates the aggressive feeding behavior of leopard frogs. The size and activity of the toadlets coupled with the aggressive feeding habits and size of R. pipiens accounts, at least in part, for the consumption of the toadlets.

Slight, occasional, temporal separation helps to reduce the competition at specific locations. For example, a spring fed cold water channel connects Lake Five to Mud Lake. R. pipiens was found in both lakes but was seldom found along the shore of the channel. On the other hand, R. pretiosa was abundant along the channel but was seldom captured or seen along the shore of the lakes.

Dumas (1964) concluded that food was not the limiting factor for either species of ranid. He concluded that in allopatric situations differential tadpole mortality was the primary mechanism of replacement of R. pretiosa by R. pipiens.

The consumption of a variety of items from more than 40 families of insects, plus snails, and spiders, support the contention that leopard frogs and spotted frogs are opportunistic feeders.

The competition for dietary items between Bufo boreas and both of the ranids is minimal (Table 20). The dependence upon specific taxa which were represented in the samples from the stomachs of the ranids implies minimal competition. The addition of temporal separation during feeding (evening and night, woods and meadows for toads, versus days and evenings, riparian and meadows for ranids) further reduces the interspecific competition.

The composition of the food of Bufo boreas, as illustrated by this study, does not differ radically from the food which has been reported from other parts of the range. (Ellis and Henderson, 1915; Tanner; 1931; Eckert, 1943; Schonberger, 1945; Burger and Brugg, 1947; Moore and Strickland, 1955; Campbell, 1970). A major exception occurs in the dependence upon specific organisms in the diet. The apparent dependence upon Formicidae which is suggested by Campbell (1970) and Schonberger (1945) may be an artifact which resulted from the small number of toads in the sample.

Accumulation of fluoride by amphibians and reptiles

At least twelve factors influence the accumulation of fluoride by an organism. These include (1) the amount of fluoride which is ingested, (2) the duration of ingestion, (3) fluctuations in the amount of fluoride which is ingested, (4) the solubility of the fluoride which is ingested, (5) the species which ingests the fluoride, (6) the age of the organism at the time of ingestion, (7) the nutritional status of the organism, (8) the influence of stress factors during digestion, (9) the amount of fluoride in the ambient environment (aquatic or terrestrial), (10) the amount of fluoride which is absorbed through the skin, (11) the amount of fluoride which is absorbed through the lungs, and (12) the response of the metabolic system of the organism to the fluoride (Shupe, 1972).

These factors work synergistically to produce variation in the amount of fluoride which is accumulated by an individual organism. Some of these are of greater importance to certain species than to others. For example, absorption through the skin is of greater importance to amphibians than reptiles.

Accumulation of fluoride by amphibians and reptiles is accomplished in two general ways. One pathway involves the direct absorption through the lining of the stomach and intestine from food and organic material which have been ingested. The life cycle, physical structure, and physiology play important roles in the susceptibility of amphibians and reptiles to fluoride.

Anurans deposit their egg masses in water. After hatching, the free swimming larvae spends several weeks in the water before metamorphosis. The adult and larval forms possess a permeable skin which must be kept moist and which makes the Anura susceptible to water and airborne fluoride. The larval stage of the Anura are herbivorous. The adults are insectivorous. The tadpoles are primary consumers in the aquatic system and are fed upon by snakes and, occasionally, fish as well as predacious aquatic insects. The adults are first level carnivores and are fed upon by a variety of reptiles, birds and mammals. The ranids represent a middle link in the food web of the terrestrial and aquatic ecological systems.

Reptiles are not restricted to the riparian habitat as a result of their amniote egg and integument. The reptilian egg possesses a hard outer shell which reduces desiccation of the egg. The egg may be carried inside the female until hatching which reduces the water loss still more.

The larval stage is omitted but reptilian eggs require more time before hatching. The subadult resembles the adult with the possible exceptions of color pattern and food utilization. Sexual maturity is the primary distinction between subadults and adults. The integument of reptiles has no respiratory function and as a result helps to reduce the loss of body water to the environment. Respiration is primarily by lungs which by structure, are more efficient than those of amphibians (Porter, 1972).

Non-accumulative effects of fluoride

Members of the classes Amphibia and Reptillia have the same general mechanism for energy production and utilization of food stuffs, as well as the same general requirements for ions, minerals, salts, vitamins, and metabolites as other classes of vertebrates (Brown, 1964). Calcium, fluoride, magnesium, phosphorous ($HPO_4^{=}$ and H_2PO_4) are essential for proper formation of bone. The requirements for these and other specific elements and ions do not differ in "any known respect" from other classes of vertebrates (Brown, 1964). The majority of the essential minerals, ions and elements are generally available from dietary and environmental sources.

Fluoride has been reported to inhibit several enzymatic reactions. Soluble ATPase is inhibited by fluoride through the reaction of the F^- with Mg^{++} or Mn^{++} which act to stimulate ATPase activity. ATPase functions in the trapping of high-energy phosphate bonds (Florkin and Jeuniaux, 1964). Hexose-l-phosphate which hydrolyzes glucose-l-phosphate, galactose-l-phosphate and p-nitropheny-l-phosphate is inhibited by fluoride. The action of enolase is also inhibited by fluoride. Enolase catalyzes the removal of water from 2-phosphoglycerate to form phosphoenol-pyruvate. The reaction redistributes energy in the molecule which may then be liberated by the hydrolic transfer of phosphate (Florkin and Scheer, 1970; Hochester and Quastel, 1963).

Cameron (1940) reported that when fluoride was present in concentrations as low as 1 ppm, the rate of larval development and the developmental stage at which Rana pipiens hatches was measurably retarded.

The accumulation of fluoride by tissue other than bone is usually slight by comparison (Wright and Dawson, 1975). Miller (1974) reported concentrations of fluoride in skin, muscle and organ tissue of 17 Rana pretiosa. The composite sample of skin tissue contained 107.4 ppm fluoride. The composite sample of organs (livers, kidneys, intestines) contained 81.4 ppm fluoride. The composite sample of muscle tissue contained 29.4 ppm fluoride. The average amount of fluoride which was contained in the femurs of these specimens was 2826.5 ppm. The lowest amount of fluoride in bone tissue was recorded from a juvenile specimen. The individual contained 318.7 ppm fluoride.

Fluoride and bone

The effect of fluoride on bone is not fully understood. The association between fluoride and bone is practically the result of the ability of fluoride to form a fluoroapatite. The exchange of fluoride into the bone involves the replacement of OH^- by F^- in the apatitic calcium phosphate. The reaction in mammalian bone is $Ca_{10}(PO_4)_6OH_2 + 2F^- \longrightarrow Ca_{10}(PO_4)_6F_2 + 2OH^-$. The exchange is rapid at first but eventually slows as the bone becomes saturated (N.A.S., 1971; Marier and Rose, 1971; Johnson, 1965).

The amount of fluoride which may be contained in various bones is dependent upon the type of bone. Cancellous bones from mammals show concentrations which are higher than more compact bone (N.A.S., 1971). Fluoride distribution within specific bones varies with the activity of the bone tissue. Areas of active bone growth yield higher concentrations of fluoride than areas of little or no growth (Shupe, 1972).

Because no fundamental structural changes have been found between the bones of amphibians, reptiles and mammals (Enlow, 1969), there is no evidence to suggest that the reactions of hydroxiapatite and fluoride to form the fluorapatite is different in amphibians and reptiles than in other animals.

Inspection of one member of each of 20 pairs of femurs, (the other member of the pair when analyzed had contained a high amount of fluoride) revealed no apparent fluorosis in the form of 'nodules' or spurs as illustrated by Hodge and Smith (1965) and Stacy, Tatlow and Sharpe (1961). The lack of apparent fluorosis is not conclusive that the condition does not exist in anurans. However, present data indicate that the bufonids, as a result of their longevity and habits, may exhibit fluorosis before the ranids. Because no specimens were collected on Tea Kettle Mountain, the zone of the highest concentration of ambient fluoride, no conclusion may be made.

Growth and accumulation of fluoride in amphibians and reptiles

The growth of amphibians and reptiles continues throughout the life of the individual. Growth is initially rapid until the individual reaches sexual maturity. Growth tends to be intermittent during the adult stage and the rate slows. The rate of growth in both larval and adult stages is influenced by environmental factors such as temperature and nutrition (Porter, 1972).

The determination of age on the basis of size is of limited accuracy for the following reasons: (1) not all eggs are deposited at the same time (some eggs are deposited very early and some very late in the breeding season which results in a difference in the size of frogs which metamorphose in the same summer), (2) individual rates of growth vary according to metabolism, temperature of the environment and amount of food which is ingested, and (3) the rate of growth slows with increased age.

The growth of Rana pretiosa illustrates the range of size which was found by capture-recapture over a period of five years in Yellowstone National Park (Turner, 1960). Force (1933) described the same pattern of growth in R. pipiens. Intermittent growth, environmental factors and individual metabolism account for the range of size which is illustrated (Figure 12).

