WATER RELATIONS IN NESTLING AND ADULT ZEBRA FINCHES, POEPHILA GUTTATA

By

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ABSTRACT

WATER RELATIONS IN NESTLING AND ADULT ZEBRA FINCHES, *POEPHILA GUTTATA*

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The problems associated with acquisition and allocation of water resources have been examined in adults of many species, but our knowledge of how neonatal animals budget their water reserves is limited. The purpose of the present study was to collect baseline data on water flux in nestling zebra finches, *Poephila guttata*. Water budgets for nestlings were derived from evaporative water loss (EWL) and total body-water turnover rate (WTR) data.

Rates of EWL were measured from individual nestlings and adults exposed to 20°C, 27.5°C, and 35°C. These data showed that EWL rates were affected by ambient temperature and age. The inability of young nestlings to thermoregulate appears to be the primary reason for this age-temperature reduction in EWL. Individual nestlings could thermoregulate by day 13, and with the onset of thermoregulatory abilities nestling EWL rates became similar to adults. Also, brood EWL was measured to evaluate the effects of huddling. At 35°C brood EWL was lower than individual EWL.

Daily WTR's were measured in nestlings and adults using tritiated water (TOH). A validation study using TOH to measure total body-water volume revealed that TOH and dry-weight measures were similar. The amount of water lost per day increases with age while weight-specific WTR's decreased. Nestling WTR's became similar to adults at 13 days of age. Although WTR's increased with age, there were differences between similarly aged broods. These data suggest that nest environments may have a major effect on WTR's.

Water budgets were constructed for nestlings and adults in order to determine the effects of age on water utilization. Daily total water intake (TWI) and WTR's increased with age. The amount of water allocated for growth increased slightly with age and showed a decrease when expressed as a percentage of TWI. Rates of EWL showed a similar pattern. The amount of water lost via excretion (ExWL) constituted the major avenue of water loss. The ExWL data suggest that nestlings in my colony were not water limited and that water was provided in excess by the adults.

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INTRODUCTION

The necessity for water to sustain life has long been recognized. Even though water is essential for life, the strategies utilized by animals to meet their water requirements vary with environmental conditions and individual needs. An animal's environment often dictates the general pattern of water resource regulation. For example, fresh-water fish must cope with the problem of gaining too much water from their environment while animals inhabiting xeric regions encounter conditions that accentuate water loss to the environment (Hill 1976). Even within the same habitat, different species may show varied responses to meeting their water requirements depending upon such factors as when they are active during the year, season, or day; what type of food they eat; and how big they are (Bartholomew and Dawson 1953, Cade 1964, Hinds and Calder 1973, Davies 1982). Similarly, different individuals within the same species may exhibit contrasting responses to water demands depending upon habitat preference, reproductive condition, age, or food choice (Kayser 1930, Smyth and Bartholomew 1966, Trost 1972, Hinds and Calder 1973). It appears that no two species have the same demands for water. Each faces a specific set of environmental and physiological conditions that dictate how much water is required and how the water will be allocated. Although we cannot predict the exact pattern of water utilization for an individual, much less for an entire species, there are some basic demands for water that each animal must address.

Terrestrial animals tend to lose water readily, and survival is dependent on the animal's ability to regulate water losses or maintain an adequate level of water intake. In general, water reserves must be available to meet the demands for evaporation, excretion, reproduction, and growth. Water lost via evaporation or excretion can be regulated (increased above minimal levels) to meet the animal's homeostatic needs (Hill 1976). While it is unclear whether the amount of water allocated for growth and exported via reproduction can be regulated, a decrease in the amount of water allocated to these functions may have negative effects, such as a reduction in growth rate or the size of the eggs produced (Mertens 1977a). In any event, the added demands for water due to growth and reproduction are temporary and usually occur during times when environmental resources are plentiful (Davies 1982). The rates of water loss through all of these pathways are affected by biotic and abiotic factors. An animal's general health and vigor would affect its needs for evaporative cooling and its ability to grow and reproduce. Environmental conditions that promote water loss can create physiological stress, and an animal's ability to survive will be dependent on how it copes with stressful situations.

Although measurement of the total water requirements of an animal can provide information on how well an animal is suited to its environment and which environmental conditions present the greatest stress, an accurate analysis of water flux data requires a consideration of the specific components of an animal's water budget.

Reliance on total water flux values may result in misleading conclusions. For example, an animal with a high rate of water flux would appear to be poorly suited to an arid environment. But even though high rates of water loss may reflect physiological stress (heat stress can cause increased rates of evaporative water loss), they may merely reflect a diet which consists of watery foods. Clearly, knowledge of an animal's total water flux is insufficient to understand its water requirements. Measurements of the specific avenues of water loss are required to interpret accurately water relations in an animal.

Many of the studies that have examined water relations in animals have focused on desert-dwelling species. One of the rationales for these studies is that because deserts are harsh environments the animals which inhabit these regions should show the greatest degree of specialization in their water resource allocation. While this assumption may not always be true, the ambient conditions in the desert, seasonally high heat and low humidity, do favor high rates of water loss from animals and a scarcity of drinking water. Some animals respond to water stress by reducing their exposure to stressful conditions, as by restricting activity to cool times of the day or seeking favorable microclimates (c.f., Dawson 1954; Calder 1964; Marder 1973; Congdon _et_ _al_. 1979, 1982; Davies 1982). Physiological responses to heat and limited water availability may include an increased ability for, or efficiency of, evaporative cooling (Trost 1972); a reduction in the amount of water lost via non-evaporative pathways (thus freeing more water for evaporative cooling) (Calder 1964); and a labile body temperature (Calder 1964, Weathers 1981).

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Animals often combine behavioral and physiological responses in order to conserve water.

The water-budgeting strategies employed by desert birds are of interest because, unlike most desert mammals, amphibians, and reptiles, birds are active during the day and so do not use burrows to escape harsh conditions (Dawson 1982). As a result, birds often encounter the hottest and driest conditions. Birds are able to survive in this environment through a combination of physiological and behavioral adjustments. Most desert birds are capable of dissipating large amounts of body heat through evaporative cooling (see Dawson 1982 for a recent review). While evaporative cooling helps to reduce heat stress, dehydration can occur if water reserves are not replenished (Mertens 1977a). The ability of birds to reduce the amount of water lost via non-evaporative pathways appears to be limited. Unlike some desert mammals, such as the kangaroo rat, *Dipodomys merriami* (Schmidt-Nielsen *et al*. 1948), birds are not able to produce highly concentrated urine (Skadhauge 1981). So, many desert birds must drink or eat foods that have a high water content in order to compensate for high rates of water loss. Due to their mobility, desert birds can search for water over large areas. Sand grouse (*Pterocles alchata* and *P. orientalis*), for instance, fly as long as 75 minutes to open water during the dry season in Morocco (Thomas and Robin 1977). In general, birds utilize a variety of approaches to cope with desert conditions and problems associated with water allocation.

One desert-inhabiting bird, the zebra finch (*Poephila guttata*), appears to be poorly suited for surviving in hot, arid regions. Zebra finches are small (12-14g) seed eaters (Keast 1958) that range throughout much of Australia except for areas in the north- and southeast (Immelemann 1965). Several observers have noted that zebra finches are most often found near water (Keast 1958), and Keartland (as cited in Calder 1964) feels that these birds may be the most water-dependent species in the Australian desert. Captive zebra finches have been reported to be capable of surviving in excess of 90 days without drinking water under moderate temperature conditions in the laboratory (Cade _et_ _al_. 1965, Lee and Schmidt-Nielsen 1971). Cade _et_ _al_. (1965) note that their captive zebra finches reduced activity levels during water stress, and this reduction in activity may have helped to reduce water demands. Due to the necessity to obtain food and water and to avoid predation, it seems unlikely that a free-living bird could remain inactive for extended periods of time (several days to weeks) during periods of water stress. Skadhauge and Bradshaw (1974) report that the urine concentrating abilities in zebra finches are not highly developed (max U/P osmotic ratio = 2.79). The ability of zebra finches to dissipate large amounts of body heat evaporatively (in excess of 100% metabolic heat production) has been reported by Calder (1964), Lee (1964) and Cade _et_ _al_. (1965). This ability to cope with heat stress is crucial to zebra finches, but it is extremely costly with respect to water reserves. The combination of the zebra finch's small body size, absence of any physiological specialization for conserving water, and potentially large demand for evaporative cooling are consistent with the observations that this species is dependent on open water.

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Further evidence of this water dependency is illustrated by the zebra finches' breeding strategy. Immelemann (1965) and Serventy (1971) note that immediately following a rainfall courtship and nesting begin. Conversely, during periods of high ambient temperatures and low relative humidities, no reproductive activity occurs (Farner and Serventy 1960). Zebra finches are opportunistic breeders which respond primarily to rainfall (Sossinka 1980a,b). In fact, Oksche _et_ _al_. (1963) report that changes in photoperiod have no effect on breeding activity. In some areas of Australia where rainfall occurs at regular intervals, zebra finches do exhibit a seasonal breeding pattern (Davies 1977, Kikkawa 1980). The survival and breeding strategies utilized by zebra finches show that species can inhabit harsh environments if sufficient resources are available. Also, coordinating breeding with the rainy season helps to assure an adequate supply of food and water for the nestlings.

The amount of water required by nestlings to meet their homeostatic and growth needs may change from day to day. As nestlings mature, changes in their physiological capabilities and overall body size should affect how much water they need. Also, nestlings are not completely isolated from the environmental conditions outside the nest. Ambient conditions that affect water utilization in adults will create similar, though moderated, demands on the nestlings (Ricklefs and Hainsworth 1968). It is unclear whether nestlings can restrict water losses when faced with water stress. Studies on neonatal thermoregulation show that some nestling birds can increase evaporative water loss in response to heat stress (Kendeigh 1939, Hudson _et_ _al_. 1974, Booth 1984, Chappell _et_ _al_. 1984). Although c

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nestlings can increase evaporative water loss in response to heat stress, it is unknown whether they can reduce evaporative water loss in response to water stress. Mertens (1977a) points out that short-term evaporative cooling may not have an adverse effect on nestling health, but long-term heat stress may result in dehydration. Because nestling birds are dependent upon their parents for food and water, nestlings are limited in their ability to replenish a water deficit. Mertens feels that the increase in water demand for evaporative cooling may reduce the amount of water available for growth. His findings on great tits (Parus major) suggest that nestlings have few options when confronted with water stress, and that their ability to regulate water losses may be limited.

The total amount of water allocated for evaporation, excretion, and growth in neonatal birds has not been examined. A few studies have measured either rates of EWL, excretory water loss, or the amount of water incorporated into new body tissues (c.f. Westerterp 1973, Hudson et al. 1974, Blem 1975). These studies provide glimpses of water resource utilization in nestling birds, but they do not give a comprehensive analysis of nestling water relations. The purpose of my study is to gather background data on water relations in nestling and adult zebra finches (Poephila guttata). These data will be used to construct water budgets for these birds at various ages. In order to assess the ability of neonates to cope with water stress, an examination of water losses and gains in hydrated birds must be completed. The specific questions which this study addresses are: 1) how much water do nestlings allocate for growth and do nestlings and adults lose via evaporation and excretion; 2) do rates of water loss via these avenues change with age; 3) if the amount of water lost via one avenue changes at any age, does the total rate of water loss reflect that change; 4) what is the total water requirement for a nestling at a given age; and, 5) how much water is required for a nestling and a brood to reach fledging age? This study will be divided into three sections. The first assesses evaporative water losses in individual nestlings, broods, juveniles and adults exposed to varied ambient temperatures. The second section presents data on total body-water turnover rates in nestling and adults. The technique used to measure water turnover rates allows for the simultaneous measurement of water allocation for growth. Therefore, by subtracting changes in body-water volume due to growth and evaporation from the total body-water turnover rate, an estimate of excretory water loss can be made. The third section utilizes the data on evaporation, excretion, and growth to establish water budgets for nestling and adult finches.

EVAPORATIVE WATER LOSS IN NESTLING, JUVENILE, AND ADULT ZEBRA FINCHES

Evaporative water loss (EWL) has been studied extensively in adult birds (c.f., Bartholomew and Cade 1963, Dawson and Bartholomew 1968, Calder and King 1974, Dawson 1982). Most of these studies have focused on the thermoregulatory benefits derived from evaporative cooling and have not considered the effects of increases in EWL on a bird's water resources. Evaporative water loss can increase up to 7.5 fold in birds (Dawson 1982), and as a result, birds that are forced to cool evaporatively can lose large volumes of water. The use of evaporative cooling to combat heat stress is common in birds, but the dependence on this method of themoregulation varies between species. Dawson (1982) states that the dependence on evaporative cooling may vary with age, body size, activity level, ability to tolerate hyperthermia, and habitat. Although the ability to cool evaporatively is important in allowing birds to survive in desert environments, the potential for dehydration may be great. A bird's ability to cool evaporatively is dependent on the ability to marshall water resources for evaporation and, perhaps, to tolerate dehydration.

Although we have begun to understand the factors that affect rates of EWL and how adults respond to environmental conditions which may increase rates of EWL, we know little about EWL in nestling birds. The findings on EWL in adults may not be applicable to neonates. For example, the inability of some altricial neonates to maintain a stable body temperature may affect rates of EWL. Also, the rapidly changing body size of nestlings will alter the relationship between EWL and the animal's surface area.

The dependence of the rate of evaporation on the temperature of the evaporating surface indicates that the rate of EWL in animals will be affected by their body temperature. Therefore, the ability of neonates to thermoregulate would be expected to affect rates of EWL. Kayser (1930), Kendeigh (1939), Bernstein (1971a), Hudson _et al_. (1974), Mishaga and Whitford (1983), Booth (1984), and Chappell _et al_. (1984) have shown that nestling birds can increase EWL rates when exposed to high ambient temperatures. Because evaporative cooling can expend large volumes of water and because small nestlings may have limited water reserves, the length of time that they can rely on evaporative cooling may be limited. Morton and Carey (1971) noted that nestling white-crowned sparrows (_Zonotrichia leucophrys_) died of apparent heat stress after 20 min of exposure to direct sunlight. Their findings suggest that evaporative cooling in nestlings may be ineffective under some conditions and that the duration of evaporative cooling bouts may be limited in nestlings. Hudson _et al_. (1974) indicate that shading of cattle egret (_Bubulcus ibis_) nestlings by their parents may be as important in reducing heat stress as evaporative cooling. These two studies suggest that even though nestlings can evaporatively cool other methods of coping with heat stress may be more reliable and less demanding on nestling water reserves.

The effects of low ambient temperatures on EWL in nestlings are poorly understood. The inability of some neonates to elevate their body temperatures in response to low ambient temperatures suggests that rates of EWL should decrease. Kayser (1930) reports that in nestling pigeons (Columba livia) rates of EWL decrease as ambient temperature and metabolic rate decrease. On the other hand, Bernstein (1971a) feels that the inability of neonatal painted quail (Excalfactoria chinensis) to thermoregulate is due to evaporative heat loss. Bernstein's data suggest that the relationships among ambient temperature, body temperature, and EWL are complex. The complexity of this relationship increases when a brood is considered. Hill and Beaver (1982) show that broods, as a unit, resist cooling through thermal inertia and the combined metabolic heat output of the nestlings. Individual nestlings within a brood may have a higher mean body temperature than an isolated nestling and a smaller exposed surface area due to huddling. These studies on nestling physiology suggest that the rate of EWL in neonates may be affected by ambient temperature, body temperature, and the presence of nestmates.

Although rates of EWL in nestlings can reach high levels, it is not known what proportion of a nestling's water budget is accounted for by EWL. The primary goal of this portion of my study is to provide data on EWL for nestling water budgets. The experiments reported in this section were designed to assess how age and a nestling's ability to thermoregulate affect EWL. Two factors that affect thermoregulation, ambient temperature and huddling, were incorporated into the measurements on EWL. The specific questions on EWL which this section addresses are: Do the rates of EWL change with age; if so, are these changes associated with developmental events; what effects do low ambient temperatures have on EWL in nestlings, juveniles, and adults; does the presence of other individuals (nestmates) affect EWL; and at what age do nestlings attain adult levels of EWL?

METHODS

General Colony Maintenance

The original breeding stock of grey and chestnut-flanked white zebra finches was obtained from a local bird breeder (David Drumm, Charlotte, MI). Subsequently, the breeding stock was supplemented with individuals reared within the colony. The birds were housed in a windowed room and exposed to a 16L:8D photoperiod. Ambient temperatures ranged between 26°C and 34°C, averaging 26°C. The birds were provided with seed (a standard finch mix containing Proso, Red, Yellow, and Japanese Millet and Canary seed; Kellogg Seed Co., WI), water (with Avitron vitamins added), a mineral block containing calcium and other salts, and grit *ad libitum*. Linatone, a linoleic acid mixture, was added to the seed. The breeding season in zebra finches is initiated by rainfall and not photoperiod (Oksche *et al.* 1963; Immelmann 1965; Serventy 1971; Sossinka 1980a,b), and fresh greens are often used by aviculturalists to stimulate breeding and provide essential vitamins for females laying eggs. In my colony all breeding pairs were offered soaked millet and fresh greens daily.

The degree of relatedness of the original birds was not known, and as a result, they were paired on a random basis. Any pairs subsequently added to the breeding stock were selected so that inbreeding was minimized. Breeding pairs were housed in individual cages (91 cm x 31 cm x 31 cm), provided with wooden nest boxes (10 cm x 10 cm x 10 cm) and offered shredded burlap as nesting material. The breeding cages were made of hardware cloth (0.64 cm mesh) with access doors at either end. The breeding birds' diet was supplemented with an egg-food mixture (hard boiled egg, unflavored gelatin, powdered milk, and high-protein baby food) instead of soaked millet approximately three days before the eggs were to hatch. The egg-food supplement was provided until the young were 10 to 12 days old. Each nestling was marked with a felt-tipped pen on either a wing or leg for identification on the day of hatching (day 0). At ten days of age, nestlings received a leg band that identified the individual and parents.

Growth and Development

Measurements on the rate of growth and observations on the timing of developmental events were made on a series of nestlings and adults from the colony. Body weights were recorded daily up to 28 days of age and then on alternate days through 48 days of age. Body weights were measured to the nearest 0.01g using a Tor-Bal (Model ST-1) balance. Primary feather growth was monitored from measurements of the 10th primary using a pair of calipers. Developmental events (opening of eyes, eruption of primary feathers, onset of ability to fly, and fledging) were noted for each bird.

Evaporative Water Loss

Two different EWL measurement systems were used in this study. The first system was used for measurement of EWL from individual birds, nestlings and adults, at each age and temperature combination. The second system was used to measure EWL from a second group of adults and broods of nestlings. The systems differed only in the EWL

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chamber design and airflow rates used. Evaporative water loss was measured gravimetrically using Drierite (W.A. Hammond Co.). Air entering a chamber was maintained at a fixed flow rate (Gilmont Flowmeters, size 2, were used) and was dried by passing it through three Drierite-filled tubes. The excurrent air passed through a series of three preweighed U-tubes containing Drierite. Glass tubing (0.64 cm OD) was used for the air lines from the upstream to the downstream drying columns to minimize water loss (Mautz 1980). A complete EWL setup (upstream and downstream drying columns and the animal chamber) was placed in a Sherer Model CEL 25-7 environmental cabinet which maintained the ambient temperature within ± 1°C. Animal chamber temperatures were monitored using a YSI (Model 401) thermistor probe and Telethermometer (Model 44TZ) or a copper-constantan thermocouple and a Honeywell Class 15 Multipoint Potentiometer. The thermistors and thermocouples were calibrated against an N.B.S. thermometer.

Evaporative water loss was measured during the second hour of a two-hour period. During the first hour the chamber was flushed with dry air at 35, 27.5, or 20°C. The flush period allowed the birds to equilibrate to chamber temperature and humidity conditions. This period exceeded the 9 min flush time required for the chamber to reach 99% humidity equilibrium at constant EWL (Lasiewski _et_ _al_. 1966a). Before and after an EWL run, the downstream drying columns were weighed to the nearest 0.1mg on a Mettler H51A analytical balance. At the end of an EWL measurement period, the bird's body temperature was measured rectally using a copper-constantan thermocouple inserted 0.5-1.0 cm. Body temperature was read from a Honeywell Potentiometer

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within 1.5 min after a bird was removed from the temperature controlled cabinet. Body weights, measured to the nearest 0.01g on a Tor-Bal balance or a Mettler P1210 balance, were recorded at the end of an EWL run.

