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# Radioecological Techniques for Herpetology, with an Emphasis on Freshwater Turtles

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## Abstract

Radioisotopes, in concert with other techniques, can address important ecological research questions. In this chapter we review the literature regarding the historic uses of radionuclides in herpetology, with an emphasis on freshwater turtles. Two of the classic techniques—the estimation of field metabolic rates using doubly labeled water and the determination of feeding rates of free-ranging animals using $^{22}Na$—have had limited success on freshwater turtles; we describe why and suggest possible alternatives. The chapter also contains sections on (1) a comparison of fallout radionuclide concentrations found in turtles to those found in other organisms; (2) the relative radiosensitivity of turtles; (3) the use of radioactive tags in studies concerned with population dynamics; (4) energetics, feeding-rate estimations, and metabolic research, including the uptake and elimination rate of specific isotopes; (5) kinship determinations using radionuclides; (6) the use of radioisotopes to determine the functional aspects of ecosystems; and (7) miscellaneous topics, including the use of radioactive tags for rate-of-passage measurements in the gastrointestinal tract. An appendix presents a short description of radionuclide kinetics. Overall, this chapter provides an introduction to the field of radioecology and, specifically, acquaints the reader with the utility of radioisotopes in herpetological research.

## Introduction

Over the past 40 years the use of radionuclide techniques in the biological and physical sciences has increased dramatically (Schultz and Whicker, 1982). A discipline called radioecology has developed that (1) uses radioisotopes as tracers to study biological or ecological pro-

cesses, (2) determines the accumulation and movement rates of radioisotopes in the environment, and (3) studies the effects of radiation on organisms, populations, communities, and ecosystems. In the study of ecosystems these techniques have led to advances in the understanding and measurement of primary productivity, food-web dynamics, nutrient cycling, and various abiotic processes (e.g., water flux, sediment movement). Population ecology has been enhanced by the development of radioactive tracer techniques that allow important parameters such as home range size and age-specific dispersal to be estimated. At the level of the individual, radiological techniques have allowed numerous physiological and behavioral questions to be addressed.

In this chapter we review the literature regarding historic uses of radionuclides in the study of freshwater turtles, particularly the yellow-bellied slider, *Trachemys scripta scripta*. We also describe the primary radiological techniques and summarize how they may be used in the study of turtle populations. It is interesting that certain radiological techniques, which have been used successfully in the study of terrestrial organisms, have not worked for aquatic turtles; we describe why and suggest viable alternatives. In general, there is a paucity in reptilian, particularly turtle, radiological research relative to the volume of work conducted on mammals and fish. Consequently, we examine radiological studies of other ectotherms and homeotherms to depict the potential use of a given technique on turtles. We also compare turtles with other organisms with respect to sensitivity to radiation and the uptake and elimination of radionuclides. The lack of data limited comparisons and forced us to concentrate on two widely studied radionuclides in mammalian species: cesium-137 and strontium-90. Strontium, a chemical analogue of calcium, accumulates mainly in bone, whereas cesium, because of its similarity to potassium, resides primarily in muscle. Both nuclides are relatively mobile within the environment and have been the subject of substantial research because of their worldwide distribution as a result of fallout from aboveground nuclear testing and inadvertent releases from nuclear production facilities.

Radioecological research usually deals with one of four classes of radioactive materials. The first is the naturally occurring primordial nuclides and their daughter products, such as the decay chains of uranium-238 and thorium-232, each of which contains radioactive isotopes of many different elements. The parent nuclides have half-lives on the order of $10^9$ years and are found at low levels in soils. The disequilibrium of the parent nuclides with their daughter products often allows their use in determining the age of biological materials and studying geochemical cycles (Ivanovich and Harmon, 1982). If nuclide migration within a "system" (as defined by the researcher and representing a single organ, an organism, a community, or an ecosystem) has not occurred for a time that is long, relative to the half-life of the parent nuclide, then the activities (the transformation rate of radioactive elements) of all the daughter nuclides should be equal to the activity of the parent (i.e., secular equilibrium). However, chemical and physical processes in most environments cause differential migrations of elements, resulting in breaks in the radioactive decay chains. Under these circumstances, a member of the series is separated from its parent and subsequently decays at a rate determined by its own half-life. The activity of the parent nuclides is no longer equal to the activities of the daughters (i.e., disequilibrium), and relationships correlated to chronology may then be developed.

The second broad area of radioecological research is centered around irradiation of organisms or communities with high-energy gamma sources. This technique has been used to determine the dose required to cause various kinds of damage to individuals and to determine effects on populations and communities. Results have been summarized for plant communities by Whicker and Fraley (1974) and for animal populations by Turner (1975).

The third area involves administering radioactive tracers to organisms to determine percent assimilation and the subsequent elimination rate of the tracer or to determine transfer rates between system compartments (Whicker and Schultz, 1982). The behavior and movement of stable elements can also be determined through the use of radioactive tracers. Such techniques document not only the movement of materials but, more important, the rate of movement; thus one can examine the functional aspects of a system. Transfer rates between system compartments can then be used in environmental-transport or bioaccumulation models to understand and predict fundamental processes. The use of this technique with regard to turtles will be described in detail later in this chapter. It is important to realize that with the sensitivity of today's instruments, the quantity of a tracer necessary for measurement is often incredibly small (typically less than $10^{-15}$ g, with a level of radioactivity comparable to that found in the household smoke alarm).

The fourth class of radionuclides used in radioecological studies is those present in elevated levels as a result of anthropogenic releases from nuclear production facilities, reactors, weapons testing, mining and milling, waste disposal, and so on. One can often make positive use of these circumstances in ecological research. For example, in the preface of *Forevermore: Nuclear Waste in America*, Barlett and Steele (1985) described a scenario in which "the turtles that creep along the banks of the Savannah River near Aiken, South Carolina, are radioactive." Although this is a sensational statement, it is true that some turtles in the area (but probably very few, if any, in the Savannah River) contain elevated levels of radionuclides as a result of the operations of the Savannah River Plant (SRP), a nuclear fuels production facility located near Aiken. Pop-

ulations of these contaminated turtles have been the subject of substantial research, advancing our knowledge of turtle ecology with respect to long-distance terrestrial movement (Morreale et al., 1984), seasonal changes in field metabolic rates (Scott et al., 1986), and radionuclide kinetics (Peters, 1986). Many of the data presented in this chapter were generated from studying these populations.

## Use of Radioisotopes in Turtle Research

We categorize our discussion of the radioecological studies of turtles into eight sections that include a compilation of radionuclide concentrations in turtles and other organisms, a discussion of the radiosensitivity of these organisms, and various topics for which radiological techniques provide a useful research tool. These include terrestrial movement in turtles, metabolic studies, feeding rates in free-ranging animals, kinship studies, ecosystem analysis, and miscellaneous topics.

Before a discussion of the various radionuclide techniques can be meaningful, some background knowledge of kinetics is essential (details are presented in Appendix 21.1). In the present context, "kinetics" refers to the transfer of radionuclides into, within, and out of a system. Radionuclide kinetics depend upon numerous extrinsic and intrinsic factors. Extrinsic factors include the type of radionuclide, geochemical characteristics of the environment, and temperature. Important intrinsic parameters are body size, age, sex, metabolic rate, and physical condition (Reichle et al., 1970). Processes whereby an organism can accumulate radionuclides are, therefore, affected by properties of the radionuclide, the organism, and the ecosystem (Whicker, 1983).

### RADIONUCLIDE CONCENTRATIONS

Radiological assessment and general understanding require determining the concentrations of isotopes within organisms. This information can give us insights into (1) the relative accumulation of isotopes by comparing radionuclide concentrations among organisms, (2) the behavior of radionuclides within a system, (3) potential food-chain transport, and (4) dose estimates to the organisms from the internally deposited nuclides. Comparisons among organisms are seldom straightforward, because it is often not known if the reported values represent equilibrium conditions. This is particularly true for $^{90}$Sr, which may take a long time to reach equilibrium in calcareous tissues. It has been shown that radionuclide concentrations can be affected by season (Scott et al., 1986), age (McClellan et al., 1962; Della Rosa et al., 1965), temperature (Nakahara et al., 1977; Storr et al., 1982), body surface area and/or mass (Richmond, 1958; Stara et al., 1971), chemical nutrient content in the environment (Agnedal, 1967; Whicker et al., 1972), and metabolic rate (Reichle et al., 1970). Concentrations of fission products in the biota from fallout also vary with year and geographical location (Whicker and Schultz, 1982).

Holcomb et al. (1971) were the first to report levels of radioactivity in the exoskeletons of turtles. They examined $^{90}$Sr concentrations from nuclear fallout in the shells of eight species ($N$ = 102) from six southeastern states. Individuals varied in $^{90}$Sr concentrations from 0 to 10.4 Bq/g bone ash. (A Becquerel, or Bq, is the International System of Units measurement of radioactivity equal to one disintegration per second.[1]) Holcomb noted a tendency for specimens from Florida ($N$ = 7) to differ from specimens of other regions in $^{90}$Sr concentrations. Jackson et al. (1974) also analyzed $^{90}$Sr levels in turtles from the Southeast ($N$ = 73) and found geographic differences in concentrations. Consistently low concentrations were found in Florida species taken from a fast-flowing spring habitat of limestone origin. The authors attributed this to the possible removal of $^{90}$Sr fallout by ion exchange and the quick transport of direct fallout away from the area by the rapid flow of water. With the exception of the limestone spring habitat, aquatic turtle species exhibited reasonably uniform concentrations.

Species differences in radionuclide concentrations can be related to differences in diet. Hanson (1967) has shown that caribou generally contain higher levels of $^{90}$Sr than other ungulates because of the caribou's consumption of lichens. Lichens absorb radionuclides from air and precipitation with remarkable efficiency and thus contain radionuclide concentrations higher than most other vegetation. There is some evidence that the gopher tortoise (*Gopherus polyphemus*) accumulates higher concentrations of $^{90}$Sr than other species of turtles. This is shown in Figure 21.1, a graph of the pooled data sets of Holcomb et al. (1971) and Jackson et al. (1974) along with $^{90}$Sr concentration data for other organisms. The mean $^{90}$Sr concentration in the ashed shell of *Gopherus* is higher than in other turtles. Jackson et al. (1974) attributed this difference to the herbivorous diet of *Gopherus*, in that $^{90}$Sr levels are generally higher in vegetation than in upper trophic levels, thus possibly explaining why the $^{90}$Sr concentrations are lower in the carnivorous and omnivorous turtles.

Fallout concentration levels of $^{90}$Sr in turtles are similar to those found in other organisms (Fig. 21.1). The exoskeleton of a "shelled mammal," the nine-banded armadillo (*Dasypus novemcinctus*), contained 0.4 to 4.6 Bq/g bone ash of $^{90}$Sr (Jackson et al., 1972). Strontium levels in an ectotherm (trout) from mountain lakes in Colorado

[1]The traditional, comparable unit of radioactivity is the picocurie (pCi), and 1 Bq = 27 pCi. For comparison, Bennett (1971) estimated the average daily dietary intake of $^{90}$Sr for humans in New York as 0.43 Bq. Strontium-90 concentrations in New York City tap water reached a peak of 0.08 Bq/l in 1963 (Eisenbud, 1973). The Department of Energy allows deer hunters to take home animals harvested on federal property if Cs body burdens are less than 3.7 Bq/g (Brisbin, pers com.).