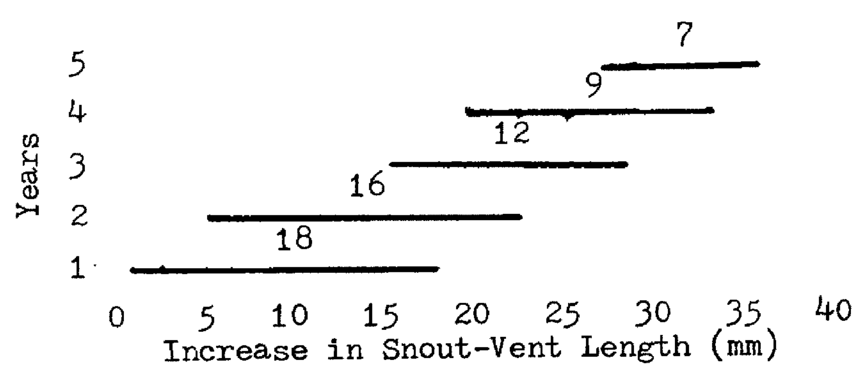


Figure 12. Growth increments of Rana pretiosa over a five-year period.

Fluoride and total length

The concentration of fluoride by an individual frog increases as the total length of that individual increases. The larger (older) animals contain greater amounts of fluoride than smaller specimens from the same site (Table 21).

TABLE - 21

SIZE DIFFERENCE (AGE) FLUORIDE ACCUMULATION BY *RANA PIPIENS* FROM MACWENNEGER SLOUGH

	Total Length (cm)			ppm Fluoride		
N	Max	X	Min	Max	X	Min
4	11.9	10.5	9.6	480.2	429.2	279.4
2	19.5	18.5	17.5	791.4	732.5	673.7

The relationship which exists between the amount of fluoride which is contained in the bone and the total length of the specimen may be described by slope of a line which has been fitted to the data. Although the correlation is not statistically significant, the curves do suggest a trend which might be confirmed by more extensive sampling. The slopes of the lines which have been fitted to specific sets of data indicate that fluoride is accumulated throughout the life of the organism. The rate of accumulation is accelerated as the result of increased exposure. The general trend of increased fluoride content in the bone in association with increased total length is illustrated in Figure 13.

Analysis of the total length and concentration of fluoride of twenty *R. pipiens* from Smith Lake illustrates several aspects of the association between fluoride and the Anura (Figure 14). Nine individuals which averaged 15.0 cm in total length (range 14.0 to 19.2 cm) contained an average of 218.0 ppm F^- (range 145.4 to 345.0 ppm F^-). Eleven individuals which averaged 12.5 cm in total length (range 11.2 to 14.0 cm) contained an average of 209.0 ppm F^- (range 158.3 to 240.1 ppm F^-). Tadpoles which

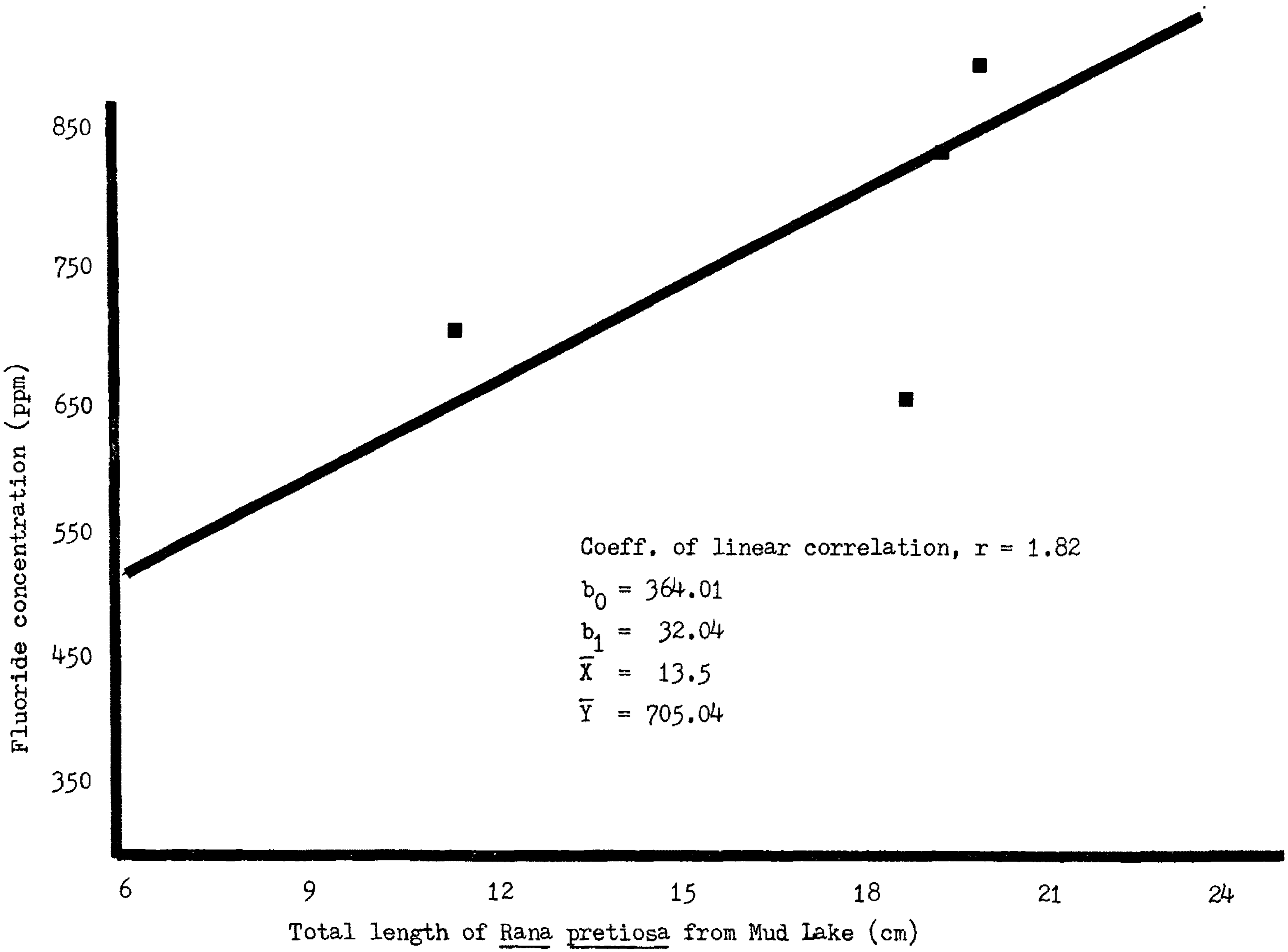


Figure 13. Relationship between total length of *Rana pretiosa* and the concentration of fluoride in bone tissue.