EWL Measurement From Individual Birds

In general, EWL was measured from birds that were 1, 4, 7, 10, 13, 16, 19, 22, 35 days of age and adults (>60 days; Sossinka 1975, 1980b reports that zebra finches are capable of reproducing at 70 days of age). Evaporative water loss was measured at three ambient temperatures (20°C, 27.5°C, 30°C) for all age groupings except one day olds at 20°C. A minimum of five nestlings was measured at each age and temperature combination. No more than two birds from the same clutch were used per age grouping and a nestling was used only once every nine days for study. At certain ages, the amount of water lost via evaporation was very low. In these cases (1 day olds at 35°C, 1 and 4 day olds at 27.5°C, and 4 day olds at 20°C), EWL from two nestlings within one chamber was measured. The nestlings were physically separated from one another within the EWL chamber by a hardware cloth partition.

The experimental temperatures were selected to provide a range of exposure that included the thermoneutral zone (TNZ) of adults, simulated "normal" brooding temperatures for nestlings, and a cold stress to the birds. The highest temperature (35°C) was selected because it was the mean body temperature of young nestlings (<6 days old) measured immediately upon removal from the nest. This temperature should simulate "normal" nest temperatures for these nestlings. Also, 35°C is stated to be just below the upper critical temperature in zebra finches (Calder 1964, Lee 1964, and Cade et al. 1965) and should elicit resting EWL rates from adult birds. The second experimental temperature, 27.5°C, is within the TNZ. It should evoke resting responses from juvenile and adult birds but pose a mild cold stress to younger birds. The lowest temperature (20°C) is below the lower critical temperature for adults. The rates of EWL at this temperature should be indicative of a response to a mild cold stress in juveniles and adults and a severe cold stress for younger nestlings.

Prior to the commencement of an EWL run, the cloaca of a bird was closed using a ligature tied around the cloacal protuberance. The ligatures were used to prevent nestlings from defecating, and data were discarded if leakage from the cloaca was evident. The birds were then placed inside a hardware cloth sleeve (grid size = 0.64 cm) which prevented them from contacting any of the chamber walls. The EWL chambers consisted of a glass tube (ID = 91 mm, length = 165 mm) with rubber stoppers (size 15) sealing both ends. The rubber stoppers filled a portion of the glass tube, so the actual animal chamber dimensions were: ID = 91 mm, length = 115 mm. Each stopper contained air ports and in one an additional glass tube was inserted to suspend the hardware cloth sleeve and to house a thermistor. The flow rate of dry air used in this portion of the study was 400-425 cc/min (measured at an average room temperature of 22°C and pressure of 735 mmHg). The Gilmont flowmeters were calibrated at room temperature using a spirometer.

EWL From Adults and Broods

Ten additional adult finches were measured at 35°C. These birds were placed in a hardware cloth sleeve with a larger grid size (1.3 cm) and were positioned above a pool of mineral oil. The birds' cloacae were not ligated, and the mineral oil served to prevent excretory water from contaminating the chambers. The durations of the equilibrium and measurement periods and flow rates remained the same as those used for ligated birds. This series of measurements was made so that comparisons between results from other studies using mineral oil could be made with my data using ligated cloacae.

The measurement of EWL from broods of nestlings required that the EWL chamber be redesigned so that four birds could be placed inside. A 0.95 l paint can with inflow and outflow ports in the lid was used. Nestlings were placed in a cup-shaped hardware cloth nest. The dimensions of the cup and hardware cloth grid size varied with the age of the birds tested (4 and 7 day olds: nest dimensions; diameter = 5.7 cm, depth = 5.7 cm, grid size = 0.64 cm; 10, 13, and 16 day olds: nest dimensions; diameter = 7.6 cm, depth = 7.6 cm, grid size = 1.3 cm). These nest dimensions approximated the size of the nest cups for similar aged broods in my colony. A 1.5 cm deep pool of mineral oil was used to trap any fecal material. Similar procedures to the ones used with individual nestlings were used except that the air flow rate was maintained between 1600-1650 cc/min (measured at an average room temperature of 22°C and pressure of 735 mmHg) and nestlings were studied at only one ambient temperature (35°C). Natural broods of four nestlings were used. Three broods were measured at each of the following ages: 4, 7, 10, 13, and 16 days. To obtain broods of four, broods were either culled to four individuals or nestlings (0 or 1 day old) were fostered into broods of three or less as soon after hatching as possible.

System Calibration and Error Control

The accuracy of the EWL system was tested by determining how much water was reclaimed from preweighed vials of water placed in the chamber. A lid that could be removed without opening a chamber was used to prevent evaporation during a 30 min pretest flush period. The average percentage of recapture was 97.0 ± 5.6% (n=10). I found that small amounts of water would be collected in the downstream drying tubes when no bird was in a chamber. As a result, periodic control runs were made to determine how much water of unknown origin was passing through the system. The average amount measured with air flow rates at 400-425 cc/min was 1.67 ± 0.86mg H_2O/hr (n=55). The average amount of water passing through the system with air flow rates between 1600-1650 cc/min was 10.02 ± 0.54mg H_2O/hr (n=3). Control runs were made for each EWL setup and the amount of water recovered was used as a correction factor for subsequent EWL runs.

When measuring EWL using trains of three drying columns, it is ordinarily assumed that the third drying column will never change weight. I found that the third drying column almost always showed a weight gain. The mean weight gain of the third column for most of the data reported here was 0.72 ± 0.53 mg (n=145). The weight gain of the third drying column averaged 2.11 ± 1.91% (n=145) of the combined weight gain of the first two drying columns. I stipulated that if the weight gain of the third drying column was more than 5% of the total weight gain of the first two drying columns the run would be discarded.

As pointed out by Lasiewski et al. (1966a,b), it is necessary to consider the relative humidity within a chamber when comparisons of differently sized animals are being made. The humidity within an open flow EWL system is dependent upon the following factors: chamber temperature, air turnover rate, and amount of water added per unit time to the air stream by the animal. Differences in water output from birds at each of the experimental ages and differences due to the three experimental temperatures will affect the relative humidity within the chambers. The relative humidity that individual birds were exposed to ranged from 0.6-15.8% (formula 3 of Lasiewski et al. 1966a). At 35°C the mean RH was 5.33 ± 2.49%; at 27.5°C mean RH was 6.39 ± 3.53%; and at 20°C the mean RH was 8.82 ± 4.88%. The mean RH for broods of nestlings was 3.52 ± 1.74%.

Statistical Analysis

Statistical comparisons of total and weight-specific EWL were made using an unweighted-means analysis of variance (ANOVA) (Snedecor and Cochran 1967). An initial one-way ANOVA was run on total and weight-specific EWL data. In these analyses there were 27 treatments (due to the empty cell for one day olds at 20°C, all of the data from one day olds were not included in the initial ANOVA's). The error term from the one-way classification was divided by the harmonic mean of the treatment sample sizes and used as the error term in a second two-way ANOVA. The use of the harmonic mean adjusted the error term so that differences in treatment sample sizes were minimized. The multiple-way ANOVA examined the two factors, temperature and age, used in this study. Differences between treatment means (including one day olds) were determined from a least significant difference (LSD) test (Steel and Torrie 1980). All mean values reported are ± one standard deviation. Bartlett's test for homogeneity of variance (Steel and Torrie 1980) was run on the total and weight-specific EWL data prior to the ANOVA's.

The results from the experiment on the effects of grouping on weight-specific EWL were analyzed using an unweighted means ANOVA. This test was set up as a two-way ANOVA with age and type of grouping (single bird or group of four) as the main effects. Comparisons between treatment means were made using an LSD test.

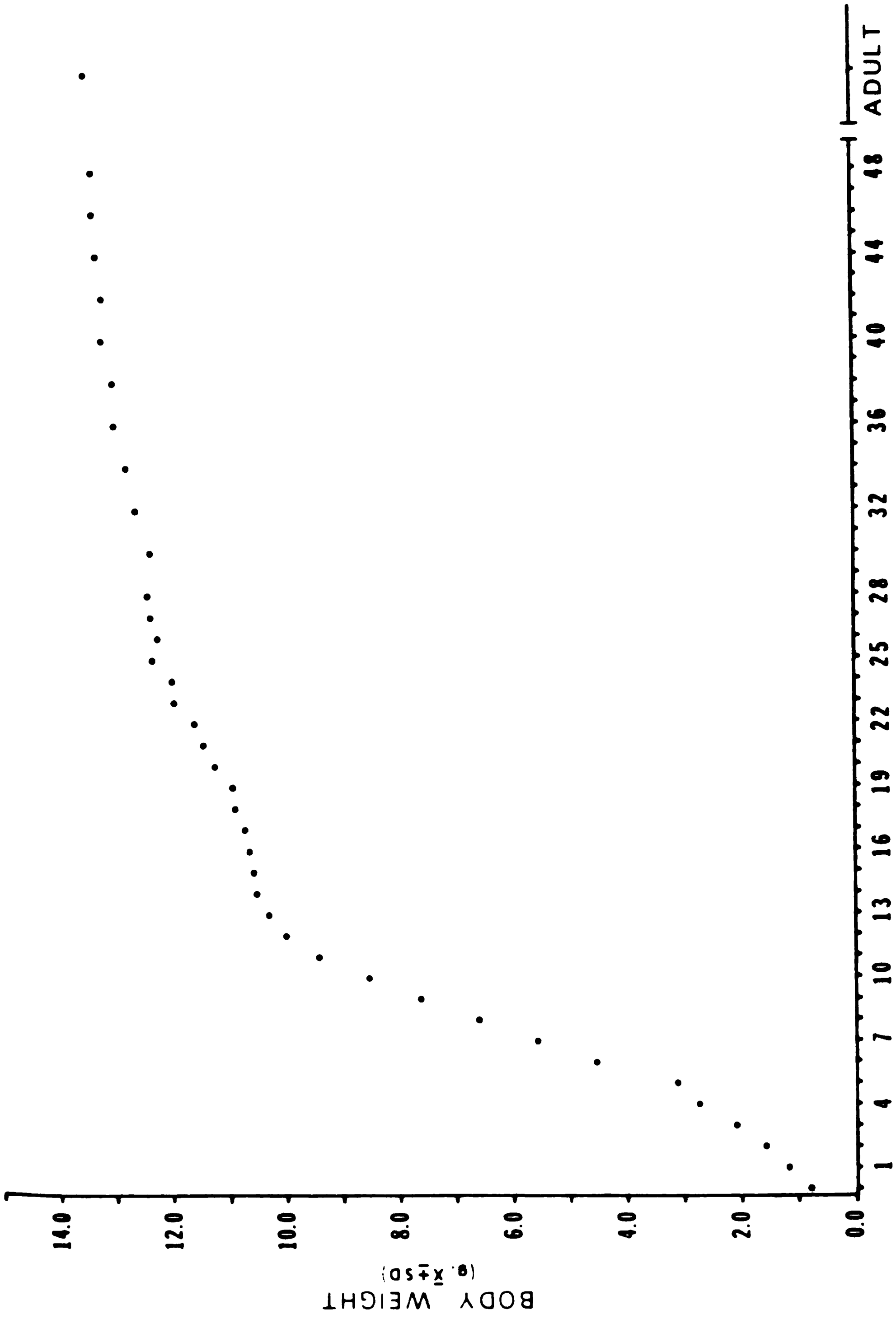
RESULTS

Growth and Development

Zebra finches are an altricial species. Eggs are laid every 24 hours until a four- to five-egg clutch is completed. Incubation normally begins with the laying of the third egg and lasts 12-13 days. Freshly laid eggs weigh 1.00 ± 0.10 g ($n=89$), and hatching occurs asynchronously over a two or three day period.

Nestlings grow rapidly after hatching until they are 12-13 days old. On the day of hatching (age = 0 days) nestlings weigh 0.78 ± 0.10g ($n=55$). The most rapid growth occurs from 3 ($W_b = 2.10 \pm 0.40$g, $n=55$) to 12 ($W_b = 9.96 \pm 1.07$g, $n=55$) days of age. The overall growth curve for zebra finches (Figure 1) is sigmoidal and is best described using the logistic growth equation (determined using the procedures outlined by Ricklefs 1967a). The calculated growth constant is 0.368 (asympotic weight = 11.30g). Nestlings selected for EWL measurements span the entire nestling phase and include specific post-fledging ages

Figure 1. Growth curve for nestling and juvenile zebra finches. (Mean ± SD and number of measurements per age are provided in Appendix A).


BODY WEIGHT
(g, $\bar{x} \pm$ SD)
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
AGE
(DAYS)
1
4
7
10
13
16
19
22
25
28
32
36
40
44
48
ADULT

(22 and 35 days old as well as adult birds). Table 1 gives the mean body weights of birds at each of the nominal experimental ages. These data are from non-experimental birds and, thus, are not subject to weight fluctuations resulting from separation from parents during experimentation. Comparisons of body weights of experimental birds versus the weights from non-experimental birds showed no significant differences ($p \geq 0.05$ in all cases).

Table 1. Mean body weight of zebra finch nestlings, juveniles, and adults for the nominal experimental ages used in this study.

Age (days)	Body Weight (g) x ± SD	(n)	Range (g)
1	1.15 ± 0.21	(55)	0.74- 1.72
4	2.74 ± 0.60	(55)	1.66- 4.35
7	5.57 ± 1.13	(55)	3.84- 7.80
10	8.53 ± 1.25	(55)	5.47-11.58
13	10.30 ± 1.05	(55)	7.57-12.80
16	10.60 ± 0.75	(51)	8.92-12.73
19	10.88 ± 0.72	(34)	9.28-12.36
22	11.55 ± 0.54	(14)	10.04-12.39
35	12.81 ± 0.66	(28)	11.29-13.87
Adult	13.47 ± 1.13	(23)	11.20-16.00

The onset and progression of feather growth and the opening of the eyes are developmental landmarks which can be used to assess physiological and morphological maturity. Primary feathers begin erupting in some individuals on day 4, and all nestlings have primary

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feather shafts by day 7. In a majority (29 out of 43 individuals) of nestlings, the primary feathers erupted on day 5. Nestlings' eyes open between 4 and 8 days of age. The majority, 42 out of 52 nestlings, opened their eyes on days 6 or 7 (16 and 26 individuals, respectively). By 13 days of age nestlings are about 50% covered by feathers. Contour feathers erupt and begin to unsheathe by 13 days and reach final length by 19 days. Sixteen-day-old birds are capable of short, uncoordinated flights, and nestlings fledge between days 19 and 22. By 19 days of age nestlings have attained 81% of mean adult weight and primary feather length (19 day olds $\bar{x} = 33.1 \pm 1.56$ mm, adults $\bar{x} = 40.9 \pm 0.91$ mm). By 22 days nestlings begin to feed on their own and can be independent of their parents by 28 days. The post-juvenile molt begins at about 28 days of age and is complete by 50 days of age. The data presented on growth and development include measurements from both color morphologies and sexes.

The body weights of a series of nestlings were examined to determine if there were any differences between color morphologies and sexes. No differences were found at any age between grey and chestnut-flanked white birds (all t-tests were non-significant, $p \geq 0.05$). Also, no differences (all t-tests were non-significant, $p \geq 0.05$) were found at any age between male and female finches (sex ascertained at maturity). Therefore, I assumed, on the basis of these data and in agreement with the findings of Cade <u>et al</u>. (1965), that no differences would exist in EWL between birds of different color or sex. No attempts were made to balance sex ratios in EWL tests at ages where sex could not, as yet, be determined (all but 35 day olds and adults).

Evaporative Water Loss

The one-way ANOVA's on total EWL (TEWL) and weight-specific EWL (SEWL) showed significant treatment effects (Table 2). The unweighted means analysis of the three-by-nine factorial showed that there was no significant interaction between temperature and age for the TEWL data. However, there was a significant temperature-age interaction for the SEWL data. Bartlett's test for homogeneity of variance indicated that the variances for both the TEWL and SEWL data were homogeneous (X^2 = 35.8, 40.1, k = 28, $0.250 \geq p \geq 0.050$, respectively).

Total EWL from birds exposed to 35°C ranged from 6.93 ± 2.18 mg H_2O/hr (1 day olds) to 67.67 ± 15.65 mg H_2O/hr (35 day olds, Table 3). Total EWL increased rapidly from 1 day of age to 13 days of age (Figure 2). The rate of water loss was not significantly different between 1 and 4 day olds, but significant increases were found at 7, 10 and 13 days of age (Table 3). Total EWL was not different between any ages ranging from 13 days old through adulthood. The data utilized for the adults represent a combination of data from ligated and non-ligated individuals. A one-way ANOVA conducted between these two groups showed that there were no differences between the treatments ($\bar{x}$ TEWL of ligated birds = 62.95 ± 12.27; n=10; $\bar{x}$ TEWL for non-ligated birds = 59.48 ± 9.85; n=10; F=0.487; df=1,18; $0.25 \geq p \geq 0.50$). Two of the measurements taken on 16 day olds were omitted from the analysis. These values were much higher than the rest of the values recorded. The large values probably resulted from a slow but steady leakage from the cloaca or from the birds being active in the chambers during the measurement period.

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Table 2. Results from the unweighted means ANOVA's on TEWL and SEWL data. (The total, treatment, and error terms are from the initial one-way ANOVA. The age, temperature, and age-temperature [AxT] terms are from the unweighted means ANOVA).

TEWL

Source	df	SS	MS	F	p
Total	151	67929.762			
Treatment	26	56404.860	2169.418	23.529	$p \leq 0.001$
Age	8	8882.436	1110.305	61.221	$p \leq 0.001$
Temperature	2	951.390	475.695	26.229	$p \leq 0.001$
A x T	16	275.779	17.236	0.950	$0.75 \geq p \geq 0.50$
Error	125	11524.902	92.199		

$$\frac{s^2}{nh} = 18.136$$

SEWL

Source	df	SS	MS	F	p
Total	151	408.976			
Treatment	26	303.836	11.686	70.653	$p \leq 0.001$
Age	8	27.140	3.393	20.514	$p \leq 0.001$
Temperature	2	19.690	9.845	59.522	$p \leq 0.001$
A x T	16	13.780	0.861	5.207	$p \leq 0.001$
Error	125	105.140	0.841		

$$\frac{s^2}{nh} = 0.165$$

Harmonic mean = 5.084

Figure 2. Mean TEWL from nestling, juvenile, and adult zebra finches exposed to 20°C, 27.5°C, and 35°C. (Solid lines connect mean TEWL at 35°C, dashed lines for 27.5°C, and dashed and dotted line for 20°C).