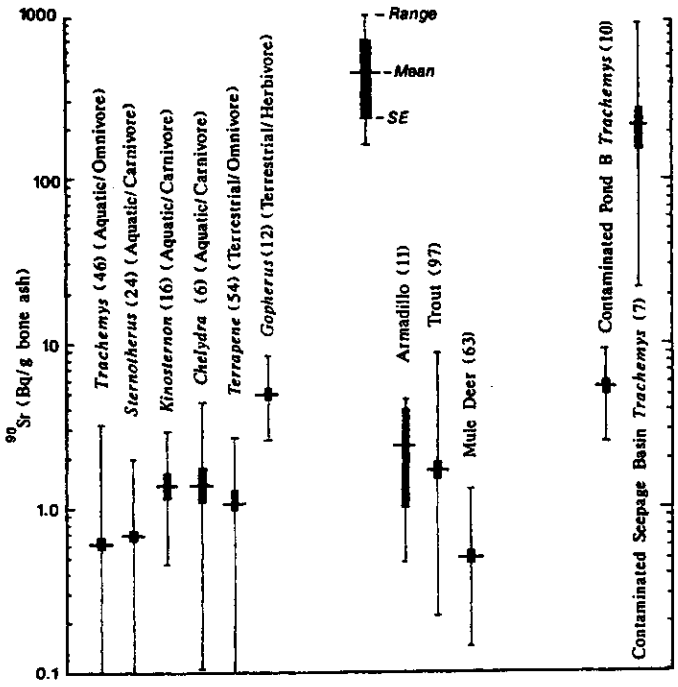


FIGURE 21.1. Fallout $^{90}$Sr concentrations (Bq/g bone ash) in turtles (from the pooled data sets of Holcomb et al., 1971, and Jackson et al., 1974), other organisms (armadillo, Jackson et al., 1972; trout, Whicker et al., 1972; mule deer, Whicker et al., 1967), and turtles from contaminated environments (Towns, 1987). Sample sizes are given in parentheses.

ranged from 0.2 to 8.8 Bq/g bone ash (Whicker et al., 1972), and the range found in Colorado deer was 0.1 to 1.3 Bq/g (Whicker et al., 1967).

Under conditions where the level of radioactivity is increased, turtles, like other organisms, assimilate higher concentrations of radioisotopes. Towns (1987) measured the Sr and Cs levels in *Trachemys* inhabiting a contaminated cooling reservoir (Pond B) on the Savannah River Plant. Strontium-90 concentrations ranged from 2.7 to 9.2 Bq/g bone ash with a mean of 5.6, three times the mean concentration found in turtles contaminated by fallout $^{90}$Sr (Jackson et al., 1974). Mean $^{137}$Cs concentrations were 0.9 Bq/g whole-body wet weight in the animals from Pond B (Towns, 1987).

Isolated contaminated seepage basins on the SRP have radioactivity levels considerably above the level of Pond B. Seven *Trachemys* from one such basin had mean $^{90}$Sr concentrations of 230 Bq/g bone ash, with a range of 20 to 930 (Fig. 21.1; Towns, 1987). Mean $^{137}$Cs concentrations were 5.7 Bq/g whole-body wet weight. To our knowledge, the highest measured $^{90}$Sr concentration in a turtle was in a 482 g female *T. scripta* captured in a contaminated seepage basin. Its radioactivity levels were above the quantitative limits of a Geiger-Müller counter (80,000 counts per minute). Subsequent analysis of the animal by the Du Pont company's Savannah River Laboratory revealed $^{90}$Sr levels of approximately 1,850 Bq/g bone ash. Analysis of $^{137}$Cs in muscle tissue yielded 63 Bq/g wet weight (Garrett, 1986). For comparison, the maximum $^{137}$Cs concentration from waterfowl collected on a radioactive leaching pond at the Idaho National Engineering Laboratory was 150 Bq/g flesh wet weight (Halford et al., 1981). Obviously, turtles are not unique in their ability to concentrate high levels of radiation. The point is that as the level of radionuclide exposure increases, so do the radionuclide concentrations in the biota. Not knowing the various levels of radiation exposure in the above discussion makes comparisons difficult. It appears from Figure 21.1 that under normal conditions turtles do not accumulate unusual concentrations (Bq/g) of radionuclides when compared with other organisms. However, the high proportion of bone tissue in turtles (≈42% of their total mass; Towns, 1987) will result in turtles' having a greater $^{90}$Sr total body burden (Bq) than unshelled animals of similar mass have. A more direct comparison is afforded by the examination of radionuclide concentrations in the biota from a single location and through the use of concentration ratios. Both concepts are explored further in the section Ecosystem Analysis, below.

### RADIOSENSITIVITY

In an attempt to determine the sensitivity of organisms to radiation, many researchers have established the "median lethal dose" by irradiating organisms and determining the dose at which 50% of them die within a specified time frame (e.g., 30 days in most mammalian studies, designated as $LD_{50[30]}$). Lethal dose experiments with reptiles and amphibians are generally carried out for a longer period to achieve 50% mortality ($LD_{50[90]}$ or $LD_{50[120]}$) because of a longer latency period (the time before which radiation effects are manifested). When animals survive for prolonged periods following irradiation, a median lethal dose based on 30 days gives falsely high $LD_{50}$ values (Sparrow et al., 1970; Willis and Lappenbush, 1975).

Differences in latency periods can be due to temperature. Cooler temperatures lengthen the mean survival time in exposed amphibians (Patt and Swift, 1948; Allen et al., 1951; O'Brien and Gojmerac, 1956) and hibernating ground squirrels (Barr and Musacchia, 1972). Other factors such as reduced radiation exposure, long mitotic cycle times, and slower rates of cell renewal are likely to lengthen the latency period (Patt and Quastler, 1963). Differences in laboratory versus field conditions and in the feeding regime of test animals also make $LD_{50}$ comparisons difficult. General trends do exist, however, and Whicker and Schultz (1982) have provided a relative ranking of radiosensitivities in various taxa (Fig. 21.2). Within taxa, the lower end of the range includes the most sensitive species and life stages, and the upper end generally represents adults of the most resistant species.

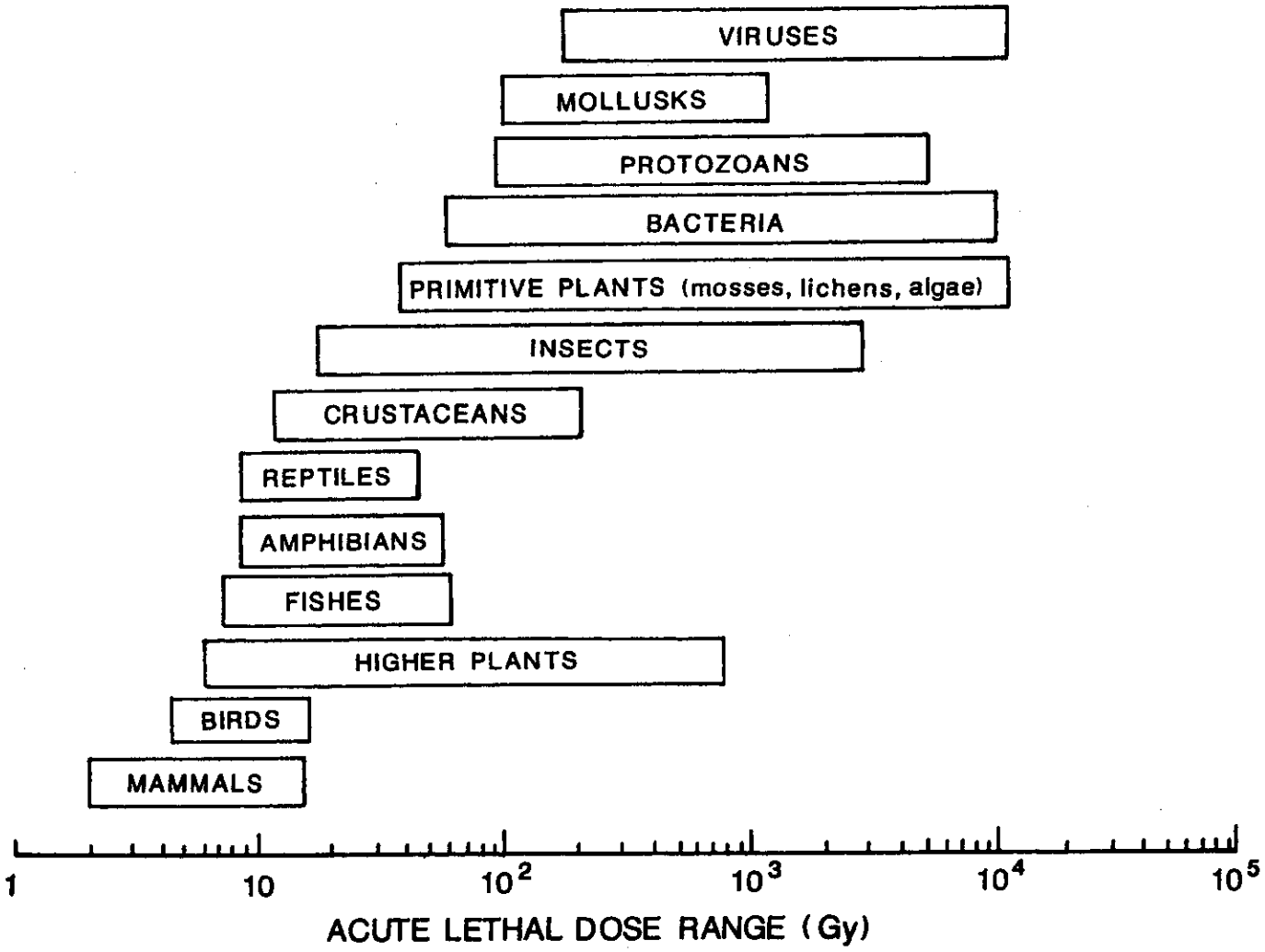


FIGURE 21.2. Radiosensitivities of various taxa. From Whicker and Shultz (1982).

Generally, reptiles are less sensitive to radiation than mammals are; however, caution should be applied when comparing individual species, because of the problems mentioned above. Comparative radiosensitivity, according to Sparrow et al. (1970), should be based on the lowest exposure required to reach a given end point without regard to the length of the postirradiation period. $LD_{50}$ values should be determined only after enough time has elapsed that most or all of the radiation-induced deaths have occurred. We have compiled the $LD_{50}$ values for turtles and representative values for other taxa (Table 21.1). Unfortunately, it is not possible to tell if the recommendations of Sparrow et al. (1970) were followed by the original researchers. It is particularly questionable on those groups of animals that were followed for only 30 days (lizards, ducks, and rodents). If radiation-induced deaths were still occurring after 30 days, then the reported $LD_{50}$ is too high. With these limitations in mind, the data from Table 21.1 suggest that if the herpetofauna were ranked according to radiosensitivity, turtles would probably occupy an upper position (i.e., they are more resistant).

An alternative approach in comparing the radiosensitivity of organisms is related to characteristics of the cell nucleus. The relationship of radiosensitivity of plants to nuclear volume, chromosomal volume, and DNA content is well documented (Sparrow, 1962; Sparrow and Sparrow, 1965). Increased sensitivity is related to a large nucleus volume, a large interphase chromosome volume, and a large amount of DNA per chromosome (Kaplan and Moses, 1964; Sparrow et al., 1967; Sparrow et al., 1968). The radiosensitivities of various amphibian species, which are known to have the greatest range of nuclear size of any vertebrate class, have been compared by Conger and Clinton (1973). Their results were similar to those found for plants: There is a positive correlation between nuclear volume and radiosensitivity.