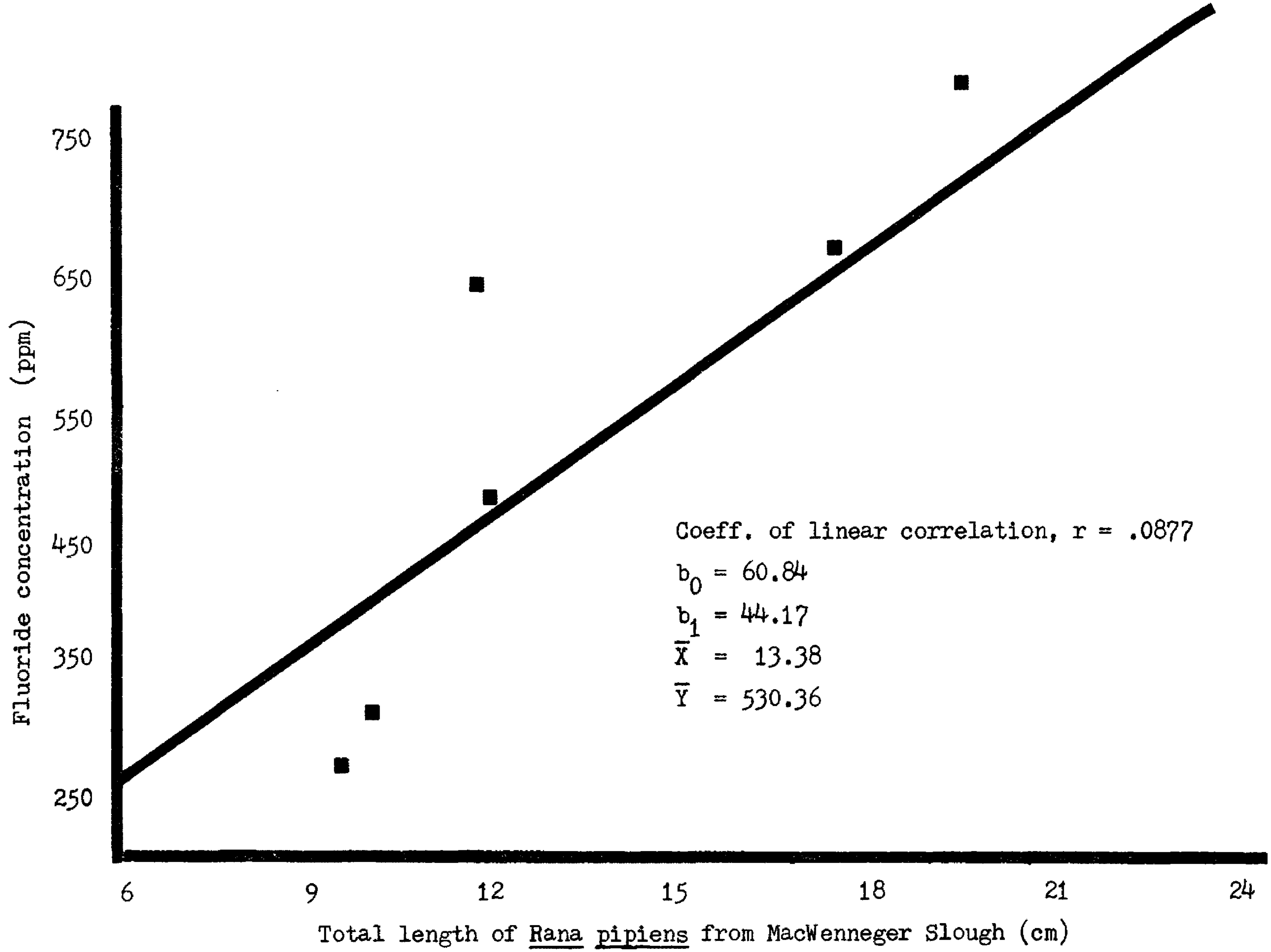


Figure 14. Relationship between total length of *Rana pipiens* and the concentration of fluoride in bone tissue.

measured less than four centimeters in snout-vent length contained an average of 61.3 ppm F^-. These data indicate that R. pipiens accumulate (1) as much as or more fluoride during metamorphosis than during any other period of its life and (2) accumulates fluoride throughout its life but at a gradually slowing rate following sexual maturity.

Inference may be made that fluoride, which is contained in the food, contributes little to the immediate concentration in the frog but over time the contribution may be significant, and that the bone is over 50% saturated with fluoride when the frog reaches sexual maturity (about 3 years of age). Miller (1974) made the same suggestions for R. pretiosa.

Bufo boreas does not follow the above pattern. Newly metamorphosed toadlets contained only slightly more fluoride than toad tadpoles, less than 100 ppm F^-, whereas, adults contained between 500 and 2000 ppm F^-.

Adult Bufo boreas consistently contained higher concentrations of fluoride than either Rana pipiens or Rana pretiosa when collected at the same locale. It is possible that the dietary habits of the bufonids may expose them to more fluoride than that of the ranids. Bufonids feed upon ground dwelling insects such as Carabidae and Formicidae (see the preceding discussion of food). Dewey (1973) reported concentrations of fluoride up to 170.0 ppm in a group of mixed Formicidae which were collected within one-half mile of the aluminum plant. Although no data are available, toads may live longer than ranids, which if true, would increase exposure to fluoride over the lifespan. Toads metamorphose at much smaller sizes than either of the ranids. Bufo boreas metamorphoses at 9.5-12 mm, Rana pretiosa at 16-23 mm and Rana pipiens at 18-31 mm (Wright and Wright, 1949). Toads breed initially at much larger size than the ranids. Bufo boreas breeds at 56-108 mm ♂ and 60-100 mm ♀ snout vent length. Rana pretiosa breeds at 44-75 mm ♂ and 46-75 mm ♀. Rana pipiens breeds at 52-80 mm ♂ and 52-108 mm ♀. Physiological processes involved in the deposition of fluoride in the bone may differ enough to account for some of the difference in observed concentrations. Differences in the permeability of the skin to fluoride may also be involved.

Exposure variation as a result of habitat

The topography of the area effects the distribution of fluoride near the aluminum plant. The low area northeast of Tea Kettle Mountain is sheltered from extensive exposure to airborne fluoride. By way of contrast, trees which have been sampled from the side of Tea Kettle Mountain which is nearest the plant and trees from the higher areas of Glacier National Park which face to the southwest have higher concentrations of fluoride than selected sites from within the sheltered area.

The microhabitat of a particular area may alter the distribution of airborne fluoride in an analogous manner, except that trees instead of mountains become the major obstruction. The microhabitat may also alter the distribution of fluoride. Tall grasses may alter the distribution of airborne fluoride only slightly when compared to the effect of trees. However, the synergistic association of such sheltering reduces the amount of airborne fluoride to which the organism is exposed.

The microhabitats which are utilized by amphibians and reptiles are varied. The density of the vegetation which covers the area may vary from no vegetation to over 280 stalks per square foot. The height of the vegetation may vary from a centimeter to several meters. Both Rana *pipiens* and *Rana pretiosa* tend to remain near the water and avoid sections of vegetation which are extremely dense (over 180 stalks per square foot) as well as extremely tall (over 100 centimeters tall) (Figure 15). *Bufo boreas* and both species of *Thamnophis* were found throughout short and tall vegetation and throughout a spectrum of density.

Comparison of control samples

The background level of fluoride has been shown to be approximately 10 ppm F^- in vegetation (Carlson and Dewey, 1971; Gordon, 1974).

The background level of fluoride in insects has been determined to be less than 10 ppm F^- (Dewey, 1973). Carlson and Dewey (1971) analyzed foliage feeding, cambium feeding, pollinating and predatory insects for fluoride content. Samples were collected within one-half mile of the aluminum plant and between 30 and 50 miles from the plant. Fluoride in control samples ranged from 7.5 ppm F^- in bumblebees and grasshoppers to 9.2 ppm F^- in damselflies. Fluoride in test samples included a range from 194 to 406 ppm F^- in bumblebees and 31.0 ppm F^- in grasshoppers to 21.7 ppm F^- in damselflies. A test group of mixed cerambycids contained 47.5 ppm fluoride and a test group of mixed elaterids contained 36.0 ppm fluoride.

The groups of insects which had been eaten by the anurans and later analyzed for fluoride content serve as samples for the amount of fluoride which occurs in insects. These data provide an indication but are not conclusive because of the composite nature of the taxa which comprised the samples. The range in the amount of the fluoride which was contained was from 8.0 ppm F^- to 52.6 ppm F^-. The average was approximately 28.0 ppm F^-. The samples were from sites which were at least forty miles