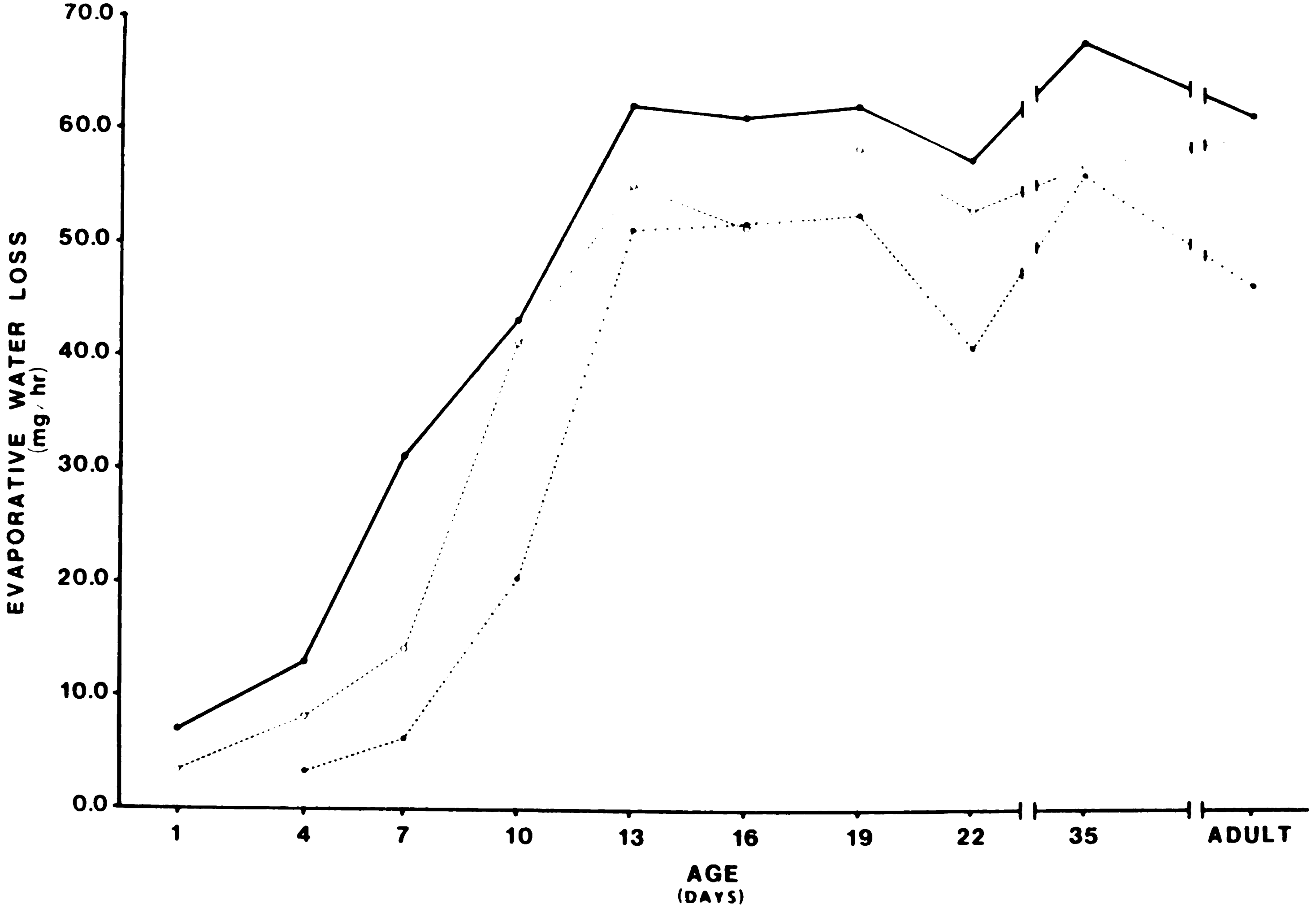

EVAPORATIVE WATER LOSS
(mg/hr)
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
1
4
7
10
13
16
19
22
35
ADULT
AGE
(DAYS)

Figure 2. Mean TEWL from nestling, juvenile, and adult zebra finches exposed to 20°C, 27.5°C, and 35°C. (Solid lines connect mean TEWL at 35°C, dashed lines for 27.5°C, and dashed and dotted line for 20°C).

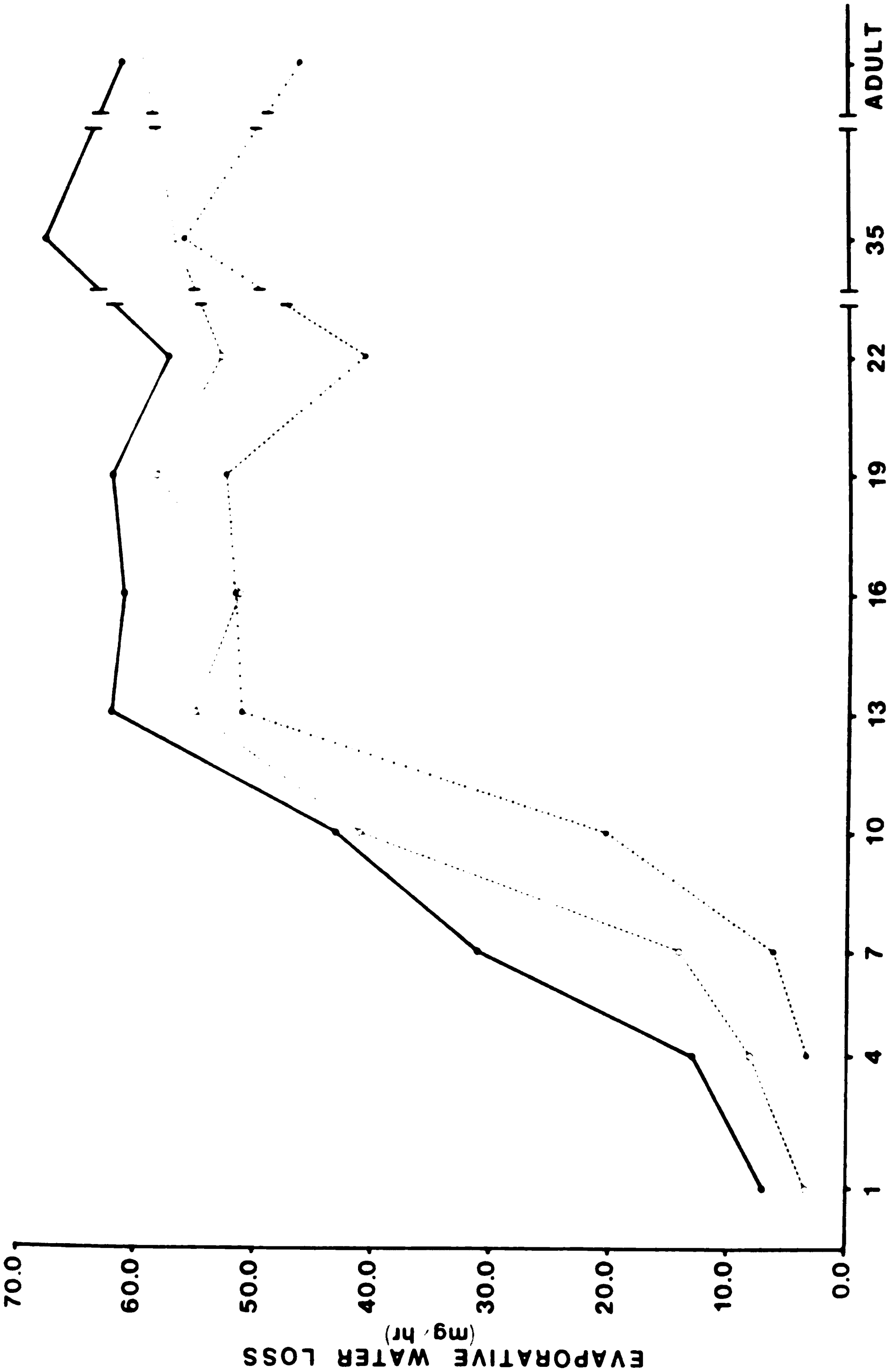

EVAPORATIVE WATER LOSS
(mg/hr)
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
AGE
(DAYS)
1
4
7
10
13
16
19
22
35
ADULT

Table 3. Mean TEWL and SEWL and mean body temperature for nestling, juvenile, and adult zebra finches exposed to 35, 27.5, and 20°C. (Any two means within a column followed by the same letter are not significantly different, α=0.05).

Age	T_a (°C)	n	Total EWL (mg/hr) $\bar{x} \pm$ SD	Weight Specific EWL (mg H_2O/g hr) $\bar{x} \pm$ SD	T_b (°C) $\bar{x} \pm$ SD
1	35*	5	6.93 ± 2.18 A	6.01 ± 1.40 HI	34.9 ± 0.5
	27.5*	5	3.31 ± 0.57 A	2.65 ± 0.41 BC	27.9 ± 0.6
	20	-	-	-	-
4	35	5	12.86 ± 2.48 AB	5.96 ± 0.51 HI	36.2 ± 0.8
	27.5*	5	8.08 ± 2.54 A	2.64 ± 0.67 BC	28.7 ± 0.9
	20*	5	3.26 ± 0.19 A	1.13 ± 0.12 A	21.3 ± 0.9
7	35	5	30.98 ± 14.74 CD	5.40 ± 1.79 GHI	37.6 ± 1.0
	27.5	5	14.01 ± 4.87 AB	3.05 ± 0.49 BCD	32.1 ± 1.3
	20	5	6.09 ± 1.93 A	1.02 ± 0.32 A	21.7 ± 0.7
10	35	5	42.89 ± 10.02 EF	6.04 ± 0.92 HI	37.4 ± 1.0
	27.5	5	40.87 ± 9.55 DEF	5.42 ± 0.95 GHI	36.4 ± 2.2
	20	5	20.05 ± 7.24 BC	2.57 ± 0.86 B	28.2 ± 5.8
13	35	6	61.79 ± 8.05 HI	6.34 ± 0.87 I	38.5 ± 0.8
	27.5	5	54.76 ± 8.20 GH	6.42 ± 0.82 I	37.7 ± 1.6
	20	4	50.79 ± 2.77 EFGH	5.62 ± 0.31 GHI	37.7 ± 2.3
16	35	3	60.79 ± 11.23 HI	6.31 ± 0.80 I	38.7 ± 0.4
	27.5	5	50.99 ± 8.28 EFGH	5.63 ± 1.20 GHI	39.9 ± 0.9
	20	5	51.11 ± 4.08 EFGH	5.39 ± 0.77 GHI	39.8 ± 0.7
19	35	5	61.81 ± 13.09 HI	6.08 ± 1.20 HI	39.0 ± 1.1
	27.5	5	58.05 ± 13.31 HI	6.37 ± 1.66 I	39.9 ± 0.7
	20	5	52.26 ± 2.21 EFGH	4.83 ± 0.12 FG	39.9 ± 0.5
22	35	5	57.06 ± 10.03 GHI	5.11 ± 1.04 GH	39.0 ± 1.3
	27.5	5	52.59 ± 6.71 FGH	5.32 ± 0.07 GHI	40.5 ± 0.6
	20	5	40.55 ± 5.54 DE	3.81 ± 0.36 DEF	40.4 ± 0.5
35	35	9	67.67 ± 15.65 I	5.64 ± 1.28 GHI	39.9 ± 0.7
	27.5	5	56.67 ± 5.34 GHI	4.62 ± 0.43 EFG	40.6 ± 0.9
	20	5	55.91 ± 8.97 GHI	4.68 ± 0.88 EFH	40.7 ± 0.8
Adult	35^{a}	20	61.21 ± 10.98 HI	4.53 ± 0.79 EFH	41.5 ± 1.4
	35^{b}	10	62.95 ± 12.27	4.62 ± 1.05	40.6 ± 1.1
	35^{c}	10	59.48 ± 9.85	4.43 ± 0.68	42.5 ± 0.9
	27.5	5	59.48 ± 16.88 HI	4.69 ± 1.41 EFG	41.2 ± 0.5
	20	5	46.24 ± 9.35 EF	3.69 ± 0.70 CDE	40.7 ± 0.6

* - values reported are means from 5 EWL runs with 2 nestlings per run. The TEWL values are reported on a per animal basis.

a - combined EWL data for all adults run at 35°C.

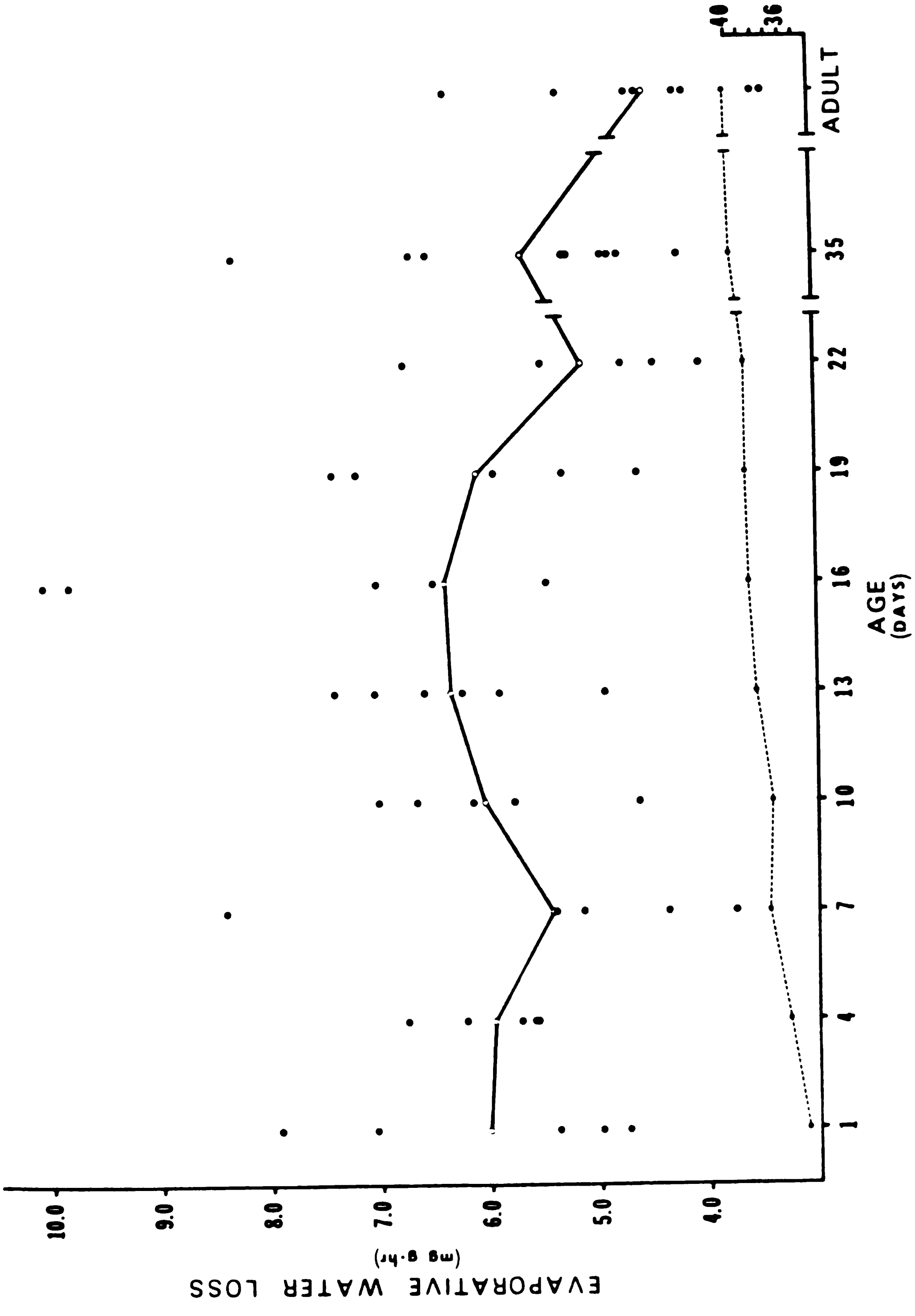
b - adults with ligated cloacae.

c - adults without ligated cloacae and placed over mineral oil.

Weight-specific EWL fluctuated with age at 35°C, but there was a general inverse relationship between SEWL and increasing age (Figure 3). The lowest level of SEWL was recorded from adults, $\bar{x} = 4.53 \pm 0.79$ mg H_2O/g·hr, and the highest rate was recorded from 13 day olds, $\bar{x} = 6.35 \pm 0.87$ mg H_2O/g·hr. There were non-significant changes in SEWL from 1 to 7 days of age and then an increase from 7 to 13 days of age. The mean body temperature (T_b) of the nestlings also increased between 1 and 13 days of age. At 1 day of age T_b is at ambient levels and by 13 days of age T_b is nearly equal to adult levels (Table 3). Weight-specific EWL decreased gradually from 13 day olds through adults, and T_b increased slightly. The results from the LSD comparisons (Table 3) show that while SEWL rates vary with age most of these rates are not significantly different. Adults and 22 day olds, the two groupings having the lowest SEWL rates at this temperature, were found to be significantly different from most of the other ages. The EWL values recorded from the two experimental adult groups were combined for EWL ANOVA's because no significant differences were found between the two groups ($\bar{x}$ SEWL for ligated birds = 4.62 ± 1.05; n=10; $\bar{x}$ SEWL for non-ligated birds = 4.43 ± 0.68; n=10; ANOVA: F=0.277; df=1,18; $0.75 \geq p \geq 0.50$).

At 27.5°C TEWL increased rapidly from 1 to 13 days of age (Table 3, Figure 2). The most rapid increase occurred between 7 and 10 days of age. No significant differences were found between 1, 4, and 7 day olds, but TEWL from 10 day olds was significantly different from these younger ages as well as from the older birds measured at this temperature. Birds, 13 days old through adulthood showed a gradual but non-significant increase in TEWL. The rate of water loss ranged from

Figure 3. Weight-specific EWL and mean body temperature (T_b) for nestling, juvenile, and adult zebra finches exposed to 35°C. (Closed circles represent individual data points, open circles represent mean SEWL at each age, and the small, closed circles represent mean T_b at each age).


EVAPORATIVE WATER LOSS
(mg g·hr)
10.0
9.0
8.0
7.0
6.0
5.0
4.0
AGE
(DAYS)
1
4
7
10
13
16
19
22
35
ADULT
40
36

3.31 ± 0.57 mg H_2O/hr (1 day old) to 59.48 ± 16.88 mg H_2O/hr (adults).

Weight-specific EWL did not increase from 1 to 4 days of age at 27.5°C (Table 3, Figure 4). The rate measured from 7 day olds was slightly elevated but none of the means for these early ages were significantly different from one another. Weight-specific EWL increased sharply from 7 to 10 days of age and there was a less dramatic (non-significant) increase from 10 to 13 days of age. At 13 days, SEWL reached its peak value, $\bar{x} = 6.42 \pm 0.82$ mg H_2O/g•hr. There was a general decrease in SEWL from 13 days through adulthood. Rates of SEWL measured from 35 day olds and adults were significantly lower than the values recorded from 13 and 19 day olds. There was only a slight increase in T_b between 13 and 16 days of age. Body temperatures of nestlings and juveniles 16 days old or older were nearly equal to adult T_b's (Table 3).

The rate of TEWL in 4 and 7 day olds exposed to 20°C did not change significantly (Table 3, Figure 2). Total EWL increased significantly between 7 and 10 and 10 and 13 days of age. The rate of TEWL was very similar among birds 13 days old and older except for birds at 22 days of age. Total EWL in 22 day olds was significantly different only from 35 day olds.

Weight-specific EWL was not significantly different between 4 and 7 day olds at 20°C. There was a significant increase in SEWL between 7 and 10 and also between 10 and 13 days of age. Weight-specific EWL values obtained from 10-day-old nestlings showed a wide range of variation (Figure 5). Corresponding with this broad range of SEWL values was a large variation in body temperature. Body temperatures ranged between 23.0 and 36.5°C at this age. Those nestlings having a

Figure 4. Weight-specific EWL and mean T_b for nestling, juvenile, and adult zebra finches exposed to 27.5°C. (Symbols are the same as described in Figure 3).