Using this concept to examine the radiosensitivity of turtles was not productive. The DNA content per cell in turtles is similar to that found in a wide variety of other

Table 21.1. $LD_{50}$ values for various taxa

| Organism | $LD_{50}$ (Sv) | Postirradiation period (d) | Reference |
|---|---|---|---|
| Turtles | | | |
| Box turtle | 10-15 | – | 3 |
| | 10.3 | 120 (adult) | 10 |
| *Chelydra* | <8 | 120 (juvenile) | 10 |
| *Chrysemys* | <10 | 120 (juvenile) | 10 |
| *Terrapene* | 8.5 | 120 | 4 |
| Lizards | | | |
| *Sceloporus* | 15 | – | 8 |
| *Uta* | 10-12 | 30 | 5 |
| | 17-22 | 30 | 6 |
| Snakes | | | |
| *Elaphe, Coluber* | 3-4 | 90 | 4,10 |
| Frogs | | | |
| *Hyla* | 11.2 | 50 | 12 |
| *Rana* | 7 | 730 | 2 |
| | 7.2 | 730 | 9 |
| | 7.8 | 150 | 12 |
| Fish | | | |
| Goldfish | 8 | – | 1 |
| Salamanders | | | |
| *Desmognathus* | 5.3 | 70 | 9 |
| *Necturus* | 0.8 | 200 | 9 |
| | <2 | 180 | 12 |
| *Notophthalmus* | 4.7 | 150 | 12 |
| Ducks | | | |
| Blue-winged teal | 7.2 | 30 | 7 |
| Green-winged teal | 4.8 | 30 | 7 |
| Shoveler | 8.9 | 30 | 7 |
| Rodents | | | |
| *Citellus* | 12.6 | 30 | 11 |
| *Ochotona* | 3.8-5.6 | 30 | 11 |
| *Peromyscus* | 9.2-11.5 | 30 | 11 |
| *Rattus* | 9.5-13.3 | 30 | 11 |
| Humans | 3-6 | 30 | 13 |

*References:* 1, Ellinger, 1940; 2, Stearner, 1950; 3, Altland et al., 1951; 4, Cosgrove, 1965; 5, Dana and Tinkle, 1965; 6, Turner et al., 1967; 7, Tester et al., 1968; 8, Willis and McCourry, 1968; 9, Sparrow et al., 1970; 10, Cosgrove, 1971; 11, Sacher and Staffeldt, 1971; 12, Conger and Clinton, 1973; 13, Whicker and Schultz, 1982.

organisms (Shapiro, 1968). Given this overlap, it is not possible to predict the relative radiosensitivity of turtles based on their nuclear volume. However, based on this very limited data base (Fig. 21.2; Table 21.1; Shapiro, 1968), turtles do not appear to differ substantially from other reptiles, amphibians, or fish in sensitivity to radiation. If a larger data base were available, we suspect that turtles would be found to be less sensitive to external radiation than most of the other herpetofauna, if for no other reason than that the carapace provides an approximate 20% shielding (Cosgrove, 1971).

### TERRESTRIAL MOVEMENT

Numerous studies concerned with population dynamics have used radioactive tagging techniques to determine home range size and dispersal distances (reviewed in Schultz and Whicker, 1982). Pendleton (1956) described the advantages of marking animals with radionuclides as (1) the animal is generally unaware of the presence of the isotope; (2) isotopes are generally easy to apply to large numbers of animals; (3) the radioactive tag becomes part of the animal, with little danger of the identifying device's being lost; and (4) accurate observation can often be made without interference by the observer. Ferner (1979) also discussed radioactive tagging in his review of marking techniques for reptiles and amphibians. Radiotags are especially useful with burrowing animals, and because of the uniqueness of this technique, we have included a brief review of the findings. Karlstrom (1957) used radioactive cobalt ($^{60}Co$) wire as a tag to recover toads (*Bufo canorus*) in the field. Breckenridge and Tester (1961) documented the subsurface vertical migration of *Bufo hemiophrys* during hibernation by using radioactive tantalum ($^{182}Ta$) tags. Both radionuclides are high-energy gamma emitters that can be easily detected in the field with a Geiger-Müller counter or scintillometer. Breckenridge and Tester (1961) could detect an animal on the surface at a distance of 6 m, and an animal under 0.5 m of water was detected 1.5 m away.

Several studies have used $^{182}Ta$ tags to examine terrestrial movement of salamanders (Madison and Shoop, 1970; Shoop and Doty, 1972; Shoop, 1974; Semlitsch, 1981a; Semlitsch, 1983). Semlitsch (1981b) stated that the effect of the radioactive tag itself on the mole salamander (*Ambystoma talpoideum*) was not a problem for short-term studies (i.e., less than a month). He did, however, document discoloration of the skin area adjacent to 1.5 MBq $^{182}Ta$ tags 40 days after implantation. At 50–80 days, the skin around the tag ulcerated. By the end of 100 days, 83% of the salamanders had lost their tags. Both Ashton (1975), who also worked with salamanders, and Hirth et al. (1969), who tracked the dispersal of three snake species from a hibernaculum in Utah, reported tag loss due to ulceration. Neither Barbour et al. (1969) nor Madison and Shoop (1970) reported problems in their use of $^{60}Co$ tags (1.7 MBq) on *Desmognathus fuscus* and $^{182}Ta$ tags (0.7–1.8 MBq) on *Plethodon jordani*. Several studies on lizard species have successfully used similar tagging techniques (O'Brien et al., 1965; Fitch and von Achen, 1977).

Terrestrial activity of aquatic turtles was monitored by Bennett et al. (1970) using tantalum pins. The pins were easily attached in holes drilled in the carapace of three species (*Kinosternon subrubrum, Trachemys scripta,* and *Deirochelys reticularia*). Twenty-seven *K. subrubrum* were radioactively tagged, of which 20 were recaptured. Individuals generally moved short distances (m/day) and then burrowed below the litter (2–11 cm deep) but occasionally moved 10 m/day. Movement ceased in December. The technique revealed that *K. subrubrum* adults normally overwinter on land away from their aquatic habitat. Fewer *T. scripta* and *D. reticularia* were tagged, but in general these two species were found not to move as far from the site as *K. subrubrum*. Ward et al. (1976) used Bennett's

technique to determine seasonal habits of the spotted turtle (*Clemmys guttata*). At the start of the study, turtles were detected at distances as great as 10 m. The technique permitted Ward to locate estivating turtles burrowed under moist, loosely matted vegetation.

Morreale et al. (1984) measured radioisotope levels in previously contaminated *T. scripta* to examine the differences in long-range movements between males and females. Contaminated radioactive sediments and food items are present in some catchment basins on the SRP. Turtles inhabiting these basins incorporate varying levels of radioisotopes into their tissues. Before 1982, individuals could migrate from unfenced, contaminated sites to nearby natural aquatic sites. Thus, turtles with abnormally large radioactivity levels found in uncontaminated sites could be assumed to represent a relocation. This observation, coupled with 16 years of mark-recapture data, allowed Morreale et al. (1984) to assess sexual differences in movement. More males moved among bodies of water than females, and males moved greater distances. Fourteen of 15 radioactive turtles emigrating from catchment basins were males. In addition, females captured within the basins exhibited higher levels of radioactivity than males, possibly an indication of their tendency to be more sedentary. These results were interpreted as evidence for differences in the sexual strategy of males and females.

Generally, more information on field movements of organisms can be gained with radiotransmitter technology than with radioactive tagging. Current transmitter techniques often allow the researcher to obtain data on parameters such as body temperature and heart rate, as well as location of the organism. In current studies of turtle movements within and among populations, it is doubtful that radioisotope tracking techniques would be superior to conventional radiotransmitter methods. The exception to this may be in the study of hatchling dispersal from the nest cavity or in the study of small species such as *K. subrubrum*. Radiotransmitters are, in general, too large to be used on such animals, whereas some form of radioactive tag might be useful.

## METABOLIC STUDIES

Perhaps the greatest potential use of radioisotopes in turtle research is in energetics and metabolic studies. Such techniques may prove profitable in two areas of interest: (1) the determination of field metabolic rates and (2) the metabolic study of specific elements from which rate constants among system compartments can be determined. The prospects for both areas are outlined below.

FIELD METABOLIC RATES. Water containing stable and/or radioactive tracers has been used to measure water, energy, and material fluxes in many species of animals (Lifson and McClintock, 1966; Nagy, 1975). Water flux through an animal can be estimated with deuterium ($^2H$) or tritium ($^3H$). Tritium is easier to measure and is used in most studies. The $^3H$ is injected into the animal and allowed to equilibrate with the body water of the animal (which takes one to four hours, depending on the organism's size). Blood samples are then periodically taken from the free-ranging animal. The $^3H$ will decline through time because of water loss due to evaporation and excreta as well as the simultaneous dilution of the $^3H$ by metabolic water production and the intake of food and liquids (Nagy, 1982). The rate of decrease in the specific activity of $^3H$ is an estimate of the water flux. The $^3H$ is analyzed with a liquid scintillation counter after microdistilling the blood samples (Wood et al., 1975). Nagy and Costa (1980) believed that the tritiated water method can give estimates of water flux within ±10% of actual rates.

By introducing water labeled with heavy oxygen ($^{18}O$), in addition to $^3H$ or $^2H$, one can estimate $CO_2$ production (i.e., metabolic rate). The doubly labeled water ($^3H_2{}^{18}O$) is injected into the animal and allowed to equilibrate with the oxygen of $CO_2$ in the blood because of the action of carbonic anhydrase (Lifson et al., 1949). The specific activity of $^{18}O$ in the body water declines faster than that of the $^3H$ because $^{18}O$ is being lost in $CO_2$ as well as in $H_2O$ (Lifson and McClintock, 1966). Therefore, the difference in the rate loss of $^{18}O$ and that of $^3H$ is an estimate of the $CO_2$ production. Oxygen-18 content of the distilled blood samples is determined by proton activation, transforming $^{18}O$ into a gamma-emitting isotope of fluorine (Wood et al., 1975). Mass spectrometry can also be used to analyze the $^{18}O$ (Boyer et al., 1961), but the method is more difficult than the proton activation technique (Congdon et al., 1978).

The doubly labeled water method has been compared with other techniques and, according to Nagy (1987), has an accuracy comparable to the tritiated water method (±8%). Once the method is validated for the taxon of interest (Congdon et al., 1978), it affords an extremely powerful means of determining the energy metabolism of animals in their natural environment (Mullen, 1973). This technique has been used in several field studies of lizards (Nagy and Shoemaker, 1975; Bennett and Nagy, 1977; Congdon, 1977; Congdon et al., 1979; Congdon and Tinkle, 1982a; Green et al., 1986) and birds and mammals (reviewed by Nagy, 1987).

The technique has proved to be of little use in metabolic studies of aquatic turtles, however, because of their extremely rapid water flux. Preliminary studies of water flux in *Chrysemys picta* and *Trachemys scripta* revealed that turtles may turn over half their body water each day (Congdon, pers. com.). The concentration of doubly labeled water, therefore, is quickly reduced to levels below the detection limits of standard instrumentation. Until the technique or the instrumentation is further refined, the effective use of labeled water may be limited to nonaquatic species.

As an alternative to the doubly labeled water technique, elimination rates of radioisotopes may be used as a relative index of metabolic activity. Odum (1961) suggested this as a method to compare laboratory and field metabolic rates in arthropod populations. It has since been used with varying success in studies of field metabolic rates in small mammals (Orr, 1967; Baker and Dunaway, 1969; Pulliam et al., 1969; Chew, 1971). In general there appears to be a relationship, on a gross level, between energy use and elimination rates of some tracer substances. Pulliam et al. (1969) found significant correlations ($r^2 > .92$) between the biological half-time of $^{65}Zn$ in mice maintained at three different temperatures and the metabolic rates as estimated by respirometers and metabolic cages (Table 21.2). Biological half-times were significantly different ($p < .01$) between temperature groups but not between individuals within any one group.