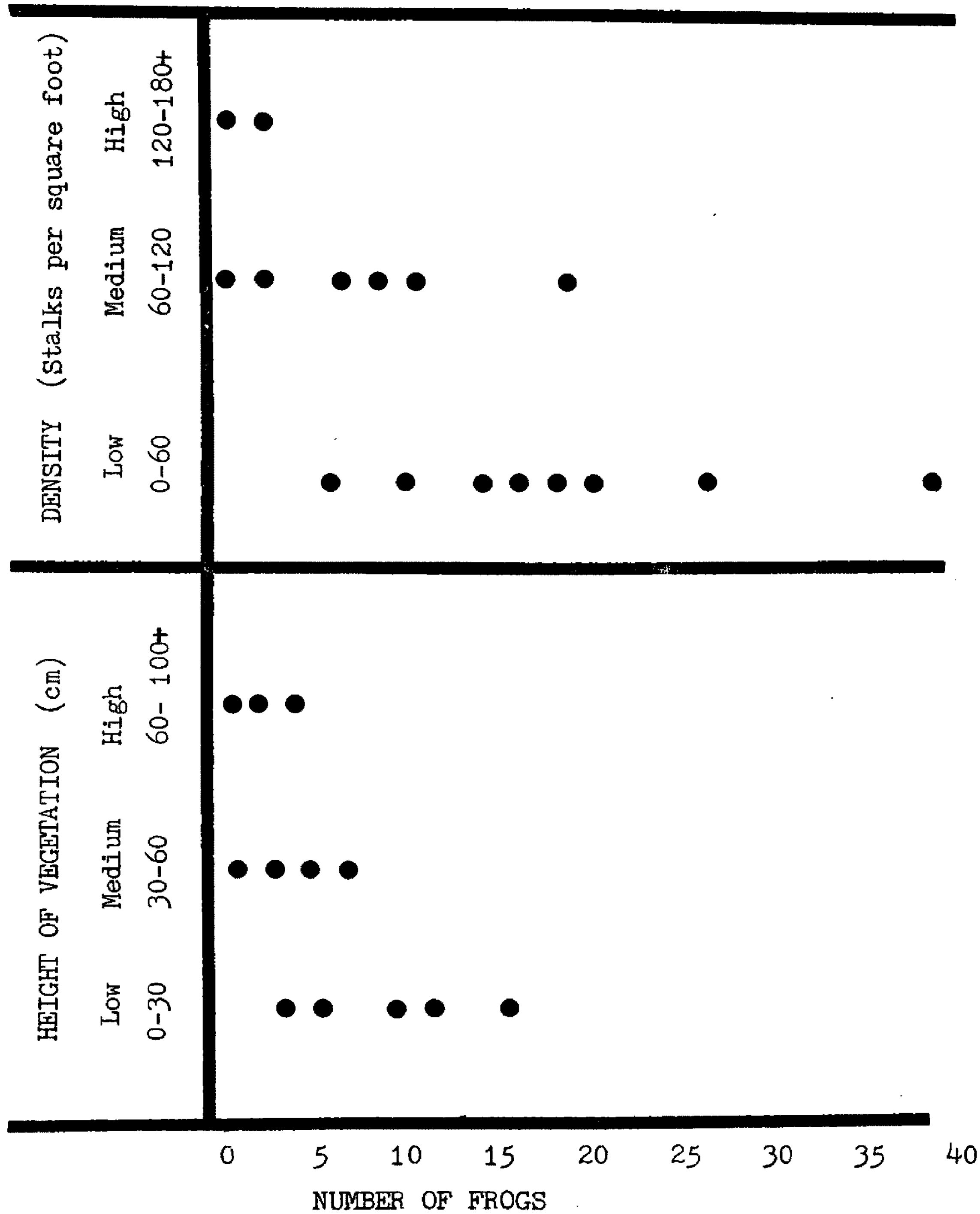


Figure 15. The relationship between the density of vegetation and the number of frogs which were present, and the relationship between the height of the vegetation and the number of frogs which were present.

from the plant. Samples which were collected at least 2 miles but no more than 10 miles from the aluminum plant contained an average of 31.3 ppm F^-. The amount of fluoride which was contained in the insects from the stomachs of those specimens which had been captured prior to 1952 provides still further control data. The three composite samples from the Anura averaged 37.1 ppm fluoride (range from 33.1-43.2 ppm F^-). These data indicate that the estimate by Carlson and Dewey (1971) for a background amount of fluoride in insects was too low. However, the data agree that insects which are collected nearer the aluminum plant contained more fluoride than those which were collected further away.

Gordon (1974) studied fluoride concentration in six species of mammals and birds which occur in the vicinity of the aluminum plant. The average concentration of fluoride in the femurs of control specimens was below 250 ppm F^-. The average concentration of fluoride in the femur bones of animals which had been collected around the aluminum plant ranged from 750 ppm F^- in grouse and ground squirrels through 1000 ppm F^- and 1100 ppm F^- in chipmunks and snowshoe hare, respectively, to 1325 ppm F^- in deer mice and 1500 ppm F^- in deer (Gordon, 1974). In all cases, the concentration of fluoride in the femur bones from experimental animals exceeded that of the control animals of the same species. Gordon (1974) reports that the "results of the fluoride analyses demonstrate that the rate of fluoride accumulation can vary considerably among species. These differences can be attributed to differences in life span, diet and seasonal activity".

Analyses of five R. pipiens, six R. pretiosa and six Bufo boreas which had been captured (and preserved in ETOH) before 1952 provide background levels of fluoride in the Anura from the northern Flathead Valley previous to the construction of the aluminum plant at Columbia Falls (Table 22). The average concentration of fluoride ranged between 158.0 and 248.3. These data are comparable to concentrations of fluoride in control specimens which were reported by Gordon (1974) (Figure 16). The notable difference between these data is that the amphibian and reptilian samples contain more fluoride than the other samples but remain below the 250 ppm level of fluoride.

TABLE - 22

FLUORIDE CONTENT OF SELECTED SPECIES OF ANURA FROM THE NORTHERN FLATHEAD VALLEY BEFORE 1952

Species	N	Total Length (cm)			Ppm F^-		
		max.	mean	min.	max.	mean	min.
Rana pretiosa	6	19.7	15.6	13.3	335.6	248.3	122.3
Rana pipiens	5	19.5	16.8	15.5	225.5	158.0	102.4
Bufo boreas	6	22.7	18.7	12.3	456.3	241.3	121.8

Comparison of mean concentration levels

The average concentration of fluoride by _R. pipiens_ from the various sites fluctuates near the average concentration of the pre-1952 specimens with the exceptions of specimens from MacWenneger Slough and Cedar Lake (Figure 17).

The area which is drained by MacWenneger Slough may contribute enough fluoride to increase the concentration in the environment of the frogs. The probable application of fertilizer which contains a fluoroapatite to the surrounding farm land contributes to the concentration of fluoride in the area and consequently in the food web. The proximity of Cedar

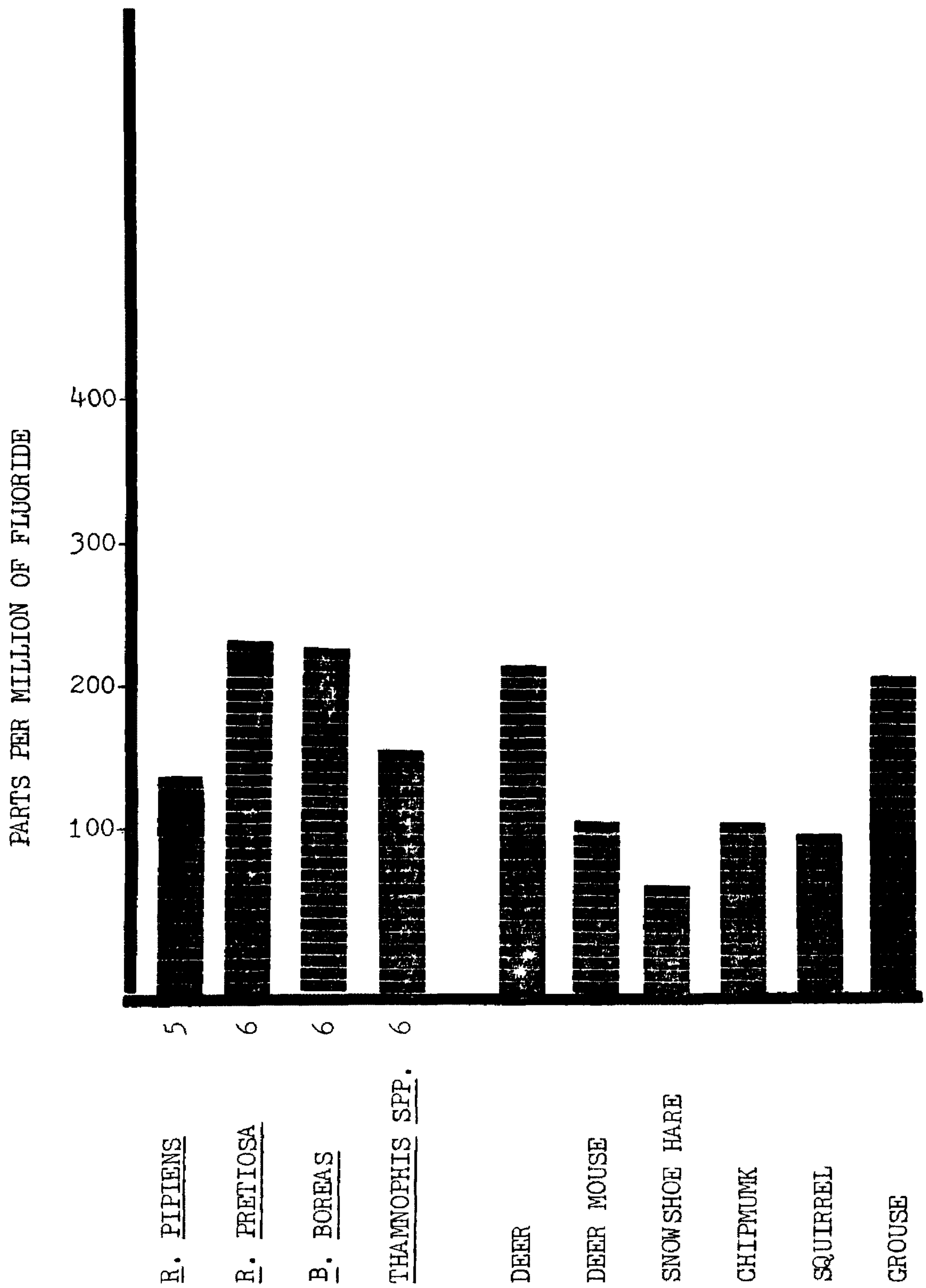


Figure 16. Comparison of pre-1952 control species to control species of Gordon (1974).