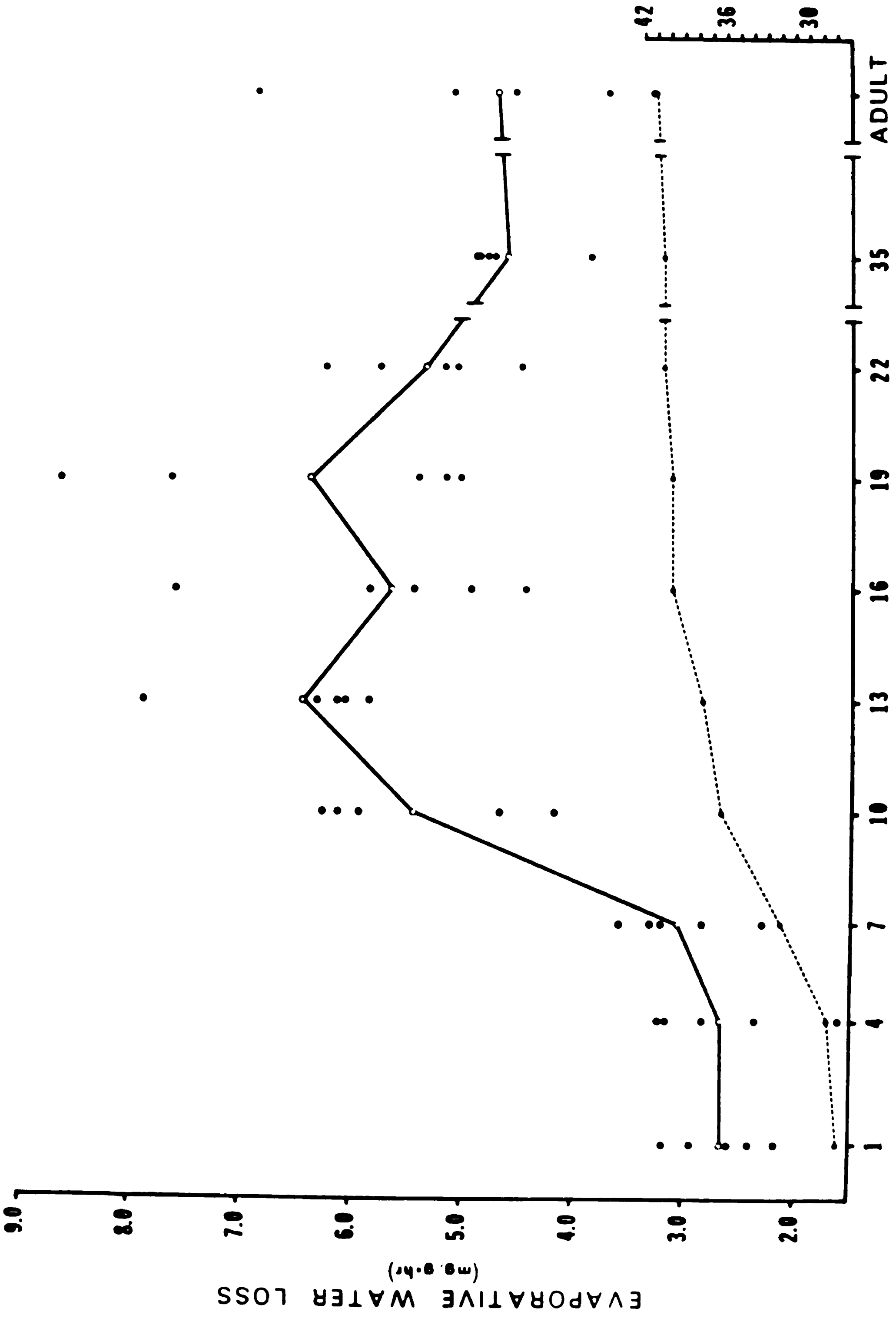
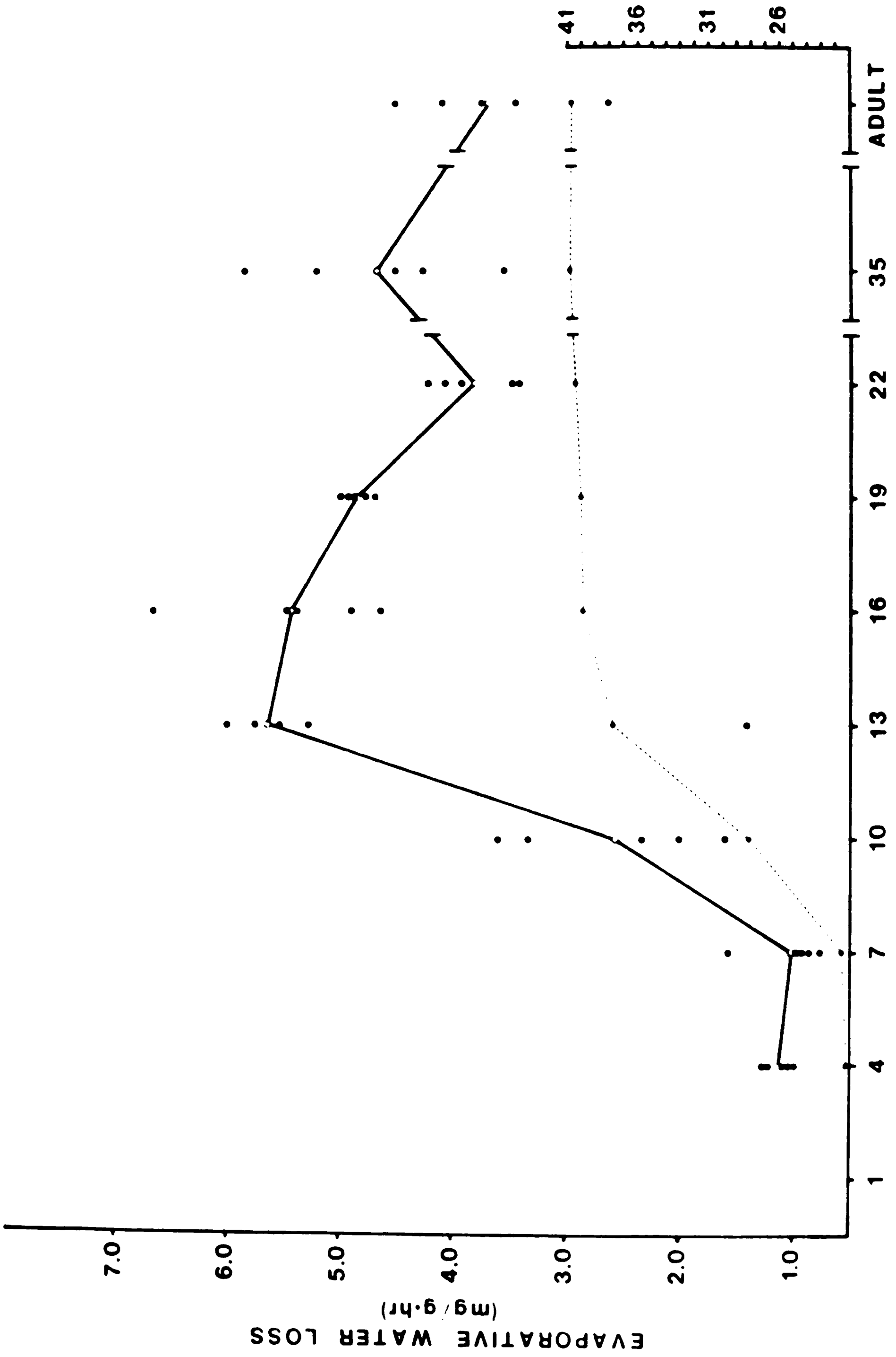

EVAPORATIVE WATER LOSS
(mg. g·hr)
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
AGE
(DAYS)
1
4
7
10
13
16
19
22
35
ADULT
42
36
30

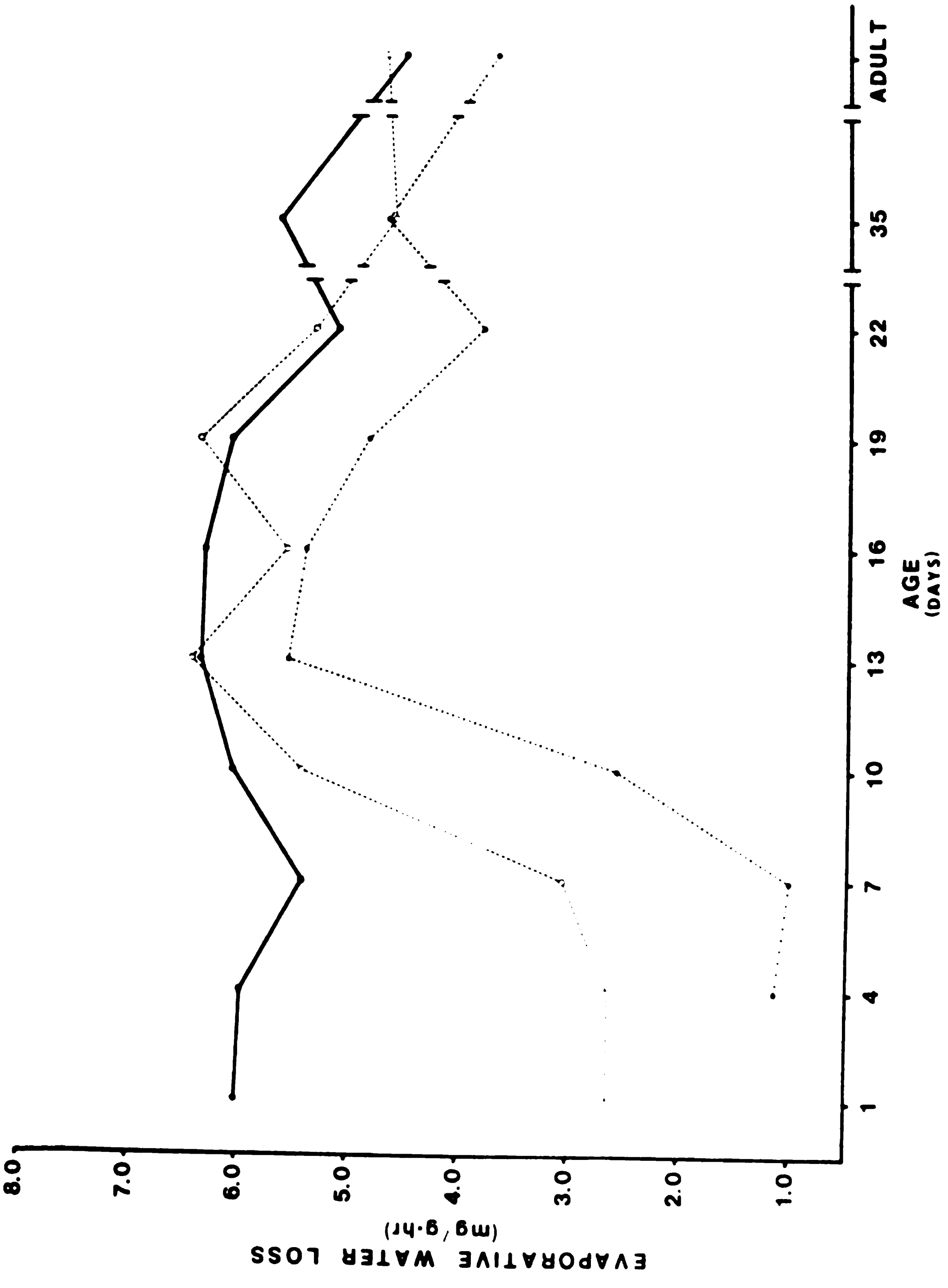
Figure 5. Weight-specific EWL and mean T_b for nestling, juvenile, and adult zebra finches exposed to 20°C. (Symbols are the same as described in Figure 3).


EVAPORATIVE WATER LOSS
(mg/g·hr)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
1
4
7
10
13
16
19
22
35
ADULT
AGE
(DAYS)
41
36
31
26

lower body temperature also had a lower rate of SEWL ($r=0.95$, $n=5$, $r^2=0.911$). Although five measurements were made on 13 day olds, only four were included in the calculation of the mean. The omitted value was from a nestling that became hypothermic during EWL measurement ($T_b=21.0°C$, SEWL=1.41 mg $H_2O/g\cdot hr$). Mean body temperature did not increase between 4 and 7 days of age in nestlings exposed to 20°C (Table 3). Although SEWL showed a non-significant increase between 7 and 10 days of age, T_b increased nearly 7°C. A similar increase in mean T_b, approximately 9.5°C, occurred between 10 and 13 days of age. At 13 days the mean T_b of nestlings exposed to 20°C is equal to that of 13 day old nestlings exposed to 27.5°C if the T_b of the hypothermic nestling is omitted. Weight-specific EWL decreased from the highest recorded rate, 5.62 ± 0.31 mg $H_2O/g\cdot hr$, from nestlings at 13 days of age to juveniles at 22 days of age. The mean SEWL at 22 days was significantly lower than values recorded from 13 and 16 day olds. There was a non-significant increase in SEWL at 35 days of age. The rate of SEWL in adults was much lower than in 13, 16, 19 and 35 day olds, but only the rates for 13 and 16 day olds were significantly different. By 16 days of age T_b approaches adult levels, and mean T_b increases less than 1°C between 16 days of age and adulthood.

As indicated by the ANOVA for the TEWL (Table 2), there were significant effects due to temperature and age. Comparing TEWL between temperatures shows that at 1 and 4 days of age a lower ambient temperature does not significantly decrease EWL even though the rate of TEWL at 20°C in 4 day olds is nearly four times lower than that of 4 day olds at 35°C. Total EWL in 7 day olds is similar at 20 and 27.5°C but the rate recorded from nestlings at 35°C is significantly

Figure 6. Mean SEWL for nestling, juvenile, and adult zebra finches exposed to 20°C, 27.5°C, and 35°C. (Solid lines connect mean SEWL at 35°C, dashed lines for 27.5°C, and dashed and dotted lines for 20°C).


EVAPORATIVE WATER LOSS
(mg/g·hr)
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
AGE
(DAYS)
1
4
7
10
13
16
19
22
35
ADULT

SEWL at 13 days. It is clear that decreasing ambient temperature lowers the rate of SEWL, but as nestlings age this effect is less pronounced. Comparing SEWL in nestlings at 27.5 and 20°C shows that similar rates of SEWL are attained three days later in 20°C birds (i.e., SEWL in 10 day olds at 20°C is not significantly different from 7 day olds at 27.5°C, and the same pattern is seen comparing 13 day olds at 20°C with 10 day olds at 27.5°C).

The results of the LSD comparisons on birds ranging from 13 days of age through adulthood showed only one significant difference (22 day olds at 20°C) between similarly aged birds at each temperature. The results obtained from nestlings exposed to 20°C, however, were consistently lower than those obtained at the other two ambient temperatures. Also, it should be noted that the measured body temperatures of birds in this group were all very similar, regardless of age. Comparisons among age-temperature combinations showed few differences in SEWL. In general, nestlings between 13 and 19 days of age had higher SEWL rates than the other age groupings. Although SEWL rates for adults at all temperatures were not significantly different from rates for most of the other birds exposed to the same temperatures, adult rates were generally lower.

EWL From Broods of Nestlings

The rate of EWL was measured from natural broods consisting of four nestlings. Natural broods are not of uniform age. However, at least two members of each brood were at the nominal experimental age, and the other two members of the brood were often one day older and younger than the nominal age. The results presented in Table 4 are the mean rates of SEWL from each brood age. Although the mean rate of brood SEWL fluctuates between ages, no significant differences were found between any of the ages (Tables 4 and 5). In 13 and 16 day old broods one of the measurements was higher than the other two. The high value for 16 day olds (6.95 mg H_2O/g·hr) was 80% above the other two SEWL rates recorded and was omitted from the calculations. The higher value for 13 day old broods exceeded the other two values by 30%, but it was not excluded from the calculations. It is possible that the nestlings in the 16 day old brood, and possibly the nestlings from the 13 day old brood, were active during the measurement period, thus increasing the rate of EWL. The body temperatures of broods at each age were nearly the same as those recorded from individual nestlings.

Table 4. Mean SEWL from broods of four nestlings exposed to 35°C.

Age (days)	SEwL (mg H_2O/g·hr)	T_b (°C)	n	Range
4	4.14 ± 0.72	35.46 ± 1.06	3	3.46-4.90
7	3.53 ± 0.46	37.13 ± 0.45	3	3.08-4.00
10	3.87 ± 0.24	38.05 ± 0.62	3	3.70-4.15
13	5.29 ± 0.95	39.03 ± 1.00	3	4.59-6.37
16	3.79 ± 0.06	40.32 ± 0.26	2	3.75-3.83

Comparisons of brood and individual nestling SEWL revealed two patterns. First, mean SEWL from individual nestlings and broods showed similar increases and decreases with age. Second, it was clear that grouping the nestlings resulted in a decrease in SEWL. The results from the ANOVA indicated that grouping had a significant effect on SEWL while age had no effect (Table 5). No interaction between age and type of grouping was demonstrated. Mean SEWL was significantly lower in 10 day old broods than in similarly aged individuals. Although brood SEWL rates were not always significantly different from individual rates, brood SEWL was, in general, 32% lower than individual rates.

DISCUSSION

Although the loss of water due to evaporation is governed, in part, by the physical properties of water, the biological causes and consequences of evaporation play a major role in the physiology of an organism. Calder and King (1974) state that the loss of water via evaporation is an inevitable consequence of the need for gas exchange to occur across a moist surface and is a potential liability to thermal and osmotic balance. In addition to losses of water across the respiratory surfaces, the skin can be a major site of evaporative water losses (c.f., amphibians and reptiles, Spotila and Berman 1976, Davis et al. 1980; birds, Marder 1983; mammals, Schmidt-Nielsen 1979). Cutaneous water losses in birds were considered to be unimportant in early studies of EWL because birds lack sweat glands (Rawles 1960, Jenkinson and Blackburn 1968). However, the significant rate of cutaneous evaporative water loss in birds and its importance in thermoregulation has been clearly demonstrated over the last 20 years (Lee 1964; Smith 1969; Smith and Suthers 1969; Bernstein 1971a,b; Marder 1983; Marder and Ben-Asher 1983). One of the major forces influencing the rate of evaporation is the temperature of body liquids exposed to the air interface. The latent heat of vaporization is

Table 5. Unweighted means ANOVA and LSD comparisons for SEWL from broods and individual nestlings exposed to 35°C. (The total, treatment, and error 1 terms are from the initial one-way ANOVA. The grouping, age, and grouping-age [GxA] terms are from the unweighted means ANOVA.)

Source	df	SS	MS	F	p
Total	37	67.208			
Tmt	9	30.254	3.362	2.547	$p \leq 0.05$
Grouping	1	8.893	8.893	22.979	$p \leq 0.001$
Age	4	1.870	0.468	1.208	$p \leq 0.25$
G x A	4	0.597	0.149	0.386	$p \leq 0.75$
Error 1	28	36.954	1.320		
Error			0.387[a]		

a) Error term used for unweighted means ANOVA.

LDS Comparisons[b]

Age	Grouping	SEWL	LSD
4	Brood	4.14 ± 0.72	AB
	Individual	5.96 ± 0.51	BC
7	Brood	3.53 ± 0.46	A
	Individual	5.40 ± 1.79	ABC
10	Brood	3.87 ± 0.24	A
	Individual	6.04 ± 0.92	BC
13	Brood	5.29 ± 0.95	ABC
	Individual	6.34 ± 0.87	C
16	Brood	3.79 ± 0.06	A
	Individual	6.31 ± 0.80	C

b) Any two means followed by the same letter are not significantly different.

dependent upon the temperature of the liquid (in animals the water is contained within the skin and tissues lining the respiratory passages, so skin and core temperatures are the best indicators of evaporative temperatures), and to a lesser degree on the ambient vapor pressure and air temperature (Calder and King, 1974). Recently, Welch (1980) found that evaporation from a wet surface, when convective heat loss is constant, shows a negative linear relationship with ambient vapor density. In a thermally homogeneous environment, evaporation is maximal when the air is dry and is zero in saturated air. Welch's work points to the importance of ambient vapor density and the complex interaction between surface and ambient conditions in influencing evaporation. Lasiewski _et_ _al_. (1966a) note that without consideration of chamber humidity conditions, comparisons of EWL measurements are difficult because vapor pressure conditions can vary greatly. Lasiewski _et_ _al_. recommend that EWL measurements be made in dry conditions (RH $\leq$ 15-20%) in order to standardize EWL measurements and, more importantly, to minimize the supression of EWL by increased water vapor pressure within the animal chamber. Unfortunately, dry conditions ignore the actual response of an animal to its environmental conditions. A few studies have investigated the effects of increased ambient vapor pressure on EWL from animals. The results of these studies are, at times, contradictory and will not be discussed at this point. The use of dry air in the present study established similar hygric conditions for all the birds measured and will allow comparisons of my data with previous studies on zebra finches and other birds in general.

Evaporative Water Loss

The effects of ambient temperature on TEWL, SEWL, and body temperature indicate that nestling, juvenile, and adult zebra finches can be divided into two response groups. The first group, nestlings ranging in age from 1 to 13 days old, is characterized by dramatic changes in the rate of EWL and T_b with increasing age at each ambient temperature (T_a). The second group--13 through 22 day olds, 35 day olds, and adult birds--exhibits relatively constant rates of EWL and body temperature. The rapid, exponential growth phase of nestling zebra finches coincides with the first group, and the rate of growth slows at 13 days of age. The accretion of body mass proceeded slowly from 13 days through adulthood. As a result of the similarities within each grouping and the differences between groupings, the following discussion will consider each grouping separately and then consider the overall trends in EWL for zebra finches.

The analysis performed on the data indicates that when EWL is expressed in weight-specific terms there is a significant interaction between age and temperature. Inspection of the data presented in Figure 6 shows that the effects of the interaction are more pronounced in nestlings ranging in age from 1 to 13 days than in the older grouping of birds. This interaction can be best understood if it is assumed that the data collected from nestlings at 35°C are the normal response of zebra finches. As previously indicated (see Methods section), 35°C was the mean T_b of young nestlings measured immediately upon removal from the nest. It would be unexpected from our knowledge of nestling thermoregulatory abilities that individual nestlings between 1 and 7 days old could maintain a T_b 14°C above ambient (the air temperature in the colony measured next to one nest box was 21°C on the day nestling T_b's were measured). Therefore, the measured T_b's represent the combined effects of brood thermogenesis and heat input from the brooding adult. If 35°C is the normal T_b of young nestlings, then the data collected at the other two temperatures reflect responses to cold stress.