A strong positive relationship between standard metabolic rate and temperature is well documented for ectotherms (Bennett and Dawson, 1976). The same relationship is true for the elimination rate of certain radioisotopes and temperature. A temperature drop from 15° to 5° C slowed the elimination of $^{137}Cs$ by a factor of 2 to 3 in freshwater fish (Kevern et al., 1964, in Hasanen et al., 1967; Hasanen et al., 1967). Hakonson et al. (1975) observed the biological half-time of Cs to increase in trout from about 100 days in summer to 850 days in winter. Gallegos and Whicker (1971) also correlated radiocesium elimination in trout with temperature. Similar relationships have been documented in marine organisms (O'Hara, 1968; Nakahara et al., 1977) and the freshwater clam (Storr et al., 1982).

As was shown in Table 21.2, Pulliam et al. (1969) obtained excellent relationships between temperature and $^{65}Zn$ elimination in the laboratory. However, when they looked at $^{65}Zn$ elimination in mice kept within outdoor enclosures, the predicted biological half-time based on the animal's mean field temperature was 12.0 days, whereas the actual measured half-time was 4.8 days. Obviously, temperature does not explain all of the variance in radionuclide elimination or metabolic rate, especially under field conditions. Elimination rates of radioisotopes have been shown to be related to food intake (Staton et al., 1974) as well as to ingestion rates of stable isotopes and analogous elements (Marey et al., 1967; Whicker, 1983). Staton et al. (1974) examined $^{137}Cs$ elimination in 32 captive snakes collected from contaminated habitats on the SRP. Elimination rates were positively correlated with caloric intake and exhibited a negative exponential relationship with body mass. Scott et al. (1986) found that the elimination of $^{90}Sr$ and $^{137}Cs$ in *T. scripta* varied according to season but was not governed solely by temperature. Free-ranging turtles that had previously incorporated $^{90}Sr$ and $^{137}Cs$ from contaminated sites were enclosed in an 18 × 20 m uncontaminated, outdoor, experimental pond with a maximal depth of 3 m, fed weekly ad libitum, and analyzed for radioactivity at two-month intervals. As expected, elimination rates were very low during winter periods, when activity was minimal and little, if any, feeding occurred (Fig. 21.3). Elimination rates increased dramatically during the spring period (April–June) but significantly decreased by a factor of 4 during the summer months, despite warmer water temperatures. The authors speculated that the higher spring elimination rates (and associated high metabolic rates) were probably related to reproductive behavior.

Table 21.2. Biological half-time of $^{65}Zn$ ($t_{\frac{1}{2}}$) and metabolic rate as estimated by respirometer ($\Delta_1$) and metabolic cages($\Delta_2$) for *Mus musculus* at three temperatures

| Temperature (° C) | N | $t_{\frac{1}{2}}$ (d) | $\Delta_1$ (kcal/g/d) | $\Delta_2$ (kcal/g/d) |
|---|---|---|---|---|
| 5 | 10 | 5.9 ± 0.7 | 0.39 ± 0.04 | 1.07 ± 0.06 |
| 10 | 10 | 6.4 ± 0.9 | 0.32 ± 0.01 | 0.94 ± 0.05 |
| 20 | 10 | 9.9 ± 0.5 | 0.22 ± 0.01 | 0.55 ± 0.05 |

*Source:* Pulliam et al., 1969.
*Note:* Means and standard deviation are given.

The elimination rate of radioisotopes, therefore, is probably a better estimator of field metabolic rates (FMR) than of basal or standard metabolic rates. Nagy (1987) stated that FMR includes "the costs of basal metabolism, thermoregulation, locomotion, feeding, predator avoidance, alertness, posture, digestion and food detoxification, reproduction and growth, and other expenses that ultimately appear as heat, as well as any savings resulting from hypothermia." All these parameters could conceivably affect the elimination of certain radioisotopes.

The usefulness in estimating an animal's metabolic rate varies with individual isotopes. Reichle and Van Hook (1970) termed tracers "biologically indeterminate" if their turnover at steady state proceeded at a rate proportional to the whole-body metabolism of the animal. The similarity between whole-body metabolism and tracer metabolism may be based on the relationship of both to body mass. Equilibrium amounts of a tracer in similarly exposed individuals from the same population are often related to body mass by the equation

$$Q_e = \gamma(W_i)^{\beta}$$

where $Q_e$ is the equilibrium amount of the tracer, $W_i$ is the body mass of individual $i$, and $\gamma$ and $\beta$ are fitted parameters (Eberhardt, 1969; Boyden, 1974; Fagerström, 1977). The metabolic allometric equation (Kleiber, 1975) is very similar:

$$M_i = a(W_i)^b$$

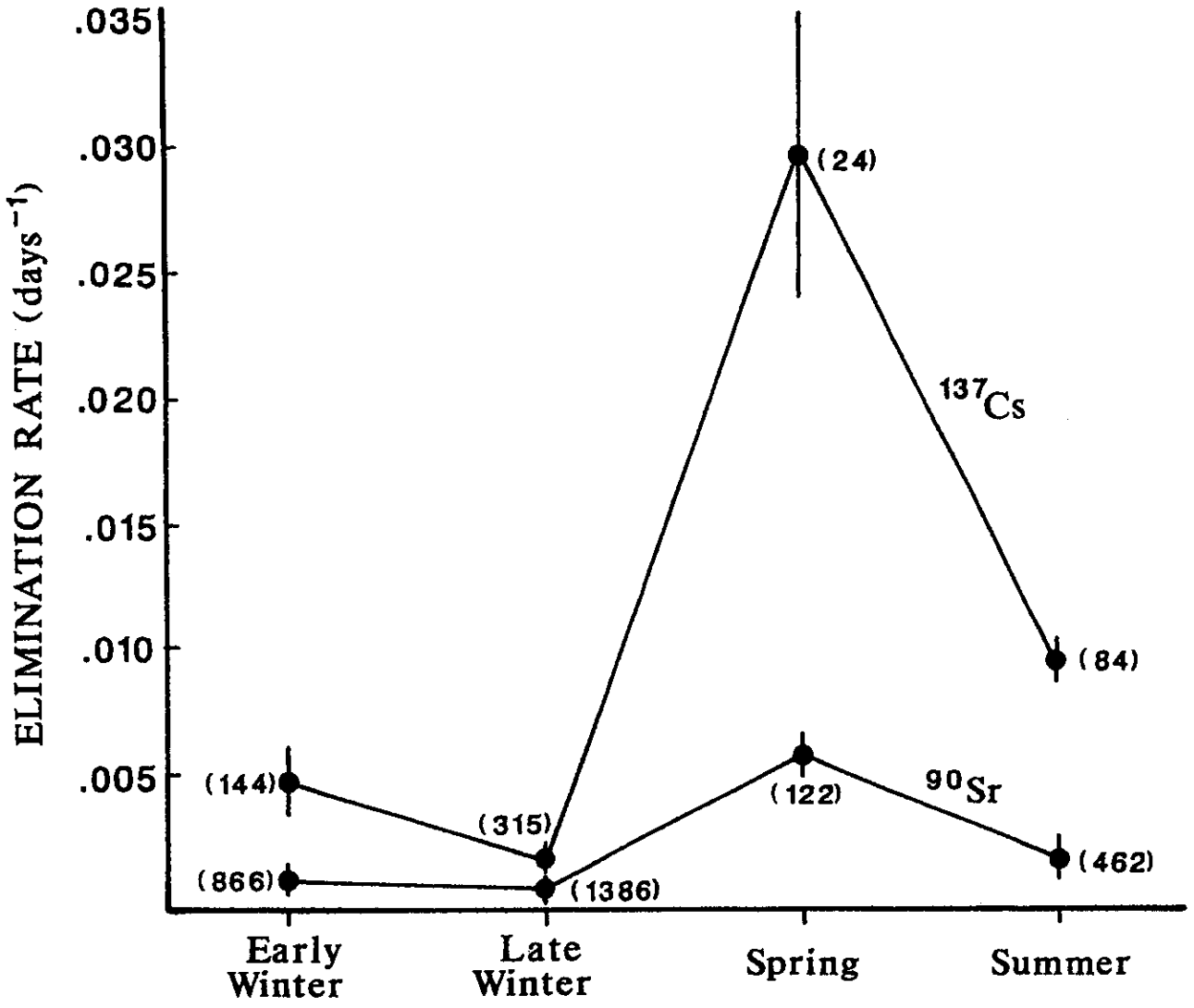


FIGURE 21.3. Seasonal differences in the elimination of $^{137}$Cs and $^{90}$Sr in *T. scripta* ($\bar{x} \pm 1$ SE). Numbers in parentheses represent the respective long-term biological half-times in days. Adapted from Scott et al. (1986).

where $M_i$ is some measure of steady-state energy flux, and $a$ and $b$ are fitted parameters. Fagerström (1977) derived equations to test whether a given tracer is biologically indeterminate and thus a feasible tracer of energy flow in ecological systems. He stated that, within a population of animals, the biological half-time, body burden, and whole-body concentration of an indeterminate tracer should be proportional to body weight raised to $(1 - b)$, 1, and 0, respectively, where $b$ is the exponent relating body weight to standard metabolic rate. The value for $b$ can be determined experimentally, or the commonly accepted value of 0.8 for $b$ can be used (Hemmingsen, 1950). If the relationships between body mass and the tracer parameters (biological half-time, body burden, and whole-body concentration) do not hold, it could be that (1) the system is not in equilibrium with respect to energy and/or the tracer, or (2) the turnover rate of the tracer is governed by the activity of a specific enzyme or organ rather than by whole-body metabolism (Fagerström, 1977). Cesium-137 and $^{65}$Zn have successfully been used in the past as realistic estimators of metabolic rate (Reichle et al., 1970) and may be particularly suited for situations where the doubly labeled water technique is inappropriate. However, we caution that at this time the relationship of metabolic rate to radionuclide elimination rate needs more rigorous experimental testing.

Stara et al. (1971) examined the metabolism of radionuclides in mammals and found a relationship between $^{137}$Cs long-term elimination and body mass. A plot of this relationship revealed that ruminants eliminate $^{137}$Cs more quickly than similar-sized nonruminants. Reichle et al. (1970) found that when the logarithm of cesium biolog-