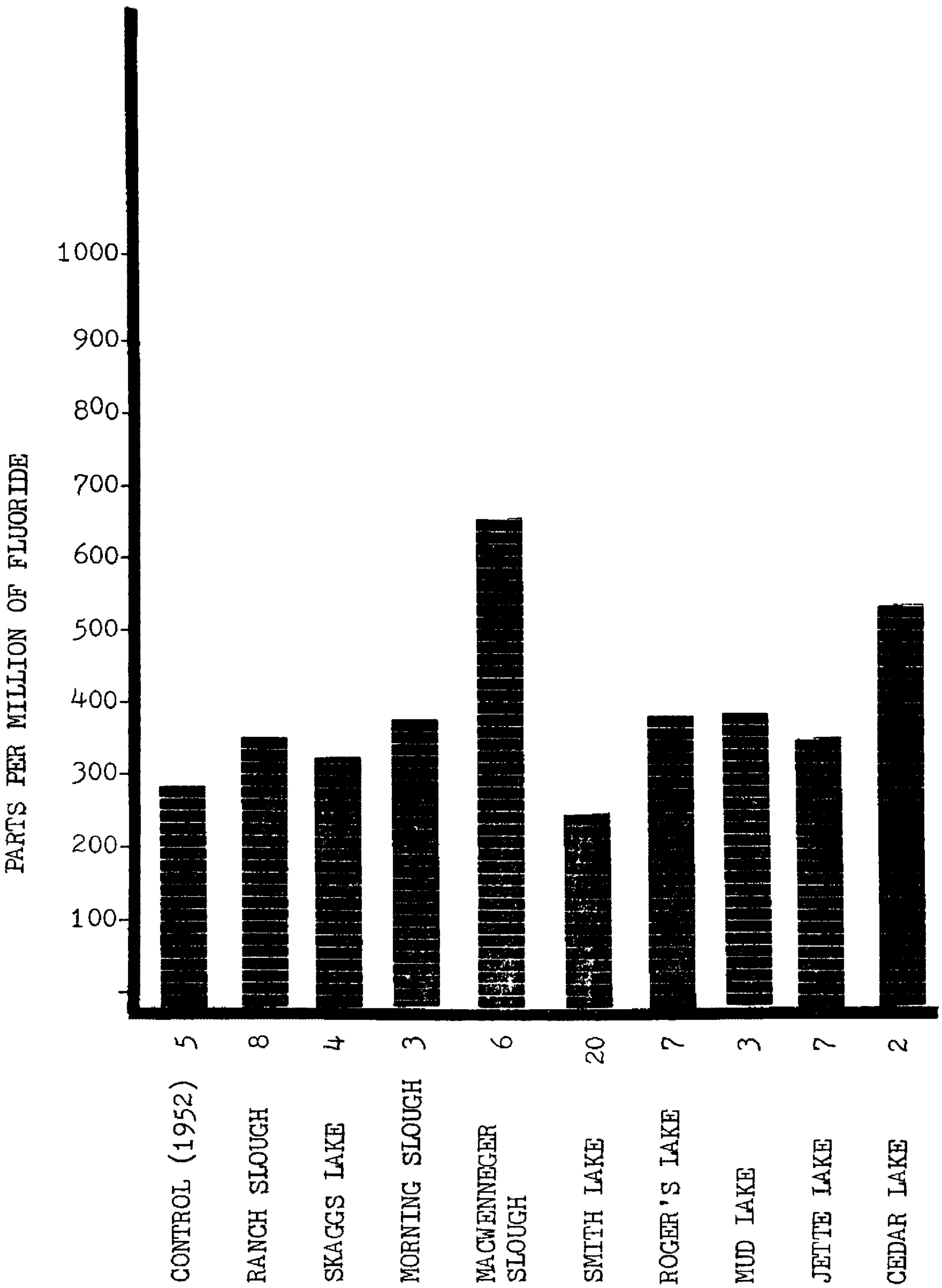


Figure 17. Mean fluoride content of the bone of *Rana pipiens*.

Lake to the aluminum plant accounts for the concentration of fluoride in frogs from the vicinity of the lake. The distribution of fluoride as shown by studies by the E.P.A. (1972) and others support this contention.

Areas such as Ranch Slough, Skaggs Lake, Morning Slough, Smith Lake, Roger's Lake and Jette Lake in which the concentrations of fluoride are close to the pre-1952 level reflect the variation which may be expected in natural systems. Slight influence of farming and airborne fluoride may be included in the concentrations but cannot be separated without detailed analyses of fluora and fauna from particular localities.

The average concentration of fluoride by R. pretiosa shows a greater fluctuation from the pre-1952 level (Figure 18) than R. pipiens does. Differences between the concentrations of fluoride by leopard frogs and spotted frogs which were collected at the same site mainly reflects differences in the ages of the specimens and differences in the number of each species which were analyzed but may also include slight differences in habitat utilization, food, and metabolism. The influence of these, however, cannot be determined in the field. Examples of differences in interspecific concentration occur at Cedar Lake, Mud Lake and, to a lesser extent at Roger's Lake

The difference between the concentration of fluoride by R. pretiosa from Lion Lake and Doris Creek corroborates the concept of sheltering by the local mountains. Lion Lake is located to the northeast of Badrock Canyon through which part of the airborne fluoride is carried. Doris Creek is situated on the southwestern shore of Hungry Horse Reservoir and is sheltered by Columbia Mountain (Map 1).

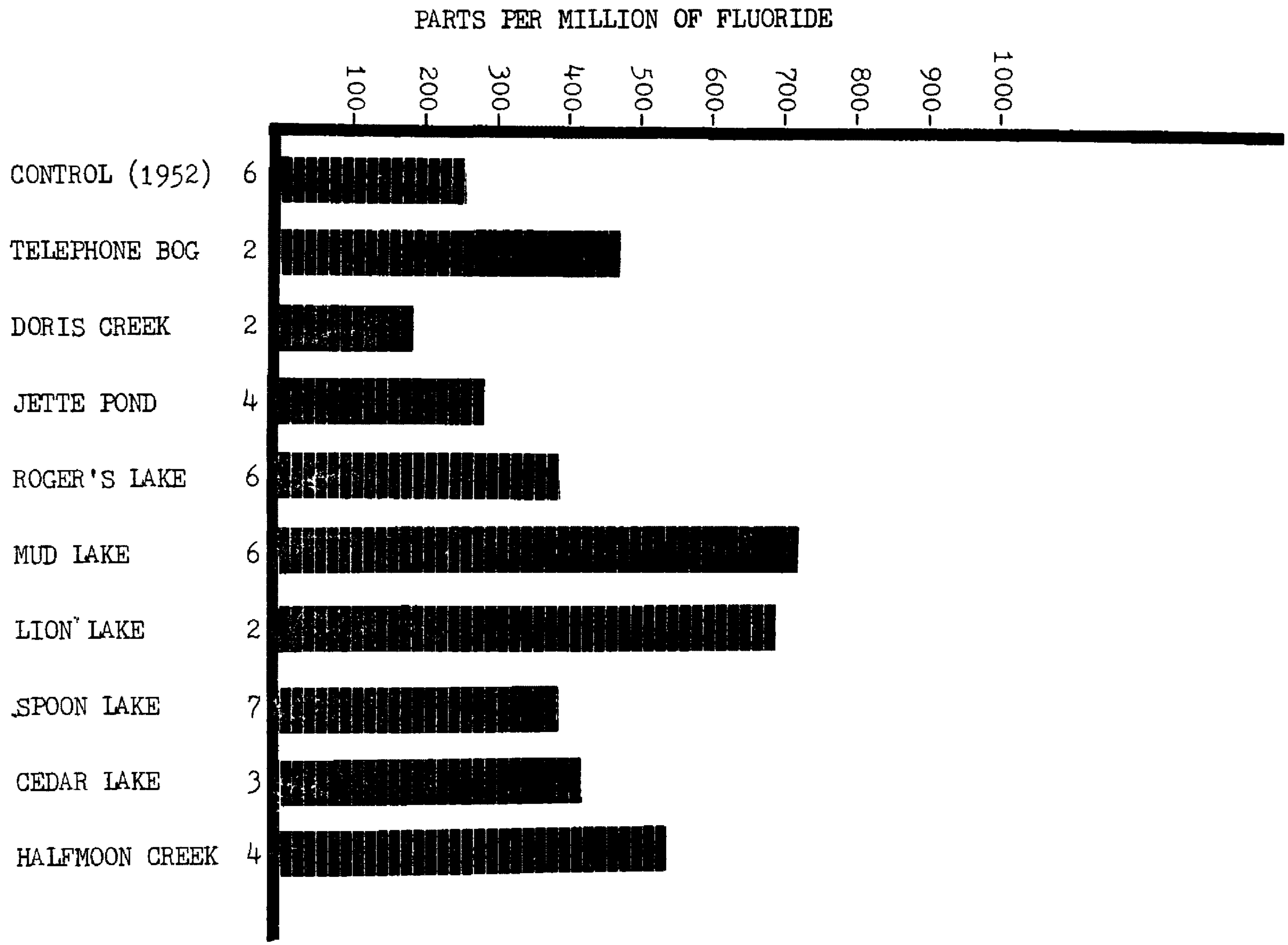


Figure 18. Mean fluoride content of the bone of *Rana pretiosa*.

The range of variation of the mean concentration of fluoride in Thamnophis spp. is similar to that of the toad (Figures 19 & 20). Specimens from Mud Lake, Spoon Lake, and Brenamen's Marsh contained higher concentrations of fluoride than specimens from other sites. The actual mean concentration of fluoride tend to be higher than those for the ranids.