1-13 Days of Age

Although TEWL at 35°C increases rapidly between 1 and 13 days of age, SEWL shows only a gradual, non-significant increase through this age range. Total EWL increases significantly between all ages in this age grouping except for 1 and 4 day olds. Evaporative water loss rates are variable in 1 day olds. This variability may be due to variation in the general health of the nestlings. During the first 24-36 hours of life, nestlings are not always fed by their parents. Nestlings that are fed during the first 24 hours appear to be more vigorous than unfed nestlings. Adults begin to feed nestlings consistently after this period and it is possible that adults are not making the behavioral transition between incubating and rearing young until most of the eggs have hatched. By 4 days of age these differences in nestling vigor are not evident.

Comparisons of EWL rates from 7 day olds at 35°C show that rates from one individual were much higher than the others (Figure 3). Weight-specific EWL and T_b for this individual were 8.4 mg $H_2O/g \cdot hr$ and 38.5°C, respectively. This nestling's body temperature was elevated several degrees above ambient, and its body weight was higher than that of the other 7 day olds. The higher rate of EWL from this nestling may have been due to the onset of thermoregulatory abilities.

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If this were the case, then the elevated body temperature could cause an increase in skin and core temperature, thus accelerating the rate of EWL. Also, the absence of any feathering at this age could contribute to this high rate of water loss. On the other hand, it is possible that this nestling was active throughout the measurement period, and the rate of EWL measured was not indicative of resting EWL rates for 7 day olds. The data collected on this nestling do not provide conclusive evidence of the interrelationship between thermoregulatory abilities and rates of EWL. The data collected at lower ambient temperatures provide stronger evidence.

The large increases in TEWL between 1 and 13 days of age coincide with the exponential growth phase for zebra finches. It is difficult to assess what portion of the increase in TEWL is due to the nestlings' larger body size. As an animal grows, its evaporative surface areas (skin and respiratory surfaces) increase. If the rate of water flux per unit area does not change, then an animal with a larger surface area will have a greater rate of water loss. It is interesting to note that during periods of slow growth (1 through 4 days of age, and at ages older than 13 days) non-significant differences in TEWL were noted for nestlings at 35°C (Figure 1 and Table 3). These data indicate how closely rates of EWL and body size are linked, but as the data for the single 7 day old at 35°C suggest, the ability to maintain an elevated, stable T_b also has an impact on TEWL.

Total and weight-specific evaporative water losses at 27.5°C are numerically lower in 1, 4, 7, and 10 day olds than at 35°C (Figures 2 and 4). Weight-specific EWL is significantly lower in 1, 4, and 7 day olds, but by 10 days of age there are no statistical differences betwee

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between nestlings exposed to 27.5 and 35°C. Total EWL is not significantly different among most ages in this age grouping. The exceptions are 7 day olds at these two temperatures. There are significant increases in TEWL and SEWL between 7 and 10 day olds at 27.5°C. Coinciding with this increase in EWL is a rise in mean T_b, 32.1 to 36.4°C. The effects of lowered ambient temperature on EWL are clear in nestlings between 1 and 7 days of age. In older nestlings the lowered temperature has less of an effect. The inability of nestlings to elevate their T_b's at low ambient temperatures when 1 to 7 days old is an indicator of the immature thermoregulatory response in the younger nestlings.

The effects of a lower ambient temperature on EWL are obvious from the SEWL data at 20°C (Figure 5). The rates of EWL are significantly lower in 4, 7, and 10 day olds at 20°C than at either of the other two temperatures. Between 4- and 7-day-old nestlings at 20°C there is little variation in SEWL or body temperature and TEWL shows only a modest increase. Nestlings at both ages were lethargic when removed from the chambers and their body temperatures indicate that they were hypothermic. Weight-specific EWL and TEWL increase markedly in 10 day olds, but the rates of water loss are still significantly lower than rates at 27.5°C and 35°C. There is large variation in TEWL, SEWL and T_b in 10 day olds at 20°C (see Figure 5). For example, in two of the nestlings, T_b and SEWL rates were 36.5 and 32.0°C and 3.59 and 3.33 mg $H_2O/g \cdot hr$, respectively, while T_b and rates of SEWL are 25.5, 24.0, and 23.0°C and 2.33, 1.60 and 2.00 mg $H_2O/g \cdot hr$ in the other three nestlings. The differences in rates of EWL and T_b that are seen at 10 days of age are no longer apparent by 13 days of age.

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Thirteen day olds exposed to 20°C show rates of EWL that are not significantly different from nestlings measured at 27.5 and 35°C. Also, the T_b of 13 day olds is similar to that of nestlings at the other temperatures. The T_b data from nestlings at 20°C indicate that the onset of thermoregulatory abilities begins around 10 days of age. Also, these data show that as the temperature of the evaporative surface (measured as T_b) increases, so does EWL.

Although the inability of young nestlings to thermoregulate affects the rate of TEWL at low ambient temperatures, TEWL increases with age until the nestlings are 13 days old. Total EWL in birds older than 13 days is relatively constant (Figure 2). This pattern of TEWL increasing with age has been reported for several species. Bernstein (1971a) states that TEWL increases with age in painted quail (*Excalfactoria chinensis*). Similar findings are reported by Medway and Kare (1959a) in chickens (*Gallus domesticus*), Kayser (1930) in pigeons (*Columba livia*), and Kendeigh (1939) in house wrens (*Troglodytes aedon*). Both Kayser and Kendeigh note that a steady rate of water loss is reached halfway through the nestling period and that this transition in the rate of TEWL occurs when the nestlings begin to thermoregulate. Even though developmental strategies vary between these precocial and altricial species, the similarities in physiological trends indicate that some developmental characteristics are uniform in neonatal birds.

While nestlings may not be able to thermoregulate at low ambient temperatures, their ability to dissipate body heat through evaporative cooling can be well developed at early ages. For example, Hudson *et al*. (1974) report that nestling cattle egrets (*Bubulcus ibis*) increase

SEWL twofold when ambient temperatures increase from 40 to 45°C. The ability of individual nestling cattle egrets to thermoregulate at high ambient temperatures precedes their ability to respond to low ambient temperatures. Other neonatal birds show large increases in EWL in response to high ambient temperatures (house wrens, Kendeigh 1939; painted quail, Bernstein 1971a; ravens, Mishaga and Whitford 1983). These studies show that the ability of neonates to thermoregulate affects EWL in two ways. First, as nestlings gain the ability to thermoregulate at low ambient temperatures, their T_b and, as a result, evaporative surface temperatures increase. Calder and King (1974) state that the rate of evaporation is dependent on the temperature of the evaporating surface. As skin and core temperatures increase, so should EWL. Second, if a nestling is capable of dissipating body heat through evaporative cooling, then the rates of EWL can double or triple depending on the level of heat stress. Bernstein (1971a) states that the high rate of evaporative heat loss in neonatal quail prevents younger birds from maintaining a stable body temperature.

As indicated, Bernstein (1971a) states that the inability of neonatal quail to thermoregulate is due to their high rates of cutaneous EWL. He further suggests that the comparatively high skin permeability of neonates is a contributing factor. Bernstein argues that the absence of adult-like plumage and high skin permeability establishes a larger vapor pressure gradient in neonates when compared with adults. One of the principle aspects of Bernstein's argument is that the body temperature of the young quail is equal to adult body temperature. I examined my data on zebra finch EWL to determine if Bernstein's hypothesis can be applied to altricial nestlings. I calculated the vapor pressure gradient between a bird and the air in the EWL chamber. In order to make this calculation, I assumed that 1) the air at the skin surface was saturated and that the temperature of this air was equal to the bird's body temperature and 2) the partial pressure of the air in the chamber was a function of the amount of water vapor added by EWL. The vapor pressure gradients which I calculated suggest two trends. First, if the body temperature of the nestlings varies with ambient temperature, then the vapor pressure gradient for an adult bird is always greater than that of a nestling. Second, if nestling body temperatures are at adult levels, then the nestlings have a larger vapor pressure gradient. Because my data on nestling body temperatures show that young nestlings can not maintain an elevated T_b at all T_a's, the second trend does not apply to my data. In order for Bernstein's hypothesis to apply to zebra finch nestlings, a young bird having a lower T_b must lose a larger volume of water than an adult. My data show that nestlings lost less water, and therefore my data suggest that body temperature governs EWL instead of EWL governing body temperature as Bernstein suggests. A more complete test of Bernstein's theory is necessary before we can determine what role skin permeability and vapor pressure gradients play in EWL and thermoregulatory abilities of neonatal birds. His results, when combined with the data from other studies, show that regulation of EWL is one of the principal factors in allowing neonates to thermoregulate.

It is apparent that the ability of a nestling to thermoregulate greatly affects its rate of EWL. During the period from 0-13 days zebra finches appear to acquire the ability to thermoregulate. Dunn

(1975) defines the age of endothermy as "...that age at which individual nestlings can keep their body temperatures at least 75% as high above an ambient temperature of 20°C as can an adult, after the same period of exposure." Applying Dunn's criterion for determining the onset of endothermy to the data of the present study indicates that nestlings are capable of endothermy at 13 days of age (mean adult T_b = 41°C, T_b indicating thermoregulation = 35.8°C; see Table 3). Also, Dunn indicates that the age of endothermy varies with respect to growth and is directly dependent on the length of the nestling period. For example, field sparrows (Spizella pusilla) and chipping sparrows (Spizella passerina) which are similar in body size to adult zebra finches (W_b = 12.0 and 12.2g, respectively) are capable of thermoregulating between 5.5 and 6.0 days of age (Dawson and Evans 1957). This is a more rapid maturation than in zebra finches (age of endothermy is 13 days). The shorter nestling period in field (8.1 days) and chipping (9.9 days) sparrows is consistent with the rapid rate of maturation. As these data and others indicate, body weight or size is not the best predictor of the age of endothermy (Dunn 1975, Ricklefs and Hainsworth 1968).

It is difficult to determine what effects an increase in body weight or size have on nestling physiology. In the simplest of terms, an increase in body weight occurs with increasing age, and as a neonate ages it matures behaviorally, morphologically, and physiologically. Therefore, an increase in body weight implies an increase in physiological maturity. The age of endothermy in individual nestlings was first associated with the attainment of a critical body mass in conjunction with the development of insulation from feathers

(Kendeigh and Baldwin 1928; Dawson and Evans 1957, 1960; Ricklefs and Hainsworth 1968; Yarbrough 1970; Morton and Carey 1971). In theory, this critical mass represents the point at which heat storage capacity increases and surface-to-volume ratio decreases sufficiently to allow the nestlings' metabolic heat production to offset heat losses while maintaining a stable T_b. Marsh (1979) found that at the onset of thermoregulatory abilities, nestling bank swallows (Riparia riparia) lack sufficient plumage to retard heat loss and that overall conductance changes very little when feathers are present. An increase in thermogenic capacity in neonatal tissues appears to play a major role in the attainment of adult-like thermoregulatory abilities. Marsh and Wickler (1982) show that levels of myofibrillar ATPase (mATPase) in the pectoralis muscle and mixed leg muscles are low prior to the onset of endothermy. As thermoregulatory abilities develop, the levels of mATPase increase primarily in pectoral muscles. The abrupt change in enzyme activity coincides with the rapid increase in peak metabolic rate at this age. The work of Marsh and Wickler shows the complex nature of the thermoregulatory response of neonates. As Marsh's (1979) work on bank swallows illustrates, nestlings are capable of maintaining high body temperatures prior to total feather unsheathing. Similarly, the data from my study show that nestling zebra finches are capable of thermoregulating when they are only 50% covered by feathers. Also, the SEWL data from zebra finches show that the highest rate of water loss occurs close to the age of endothermy. The ability of the nestlings to maintain a high T_b when they lack complete feathering may create conditions that accentuate EWL. At 13 days of age, the skin surface temperature would be high. The absence of a total feather covering and a high body temperature in 13 day olds appear to be correlated with the high rate of SEWL.

The rate of EWL in neonatal birds appears to be independent of developmental strategy and is affected by age, ambient temperature, and the ability to thermoregulate. Comparisons of trends in EWL in altricial and precocial neonates reveal that TEWL increases with age and that lower ambient temperatures reduce rates of water loss. Although the effects of either high or low T_a's on EWL during ontogeny have been examined, no studies have examined EWL over a wide range of T_a's on one species. Also, the ability of neonates to maintain a stable T_b plays an important role in EWL. As skin and core temperature rise so does the driving force behind EWL. Although Bernstein's (1971a) data on quail chicks show that high rates of EWL may prohibit thermoregulation, no comparable data are available on altricial species. In order to gain a more complete understanding of EWL in neonates, broader studies which include a consideration of high and low T_a's, metabolic rate, and different habitat and nest types must be conducted.

13 Days - Adults

Birds within this age grouping all exhibit similar rates and patterns of EWL. The rate of TEWL shows only a slight increase between 13 days of age and adulthood. Although this pattern is observed in birds exposed to all three T_a's, there are some differences between each temperature group. Weight-specific EWL reaches peak rates in 13 day olds and shows a general decline with increasing age, an opposite trend compared to TEWL. The interaction between age and T_a on SEWL and the effects of age and T_a on TEWL must be considered in terms of the changes that occur when a bird progress from being a nestling to a fully independent bird (adult).

The rate of TEWL in nestlings, juveniles, and adults exposed to 35°C shows little change with age (Figure 2). The LSD comparisons of mean TEWL at each age show no difference. This static rate of TEWL further supports Calder (1964), Lee (1964), and Cade _et al_. (1965) in their assertion that 35°C is within the thermal neutral zone.

The decrease in SEWL between 13 days of age and adulthood may reflect changes in the rate of cutaneous EWL and activity level in the nestlings. Sixteen day old nestlings are almost completely covered by feathers. If feathers help to reduce the rate of cutaneous EWL, then it would be expected that SEWL would decrease between 13, 16, and 19 days. No significant decreases were found. Nestlings at 16 and 19 days of age are more active within the nest. The possible reduction in EWL due to feathers may be offset by an increase in respiratory EWL associated with an increase in metabolic rate due to activity (Calder 1964). Nestlings at these two ages often move about and practice flying within the nest. The measurement of EWL in this study does not include any consideration of activity within the animal chambers. Because nestlings are more active at these ages, it is possible that they are not at rest within the EWL chamber.

Once nestlings fledge, the rates of EWL are relatively constant. The slight changes in rates of TEWL and SEWL may be due in part to changes in the degree of cornification of the skin. McNabb and McNabb (1977) report that cornification of the skin in quail (_Coturnix c. japonica_) increases with age. The rate of water flux across the skin decreases as the degree of cornification increases (McNabb and McNabb

1977). Also, while 19 day old nestlings are about 81% of adult body weight, bill length and tarsus lengths are 91 and 99% of adult lengths, respectively. These data indicate that there is little change in body size in birds 19 days or older. The increase in body weight is probably due to fat deposition. An increase in body weight without a concomitant increase in body size will distort a weight-relative measure.

The overall trends in EWL from birds exposed to 27.5°C are similar to those observed in birds at 35°C. Total EWL increases with age, but none of the differences is significant. It is interesting to note that at this temperature the developmental changes that occur in nestlings at 13, 16, or 19 days of age do not produce any changes in TEWL rates. The lack of an effect other than a slight suppression in TEWL at 27.5 versus 35°C may result from two conditions. First, the developmental changes that occur as a nestling matures, acquires feathers, and then begins to fly may have no bearing on EWL rates. Second, the slowing of growth between 13 and 19 days reduces changes in EWL that are tied to body size. Weight-specific EWL rates from 13 day olds through adults at 27.5°C are similar to rates from birds at 35°C. The sharp drop in SEWL at 22 days may result from the increase in body weight observed in these juvenile birds (Figure 1). Body weight reaches a plateau at 13 days of age and increases only slightly by 19 days. Twenty-two day olds show an increase of about 0.6g over 19 day olds. Weight-specific EWL is inversely related to body weight in adult birds (Bartholomew and Cade 1963), and it is likely that this relationship applies to neonates. As nestling body weight increases, the rate of SEWL should decrease. The rate of SEWL in adults at

27.5°C is nearly equal to that from 35°C birds (Table 3). This similarity in SEWL further indicates that 35°C does not pose a heat stress and, conversely, that 27.5°C does not pose a cold stress in adult zebra finches. The differences in SEWL between 35 and 27.5°C at most of the ages may be a consequence of where the lower temperature (27.5) falls within the TNZ. Weathers (1981) states that as T_a's increase within the TNZ there is an uncoupling of T_b and metabolic rate in some small birds. This uncoupling allows T_b to increase without a concomitant increase in metabolic rate. The increase in T_b expected from Weathers' predictions would be at its highest level at ambient temperatures just below the upper critical temperature. My data show that T_b does not vary between 35 and 27.5°C, but differences in skin temperatures may account for the higher rate of EWL in adults at 35°C. If zebra finches are capable of making this metabolic adjustment, then it is possible that the observed differences are due to changes in the relationship between T_b, oxygen consumption, and EWL.

The response to cold stress involves an opposite interaction between metabolic rate and T_b. At lower air temperatures T_b remains stable and oxygen consumption increases with thermogenic demand. The lowest T_a in this study (20°C) is below the lower critical temperature in zebra finches (Calder 1964). Even though this temperature represents a cold stress to the birds, the patterns of TEWL and SEWL at 20°C are very similar to those at 27.5 and 35°C (Figures 2 and 6). The rates of TEWL from birds exposed to 20°C are stable at 13, 16, 19, and 35 days of age. As noted in birds exposed to the other T_a's, there is a sharp decrease in TEWL and SEWL at 22 days. This decrease is the most pronounced at 20°C and represents the lowest level of TEWL recorded for this overall age grouping. The rate of TEWL at 20°C in adults is significantly lower than the rates at 27.5 and 35°C. Presumably, this lower rate reflects physiological changes in response to cold stress. Although 20°C is probably only a mild stress to adult birds, the decrease in TEWL could result from a decrease in peripheral blood flow, a decrease in skin temperature or a larger dead air space beneath the feathers. As a bird fluffs its feathers in response to cold, the air space between the outer feather surface and the skin increases. This larger dead air space would reduce the exchange of moist air with the environment. While EWL rates in young nestlings show a strong age-temperature dependence, EWL rates in older nestlings, juveniles, and adults show a slight temperature dependence and are relatively stable throughout this age range.

Evaporative water loss in zebra finches ranging from 13 days of age through adulthood is characterized by relatively consistent rates of loss even though there are major ontogenetic changes (inability to thermoregulate, body size, and feathering) occurring during this period. As nestlings mature and approach fledging age, the ability to control or regulate EWL is essential. The nest environment is relatively stable when compared to conditions encountered outside the nest. Upon leaving the nest, a bird may be exposed to sun and wind conditions which are absent in the nest. The ability to make the behavioral and physiological adjustments to this new environment may determine if a fledgling will survive.

Evaporative Water Loss From Broods of Nestlings

One aspect of nestling EWL that is difficult to quantify is the effect of other nestmates on EWL rates. Previous studies on oxygen consumption in neonatal birds have shown that groups of nestlings can thermoregulate as a unit at lower temperatures and at an earlier age than individual nestlings can (Mertens 1969, 1977a,b; Yarbrough 1970; Diehl and Myrcha 1973; Westerterp 1973; Hudson *et al.* 1974; O'Connor 1975; Dunn 1976, 1979; Rowlands and Prange 1979; Western 1979; Clark and Balda 1981; Hill and Beaver 1982). Hill and Beaver (1982) divided the nestling life stage into two phases. The first is characterized by the lack of an ability in nestlings to increase metabolic rate in response to lowered T_a. They point out that the presence of nestmates is important because the combined body mass, especially when the nestlings are huddled, slows the rate of cooling. This slower rate of cooling produces the effect of brood thermostability. The second phase is characterized by the nestlings' ability to exert control over their T_b's and, thus, maintain a high, stable T_b. One aspect of this maintenance of an elevated T_b and, thus, increased rate of oxygen consumption is that the cost per individual within the brood is less than the energetic cost for a single nestling to maintain the same T_b. The previous studies on nestling thermoregulation point to the energetic savings incurred when the nestlings function as a unit and the ability of the nestling group to control their environment.