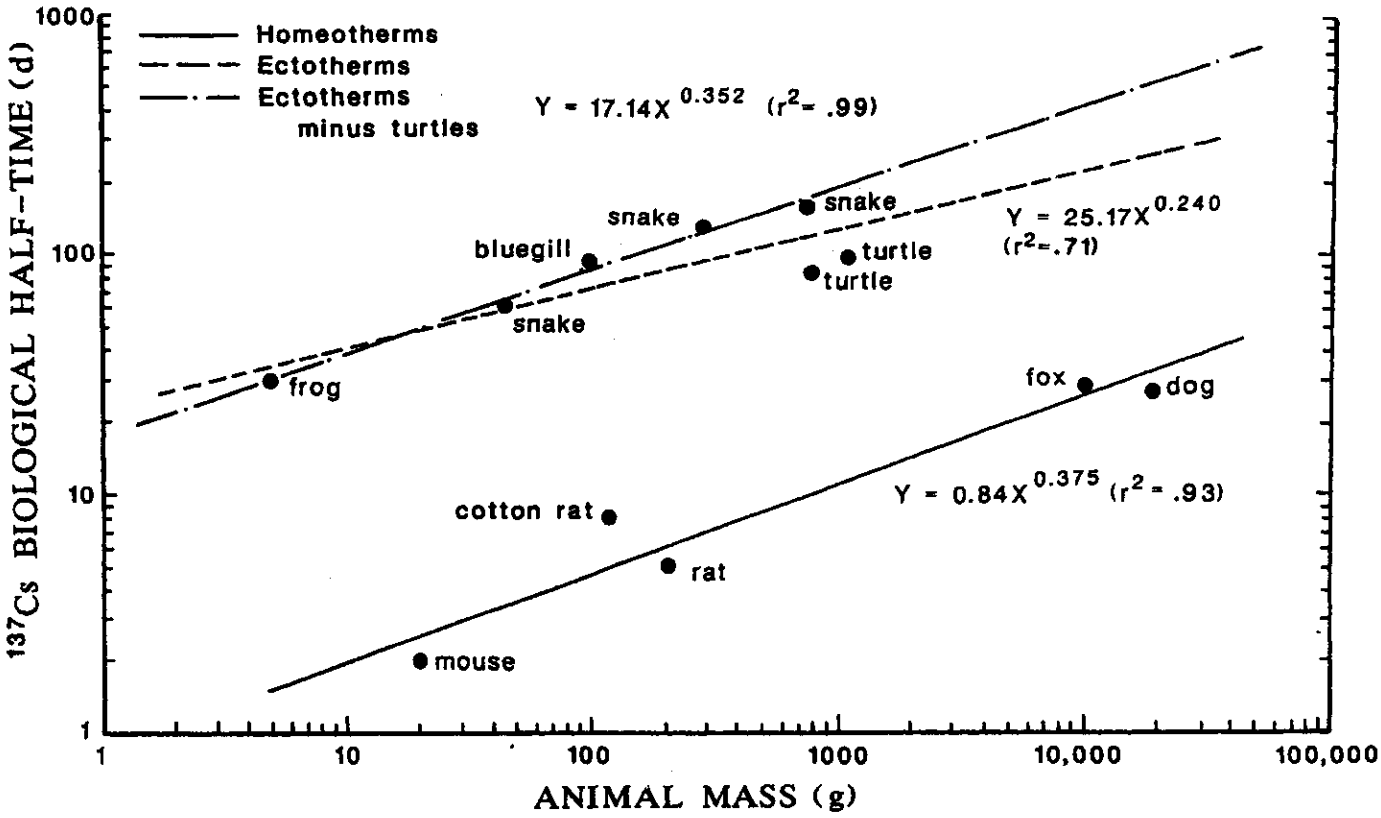


FIGURE 21.4. Relation between animal body size and $^{137}Cs$ biological half-time. Regressions given for homeotherms, ectotherms, and ectotherms minus turtles. Data are from Kitchings et al. (1976) and Table 21.3.

ical half-times is plotted as a function of the logarithm of animal mass, various groups of organisms fall into discrete patterns. Regressions yielded the general formula

$$\hat{y} = AX^b$$

where $\hat{y}$ is an estimate of the $^{137}Cs$ biological half-time, $A$ is the $y$-intercept of the regression line, $X$ is the mass of the animal in grams, and $b$ represents the slope of the regression line. The $^{137}Cs$ biological half-times for invertebrates (other than insects) and cold-blooded vertebrates were regressed together against body weight to yield

$$\hat{y} = 38.02X^{0.139}$$

Insects and warm-blooded vertebrates fell along a common regression line:

$$\hat{y} = 3.46X^{0.206}$$

Because the invertebrates have a large influence in the above regressions, we have compared the mass/(Cs half-time) relationships for vertebrate homeotherms and ectotherms separately (Fig. 21.4). The mammalian data come from Kitchings et al. (1976), who reviewed the literature and listed the masses of various animals with their respective cesium elimination rates. The ectotherm data come from portions of Table 21.3 (i.e., data sets where both $^{137}Cs$ half-times and animal masses were reported by the original authors). The snake data reported by Staton et al. (1974) were averaged for animals <100 g, 100–500 g, and >500 g. The cesium half-times reported by Kolehmainen (1972) for the bluegill were normalized to 25° C using a $Q_{10}$ of 2 (i.e., a 10° C increase in temperature doubles the elimination rate). Significant regressions were obtained for both groups (mammals, $p < .01$, $r^2 = .93$; ectotherms, $p < .01$, $r^2 = .71$). Analysis of covariance was used to test for differences between the two regressions and revealed significant differences between the intercepts ($p < .01$) but not between the slopes ($p = .17$). As suggested by Beauchamp and Olson (1973), one-half of the error mean square was added to the logarithm-transformed intercept estimate before converting the regression equations to their untransformed values. This corrects for possible bias obtained from using the antilogarithms and results in the following allometric equations (Fig. 21.4):

$$\hat{y} = 0.84X^{0.375} \text{ for mammals}$$

$$\hat{y} = 25.17X^{0.240} \text{ for ectotherms} \qquad (21.1)$$

Table 21.3. Long-term biological half-times ($t_{1/2}$) of $^{137}Cs$ in various organisms

| Organism | $t_{1/2}$ (d) | Comment | Reference |
|---|---|---|---|
| Invertebrate | | | |
| Oyster | 70-90 | – | 9 |
| Fish | | | |
| Bluegill | 187 | – | 7 |
| Perch | 175-200 | 15° C[a] | 2 |
| Carp | 174 | 12.5° C | 1 |
| | 98 | 20° C | 1 |
| Trout | 25-80 | 15° C[a] | 2 |
| | 74 | 5° C | 4 |
| | 69 | 12.7° C | 4 |
| | 49 | 18.3° C | 4 |
| Reptiles and amphibians | | | |
| Snakes | 24-430, $\bar{X}$ = 131 | Five species | 8 |
| *Trachemys scripta* | 315 | Winter | 12 |
| | 24 | Spring | 12 |
| | 84 | Summer | 12 |
| | 64 | Yearly average | 12 |
| | 96 | Summer | 11 |
| *Hyla* | 30 | Captive, unfed | 10 |
| Mammals | | | |
| Mice | 6 | Oral dose | 6 |
| Rats | 13 | Oral dose | 6 |
| Dog | 43 | IV dose | 6 |
| Mule deer | 14 | $^{134}Cs$ | 3 |
| Reindeer | 6-19 | –[b] | 5 |
| Monkey | 40 | IV dose | 6 |
| Man | 60-160 | IV dose | 6 |

*References:* 1, Kevern et al., 1964, in Hasanen et al., 1967; 2, Hasanen et al., 1967; 3, Hakonson and Whicker, 1969; 4, Gallegos and Whicker, 1971; 5, Holleman et al., 1971; 6 Stara et al., 1971; 7, Kolehmainen, 1972; 8, Staton et al., 1974; 9, Cranmore and Harrison, 1975; 10, Dapson and Kaplan, 1975; 11, Peters, 1986; 12, Scott et al., 1986.

[a]Older fish had longer Cs $t_{1/2}$.

[b]Cs $t_{1/2}$ varied with potassium intake.

Equation 21.1 overpredicts the mean $^{137}Cs$ half-time in turtles, as determined by Peters (1986) and Scott et al. (1986), by about 40%, although the prediction is still within their 95% confidence interval. This could be due to the low predictive power of the regression ($r^2$ = .71) or may reflect the presence of the turtle's shell and its effect on the reduced flesh mass to total mass ratio when compared with that of other organisms. The shell has previously been considered metabolically inert (Benedict, 1932; Hughes et al., 1971). Inclusion of such inert material, which constitutes ≈40% of adult *T. scripta*'s mass (Towns, 1987), could lead to an overestimation of the cesium biological half-times. However, reducing the turtles' total mass by 40% and then estimating the $^{137}Cs$ half-time according to equation 21.1 do not totally compensate the initial overprediction. Bennett and Dawson (1976) did not find significant differences in the resting metabolic rates (RMR) among lizards, snakes, and turtles. This suggested that either the shell is not metabolically inert or the metabolic rate of the other tissues in turtles is high enough to compensate for the inert characteristics of the shell. Bennett and Dawson (1976) suggested that the former explanation is more plausible. Towns (1987) found that 23% of *Trachemys*'s total $^{137}Cs$ body burden is in the shell and bones ($N$ = 14), indicating that the shell is at least partially labile to potassium-analogous elements.

It is obvious from Figure 21.4 that the two turtle data points are below those for the other ectotherms and influence the regression line. If the turtle data are eliminated from the analysis, the $r^2$ value increases from .71 to .99, and the probability value decreases from .018 to .001 (Fig. 21.4). This change also increases the similarity between the slopes of the ectotherm and homeotherm regression lines ($p$ values increase from .17 to .78). The ectotherm regression equation, minus turtles, becomes

$$\hat{y} = 17.14X^{0.352} \qquad (21.2)$$

It is interesting that the slopes of the homeotherm and ectotherm regressions are equal. This disagrees with what has been reported in the literature. Our data set, however, contains the widest range of ectothermic vertebrate taxa analyzed. Reichle et al. (1970) regressions included fish (perch, trout, bluegill, and carp), invertebrates, and one amphibian. Admittedly, our sample size is small, and more data are needed.

It is evident from Figure 21.4 that the biological half-time of $^{137}Cs$ in ectotherms is greater than in similar-sized homeotherms. A 250-gram ectotherm would retain $^{137}Cs$ between 14 and 17 times longer than a similar-sized homeotherm (depending on whether equation 21.1 or 21.2 is used, and assuming the ectotherm is in a 25° C environment). This compares to Nagy's (1987) finding that the FMR of iguanid lizards is about 17 times lower than the FMR of similar-sized mammals.

Radioisotopes such as $^{137}Cs$ may be useful relative indexes of metabolic rates but have not been adequately studied or validated to the extent and usefulness of the doubly labeled water methodology. This area needs more research. The comment of Odum and Golley (1963), who discussed the use of radionuclides as estimators of metabolic rates, is still pertinent: "The approach, even if difficult, is well worth investigation because the stakes are very high. At the present time we can see no other way to measure metabolic rate in completely free-living populations. Even approximate guesses are better than wild guesses. Until we are able to estimate these energy transformations we cannot hope to understand, much less manage intelligently, the ecosystems in which man, himself, is an integral part."

METABOLISM (UPTAKE AND ELIMINATION) OF SPECIFIC RADIOISOTOPES. To examine accurately the consequences of radioactivity in the environment, it is necessary to understand the transport and bioaccumulation of radioisotopes within systems. The complex properties of individual radionuclides, organisms, and the fundamental processes of ecosystems can lead to a wide spectrum of scenarios rang-

tic estimates of intake (Gallagher et al., 1983). Doubly labeled water is sometimes used to estimate food consumption (Minnich and Shoemaker, 1970), but as was discussed above, the technique has had limited success in the study of aquatic turtles. Mullen (1973) cautions that the doubly labeled water technique may be of little use in estimating food intake in the field because the diverse diet of most organisms prohibits a knowledge of the dry diet yield of metabolic $CO_2$.

Several recent studies have used the turnover of $^{22}Na$ to measure food intake in free-ranging animals (Green and Dunsmore, 1978; Williams and Ridpath, 1982; Gallagher et al., 1983; Green et al., 1986). Stable Na turnover can be estimated by measuring the elimination rate of $^{22}Na$ (Buscarlet, 1974), which is dependent on the amount of stable Na in the diet (Fairbanks and Burch, 1968). However, reliable estimates of food intake can be obtained only if the Na content of the diet is known and if the major source of Na is dietary. Dunson, who used radioisotopic methods to examine sodium flux, found that active uptake of Na from the water column occurs in at least two species of freshwater turtles (Dunson, 1964; Dunson and Weymouth, 1965; Dunson, 1966; Dunson, 1969). In *T. scripta* the cloacal region (cloacal bursae and cloaca) accounts for 48% to 68% of the sodium influx rate (Dunson, 1967). It is therefore unlikely that $^{22}Na$ turnover can be used to estimate food consumption in aquatic turtles.