Transmission of fluoride along the food chain

The accumulation of fluoride in animals which are carnivorous throughout their life has been noted by Carlson and Dewey (1971), and Gordon (1972).

The food web of the riparian community includes both terrestrial and aquatic organisms. The complexity of the interrelationships is compounded because many organisms function on several trophic levels. Frogs and toads are herbivorous as larvae and carnivorous as adults; turtles are both herbivorous and carnivorous throughout their lives. Similar distinctions based on food habits can be made for a variety of insects.

The comparison of the amounts of fluoride which are contained in organisms from several trophic levels at the same locality illustrate the tendency of organisms from higher trophic levels of the riparian community to concentrate fluoride. Selected groups of specimens from Roger's Lake, (Figure 21), Jette Lake, Jette Pond, (Figure 22) and Mud Lake, (Figure 23) provide examples of this phenomenon.

A group of miscellaneous food items (leeches, snails, and aquatic insects) from Roger's Lake contained less than 50 ppm of fluoride. Insects which have been removed from the stomach of the anurans contained

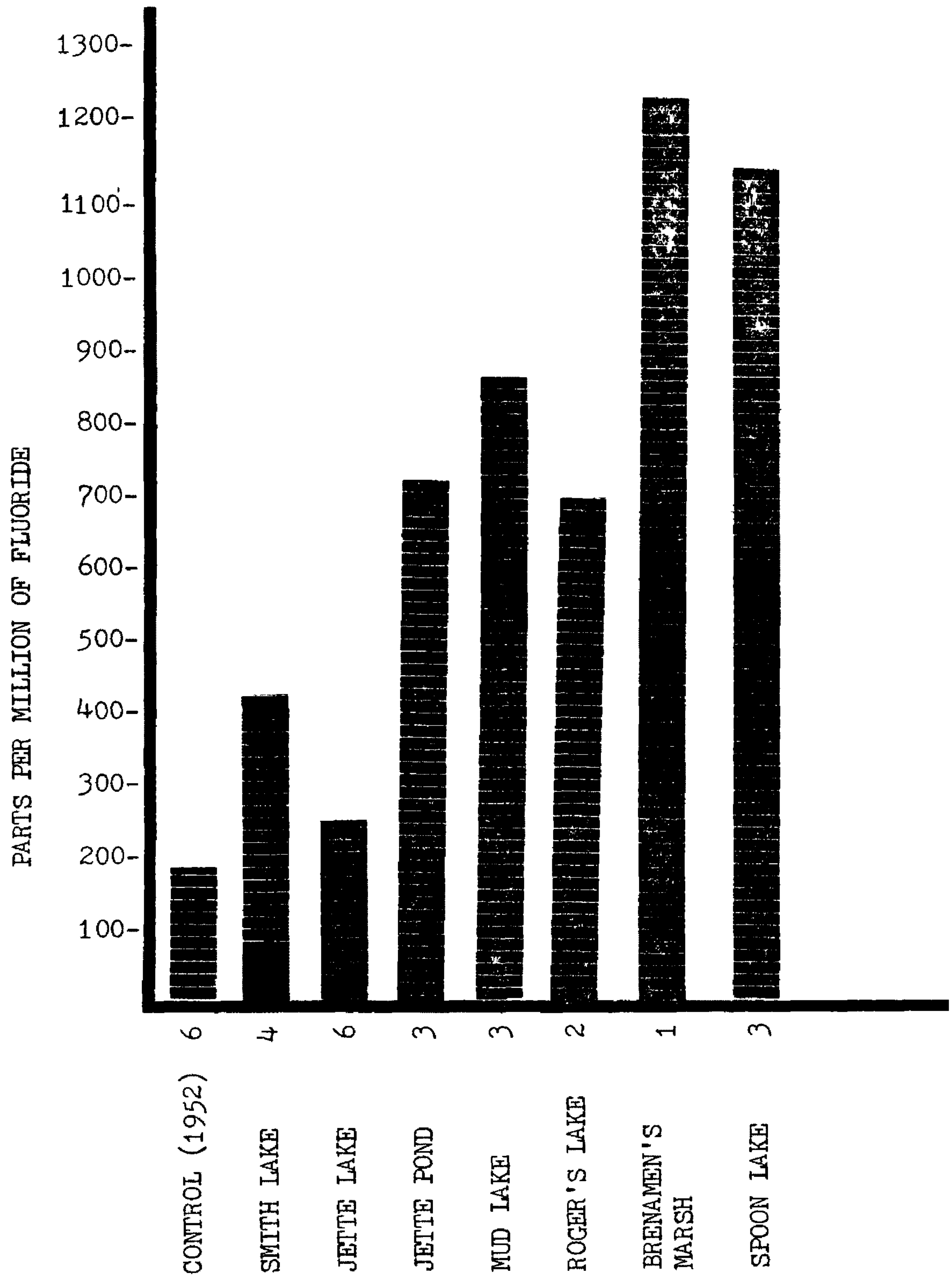


Figure 19. Mean fluoride content of the bone of *Thamnophis* spp.

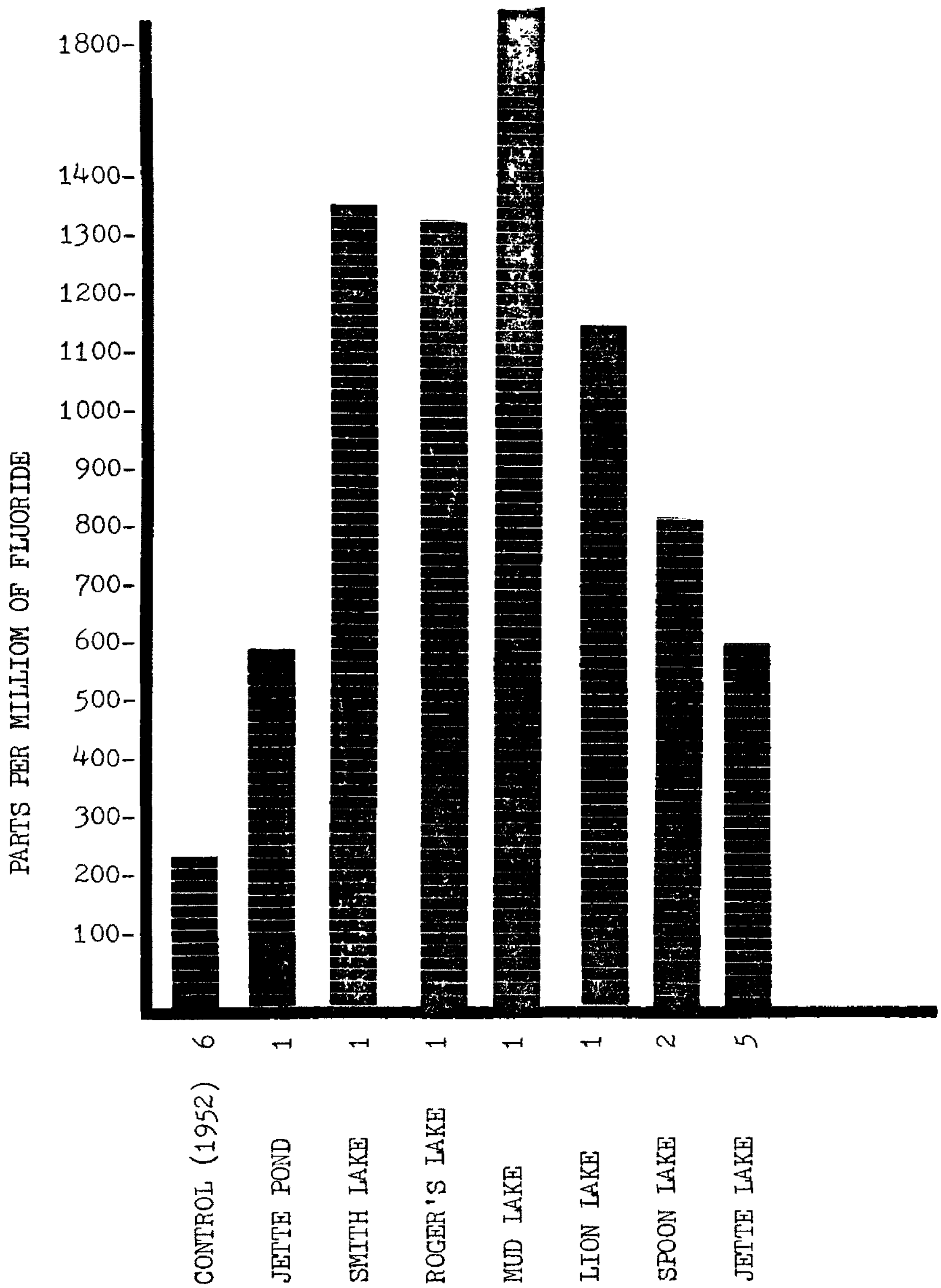


Figure 20. Mean fluoride content of the bone of *Bufo boreas*.