The close tie between the rate of evaporative water loss and the ability of a nestling to thermoregulate suggest that a brood, as a unit, should show evaporative responses similar to their thermoregulatory responses. Although the present study does not specifically address the response of broods to lowered T_a's, the data from individual nestlings show that EWL should track mean brood body temperature and not air temperature. Nestlings within a brood have a higher T_b than isolated nestlings at the same air temperature. Although a higher T_b in itself would cause an increase in the rate of EWL, the reduction in surface area of huddled nestlings would cause a reduction in cutaneous EWL. Bernstein (1971a) reports that cutaneous EWL accounts for 66% of the total EWL from neonatal quail. Other things being equal, reducing the cutaneous evaporative surface area will reduce the overall EWL of a nestling. The data on the energetic savings by individuals within a brood and the magnitude of cutaneous EWL in some neonates suggest that huddling may have a greater physiological impact than previously believed.

The data collected on EWL from broods of nestlings exposed to 35°C show that the effects of huddling reduce the rate of EWL per individual by approximately 32%. Nestlings in the wild are often exposed to a wide range of environmental conditions, and the nest is important in stabilizing the conditions faced by the nestlings. Within a nest humidity levels are often higher than in surrounding air (Walsberg 1983). The increased level of water vapor is the result of the water added to the air by the nestlings and brooding adults, the low rate of air turnover in the nest (Walsberg 1983), and in some species, like a zebra finch, the presence of fecal material within the nest (Ricklefs and Hainsworth 1968). Walsberg (1983) has tested whether brooding adults can regulate nest humidity. He found no evidence to support this hypothesis. His data did show that nest construction often inhibits the exchange of air within the nest, and the slow turnover rate of water vapor allows nest humidity to increase. An increase in humidity reduces the rate of EWL (Welch 1980) and would result in an additional water savings to the individual nestling.

The data collected on brood EWL in this study provide useful information on the extent of EWL reduction due to huddling. It must be remembered that the experimental conditions, specifically, low relative humidities and hardware cloth nests, do not represent conditions in the wild. The rates of EWL in wild nestlings may be lower than those reported here due to higher nest humidities and differences in nest structure and microclimate. Nevertheless, under the conditions used in the present study the effects of huddling on EWL have been demonstrated.

Evaporative Water Loss From Adult Birds

Rates of EWL have been measured from zebra finches by four other authors (Calder 1964, Lee 1964, Cade *et al*. 1965, Bernstein 1971b). Except for the work by Bernstein, the other studies measured oxygen consumption or carbon dioxide production as well as EWL. In these studies mineral oil was used to trap any fecal material excreted during the measurement period. Because my initial measurements on adult zebra finches used ligatures rather than mineral oil, comparisons between my data and the results reported by Calder, Cade *et al*., and Lee may not be valid. It is possible that the ligatures affected EWL in some unforeseen manner. On the other hand, the other researchers utilized gas analysis systems that were designed primarily for O_2 or CO_2 measurements and not EWL. Bernstein (1971b) designed a system which allowed partitioning of respiratory and cutaneous water losses. His system required that the bird be restrained throughout the measurement period. Restraint is known to affect metabolism and thermoregulation (McEwan 1975, Hayashi and Nagasaka 1981), and as a result, Bernstein may not have measured resting EWL rates. I feel that comparisons of his data and my own are confounded by the possible effects of restraint. In order to compare my data with those from the other studies on zebra finches and other studies on avian EWL, I measured EWL from a series of adults that were placed over mineral oil without cloacal ligatures. The rate of EWL from ligated and mineral-oil birds were nearly identical (SEWL for ligated birds was 4.62 ± 1.05, n=10; SEWL for mineral-oil birds was 4.43 ± 0.68, n=10). These results indicate that the use of ligatures did not affect EWL in adult finches and most likely had no effect on rates of EWL at any of the other ages.

The rates of SEWL reported on adult birds by Calder, Cade _et_ _al_., and Lee are higher than the rates reported in the present study. Although their values are higher, the effects of varied ambient temperatures on EWL are similar. Cade _et_ _al_. (1965) found that SEWL was relatively constant between 2 and 37°C, averaging 8.6 mg H_2O/g·hr. A small increase in SEWL was found between 20 and 35°C, 8.1 mg H_2O/g·hr to 9.4 mg H_2O/g·hr, respectively. This rate of increase is similar to that reported in my study. Above 40°C, SEWL rises to 24 mg H_2O/g·hr, a 2.5-fold increase in water loss. Lee (1964) and Lee and Schmidt-Nielsen (1971) report similar findings. Weight-specific EWL remained constant from 24 to 35°C (at 25°C SEWL = 6.85 mg H_2O/g·hr) and at 39°C the rate of SEWL is nearly double the rate recorded at 25°C (Lee 1964). Calder (1964) reports that SEWL decreases between 20 and 30°C, SEWL is approximately equal to 14.3 mg $H_2O/g \cdot hr$ at 20°C and 8.9 mg $H_2O/g \cdot hr$ at 30°C. Weight-specific EWL increases to 15.9 mg $H_2O/g \cdot hr$ at 36°C. It is unclear why Calder's data show a very different response to cold temperatures. The substantial increase in SEWL at low temperatures is not seen in other studies on zebra finches. It is possible that the lower temperatures may have been more stressful to his birds.

In comparing the data from the present study with those reported by Calder (1964), Lee (1964), Cade *et al*. (1965), and Bernstein (1971b) there are two consistent trends which appear. First, all of the studies on zebra finches show similar responses to changes in ambient temperature. As air temperatures rise above thermoneutrality, the rate of EWL increases with thermoregulatory demands. When a bird is exposed to lowered ambient temperatures, EWL appears to change very little from resting rates. Although it might be expected that EWL would increase with metabolic rate in response to cold temperatures, there is little evidence to support this assumption. Welch (1980) shows that rates of EWL vary from species to species at air temperatures below the TNZ. He feels that each species has a characteristic evaporative response to cold temperatures. The second trend is that the rate of EWL from adult finches in my study is 51 to 95% lower than the rates reported in the other zebra finch studies. Rates of EWL are variable in other species of wild birds. For example, EWL can differ by twofold between species with similar body weights (see *Cardinalis sinuata* and *Calamospiza melanocorys*, Table 6) or between different individuals of the same species (c.f., *Piplo fuscus*, Table 6). Some of these differences may be due to habitat selection (Trost 1972) or

Table 6. Evaporative water loss from adult passerines ranging in body weight from 10g to 75g.

Species	W_b (g)	T_a (°C)	TEWL mg H_2O/hr	Daily TEWL (g/day)	$\%W_b$ $\left(\frac{g \cdot day^{-1}}{W_b}\right)$	SEWL (mg H_2O/g·hr)	Source
Troglodytes aedon	10.	35	150.0	3.60	36.00	15.00	Kendeigh 1939
Lonchura malabarica	10.5	30	73.50	1.764	16.80	7.00	Willoughby 1969
Poephila guttata	11.5	30	97.75	2.346	20.40	8.50	Cade et al. 1965
Amphispiza bilineata	11.6	35	54.64	1.311	11.30	4.71	Weathers 1981
Poephila guttata[a](1)	11.69	25	80.08	1.922	16.44	6.85	Lee and Schmidt-Nielsen 1971
Spizella passerina	11.82	25	32.35	0.776	6.57	2.76	Dawson et al. 1979
Poephila guttata[a]	12.00	30	106.32	2.552	21.26	8.86	Calder 1964
Spizella breweri	12.19	25	56.32	1.352	11.09	4.62	Dawson et al. 1979
Poephila guttata[a]	12.76	27.5	59.48	1.428	11.19	4.69	Present Study
Poephila guttata[a]	13.41	35*	59.48	1.428	10.65	4.43	Present Study *with mineral oil
Poephila guttata[a]	13.69	35	62.95	1.511	11.04	4.62	Present Study
Spizocarys starki	16.0	28.5	62.40	1.498	9.35	3.90	Willoughby 1969
Eremopterix verticalis	16.0	27	89.60	2.150	13.44	5.60	Willoughby 1969
Carpodacus mexicanus[b]	18.8	25	135.36	3.249	17.28	7.20	Bartholomew and Dawson 1953
Carpodacus mexicanus[b]	20.4	35	64.46	1.548	7.58	3.16	Weathers 1981
Emberiza hortulana	22.0	25	153.56	3.685	16.75	6.98	Wallgren 1954
Zonotrichia leucophrys	23.2	25	132.24	3.174	13.68	5.70	Bartholomew and Dawson 1953
Emberiza citrinella	26.0	25	144.56	3.469	13.34	5.56	Wallgren 1954
Carpodacus cassini	28.1	<30	137.69	3.305	11.76	4.90	Weathers et al. 1980
Passer domesticus	29.0	25	205.9	4.942	17.04	7.10	Kendeigh 1944
Psittirostra cantanus	31.0	25	90.52	2.173	7.01	2.92	Weathers and Van Riper 1982
Eremophila alpestris	32.0	30	185.6	4.454	13.92	4.60	Trost 1972
Cardinalis sinuata	32.0	27.5	115.20	2.765	8.64	3.60	Hinds and Calder 1973
Calamospiza melanocorys	32.6	30	229.83	5.516	16.92	7.05	Wunder 1979
Psittirotra baillevi	34.8	25	136.42	3.274	9.41	3.92	Weathers and Van Riper 1982
Piplo megalonyx	35.4	25	194.70	4.673	13.20	5.50	Bartholomew and Dawson 1953
Piplo aberti	38.2	25	122.24	2.934	7.68	3.20	Bartholomew and Dawson 1953
Piplo fuscus[c]	39.3	25	98.25	2.358	6.00	2.50	Bartholomew and Dawson 1953
Mimus potyglottus	39.6	25	91.08	2.186	5.52	2.30	Bartholomew and Dawson 1953
Carduelis cardinalis[d]	40.0	30	128.0	3.072	7.68	3.20	Dawson 1958
Lanius lanius	40.8	25	97.92	2.350	5.76	2.40	Bartholomew and Dawson 1953
Carduelis cardinalis[d]	41.3	27.8	152.81	3.667	8.88	3.70	Hinds and Calder 1973
Piplo fuscus[c]	44	31	220.0	5.280	12.00	5.00	Dawson 1954
Agelaius phoeniceus	46.3	30	103.71	2.489	5.38	2.24	Weathers 1981
Cinclus mexicanus	50.2	25	110.44	2.651	5.28	2.20	Murrish 1970
Toxostoma rufum	74.7	25	149.4	3.586	4.80	2.00	Bartholomew and Dawson 1953

(1) a superscript letter designates repeated samples from a single species.

daily activity patterns (Dawson and Fisher 1982). When comparisons between species are made, a consideration of habitat, activity patterns, and age is necessary to interpret accurately differences in EWL rates.

Although differences in habitat preferences and activity patterns affect EWL, the rates of EWL from adult passerines are more similar than rates between passerine and non-passerine species (Crawford and Lasiewski 1968). I derived two prediction equations for adult passerines weighing between 10 and 74g using the data presented in Table 6. The equations are:

$$\text{TEWL} = 22.96w^{0.483} \quad \text{and} \quad \text{SEWL} = 23.39w^{-0.524}$$

where w is in grams (n=36 for both equations and r=0.592 and -0.631 for TEWL and SEWL, respectively). Crawford and Lasiewski (1968) present an equation which predicts TEWL per day. When their formula is adjusted to provide hourly rates, the values obtained are higher than predicted using my TEWL formula (for example, using my formula, TEWL for a 13.5g bird = 80.71 mg H_2O/hr, while Crawford and Lasiewski's predicts 114.52 mg H_2O/hr). The higher values predicted by Crawford and Lasiewski's formula reflect the limited EWL data available to them. Their study could not incorporate a large number of species from different habitats and as a result some of the natural variation in EWL rates is not expressed. The data in Table 6 illustrate the differences in EWL rates that are possible in similarly sized species. The variability in EWL observed within and between species of birds shows that this physiological response can be easily influenced by biotic and abiotic factors. The amount of water which an animal must allocate for evaporation varies depending on environmental conditions and the physiological capabilities of the animal. My study has shown that EWL in nestlings is affected by age, thermoregulatory abilities, ambient temperature, and the presence of nestmates. The amount of water lost via evaporation in nestlings is governed by a complex interaction among these factors. Also, I have shown that EWL in adults varies due to differences in body size and habitat.

Evaporation of water plays an important role in the physiology and survival of an animal. The importance of evaporation is not limited to thermoregulatory considerations. The drain which evaporation places on water resources can affect body-water volume and osmoregulation. The ability to regulate evaporative water losses may be critical to animals in hot, dry environments. These species may be forced to cool evaporatively and at the same time avoid dehydration. The ability of neonates to regulate EWL may affect their chances for survival during post-natal development. Because evaporation is dependent upon the temperature of the evaporative surface, an animal's deep body and skin temperatures are directly related to the loss of water via evaporation. Rates of water loss can be affected by varying the temperature of specific evaporative surfaces. The loss of heat via evaporation can be beneficial, as in evaporative cooling, or it can be detrimental to maintenance of a stable T_b, as in neonatal quail (Bernstein 1971a). Schmidt-Nielsen _et_ _al_. (1970) found that during inspiration the nasal passage surfaces lose water as the inspired air is humidified. As a result, the skin surfaces cool, and during expiration moisture in the air leaving the animal condenses on these cool surfaces. This counter-current heat exchanger may help to cool blood going to the animal's brain or to reclaim water added to the inspired air. A few species of birds are capable of reclaiming respiratory water losses, and it is possible that other species which have not been examined can do this as well. During exposure to cold, many animals reduce blood flow to the skin surfaces. In so doing, they decrease the skin temperature and indirectly are reducing the rate of EWL.

Because evaporation occurs constantly, a certain proportion of an animal's body water must be relegated to evaporation. The amount of water required is affected by a host of factors including: air temperature, body temperature, age, humidity, presence of other individuals and perhaps amount of plumage. Although each of these factors may have a specific effect on EWL, their combined interaction governs the rate of water loss. The data collected in the present study indicate that as a nestling matures (ages), its rate of EWL becomes less sensitive to changes in air temperature. Also, huddling in the nest helps to reduce evaporative water losses. The acquisition of thermoregulatory abilities and plumage are associated with a stabilization of EWL. As an animal becomes better able to control its internal environment, it is less affected by fluctuations in its external environment. Evaporative water loss from neonates may be a factor in prohibiting maintenance of a stable T_b, but more data are needed before this interaction can be fully explained.

TRITIATED WATER MEASUREMENTS OF NESTLING AND ADULT WATER FLUX

The total amount of water that adult birds lose per day has been used to provide an indication of homeostatic demands, how well the animal is suited to its environment, and how the animal responds to changes in its environment. The information gathered on adults has been invaluable in helping to determine patterns of water utilization in different species and habitats. Although we have learned a great deal about water flux in adult birds, our knowledge of water turnover rates (WTR) in neonatal birds is very limited. Recent studies on altricial nestlings indicate that many physiological processes are immature at hatching. For example, the ability of nestlings to regulate water losses via evaporation increases with age (Hudson *et al*. 1974, the present study). Because WTR's represent the sum of water losses via specific pathways (EWL and excretion), they should reflect the immaturity of any physiological system that affects water loss. Although WTR's are a crude measure of the immaturity of any specific physiological system, they do provide an indication of how the whole animal manages its water resources.

One of the difficulties commonly encountered when studying the physiology of neonates is the interruption of parental care and feeding and absence of contact with nestmates during the experimental period. Nestlings can be removed from the nest for short periods of time, without apparent ill effect, but accurate measurements of daily

WTR's by many methods (e.g., gravimetry) would require an unacceptably long period of separation. The ideal experimental design would involve the measurement of WTR's while the nestling is in its native nest environment. The importance of the nest environment has been demonstrated in recent studies on thermoregulation in nestlings (c.f., Hill and Beaver 1982). In addition to the importance of the nest environment to thermoregulation, when a nestling remains in the nest during the experimental period it should receive normal care and attention from its parents. In order to measure WTR's from a nestling remaining in its nest, a biological marker must be used. Tritiated water (TOH) has been used successfully to measure whole-animal WTR's in captive and free-living adult animals (c.f., Holleman and Dieterich 1973; Yousef et al. 1974; Nagy 1975; Grubbs 1980; Weathers and Nagy 1980; Degen et al. 1981, 1982; Alkon et al. 1982; Cooper 1983; Karasov 1983; Williams and Nagy 1984a) and should be ideal for use with nestlings. The use of TOH to measure WTR's has two benefits over other methods. First, TOH is similar to water in its biological activity (Nagy and Costa 1980), and as a result, no disturbance of normal physiological functioning should occur. Second, methods based on TOH can be used in free-living animals, thus allowing examination of water relations in natural conditions.

Although TOH is a powerful tool for studying water relations in animals, there are some difficulties associated with its use. Measurements of WTR's using TOH are dependent on certain assumptions concerning the biological activity of tritium. If these assumptions are violated, the WTR measurements can not be accurate. A discussion of the assumptions and conditions necessary for the accurate use of

TOH is presented in the following section. Although previous studies on adult birds have shown that TOH measurements of WTR's and total body-water volumes (TBWV) are accurate, it has not been demonstrated that accurate measurements on nestlings can be obtained. In order to use TOH to measure WTR's in nestlings, it will be necessary to determine whether TOH measurements in nestlings are accurate.

The daily WTR data collected in this portion of my study will be used in the compilation of water budgets for nestlings and adults. The specific questions which this section of my study addresses are: Do TBWV's or daily WTR's change with age; do differences in nestling body size affect WTR's at a given age; what percentage of body weight or TBWV do zebra finches lose via water flux per day; how much water does a brood of four nestlings require per day and to reach fledging age?

Assumptions and Conditions for TOH Measurements of WTR and TBWV

The accurate measurement of WTR's and TBWV's using TOH requires that certain assumptions and conditions concerning the animal's water relations and the biological activity of TOH be made. Lifson and McClintock (1966) state that accurate interpretation of WTR and TBWV data depends upon the following assumptions: 1) the animal must have a constant body-water volume, 2) the rates of water influx and efflux must be equal, 3) the isotope must label body water only, 4) the isotope must leave the body only in the form of water, 5) the specific activity of the water lost from the animal must be the same as the specific activity of the animal's body water, and 6) the animal must not gain the isotope from the environment. These assumptions and conditions represent ideal situations which may be impossible to meet. Lifson and McClintock recognized the difficulties in meeting all of these assumptions and presented methods to correct for errors. Recently, Nagy and Costa (1980) have re-examined Lifson and McClintock's assumptions and have presented simplified methods for quantifying errors which result from violation of these assumptions.

The first two requirements which Lifson and McClintock (1966) outline stipulate that an animal must be in water balance. When an animal maintains constant body-water volume, its rates of water influx and efflux are equal. It has been shown in several studies that body-water volumes are not static and that they are affected by a variety of factors (c.f., Degen 1977, Campbell and Leatherland 1980, Grubbs 1980, Weathers _et al_. 1980). Nagy and Costa have modified Lifson and McClintock's formula for determining WTR's so that changes in body-water volume can be incorporated. Even if corrections can thus be made for changes in TBWV, the accuracy of WTR and TBWV estimates remains dependent upon the biological behavior of TOH.

The last four conditions which Lifson and McClintock present describe ways in which TOH must behave biologically if inaccuracies in WTR or TBWV estimates are to be avoided. The underlying assumption in the use of TOH to measure WTR's is that the labeled water has the same biological activity as unlabeled water. If this assumption is valid, then any water lost by the animal should have the same proportion of TOH as the remainder of the body water. The labeling of compounds other than water would cause errors in WTR estimates because the isotope would be lost from the body-water pool. Conversely, if the animal were to gain the isotope from the environment, the actual rate of water flux would be greater than that calculated on the basis of injected TOH alone.

When TOH is used to measure WTR's and TBWV's in neonates, some of the assumptions which Lifson and McClintock present may not be met. For example, body-water volumes increase with age in neonates (Blem 1975); the production of new tissues increases the probability that tritium will be incorporated into tissues; and the closed environment of the nest may permit nestlings to rebreathe TOH lost via evaporation. The methods to be used to quantify these errors involve determination of the TBWV at the time of injection and at the end of the experimental period, measurement of dry-tissue tritium activity, and the monitoring of uninjected nestlings for TOH uptake.