The study of turtles inhabiting radioactively contaminated habitats may provide the best estimates of food intake in the field. Consumption rates can be determined by using the relationship between intake and elimination of a radionuclide when the concentration of the radionuclide in the animal is in equilibrium with its food source. The technique was first suggested by Davis and Foster (1958) and has since been used for numerous species (insects, Crossley, 1963; carp, Kevern, 1966; reindeer, Holleman et al., 1971; bluegill, Kolehmainen, 1972; trout, Hakonson et al., 1975). In its simplest form the technique uses the following model, which is also discussed in Appendix 21.1:


where $R_{in}$ is the intake rate of the isotope (Bq/time), $k$ is the loss rate constant (time$^{-1}$), and $q$ is the total body burden (Bq).

The change in $q$ over time ($dq/dt$) can be written as

$$\frac{dq}{dt} = R_{in} - kq$$

By integrating this equation and evaluating at time = 0,

$$q_t = \frac{R_{(in)}}{k}(1 - e^{-kt}) + q_0 e^{-kt} \qquad (21.3)$$

where $q_0$ is the radionuclide content of the organism at $t = 0$, and $q_t$ is the radionuclide content at any time $t$.

The intake of a radionuclide can also be written as

$$R_{in} = RCa \qquad (21.4)$$

where $R_{in}$ is the intake rate, $R$ is the rate of food consumption (g/day), $C$ is the concentration of radionuclide in food (Bq/g), and $a$ is the assimilation fraction (i.e., percentage of the ingested contaminant actually incorporated into the body).

If we substitute equation 21.3 into equation 21.4 and rearrange terms, we can determine the rate of food consumption ($R$) in g/day:

$$R = \frac{(q_t - q_0 e^{-kt})k}{aC(1 - e^{-kt})}$$

Estimates of $k$, $q_t$, $q_0$, $a$, and $C$ are required to solve for food intake rates and are obtained through techniques described in Appendix 21.1. A knowledge of radionuclide kinetics (uptake and retention) within the animal and the radionuclide concentrations of the food sources is therefore necessary. Cadwell and Schreckhise (1976) used a convolution integral to determine the consumption rate of $^{32}P$-labeled blue grama grass by grasshoppers. Their technique does not require that the concentration of radionuclide in the animal be in equilibrium with its food source. Successive calculations of the consumption rates were made at various points in time as the body burden changed because of continued feeding on labeled forage. Radionuclide-contaminated environments represent ideal situations where food consumption rates could be determined for free-ranging aquatic turtles.

### KINSHIP DETERMINATION

Field attempts to identify the young of a particular female of any organism are extremely difficult and time-consuming. In one long-term study of turtle populations at the E. S. George Reserve in Michigan, more than 1,500 man-hours are spent each nesting season in an attempt to associate individual females with their nests and subsequently with their hatchlings (Congdon et al., 1983b, 1987). Radioisotopes could conceivably reduce the labor necessary to acquire the same information and are potentially less disruptive. Radionuclides have been used in small-mammal populations to determine matrilineal kinship (Wolff and Holleman, 1978; Tamarin et al., 1983). Labeling captured females with a mixture of several gamma-emitting radioisotopes (each of which has a characteristic emission spectrum) allows subsequent identi-

fication of trapped offspring. The technique works well in small mammals because there is transfer of nuclides across the placenta or through milk to the young. It is unknown at this time whether there is sufficient material transfer from a reproductive female turtle to her follicles and embryos. However, our preliminary data indicate that Sr and Ca radioisotopes are transferred from females to their embryos. If feasible, the technique, in conjunction with the drift-fence capture of hatchlings, would allow identification of hatchling-mother relationships. It could also prove useful in the identification of individual nest predators.

### ECOSYSTEM ANALYSIS

Functional ecology (i.e., energy flow, nutrient cycles, and biological regulation) has lagged behind descriptive ecology. Functional ecology hinges on measuring the rate of change per unit of time, that is, measuring the degree of energy flow, not just the standing crop (Odum, 1962). Radiotracers have greatly extended our ability to analyze the functional aspects of ecosystems by providing a means of detecting actual movements of materials and of estimating cycling or turnover rates within the systems.

Such analysis can often give insights to an organism's particular niche within an ecosystem. Pendleton and Grundmann (1954) were among the first to use radioactive tags on an ecosystem level by examining an insect-predator complex and plant pollinator relationships. Odum and Kuenzler (1963) used $^{32}P$ to determine arthropod trophic-level positions and predator-prey interactions in an old-field ecosystem. They labeled three dominant species of plants and then examined the subsequent amounts and distribution of tracer in arthropod populations. By plotting the concentration of tracer per unit of biomass against time, graphic separations of certain trophic and habitat groups became evident. In addition to isolating specific sections of a food web, the authors were able to determine the energy source being used by specific heterotrophic populations. Similarly, Crossley (1963) used $^{137}Cs$ to examine insect-plant relationships, and Coleman and McGinnis (1970) used $^{65}Zn$ to describe the fungus-arthropod food chain quantitatively.

Gallegos et al. (1970) documented a somewhat unexpected non-food-web intake pathway of Cs in rainbow trout. They determined that the major source of $^{137}Cs$ for a population of trout living in a high mountain lake was not through food items but by ingestion of bottom sediments. Evidence for this phenomenon included the following: (1) The major food items of the trout did not contain enough $^{137}Cs$ to account for the observed levels in the fish, (2) surface sediment and detritus were sufficiently high in $^{137}Cs$ that less than 0.1 g would need to be ingested daily to account for the observed levels in the trout, and (3) sediment and detritus were observed in the intestinal tracts of the fish. It is interesting that in 1970 the levels of $^{137}Cs$ in the trout dropped to one-third the levels in 1969. Gallegos et al. (1970) attributed this to a significant reduction in the trout's primary food source, *Gammarus*, with a subsequent shift to *Daphnia*. *Daphnia* occur nearer the surface in open water and do not evade predation by swimming toward the sediment or vegetation as do *Gammarus*. This change in diet and subsequent feeding behavior of the trout probably reduced their intake of sediment and detritus.

Table 21.6. Concentration ratios (CR) for Pond B components

| Component | $^{137}Cs$ | $^{90}Sr$ |
|---|---|---|
| Filtered water | 1.0 (0.76 Bq/l) | 1.0 (0.14 Bq/l) |
| Sediment (0-3 cm) | $1.6 \times 10^4$ | $6.0 \times 10^2$ |
| Aquatic macrophytes | $2.4 \times 10^3$ | $6.4 \times 10^2$ |
| Benthic macroinvertebrates | | |
| Insect nymphs and larvae | $8.0 \times 10^2$ | $5.2 \times 10$ |
| Gastropods (snails) | $1.2 \times 10^2$ | $5.4 \times 10^3$ |
| Fish (five species) | $6.4 \times 10^3$ | $4.6 \times 10^3$ |
| Turtles (*Trachemys*) | $1.3 \times 10^3$ | $1.0 \times 10^4$ |
| Waterfowl (coots) | $2.5 \times 10^3$ | -- |
| Frog (*Rana*)[a] | $4.8 \times 10^2$ | -- |
| Snake (*Natrix*)[b] | $1.0 \times 10^4$ | -- |

*Source:* Whicker et al., 1984.

*Note:* CR = (Bq/kg organism wet weight)/(Bq/l filtered water).

[a]S. G. McDowell and R. U. Fischer, unpubl. data.

[b]T. G. Hinton and H. Zippler, unpubl. data.

Other research using radiotracers to analyze the functional aspects of ecosystems includes work by Richardson and Marshall (1986), who used $^{32}P$ to quantify the inflow, storage, and export of phosphorus in peat land, and Whicker et al. (1984), who are examining the dynamics of radioisotopes within the previously mentioned Pond B, a contaminated warm monomictic lake on the SRP. Whicker et al. (1984) have preliminary data on $^{137}Cs$ and $^{90}Sr$ concentrations within various compartments of Pond B (i.e., sediments, water column, macrophytes, benthos, fish, turtles, and waterfowl). Because the data are from a single system, many of the problems associated with comparing data from different sources (as discussed in the section Radionuclide Concentrations, above) are eliminated.

We have presented the data of Whicker et al. (1984) in Table 21.6), in the form of concentration ratios (CR). The CR for aquatic organisms is defined as

$$CR = \frac{\text{Bq/kg organism (wet mass)}}{\text{Bq/l water (filtered)}}$$

Concentration ratios are useful in making comparisons among organisms as they normalize for variations in the radionuclide concentration of the water. Concentration ratios indicate the propensity for a radioisotope to concentrate within specific organisms.

Several important points can be gleaned from Table 21.6. First, there are fundamental differences in how Cs and Sr are distributed in the system. When compared with the concentration in the water, Cs levels are four orders of magnitude greater in the sediments and three orders of magnitude higher in the macrophytes. Strontium-90 concentrations, however, are the same in the sediments and macrophytes and are only two orders of magnitude greater than the water. Recall that $^{137}Cs$ mimics potassium, and $^{90}Sr$ behaves like calcium. This is evident in the benthic macroinvertebrates. The $^{137}Cs$ CR in snails and insect nymphs are about the same, but the $^{90}Sr$ CR for the snails is three orders of magnitude greater than that of the insects, probably because of the greater quantity of calcareous tissues in snails. The same analogy can be extended to the turtle, which has the highest $^{90}Sr$ CR but has a $^{137}Cs$ CR comparable to that of fish and waterfowl. In the section Radionuclide Concentrations, above, we stated that $^{90}Sr$ concentrations (Bq/g bone ash) in turtles do not differ greatly from those of other organisms. However, if animals are compared on a whole-body basis (as we have presented the CR), then the turtle's propensity to accumulate $^{90}Sr$ is evident.

Whicker (1984) stated that high concentration ratios are generally associated singly or in combination with low availability of nutrient element analogues, longevity of biological tissues, high assimilation of the radionuclide, and long retention time of the radionuclide. If we look at some of the biological characteristics of *Trachemys*, it becomes apparent that it is quite possible for them to have high CR values: (1) They have a long life span of 20 to 30 years (Gibbons and Semlitsch, 1982), which gives them sufficient opportunity to accumulate radionuclides; (2) they have a high percentage of slowly developing bone tissues, which with chronic input could achieve high concentrations of long-lived calcium-analogous radionuclides (i.e., $^{226}Ra$, $^{90}Sr$); and (3) indications exist that their assimilation of radionuclides is high, and retention times are long, particularly for isotopes that are nutrient analogues of calcium (Tables 21.4 and 21.5). These observations demonstrate the importance of how an organism's biological properties can affect its accumulation of radionuclides.

It is also interesting that a trophic-level effect is suggested from the cesium CR in Table 21.6; insects, snails, and frogs are on one order, followed by turtles, waterfowl, and fish on the second order, and the totally carnivorous brown water snake (*Nerodia taxispilota*) resides on top. Cesium is unique among radionuclides in that increased concentrations often occur in the upper trophic levels. A ninefold increase in $^{137}Cs$ in the plant→mule deer→cougar food chain was documented by Pendleton et al. (1965). An approximately twofold increase at each link in the lichen→caribou→wolf chain was reported by Hanson (1967), and an increase by a factor of three has been shown in humans over that of their food (McNeill and Trojan, 1960). Although cesium concentrations in aquatic food chains generally do not increase as dramatically as has been documented in terrestrial systems (Reichle et al., 1970), Whicker et al. (1972) documented a trophic relationship of 3.3 for a predator-prey association in freshwater trout. It will be interesting to see if the trend in the Pond B system continues as more data are collected.