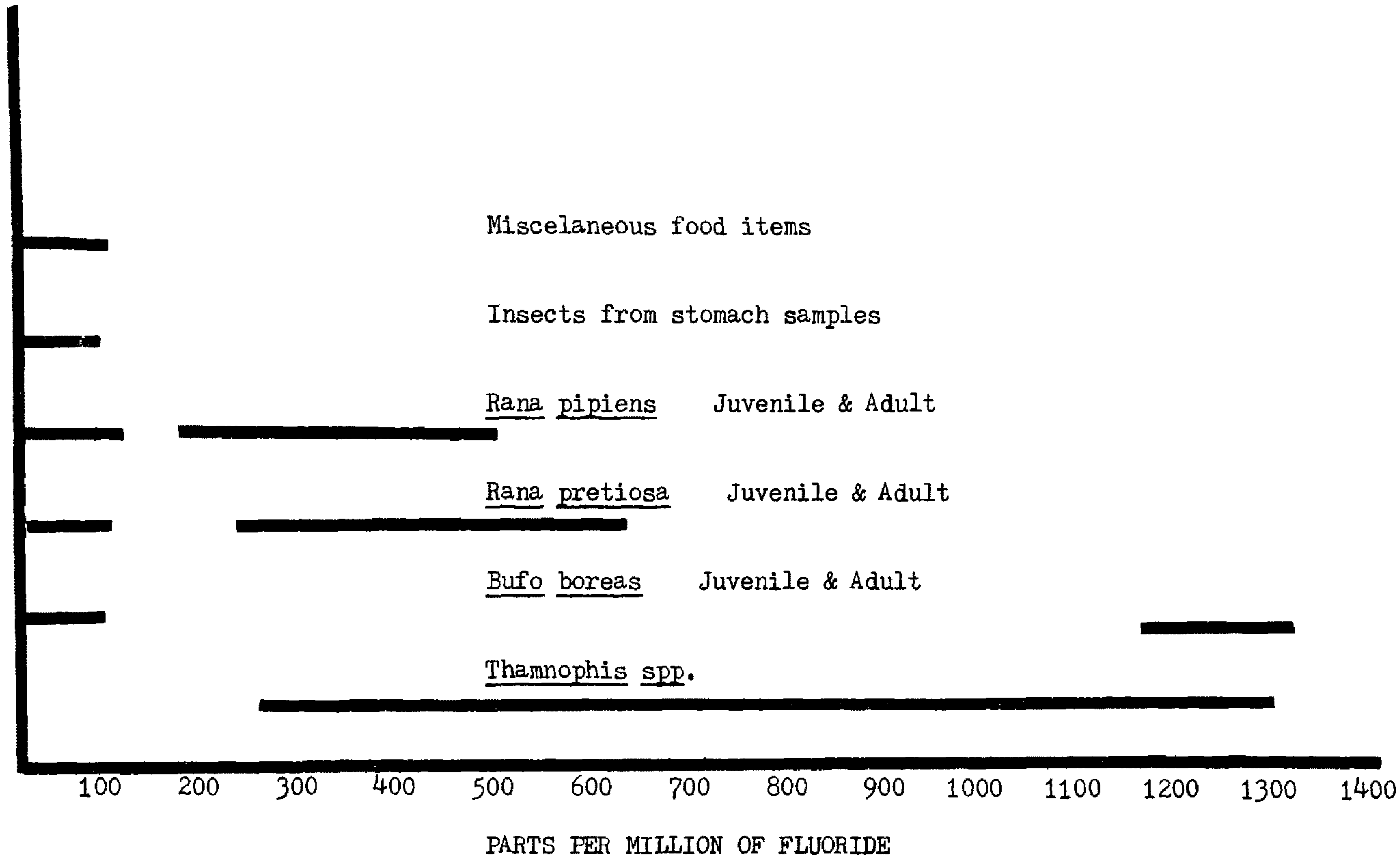


Figure 21. Fluoride content of selected species from Roger's Lake.

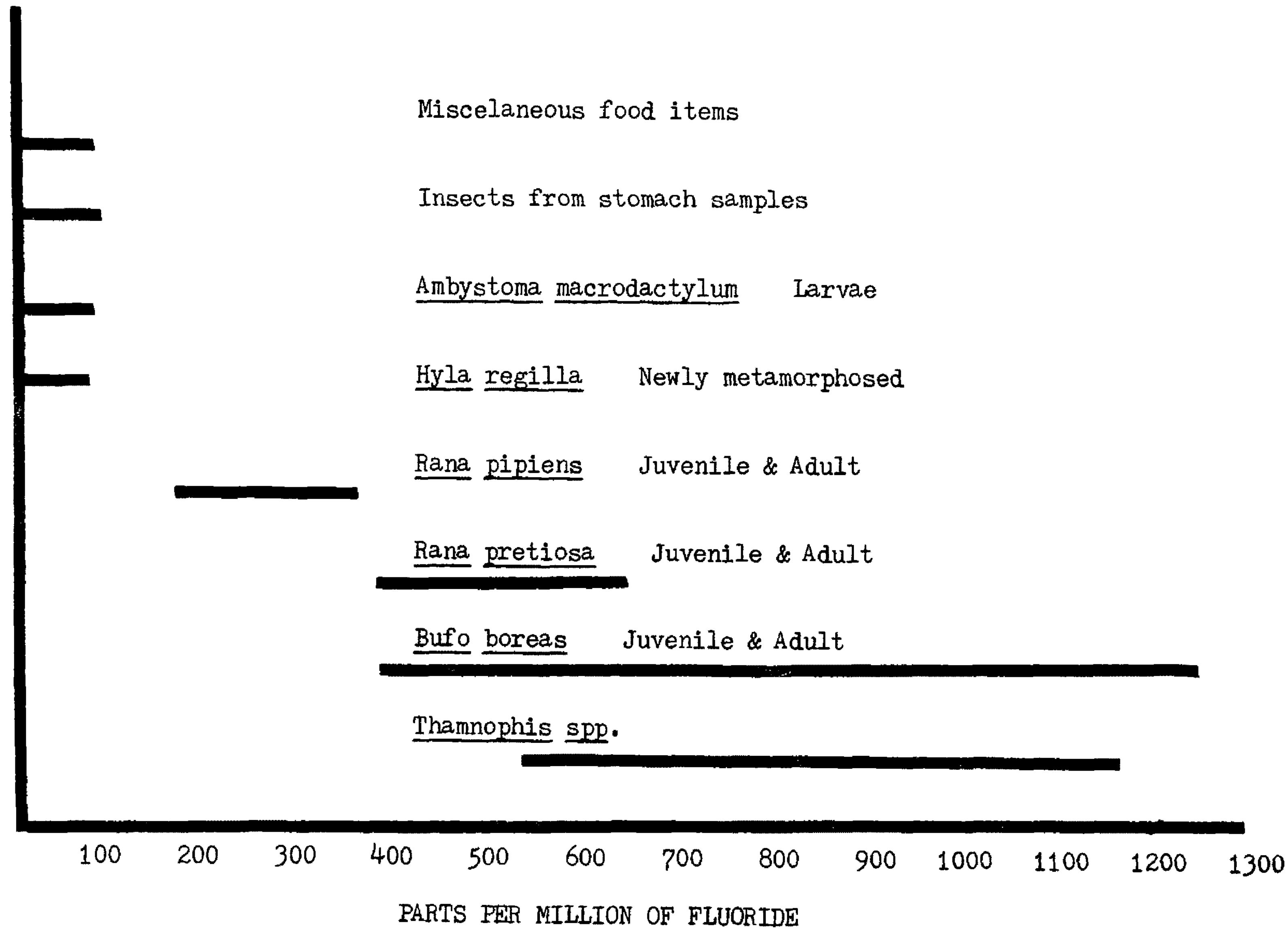


Figure 22. Fluoride content of selected species from Jette Pond and Jette Lake.