METHODS

TOH Injection and Blood Sampling

Nestling and adult birds were injected with TOH (specific activity = 0.25 µCi/µl H_2O, New England Nuclear) using a calibrated 50 µl microsyringe (Hamilton, NV). The syringe was calibrated gravimetrically using distilled water (the error in the accuracy of delivery was determined to be less than 3%). Injections were made into the peritoneal cavity, and the standard dose was 1 µCi/g body weight (4 µl of TOH/g). Adult birds weighing more than 12.5g received a maximum injection of 50 µl (12.5 µCi). The amount of water injected was less than 0.5% of the bird's body weight, therefore the injectant should not have affected body-water volumes.

Prior to injection and blood sampling, a bird was lightly anesthetized (Metofane, Pitman-Moore, NJ) so that it would remain calm and not struggle during the procedure. In general, there was little difficulty in injecting birds 7 days old or older. At these ages, the needle was inserted through the skin just posterior to the rib cage and penetrated the abdominal wall caudal to the liver. Four-day-old nestlings were difficult to inject because of their small body size (W_b = 2.71 ± 0.6g) and because the nestlings appeared to have a large amount of abdominal fluid. During some early injections, fluid flowed back out the needle track. The injection procedure was modified in order to prevent this backflow of fluid. I found the greatest success was achieved by inserting the needle under the skin in the pectoral region and then penetrating the abdominal wall caudal to the liver. All birds recovered rapidly from the anesthesia and were active within 10 min.

Three hours were allowed for the TOH and body water to reach equilibrium. The length of time required for TOH to reach equilibration in birds is not clear. Degen *et al.* (1981) report that the specific activity of blood becomes stable after 45 min in sand grouse (*Ammoperdix heyi*) and chukars (*Alectoris chukar*). Degen *et al.* used intravenous and intramuscular injections which Smith and Sykes (1974) feel may result in faster equilibration times than intraperitoneal injections. Other studies using either intramuscular or intraperitoneal injections report equilibration times exceeding 45 min (3-4 hr, Ohmart *et al.* 1970; 2 hr, Stephenson 1974; 1 hr, Weathers and Nagy 1980; 1-2 hr, Alkon *et al.* 1982; 0.75-1 hr, Degen *et al.* 1983). It was not possible to determine an accurate equilibration time for the younger nestlings because sequential blood samples could not be obtained. I used an equilibration period which exceeded the 1-2 hr period commonly used in order to ensure that the nestlings had reached equilibrium. During the equilibration period, young nestlings (4, 7, and 10 days old) were placed in an incubator at 35°C. Birds at the other experimental ages (13 and 16 days old and adults) were placed in plastic containers or a small holding cage at room temperature during the equilibration periods.

After the equilibration period, blood samples were drawn for scintillation counting and blood-water volume determinations. Heparinized capillary tubes (ID=1.15 mm) were used to collect the blood. The capillary tubes were calibrated using distilled water, and the volume of blood collected was determined from a linear measurement of the blood within the tube. The calibration results showed that linear measurements closely approximated gravimetric measures. I derived the following equation to convert linear to volume measurements:

$$y = 0.0113x + 0.2754$$

where x = linear measurement in mm and y = volume in μl ($r=0.999$, $n=27$). Blood was obtained by rupturing a femoral artery and vein with a blood lancet in birds 10 days old or older. These vessels are visible on the medial surface of the leg and are most easily located just proximal to the ankle joint. The amount of bleeding from the wound could be controlled easily. A slight amount of pressure would stop the bleeding, and wounds rarely reopened. The circulatory development in the legs of young nestlings is poor, and as a result blood could not be obtained by rupturing femoral arteries or veins. In order to obtain blood samples from 4- and 7-day-old nestlings, either a leg or a wing was amputated. Prior to amputation the nestlings were anesthetized using Metofane. Sufficient quantities of blood could be gathered using this approach, but blood could be collected only once from these nestlings. Immediately following the final blood collection, all birds were killed using Halothane (Halocarbon Laboratories, NJ).

The amount of blood used for scintillation counting ranged between 20 and 40 µl. Usually, volumes outside of this range were not counted due to the scintillation counter's limitations. Samples for blood-water volume determinations were collected in preweighed capillary tubes. After filling, the capillary tubes were reweighed and dried at 65°C until constant weight was achieved.

The whole-blood samples were prepared for scintillation counting following the general procedures outlined by Kobayashi and Maudsley (1974). Whole blood is a strong quenching agent, and it is necessary to treat the blood chemically to reduce the degree of quenching. A tissue solubilizer (TS-1, Research Products International) was used to digest the blood, and hydrogen peroxide was used as a bleaching agent. The TS-1 was added to the blood within a scintillation vial (borosilicate glass with foil-lined, plastic caps: RPI) at 30-times the blood volume. The vial was placed in an incubator at 55°C for 1 to 2 hr. This time period was sufficient for all clots to be digested. After digestion, 30% hydrogen peroxide was added at 3-times the blood volume. Ten milliliters of a xylene based scintillation cocktail (3a70b, RPI) were added to the vial. This scintillation cocktail is designed for use with aqueous samples. Kobayshi and Maudsley (1974) suggest that adding an ascorbic acid solution and storing the vials in the dark will improve counting accuracy. The release of oxygen from hydrogen peroxide can cause chemiluminescence, and ascorbic acid (3 drops of a 15% solution were added to each vial) acts as an oxygen scavenger. Storing vials in the dark reduced any light-induced chemical reactions.

Blood samples were analyzed on a Beckman LS-7500 Liquid Scintillation Counter (LSC). Two ten-minute counts were made on each sample. The LSC was programmed to provide counts per minute (cpm) and H# (a measure of sample quenching) for each sample. A quench curve was established for this study using tritiated toluene (a beta particle source which does not cause quenching). The labeled toluene had a certified specific activity [decays per minute (dpm/ml) from the supplier, New England Nuclear]. One-hundred microliters of tritiated toluene were added to each of eight vials containing 10 ml of scintillation cocktail. The vials were counted to obtain the initial cpm, and then a specific amount of the quenching agent (a TS-1-blood-H_2O_2 solution) was added to each vial. The amount of quenching solution added was equivalent to 10, 20, 30, 40, 50, 60, and 70 µl of whole blood. The vials were recounted, and the ratio of final to initial cpm (counting efficiency) was calculated. The H# provided by the LSC for each vial was plotted against the counting efficiency (EFF). The resulting standard curve provided a method of determining EFF from the H# provided for any sample. The dpm of any sample could be calculated once the EFF was known. The formula used to calculate dpm was dpm = cpm/EFF.

The maximum counting efficiency for tritium is usually between 0.5 and 0.6 (Kobayashi and Maudsley 1974). The Beckman LS-7500 has a tritium counting efficiency of 0.56 (determined using a Beckman tritium standard with certified dpm and zero quenching). The maximum counting efficiency obtained with the RPI scintillation cocktail was 0.445. Typically, the counting efficiencies for blood samples measured in this study ranged from 0.15 to 0.25.

Total Body-Water Volume Measurements

Total body-water volumes were measured in 4-, 7-, 10-, 13-, and 16-day-old nestlings and adults. One-, 19-, and 22-day-old birds were not used in this experiment. One day olds were considered to be too small to survive the rigors of anesthesia and injection. Nineteen and 22 day olds were not studied because zebra finches fledge during this age range, and therefore, the dynamics of the brood as a physiological unit no longer exists.

Four nestlings and adults at the nominal experimental ages were selected at random from the colony. No more than two nestlings from the same brood were used at any age. Birds were injected with TOH, and after the 3 hr equilibration period blood samples were drawn. The procedures previously outlined for TOH injection and blood sample collection and preparation were followed.

The primary goal of this portion of my study was to determine if TOH accurately estimates body-water volumes in nestlings. In order to evaluate the accuracy of TOH, an independent measure of TBWV was needed. Thus, after blood samples were collected, the birds were killed (Halothane was used), and the carcasses were prepared for dry-weight analysis. Previous studies on birds and mammals have indicated that TOH and dry-weight estimates of TBWV are usually within ± 5% (Degen *et al*. 1981). I used this range as a guideline for evaluating the accuracy of TOH estimates in the present study.

The use of radioactively-labeled water required that a closed drying system be used in order to collect labeled water vapor from the carcasses. Hot air (65°C) was pumped into a PVC drying chamber and the excurrent air passed through a series of drying columns. A series of U-tubes placed in ice was used to condense the water within the excurrent air stream. Additionally, air leaving the U-tubes passed through a final glass tube packed with Drierite. Carcasses were dried until constant weight was achieved (48 to 72 hr). The dry-weight measure of TBWV included the amount of water contained in blood samples collected for scintillation counting and blood-water volume determinations.

Total body-water volumes are determined from data on TOH by measuring the dilution of the injected TOH into the body-water pool. The specific activity of the injected TOH was determined from a series of standards, and through analysis of a blood sample the specific activity of the body water was determined. The equation used to calculate TBWV (ml) was similar to equations presented by Degen _et al._ (1981) and Stephenson (1974). The equation I used was:

$$\text{TBWV (ml)} = \frac{I_o}{C_o} \times 0.001$$

where I_o is the total dpm injected minus the amount of TOH (dpm) lost via evaporation and excretion during the equilibration periods and C_o is the dpm/μl blood water corrected for background tritium activity. The amount of TOH lost during the equilibration period was estimated by multiplying the weight loss of the bird during this period by the mean dpm of the blood water. Background tritium levels were measured in an uninjected adult bird within the colony. The percent blood-water volume varies with age and between individuals in nestling and adult zebra finches. At least one blood sample per bird was dried to constant weight so that the percent blood water could be used to calculate C_o.

Water Turnover Rates

Water turnover rates were measured on nestling and adult finches over a 24 hr period. Four nestlings from two broods at each of five different ages and four adults were measured. Two of the nestlings in each brood examined were the same age, and experimentation began when these nestlings reached the nominal ages of 4, 7, 10, 13, or 16 days. The use of TOH to estimate WTR's requires that the initial and final specific activity of the animal's body water be known. After the 3 hr equilibration period, a single blood sample was drawn from birds 10 days old or older. It was not possible to obtain two blood samples from 4- and 7-day-old nestlings; so, I estimated the initial specific activity of the blood at these ages. The initial specific activity of the blood in these nestlings was estimated using the amount of tritium injected and the percent body-water volume of similarly aged nestlings. All birds were returned to the colony following initial blood collection. Nestlings were placed in their original nests, and adults were placed in a small flight cage. Final blood samples were drawn 24 hr after the birds were returned to the colony.

One of the major goals of this portion of my study was to provide a means of estimating excretory water loss from nestling zebra finches. Water turnover rates are a measure of overall water exchange with the environment, and by correcting WTR's for changes in TBWV and

EWL excretory water loss can be estimated. Changes in nestling TBWV over the 24 hr experimental period were calculated from the data collected for WTR determination. The daily EWL rates in nestlings were estimated using the data previously collected on broods.

In order to obtain realistic estimates of excretory water loss, the ambient conditions for EWL and WTR measurements need to be similar. Accordingly, both types of measurement were carried out on broods of four nestlings established by culling extra nestlings or fostering appropriately aged nestlings into a brood. As already described, brood EWL was measured at 35°C (the average nest temperature in my colony) and with a flow of dry air. To create similar conditions for WTR measurements, dry air was pumped into the nestboxes of nestlings under study. Incurrent air was dried by passing it through a series of U-tubes packed in ice and then through two glass tubes filled with Drierite. The air flow into the nestbox was maintained at approximately 800 cc/min (a needle valve calibrated against a Gilmont flowmeter was used). Air entered a nestbox through ports on the bottom and two of the sides. Dry air was pumped through the nest beginning 24 to 36 hr prior to WTR measurement and continuing until the measurement was complete. It was hoped that the parents would become accustomed to the conditions in the nest and not show any behavioral changes during the experimental period. Also, it was assumed that the nestlings would receive normal amounts of food and attention from their parents during the experimental period.

Rates of excretory water loss in adults were estimated using the average SEWL rate for adults studied at 27.5 and 35°C. During WTR measurements, adults were placed in a cage in which humidity levels could be regulated. A box with windows on five sides and inflow and outflow ports was placed over the cage housing the adults. Dry air was pumped into the cage at about 1600 cc/min. Adults were placed in the cage 2 to 3 days prior to experimentation. During the experimental period, adults had free access to food and water.

Lifson and McClintock (1966) note that errors in WTR estimates can occur if tritium becomes incorporated into tissues or if tritium is gained from the environment. Potential errors resulting from these situations were quantified in the present study. Tissue samples from all birds used for WTR determinations were analyzed for tritium content. The procedures used to prepare dried tissue samples for analysis were the same as those outlined for blood samples. There is potential for nestlings to gain tritium from the environment because of the slow rate of air turnover in the nest and because zebra finch nestlings defecate in the nest. Nagy and Costa (1980) have noted that tritium uptake can occur if labeled water vapor is breathed. Pinson and Langham (1957) found that TOH readily crosses cutaneous surfaces, and therefore, contact with fecal material containing TOH can result in the cutaneous uptake of tritium. The two uninjected nestlings of a brood were used as controls for tritium uptake. At the end of the WTR measurement period, blood samples were taken from these control nestlings. The average amount of tritium measured in the control nestlings was assumed to be the amount of TOH gained from the environment by the experimental nestlings.

Water turnover rates were calculated using a formula similar to those presented by Degen _et al_. (1981) and Nagy and Costa (1980). The WTR for a bird maintaining constant water volume (only adults in this study) was estimated using the following equation:

$$\text{WTR (ml/day)} = W_o \ln (C_o/C_t)$$

where W_o is the TOH space (TBWV) at equilibration time (t_o), C_o is the specific tritium activity (dpm) of 1 µl of blood at t_o, C_t is the specific tritium activity of 1 µl of blood after 24 hr (t_f) corrected for background and tissue tritium levels, and t is the elapsed time (t_f - t_o) in days. Water turnover rates for birds not maintaining a constant TBWV (nestlings in this study) were calculated using the following equation:

$$\text{WTR (ml/day)} = \frac{(W_t - W_o)\ln(C_o \cdot W_o/C_t \cdot W_t)}{\ln(W_t/W_o)t} .$$

Symbols are the same as previously defined, and in addition, W_t is the TBWV after t days and C_t has been corrected for TOH gain from the environment. The final TBWV (W_t) was determined by dry-weight analysis.

Statistical Analysis

Statistical comparisons of %blood-water volume, daily WTR expressed as a percentage of body weight and percentage of body-water volume were made using one-way ANOVA's (Steel and Torrie 1980). Comparisons between mean values were made using an LSD multiple-way comparison ($\alpha = 0.05$). Percentage data may show non-normal distributions and as a result often require transformation (Steel and Torrie 1980). Steel and Torrie recommend that an arcsin $\sqrt{\%}$ transformation be used if the majority of the data fall between 0 to 20% or 80 to

100%. The data for percent blood-water volume and WTR as a percentage of body weight were transformed to the arcsin $\sqrt{\%}$. A paired t-test (Steel and Torrie 1980) was used to compare TOH and dry-weight measurements of TBWV.

RESULTS

Total Body-Water Volume

The two methods used to measure TBWV's produced very similar results (Table 7). No statistical differences were found between TOH and dry-weight measurements of TBWV (paired t-test, $t=1.129$, $df=23$, $0.3 \geq p \geq 0.2$). There was a general tendency for TOH to underestimate dry-weight TBWV's (14 out of 24 measurements).

The TBWV data suggested that the percent body-water volume of zebra finches changes with age. I examined this trend using non-experimental birds. I chose to use non-experimental birds because changes in TBWV can occur during the TOH equilibration period. The TBWV's of five birds at each of 10 ages (1, 4, 7, 10, 13, 16, 19, 22, and 35 days of age and adults) were measured using dry-weight analysis. The percent body-water volume (%TBWV) of nestlings decreases significantly (Table 8) from 1 to 13 days of age (Table 9). Percent body-water volumes of 13, 16, 19, 22, and 35 day olds were not different. Significant differences were found between adults and 13 and 22 day old birds. The %TBWV data collected from the non-experimental birds were compared to the data collected using TOH. No differences were found in %TBWV between experimental and non-experimental birds at any age (t-test, $0.4 \geq p \geq 0.2$ in all cases).

Table 7. Comparison of TOH and dry weight measurements of total body-water volume in nestling and adult zebra finches.

Age	TBWV (ml)		% (TOH/Dry-weight)
	TOH	Dry-weight	
4	1.713	1.726	99.25
	2.507	2.527	99.21
	2.480	2.419	102.54
	1.942	1.960	99.08
7	3.452	3.530	97.79
	3.337	3.407	97.95
	4.249	4.271	99.49
	4.069	4.070	99.98
10	5.405	5.464	98.92
	6.031	6.153	98.02
	5.172	5.339	96.87
	5.516	5.800	95.10
13	6.327	6.116	103.45
	5.943	5.806	102.36
	4.804	4.905	97.94
	6.160	5.874	104.87
16	7.073	6.784	104.26
	6.115	5.984	102.19
	6.447	6.221	103.63
	5.678	5.902	95.53
Adult	8.053	7.838	102.74
	8.450	8.120	104.06
	7.830	7.919	98.88
	8.064	7.912	101.92
			$\bar{x}$ = 100.25

Table 8. Results of the one-way ANOVA on %TBWV.

Source	df	SS	MS	F	p
Total	49	2010.608			
Age	9	1897.059	210.782	74.245	$p \leq 0.001$
Error	40	113.549	2.839		

During the preliminary experiments for this section of my study, I found that the percent blood-water volume (%BlWV) decreased with age (Tables 10 and 11). Mean %BlWV was the highest in 4 day olds and lowest in adults. The %BlWV decreased significantly between 4 and 7 and 13 and 16 days of age and between 16-day-olds and adults. Seven,

Table 9. Mean %TBWV of nestling, juvenile, and adult zebra finches with LSD comparisons (n=5 at all ages).

Age	$\bar{x}$ %TBWV	LSD[1]
1	81.90 ± 2.02	F
4	79.67 ± 1.42	E
7	76.13 ± 0.66	D
10	72.29 ± 0.87	C
13	67.23 ± 0.78	B
16	65.59 ± 1.03	AB
19	65.40 ± 1.38	AB
22	66.91 ± 1.34	B
35	65.68 ± 1.32	AB
Adult	64.72 ± 3.73	A

[1] Any two means with the same letter are not significantly different.

Table 10. Percent blood-water volume in nestling and adult zebra finches.

Age	n[1]	%BlWV[2]	LSD[3]
4	5	89.83 ± 0.46	E
7	4	84.42 ± 0.43	D
10	3	82.54 ± 1.51	CD
13	4	81.27 ± 0.20	C
16	4	79.12 ± 0.45	B
Adult	6	73.18 ± 2.40	A

[1] Number of birds sampled. More than one determination was made per bird in most cases (total number of determinations = 53).

[2] Values reported are the mean of the sample means.

[3] Any two means having the same letter are not significantly different.

Table 11. Results from the one-way ANOVA on %BlWV. (The data were transformed to the arcsin $\sqrt{\%}$).

Source	df	SS	MS	F	p
Total	25	480.457			
Age	5	464.382	92.876	115.517	$p \leq 0.001$
Error	20	16.075	0.804		

10, and 13 day old nestlings had similar %BlWV's. No significant differences were found between 7 and 10 and 10 and 13 day old nestlings but 7 and 13 day olds were significantly different.

Water Turnover Rates

Daily WTR's vary with age and, thus, body size in zebra finches. The WTR's for nestling and adult zebra finches ranged from 2.05 ml/day in 4-day-olds to 6.55 ml/day in adults (Table 12). Due to the variation in body size within age groupings, no statistical tests were made on the actual WTR's. Instead, statistical comparisons were made on weight-relative expressions of WTR's. Although no statistical tests were made on the WTR data, the following trends do appear. First, WTR's generally increase from 4 to 13 days of age. Water turnover rates in birds 13 days old and older appear to change very little. Second, with certain age groupings (7, 10, and 13 days of age), the WTR's of nestmates are similar while the WTR's between the broods selected appear to be different.