In this section we have tried to show how radionuclide techniques and measurements can be useful in understanding ecosystems. The next logical step is the construction of mathematical models that can be used to simulate biological processes. (Some fundamental radionuclide modeling concepts are discussed in Appendix 21.1.) For an excellent example of a radionuclide model, see Whicker and Kirchner (1987). They have developed a dynamic simulation model of the transport of radionuclides from fallout through the agricultural food chain to man. Such models are useful for predicting the dynamics of radioisotopes within ecosystems. In doing so, they can further our knowledge on the transport and cycling of stable isotopes as well as on the biology and interactions of individual organisms. The role of aquatic turtles in ecosystem function has not been thoroughly examined and deserves consideration for future research. Reichle et al. (1970) stated, "It is not that we lack sophisticated mathematical techniques to develop predictive models of ecosystem processes, but rather that we have neither sufficiently detailed nor widely representative radioecological data with which to work." This is particularly true with respect to the herpetofauna.

### MISCELLANEOUS STUDIES

Numerous physiological studies use radioisotopes but, for the most part, are beyond the scope of this chapter. In a review of physiological methods in studying reptiles, McDonald (1976) discussed techniques that use radiotags $^{51}Cr$, $^{59}Fe$, and $^{131}I$ for the determination of blood or plasma volumes. As was mentioned previously, $^{22}Na$ has been used to identify the sites of active transport of Na in freshwater turtles (Dunson, 1969). Schwartz and Flamenbaum (1976) used radioisotopes of Na and Cl to identify heavy metal–induced alteration of sodium transport in *Trachemys scripta* urinary bladders. They found that heavy-metal salts inhibit the active transport of sodium without altering passive ion fluxes. In a study of intestinal epithelial proliferation in *Chrysemys picta*, Wurth and Musacchia (1964) used tritiated thymidine to examine temperature effects on epithelial mitosis and cell replacement. Total cell replacement was estimated to be eight weeks in turtles maintained at 20° to 24° C. The above studies indicate the potential usefulness of radioisotope techniques when ion transport mechanisms and cellular physiology are of primary interest.

Radioactive tags have also been successfully used in rate-of-passage measurements in the gastrointestinal tract. Absolute measurements of passage rates require markers that do not separate from the labeled fraction and are easily recovered. Solid markers such as powdered Brazil nuts, rubber pieces, glass beads, and plastics have been used in the past (Kotb and Luckey, 1972), but with questionable results, for it is not known whether they move with the fraction they are intended to label (Uden et al., 1980). Dyes used to stain feed are generally appreciably absorbed from the gut and thus bias the results (Kotb and Luckey, 1972). Uden et al. (1980) reviewed many of the historic methods of marking digesta and found that $^{51}Cr$ applied to prepared plant fibers proved to be successful. The preparation of both solid and liquid Cr markers is described by Uden et al. (1980).

Two other radionuclide techniques deserve mention: autoradiography and neutron activation. If a radioactive substance is placed on a photographic film, the ionizing radiation will expose the film and produce an image when the film is developed. The technique is valuable in determining the distribution of a radiotracer within a sample. Isotopes that emit soft beta particles (e.g., $^{45}Ca$, $^{14}C$) work best. Schultz and Whicker (1982) reviewed applications of autoradiography (see also Gude, 1968; Baserga and Malamud, 1969; Rogers, 1973). We did not find any reference to the use of this technique in the chelonian literature. However, one appropriate use would be in determining the movement of calcium within the carapace.

Neutron activation is a powerful technique that uses stable elements placed in a neutron field (beam), which then transforms the stable nuclide into a radioisotope. Hakonson et al. (1975) examined the kinetics of cesium within a public lake in Colorado by spiking the lake with stable cesium ($^{133}Cs$). Subsequent samples from the lake were neutron-activated, transformed into radioactive $^{134}Cs$, and then measured by gamma spectrometry. The technique allowed the study of cesium kinetics in a situation in which the addition of radioactive cesium was not desirable. The technique, however, requires access to a neutron beam (i.e., a nuclear reactor or a Pu-Be source), thus complicating the research methodology.

## Conclusions

The use of radioisotopic techniques in the study of freshwater turtles has tremendous potential. When compared with the volume of work on mammals, the paucity of turtle research involving radionuclides is obvious. Despite the lack of data, general trends do exist:

1. Turtles are similar to other organisms in their concentration of fallout $^{90}Sr$ and $^{137}Cs$, when compared on the basis of concentration (activity per gram of ashed bone).
2. Turtles have the capacity to accumulate large total-body burdens of radionuclides because of their
   a. long life span;
   b. high percentage of slowly developing bone and shell tissue, which with chronic input could achieve high concentrations of long-lived bone-seeking isotopes;
   c. tendency to assimilate a high percentage of ingested radioisotopes;
   d. ectothermic nature, which has implications for longer radionuclide retention times and for seasonal shifts in elimination.
3. Although the data are scant, turtles do not seem to be unusually sensitive or resistant to radiation when compared with amphibians and fish.
4. Turtles, especially hatchlings, are good candidates for radiotagging experiments to study movement. The hard carapace should eliminate some of the problems encountered with radiotags placed on salamanders and lizards.
5. Because of the rapid turnover of body water and absorption of Na via the cloacal region in aquatic turtles, doubly labeled water and $^{22}Na$ techniques used to determine field metabolic and feeding rates in other taxa have had limited success in the study of aquatic turtles. Elimination rates of other radioisotopes may offer viable alternatives in determining field metabolic and feeding rates.
6. The use of radioisotopes to determine kinship relationships seems to be a likely, yet presently unexplored technique.
7. The lack of data on environmental transport and bioaccumulation of radioisotopes is probably greater for the herpetofauna than for any other group of vertebrates. The knowledge gained from radionuclide transport can contribute to our understanding of fundamental processes and help us better define the function of freshwater turtles within ecosystems.

Obviously, radionuclide techniques are not a panacea. Many questions are best addressed using other methods. The disadvantages of radioisotopic work include state and federal licensing requirements. Approval by these agencies often hinges on the applicant's knowledge of radioisotopes and their safe use. The use of radioisotopes by the untrained is a hazardous situation at best; there is potential for personal harm, contamination of equipment and laboratories, and the accumulation of meaningless data due to poorly calibrated equipment. However, the quantities of radionuclides needed for today's tracer experiments are often incredibly small, less than what many people wore on their wrist when radium was commonly used in luminescent watches. With proper planning, the use of radioisotopes constitutes an extremely powerful tool that, in concert with other techniques, can

help answer important questions in freshwater turtle research.

**Acknowledgments**

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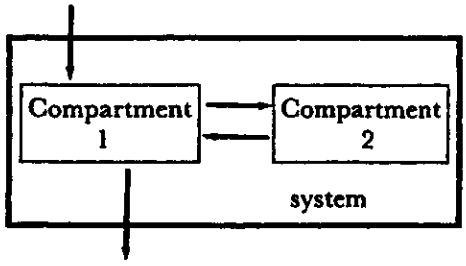
## Appendix 21.1. Radionuclide kinetics

The movement of radionuclides through a system is often depicted with box-and-arrow diagrams. The system is generally composed of one or more compartments, represented by boxes, and associated radionuclide transport processes, depicted as arrows. The compartments are system components that have uniform and distinguishable transport kinetics (Atkins, 1969). An open, two-compartment system with interchange might be represented as follows:




Examples of various transport processes include aerial deposition, resuspension, sorption, desorption, ingestion, excretion, molting, secretion, and decomposition (Whicker and Schultz, 1982). Each transport process in a first-order system has an associated rate constant. The determination of the various rate constants is the cornerstone of radionuclide kinetics. Rate constants allow one to explore the dynamics of the system by determining rates of flow among compartments. These rates govern compartmental increases and decreases in radionuclide burdens over time. Odum (1962) compared the power of isotopes in ecological research to the power of the microscope in biology. The microscope allows the examination of structure, whereas isotopes, through the determination of rate constants, allow the examination of function.

The amount of radionuclide within a compartment is generally symbolized by $q$, and the primary factors that determine $q$ are the rates of input to the compartment ($R_{in}$) and output from it ($R_{out}$; Whicker and Schultz, 1982). Radionuclide loss from a compartment generally obeys first-order processes where loss rate is proportional to compartment inventory. Thus:

$$R_{out} = -kq$$

where $k$ is the rate constant with units of time$^{-1}$. Schematically, this is represented as:


The change in radionuclide inventory ($q$) in the above system with respect to time would be

$$\frac{dq}{dt} = R_{in} - kq$$

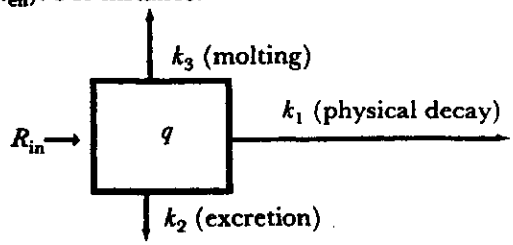
All radioisotopes have an associated physical half-life, $t_{1/2}$, which represents the time required for half of the original material to decay. Radioisotopes decay exponentially according to

$$q = q_0 e^{-kt}$$

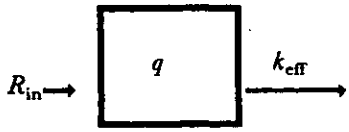
where $q$ is activity at time $t$, $q_0$ is the initial activity, and $k$ is the rate constant. The rate constant ($k$) is related to the physical half-life of the isotope by

$$t_{\frac{1}{2}} = \frac{\ln 2}{k}$$

Isotopes also have a biological half-time within compartments that represents the time required for half of the material to be lost by means other than physical decay (e.g., excretion, transport, etc.). The rate constants of all mechanisms that reduce an isotopic inventory within a compartment can be combined into the effective rate constant ($k_{eff}$). For instance:




could be reduced to



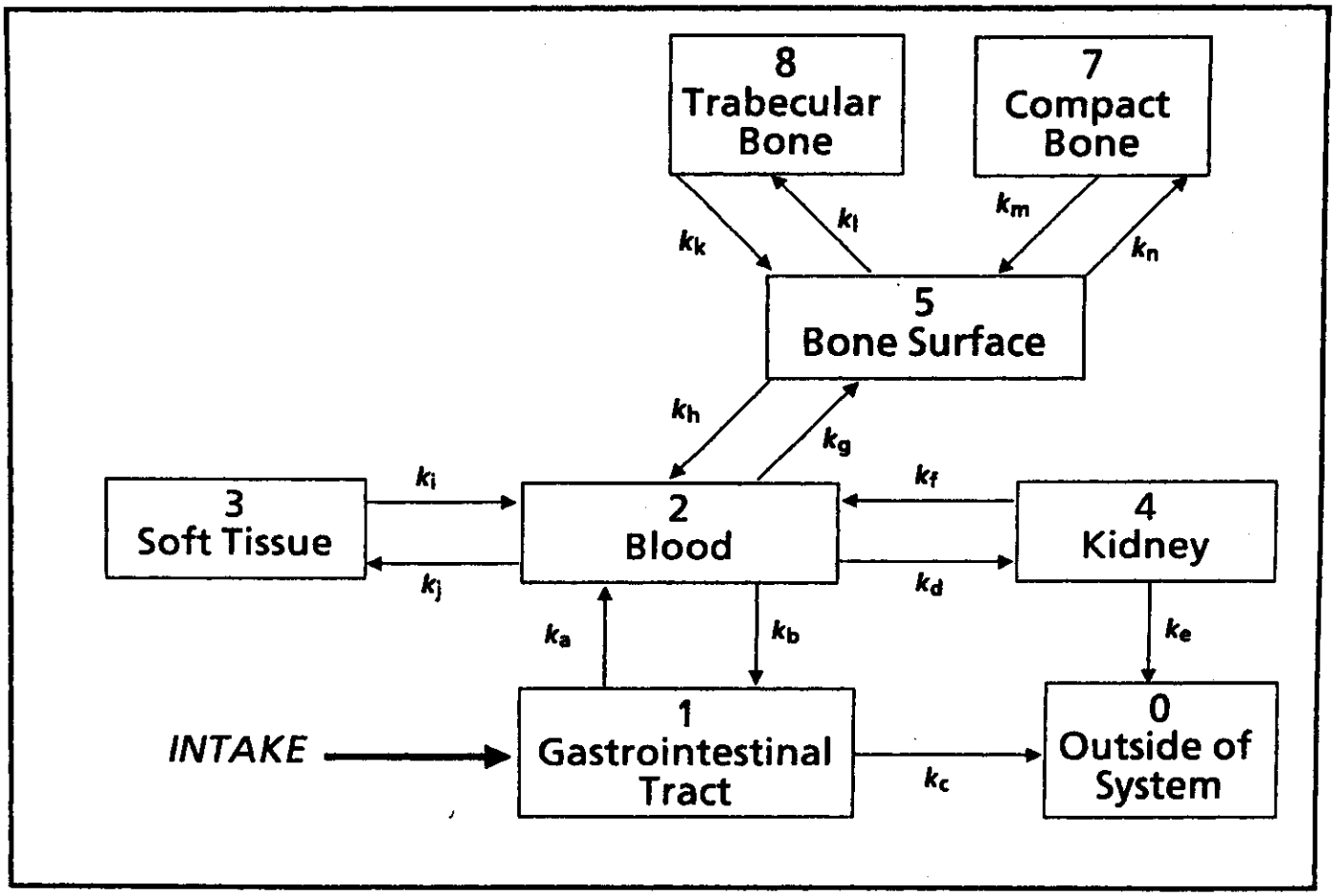



FIGURE 21.5. Model of the possible distribution and movement of strontium in a turtle (adapted from Schreckhise, 1974). Each letter designates a particular pathway.

where $k_{eff} = k_1 + k_2 + k_3$. The effective half-life then becomes

$$t_{\frac{1}{2}eff} = \frac{\ln 2}{k_{eff}}$$

If modeling the movement of radionuclides within a system is of interest, it soon becomes apparent that isolating and quantifying each individual rate process is a difficult task. Consider the movement of radionuclides within a single organism. For example, the anatomical and physiological kinetics of strontium in the turtle could be illustrated as in Figure 21.5. The accurate determination of each rate constant in such a complex system would require numerous experiments and a rather complicated mathematical model. Often the more useful models are those that are sufficiently realistic to answer specific questions or to help understand a particular process, yet are still computationally manageable (Whicker and Schultz, 1982). Two questions often asked in radioecology research are (1) how much of the ingested contaminant is actually incorporated into the body (percent assimilation), and (2) how long does the assimilated contaminant subsequently stay in the body (the biological half-time or elimination rate)? Answers to these questions can be estimated, without knowing every individual rate constant in Figure 21.5, by orally administering a known quantity of tracer to the turtle and examining its subsequent elimination. This is accomplished by performing a series of whole-body radiation measurements on the live animal over time. A graph of the elimination of $^{85}Sr$ in a representative individual *T. scripta* following an acute dose is presented in Figure 21.6. The data can be fit to a two-component elimination model by nonlinear regression techniques (SAS Institute, 1982), in which the fraction of the ingested dose remaining in the turtle (i.e., the amount of $^{85}Sr$, or $q_t$) at any time $t$ is expressed mathematically as

$$q_t = A_1 e^{-\lambda_1 t} + A_2 e^{-\lambda_2 t} \qquad (21.5)$$

The short component (in relation to time) is generally an estimate of that portion of the initial amount of tracer

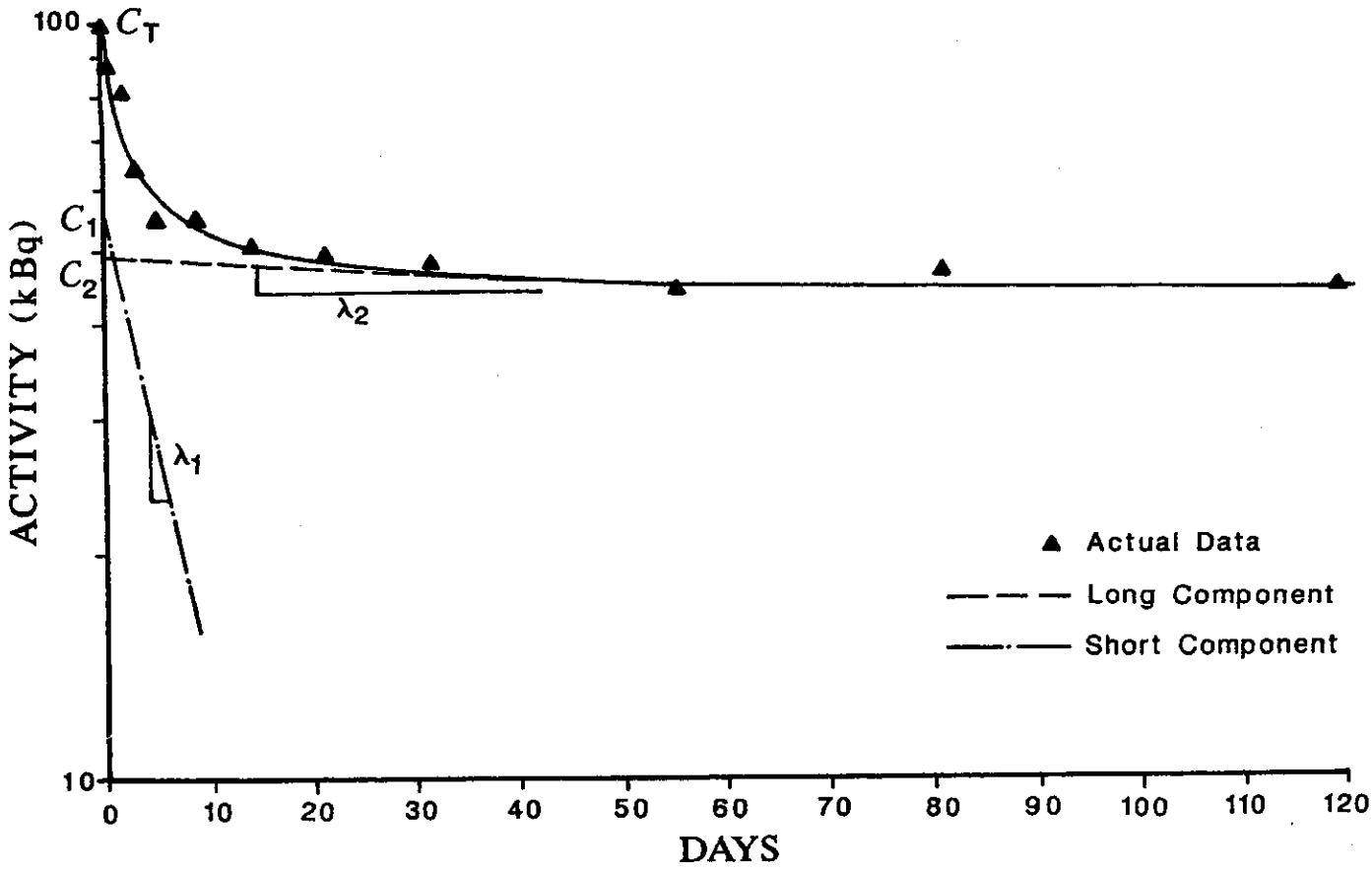


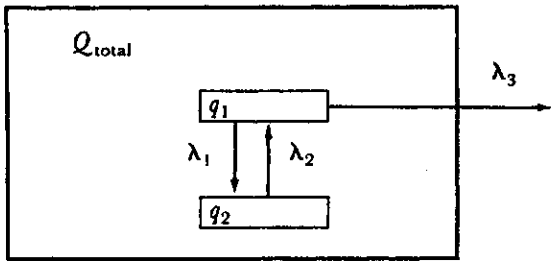

FIGURE 21.6. Elimination of $^{85}Sr$ from *T. scripta* following an acute oral administration. Elimination rates for the respective components are estimated from $\lambda_1$ and $\lambda_2$. $C_1$ and $C_2$ are $y$-intercepts; $C_T$ is the initial amount of tracer administered. Percent assimilation is estimated as $C_2/C_T$.

that is rapidly excreted by gut clearance. The fractional amount ($A_1$) is determined by dividing the $y$-intercept of the short component ($C_1$ in Fig. 21.6) by the initial amount of tracer administered ($C_T$ in Fig. 21.6). The rate constant for gut clearance is the slope of the line for the short component ($\lambda_1$; in this example, 0.018/day). The long component of the curve represents material actually incorporated into body tissues. The $y$-intercept of the long component ($C_2$) divided by $C_T$ is an estimate of the percent assimilation ($A_2$). The slope of the long component ($\lambda_2$) estimates the rate at which the assimilated tracer is subsequently eliminated from the body. $A_2$ and $\lambda_2$ are the values most often compared in the radioecology literature and are the parameters that can answer the two questions presented earlier. In the above example the turtle lost 52% of the $^{85}Sr$ through initial gut excretion of nonassimilated material with a half-time of 3.8 days ($\ln 2/\lambda_1$). It assimilated 48% of the ingested $^{85}Sr$ with a long-term biological half-time in excess of 1,000 days, resulting in this equation:

$$q_t = 0.52e^{-0.018t} + 0.48e^{-0.0006t}$$

Obviously, this simplified model is not a true physiological representation of the animal. However, the simpler model allows one to estimate the parameters $A_2$ and $\lambda_2$ (i.e., percent assimilation and rate of elimination), processes that are vital to the understanding of isotope kinetics within the animal.

Remember that transport models are generally quantitatively simple representations of real, complex systems (Whicker and Schultz, 1982). In equation 21.5 we have followed the notation of Whicker and Schultz (1982) by using the symbol $\lambda$ as an estimate of the true rate constant ($k$). The $\lambda$ values, although having the same dimensional units as $k$ values ($t^{-1}$), are derived parameters that are not necessarily equal to the real rate constants of a system (Whicker and Schultz, 1982), the real rate constants being the $k$'s in Figure 21.5. In the above example the simplified two-component elimination model could be depicted as




$Q_{total}$ is the quantity of $^{85}Sr$ determined by whole-body counting of the turtle at various times and corresponds to $q_t$ in equation 21.5. Compartment $q_1$ is a simplification of the first four compartments in Figure 21.5 (gastrointestinal tract, blood, soft tissue, and kidney), with compartment 1 (gastrointestinal tract) of primary importance. Compartment $q_2$ is a simplification of the bone compartments, where most of the Sr resides. The $k$ values in Figure 21.5 are related to the estimated rate constants $\lambda_1$, $\lambda_2$, and $\lambda_3$. The differential equations for the quantity of tracer in each compartment are

$$\frac{dq_1}{dt} = q_2\lambda_2 - q_1(\lambda_1 + \lambda_3) \tag{21.6}$$

$$\frac{dq_2}{dt} = q_1\lambda_1 - q_2\lambda_2 \tag{21.7}$$

The numerical values for the parameters in equations 21.6 and 21.7 can be estimated efficiently by a computerized curve-fitting routine that adjusts the parameters to the best fit of the data (Whicker and Schultz, 1982). If required for a specific question, further experimentation and more detailed modeling could be used to refine our conceptual and mathematical representation of the system. More thorough and detailed presentations of radionuclide kinetics can be found in Atkins (1969), Wagner (1979), and Whicker and Schultz (1982).