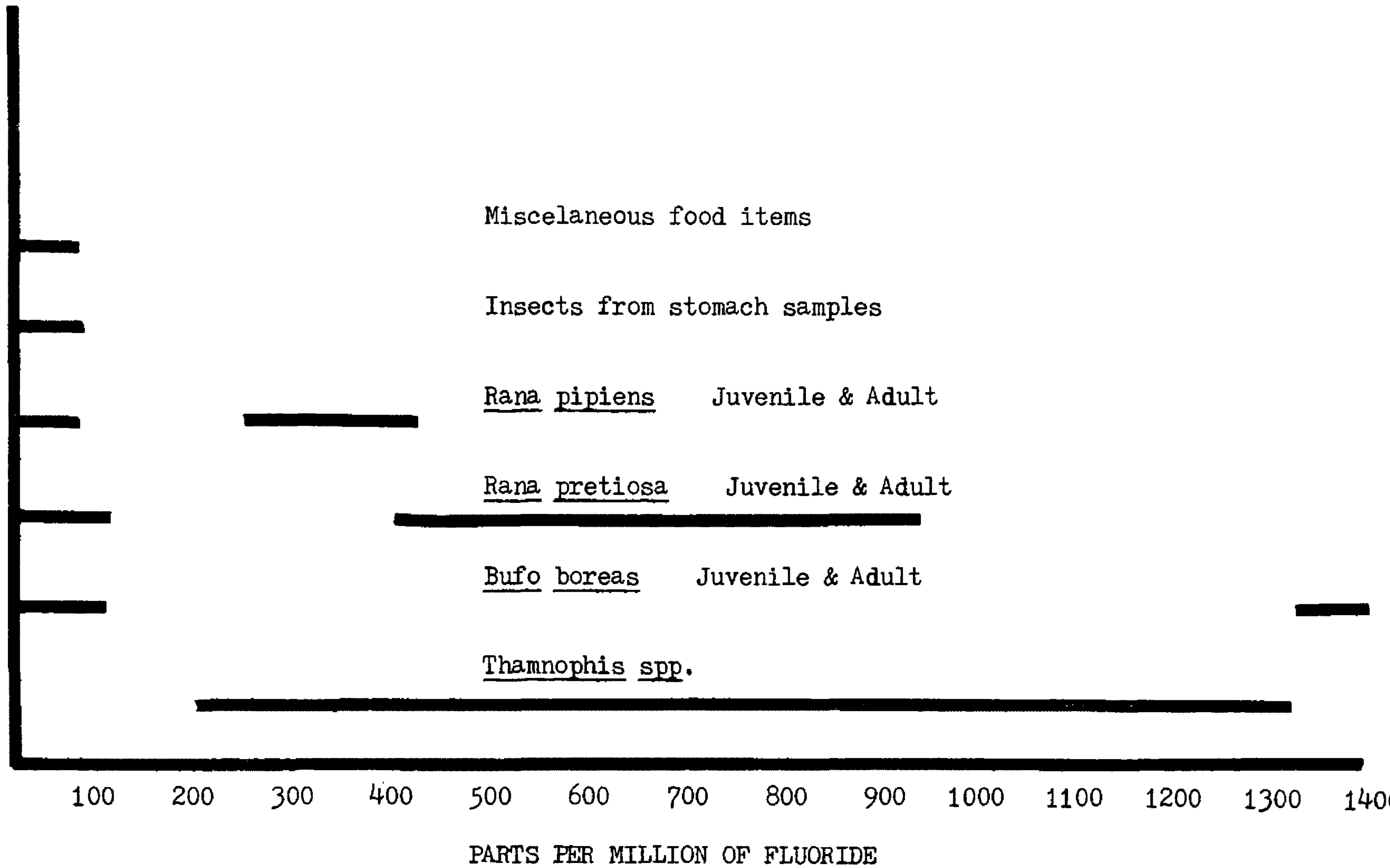


Figure 23. Fluoride content of selected species from Mud Lake.

less than 50 ppm F^-. Tadpoles of _R_. _pipiens_, _R_. _pretiosa_ and _B_. _boreas_ contained less than 100 ppm F^-. These organisms are primarily herbivorous. Adult frogs contained 140^+ ppm F^- to almost 600 ppm F^-. Adult toads contained up to 1200 ppm F^-. These organisms are primarily insectivorous. A group of juvenile and adult _Thamnophis_ _spp_. contained between 275 ppm F^- and 1200 ppm F^-. Garter snakes prey upon a variety of invertebrates, insects and small vertebrates.

Herbivorous and carnivorous animals from Jette Lake and Jette Pond follow an analogous pattern (Figure 22). The primary herbivorous organisms (leeches, snails, aquatic and terrestrial insects, and larvae of _Ambystoma_ _macrodactylum_, _R_. _pretiosa_, _R_. _pipiens_, _B_. _boreas_ and _Hyla regilla_) contained less than 100 ppm F^-. The carnivorous forms (juvenile and adult _R_. _pipiens_, _R_. _pretiosa_ and _B_. _boreas_) contained between 150 ppm F^- and 1220 ppm F^-. The toads extend the upper limit of the range because they tend to accumulate fluoride throughout their lives and tend to live longer than the ranids. Members of the two species of _Thamnophis_ had accumulated between 545 ppm F^- and 1100 ppm F^-.

Further corroboration of the transfer of fluoride between trophic levels is provided by specimens from Mud Lake (Figure 23). Herbivorous forms, such as snails, slugs and insects and larval forms of _R_. _pipiens_, _R_. _pretiosa_ and _B_. _boreas_, contained less than 100 ppm of fluoride. Primary carnivores, juvenile and adults _R_. _pipiens_ and _R_. _pretiosa_, contained between 200 ppm F^- and 900 ppm F^-. One _Bufo_ _boreas_ contained over 1700 ppm F^- which reflects the longevity of the species. The amount of fluoride which was present in secondary carnivores, _Thamnophis_ _spp_., ranged between 250 ppm F^- and 1350 ppm F^-.

The concentration of fluoride by members of succeeding trophic levels raises the question of whether standards based upon vegetation are adequate to protect animals. The accumulation of fluoride by vegetation has resulted in measurable damage to economically important species of trees (Carlson and Hammer, 1974). The accumulation of high amounts of flouride and fluorosis have been reported in a number of herbivorous species including deer (Gordon, 1974) and cattle (Suttie, 1964; Sharpe, 1972; Shupe and Alther, 1966). It is reasonable to assume that the accumulation of fluoride beyond a non-specified level may adversely influence the normal activity of members of the riparian ecosystem.

CHAPTER VI

SUMMARY

Sympatric and allopatric populations of _R_. _pipiens_ and _R_. _pretiosa_ illustrate the versatility and limitation of each species, in addition to providing information on the dynamics of these populations. Several generalizations may be stated which help to define the types of interactions and mechanisms which reduce the interactions which may be expected. In sympatric situations competition for food occurs. This is supported by the similarity in the taxonomic composition and the number of prey as recorded from the stomach samples. Both frogs actively feed during the day and early evening. No real differences occur intraspecifically in the types of food items which are utilized between sympatric and allopatric situations. Most differences which do exist reflect the distribution of the insects more than the feeding habits of the frogs. Competition for food is slightly reduced by the ability of leopard frogs to eat larger prey, by its relatively aggressive feeding habits, and by the large number of prey which are available. Competition for space occurs in some situations. The simultaneous collection in the riparian habitat of both ranids attests to this. Slight separation between the species is achieved as a result of the ability of leopard frogs to inhabit warmer water and the ability of spotted frogs to inhabit cooler water. The distribution of each species is influenced by temperature.

Bufo boreas does not compete directly with the ranids for food as a result of temporal separation which occurs. Toads feed primarily from the ground in the terrestrial sector whereas the ranids feed from plants and are found primarily in the riparian sector. More temporal separation is achieved as a result of the preference of the boreal toad for cooler temperatures. Further separation occurs as the result of the periods of activity of the species. The ranids are more diurnal and crepuscular whereas the bufonids are more crepuscular and nocturnal.

The accumulation of fluoride by amphibians and reptiles is governed by several principles. First, those animals which are exposed to higher concentrations of fluoride accumulate more. Second, those species which live longer continue to accumulate fluoride throughout their lives. Third, larger (older) specimens contain more fluoride than smaller (younger) specimens from the same locale. Fourth, food organisms contribute to the concentration of fluoride in the bone.

It is interesting to note that toads tend to contain more fluoride than frogs of the same size from the same site. Frogs tend to contain approximately $\frac{1}{2}$ of the probable total concentration of fluoride at metamorphosis. Toads tend to contain less than $\frac{1}{4}$ of the probable total concentration of fluoride. The rate of feeding, rate of growth, longevity, metabolism and permeability of the skin of each species influences the the uptake and retension of fluoride.

Turtles are, for the most part, herbivorous and they tend to accumulate relatively large quantities of fluoride in their bones. Snakes are carnivorous and also tend to accumulate relatively large quantities of fluoride in their bones. It should be noted that the longevity and metabolism of the organism greatly influences the total amount of fluoride in the bone tissue. Turtles live many years and accumulate fluoride throughout the period even though the amount of fluoride in their food is low. Snakes have shorter life spans but their food contains more fluoride. The presence of large amounts of fluoride in snakes which are carnivorous throughout their lives indicates that fluoride is passed between trophic levels and that each successive level concentrates a bit more fluoride.

Amphibians and reptiles are of limited use in establishing the extent of fluoride pollution in a particular area. Because the ranids are restricted to moist environments, the locations which are available to be sampled may not reflect the range of pollution as shown by other species which are more ubiquitous. The boreal toad accumulates fluoride in different amounts than the other species of Anura which were studied, as do the turtles and snakes. The habitats which are utilized by amphibians and reptiles may afford extensive shelter from airborne fluoride. Therefore dependence upon one species of amphibian or reptile may not illustrate the extent of pollution in an area. However, the use of multiple species of amphibians and reptiles will illustrate general trends and corroborate studies of other organisms, both plant and animal.

Because they are transitional between major habitats and are, as adults, first and second level carnivores, the herpetofauna of an area is useful in the establishment of the transfer of pollutants between tropic levels and between members of food webs.

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