The daily WTR's presented in Table 12 have been corrected for TOH gain from the environment. The average amount of TOH gained by the two control nestlings in each nest was used as a correction factor for the WTR's of the other two nestmates. The average amounts of TOH gained from the environment for the four control nestlings at each age are presented in Table 13. Although these data are highly variable, especially at the younger ages, they do show that there is some TOH uptake within the nest. The TOH gain from the environment represents, on the average, 2.61 ± 1.39% of the daily WTR's for nestling finches.

Table 12. Daily WTR's for nestling and adult zebra finches. At each age, except for adults, the first two nestlings listed are from the same brood, and similarly, the second two nestlings listed are nestmates from a different brood.

Age (days)	W_b (g)	WTR[1] (ml/day)	WTR%W_b	WTR%TBWV
4	4.75	4.459	93.87	127.00
	2.61	2.048	78.47	100.90
	3.45	2.518	72.99	94.93
	3.99	2.742	68.73	89.01
7	5.64	2.632	46.67	66.70
	6.04	3.088	51.13	70.67
	8.46	5.369	63.46	87.66
	5.69	5.292	93.00	133.08
10	8.36	3.096	37.03	54.91
	8.25	3.577	43.36	62.21
	7.60	3.865	50.86	73.45
	8.51	3.875	45.54	67.22
13	10.15	5.575	54.93	80.77
	10.58	5.831	55.11	81.10
	8.59	4.434	51.62	77.05
	8.47	4.585	54.13	81.60
16	8.63	5.372	62.23	92.72
	7.80	4.484	57.49	83.11
	11.58	5.747	49.63	75.19
	11.13	5.201	46.73	70.27
Adult	10.37	5.548	53.50	77.20
	10.97	5.258	47.93	72.08
	10.59	5.176	48.88	61.55
	10.90	5.300	48.62	77.18

1) Daily WTR's are corrected for TOH gain from the environment at all ages except for the adults.

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Table 13. Mean volume of TOH gained by the control nestlings.

Age	$\bar{x}$ TOH gain (μl)
4	104.33 ± 41.55
7	91.98 ± 76.56
10	150.87 ± 24.44
13	50.74 ± 11.65
16	90.06 ± 0.50

The expression of WTR's in weight-relative terms helps to reduce the effects of differences in body weight and to show what percentage of a bird's body weight is lost due to water flux per day. Water turnover rates expressed as a percentage of body weight ($WTR\%W_b$) ranged from 93.87% in a 4-day-old to 37.03% in a 10-day-old (Table 12). There was a significant decrease in $WTR\%W_b$ with increasing age (Table 14). Mean $WTR\%W_b$ were similar in 4 and 7 day old nestlings. Mean $WTR\%W_b$ in 4-day-olds was significantly different from all other ages (Table 15). Although mean weight-specific WTR's were the lowest at 10 days of age, no significant differences were found in $WTR\%W_b$ in birds 10 days old and older. In two of the nestlings, a 4- and 7-day-old, WTR's represented a water loss equivalent to 93% of their body weights.

The rate at which a nestling turns over its body water pool per day can be determined from the WTR and final TBWV data collected in this study. The percent total body-water volume turnover rate (WTR%TBWV) for each of the birds examined in this study is presented in Table 12. The mean WTR%TBWV varied with age (Table 16) and, in general, decreased with increasing age (Table 17). Four day old nestings had the highest mean WTR%TBWV while 10-day-olds had the

Table 14. Results from the one-way ANOVA on $WTR\%W_b$. (The $WTR\%W_b$ data were transformed to the arcsin $\sqrt{\%}$).

Source	df	SS	MS	F	p
Total	23	2286.025			
Age	5	1170.402	234.080	3.793	$0.025 \geq p \geq 0.01$
Error	18	1115.623	61.979		

Table 15. Mean (± SD) $WTR\%W_b$ of nestling and adult zebra finches with LSD comparisons.

Age	$\bar{x}$ $WTR\%W_b$	LSD[1]
4	78.52±10.99	C
7	63.57±20.87	BC
10	44.20± 5.72	A
13	53.95± 1.61	AB
16	54.02± 7.12	AB
Adult	47.04± 6.46	AB

[1] Any two means with the same letter are not significantly different.

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lowest. The mean WTR%TBWV's for birds 13 days old and older were not significantly different. Three of the nestlings examined, two 4-day-olds and one 7-day-old, had WTR%TBWV which exceeded 100%, and all birds turned over at least 50% of their body-water pool per day.

DISCUSSION

Water turnover rates are physiological variables that are sensitive to changes in an animal's environment, health, and diet (Walsberg 1975; Alkon et al. 1982). As conditions that affect water availability to the animal or rates of water loss from the animal fluctuate, WTR's will reflect these changes. An animal's WTR may indicate whether physiological or behavioral adjustments are being made in response to changes in the environment. For example, two of the strategies used by desert-dwelling animals to cope with heat stress are to utilize evaporative cooling to dissipate excess body heat and to retreat to a burrow where the microclimate is less harsh. If heat stress is the only change in the environment, then the WTR of an animal relying on evaporative cooling would increase due to the increase in EWL while the WTR of an animal seeking shelter would not change because behavioral adjustments were made. An increase in WTR is not proof that an animal is stressed physiologically. If an animal switches food sources to one that contains large amounts of water, then the daily WTR may increase because of excess water intake and the need to regulate body-water volume.

Water turnover rates are determined in one of two ways. The first approach involves quantification of water loss via specific avenues (e.g., evaporation) and then summing all water losses to give an indication of the animal's total body water flux. Measurements of w

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water losses via specific avenues are made under controlled laboratory conditions, and often the experimental procedure is designed to measure minimal rates. The second approach involves the use of a marker which is biologically similar to water. An animal's WTR is determined directly by quantifying the rate at which the marker is lost over time. This approach only gives a measure of the animal's overall rate of water loss. Depending on the design of the study, information can be gathered using a biological marker on water loss in free-living or active animals. The information provided by each method of determining WTR's is important to the understanding of an animal's water relations. The first approach provides information on how environmental parameters (e.g., temperature or humidity) affect water loss via specific pathways. These studies help to define the limits of physiological functioning by exposing the animals to stressful conditions. The second approach gives an indication of how the whole animal and not just one system responds to environmental factors. The combination of information and insights gained from both of these approaches reveals how an animal responds to its environment and marshalls its water resources. Most of the information on WTR's has been gathered from studies on adult birds. These studies have shown that WTR data are useful in describing the physiological interaction between an animal and its environment. The ability to examine an animal's physiology by monitoring WTR's provides a powerful tool for studying neonatal animals. Although WTR's have not heretofore been measured in nestling birds, the potential for WTR's to help describe the development of a nestling's water regulatory abilities and how nestlings respond to their environment is great.

Total Body-water Volume

Dry-weight and TOH measurements of TBWV were used to validate the accuracy of TOH estimates of WTR's in nestling and adult zebra finches. I chose to use TBWV for TOH validation testing for three reasons. First, measurement of TBWV is a fundamental step in WTR calculation, and as a result, the validation test directly assesses the accuracy of TOH in one part of the WTR calculation. Second, TBWV can be measured in the same individual using both TOH and dry-weight analysis. These two independent measures of TBWV can be compared to one another as a means of assessing the accuracy of either technique. Third, because TOH and dry-weight measurements of TBWV have been used in validation testing by other researchers, I was able to evaluate the procedures developed for nestlings in the present study by comparing the accuracy of TOH measurements of TBWV between my study and others.

The TOH and dry-weight measures of TBWV from individual birds are presented in Table 7. No significant difference was found between dry-weight and TOH measurements of TBWV. I found that TOH measurements of TBWV were, on the average, 100.25 ± 2.85% of dry-weight measurements. The results from the validation testing show that TOH measures of TBWV are accurate in nestling and adult zebra finches. Further, these results imply that TOH measurements of WTR's in nestlings should be accurate.

Other studies utilizing TOH have reported accurate estimates of TBWV's and WTR's. For example, Degen *et al.* (1981) report that TOH estimates of TBWV's in chukars (*Alectoris chukar*) receiving intramuscular or intravenous injections were 99.0 ± 0.7% and 100.2 ± 2.2% of dry-weight measurements, respectively. Similarly, Culebras *et al.*

(1977) state that TOH estimates averaged about 98% of dry-weight measures in white rats (*Rattus norvegicus*). Karasov (1983) determined that TOH estimates of water influx in ground squirrels (*Ammospermophilus leucurus*) were, on the average, 0.1 ± 9.3% higher than gravimetric measurements. The results from the validation test which I completed are similar to those from other studies. I have shown that TOH measurements of TBWV in nestling zebra finches are accurate and that the procedures used in my study yield valid results.

The %TBWV decreased steadily in nestlings from 1 to 13 days of age (Table 9). Nestling %TBWV's decreased about 14% through this age range. Increases in TBWV occur in growing animals due to the addition of new tissues. The %TBWV decreases as neonates grow because of differences in the water content of tissues being produced. Ricklefs (1967b) has shown that during ontogeny different types of tissue (for example, skeletal and cardiac muscle, liver, and gastrointestinal tract tissues) are produced at varying rates and times. At hatching, nestling red-winged blackbirds (*Agelaius phoeniceus*) and barn swallows (*Hirundo rustica*) have very little skeletal muscle and, proportionally, large amounts of digestive tissues. Ricklefs shows that digestive tissues have a high water content while muscle tissue has a low water content. During the rapid growth phase, tissues with low water content are added. The timing of the onset of tissue growth and differences in the type of tissue accrued cause an increase in TBWV but a decrease in %TBWV.

The %TBWV changed very little in birds 13 days old or older. Although some significant differences were found between age groupings, the %TBWV varied less than 2.5% from 13 days through adulthood. The data collected on growth in zebra finches indicate (Figure 1) that the rapid growth phase in nestlings is completed by 13 days. The stability in %TBWV observed in birds 13 days old and older is consistent with the growth data. The data that I have collected on TEWL, thermoregulatory abilities, body weight, and %TBWV suggest that 13 days of age marks a turning point in the development of zebra finches. Nestlings at this age show adult-like physiological responses and morphological development.

The %TBWV for adult birds of diverse species averages about 60% (Skadhauge 1981). The value for %TBWV reported in the present study is close to the mean for adult birds in general, and is in agreement with the %TBWV (63%) reported by Skadhauge and Bradshaw (1974) for adult zebra finches.

Coinciding with the decrease in %TBWV in nestlings was a decrease in percent blood-water volume (Table 10). Medway and Kare (1959b) report that blood and plasma volumes expressed as percentages of body weight decrease with increasing age in chickens. The %BlWV calculated from their data vary with age and show only a slight age related decrease. Wang and Hegsted (1949) report that there is an increase in hematocrit with age in white rats and that plasma volume does not increase as rapidly as body weight. Hughes (1984) reports similar findings from work on nestling glaucous-winged gulls. The findings of Wang and Hegsted, Medway and Kare, Hughes and the present study indicate that blood-water volumes vary with age in some growing animals.

Variation in %BlWV can affect the accuracy of TOH measurements. Calculation of TBWV's or WTR's depend on the determination of the specific activity of tritium in a known volume of body water. When blood is used as a means of sampling the body-water pool, the measurements are based on the water content of the blood. If whole blood is used, then the amount of water contained in the blood sample must be determined. My data show that it was necessary to monitor the %BlWV of all birds because of the variation between individuals and ages. Even if plasma samples are used, the amount of water within the sample should be determined. Stephenson (1974) reports that plasma in adult song sparrows (*Melospiza melodia*) is 94.75 ± 0.9% water. It is possible that the percent plasma-water volume may vary with age; so, in order to achieve the most precise measurements using TOH, determinations should be made of the water content of any sample to be analyzed.

Daily Water Turnover Rates

Daily WTR's generally increased from 4 to 13 days of age (Table 12). The data reported in Table 12 show that WTR's can be highly variable within an age grouping. For example, at 4 days WTR's range from 2.05 ml/day to 4.46 ml/day. The differences in body size of the nestlings could contribute to this variability. The body weights of the two 4-day-old nestlings previously mentioned were 2.61g and 4.75g, respectively. These results suggest a positive relationship between WTR's and body size. Degen *et al*. (1981) have shown that WTR's increase with body size in adult birds, and some of my findings support this trend. But, it can be seen in Table 12 that at some ages (7 and 10 days old) the heavier nestlings have lower daily WTR's. This contradiction of the expected body weight-daily WTR relationship may be the result of differences in physiological maturity between similarly aged individuals or differences in the behavior, or other parameters in the nest environment, among broods.

It is possible that the ability to regulate water losses also develops with age, and that a larger nestling may be more mature physiologically. On the other hand, the data in Table 12 indicate strongly that WTR's may be affected by differences between nests. At 4, 7, 10, and 13 days of age nestlings from one of the two broods examined had very similar WTR's. For example, at 10 days two of the nestlings (both were nestmates) had WTR's of 3.1 ml/day and 3.68 ml/day while the other two nestlings (both were also nestmates) had WTR's equalling 3.9 ml/day. It is unknown how nest microclimate, feeding rates, presence of nestmates, parental care, and other nest environmental conditions affect WTR's. It can be assumed that each brood encounters a unique combination of factors which affect nestling physiology. It is interesting to note that my data suggest that internest differences are greater than intranest differences. Due to the differences in nestling maturity, body size, and nest conditions, it is difficult to assess to what degree WTR's change with age in zebra finches.

In general, weight-specific WTR's ($WTR\%W_b$) decrease with increasing age in early-age nestling zebra finches (Table 15). Weight-specific WTR's were the highest in 4 day olds (78.52 ± 10.99%) and lowest in 10 day olds (44.20 ± 5.72%). Even though there was a decrease in $WTR\%W_b$ with age, all nestlings lost an amount of water equivalent to at least 50% of their body weight per day. Two nestlings, 4 and 7 days of age, lost a volume of water equivalent to 93% of their body weight per day (Table 12). Due to the lack of data on WTR's in neonatal birds, it is difficult to judge whether these two nestlings have abnormally high WTR's. The high value for the 4 day old exceeds the second highest value by 20% while the high value for the 7 day old exceeds the second highest value by 46%. The two higher values recorded at both 4 and 7 days were from siblings, and as previously argued, it is possible that differences in nest conditions contributed to the variability in WTR's between broods at each of these ages. If the high values are omitted from the calculation of mean WTR%W_b, the means for 4- and 7-day-old nestlings becomes 73.4 ± 4.9% and 53.8 ± 8.7% (n=3 in both cases), respectively. Although no statistical tests were made excluding these two high values, their omission changes the overall pattern of change of WTR%W_b for nestling and adult zebra finches. The average WTR%W_b would decrease between 4 and 7 days but would change very little from 7 days of age through adulthood. Even though the WTR%W_b for these two nestlings are higher than ones recorded for other similarly aged nestlings, I do not feel that there is sufficient reason to exclude these values. Much of the data on nestling physiology indicate that responses are highly variable, and my results on EWL show the extent of the variability that can occur in nestling zebra finches.

Although WTR%W_b were the lowest in 10-day-old nestlings, no significant differences were found between birds 10 days old or older. The WTR%W_b's of nestling zebra finches appear to reach adult levels at 10 to 13 days of age. I have indicated previously that by 13 days of age zebra finch nestlings exhibit adult-like physiological responses (thermoregulatory ability, rates of EWL),and the WTR%W_b data indicate that the rate of water utilization in young birds may be similar to that of adults.

When WTR's are expressed as a percentage of TBWV (WTR%TBWV), it is apparent the zebra finches lose a large proportion of their body-water per day (Table 17). As expected, nestlings that had high WTR%W_b's also had high WTR%TBWV's. Two of the 4-day-old nestlings and one 7 day old turned over in excess of 100% of their body-water pool in one day. The percentages of body-water turnover for the nestlings examined in the present study appear to be high (all birds 16 days old or younger lost at least 55% of their body-water pool per day, see Table 12). Due to the lack of other data on WTR's in altricial nestlings, it is difficult to determine whether the WTR%TBWV's in zebra finches represent usual rates for nestling birds. The WTR%TBWV for the adults measured in this study averaged 72.00 ± 7.37%. A low rate of water loss, expressed as WTR%W_b or WTR%TBWV, often is cited as an indication that a species is well suited to a xeric environment (Degen _et al_. 1982). Weathers and Nagy (1984) found that black-rumped waxbills (_Estrilda troglodytes_) have low daily WTR's. The WTR%TBWV for waxbills, a xeric species from Africa, is approximately 31.3% and is considerably lower than in zebra finches. Weathers and Nagy's data suggest that, in comparison to another xeric species, zebra finches may not be suited to a desert environment. If the WTR%TBWV in adult zebra finches is greater than the rate in other desert species, then by implication, zebra finch nestlings may have unusually high WTR%TBWV's for a desert species. I have presented previously some of the findings which indicate that zebra finch survival and successful breeding in the desert are dependent upon open water. The data which

I have collected on water flux in zebra finches appear to offer physiological evidence which supports the natural history data concerning this species' dependence on water. It should be noted that the experimental conditions for the measurement of WTR's in the present study were not designed to assess the response of zebra finches to water stress. Adults had free access to food and water, and although there was sufficient cage space for the birds to fly, high levels of activity could be avoided. Given the environmental conditions in my colony, it is possible that the high WTR%TBWV's of adults may reflect an excess of water in the bird's diet rather than an inability to reduce total body-water losses. In general, I feel that the adults did not face stressful conditions; so, any conclusions drawn from my data concerning the ability of zebra finches to survive in the desert must be made carefully.

My data have shown that daily WTR's in nestling birds are affected by age (body size) and nest environment. The interactions between changes in surface-to-volume ratios, maturation of thermoregulatory abilities, and general morphological development associated with increasing age are complex, and their effects on daily WTR's are unclear. The biotic and abiotic conditions within a nest also affect WTR's and must be considered when examining nestling water flux. Due to the differences in morphological and physiological maturity in nestlings and the effects of the nest environment, it is clear that patterns of water flux in neonates cannot be completely explained from using our knowledge of water flux in adults. Future studies of water flux in neonates will require a more detailed examination of the interaction between morphology, physiology, and the environment.

Water turnover rates have been determined for adult zebra finches in three other studies. Two of these studies (Calder 1964, Lee 1964) derived WTR's from measurements of water losses via evaporation and excretion. The third study, Skadhauge and Bradshaw (1974), measured WTR's using TOH. Lee (1964) estimated that adult zebra finches lost 2.19 ml/day. The rate of whole-body water loss calculated from Calder's (1964) data was 3.83 ml/day. Skadhauge and Bradshaw (1974) report that WTR's in zebra finches were 6.38 ml/day. The daily WTR's reported by Lee and Calder are lower, and the value reported by Skadhauge and Bradshaw is slightly higher than the mean daily WTR for adult zebra finches ($\bar{x} = 5.57 \pm 0.65$ ml/day) reported in the present study. The differences in WTR's reported in all of these studies are due, in part, to the approach used to quantify rates of water flux.

The approach used by Calder (1964) and Lee (1964) to quantify WTR's provides a different view of water flux than does the approach used by Skadhauge and Bradshaw (1974) and myself. The measurement of water losses via specific avenues often requires that animals are confined, at rest, and not stressed, while the use of TOH allows an animal free activity during the measurement period. As a result of the fundamental differences in the strategies used to quantify WTR's, the information that each approach provides is different. The measurement of water losses via specific pathways yields an estimate of the minimal WTR. Water flux measurements using TOH yield estimates of WTR's in animals that are active and experiencing natural environmental conditions. The TOH measurements of WTR's in adult zebra finches are 1.5 to 2.9 times greater than the values reported by Calder (1964) and Lee (1964). The differences in WTR's between the two approaches used indicate the potential increase in water flux between active and inactive birds.

Degen *et al.* (1982) have summarized much of the existing literature on TOH measurements of WTR's in birds. They report data on 14 species including zebra finches. In general, Degen *et al.* show that WTR's increase with body size while weight-specific WTR's decrease. Bartholomew and Dawson (1953) previously have reported that WTR's calculated from specific avenues of water loss exhibit the same trends. Although the estimate of the amount of water lost per day may vary between these two approaches, the general relationships between body weight and water flux still apply. Inspection of the data presented by Degen *et al.* reveals the following points: 1) the WTR values which they report for zebra finches (taken from Skadhauge and Bradshaw 1974) and song sparrows (taken from Stephenson 1974) have not been calculated in the same manner as the other values in their table, and 2) only two passerine species (zebra finches and song sparrows) are included. Degen *et al.* report that the WTR in zebra finches is 4.6 ml/day while Skadhauge and Bradshaw report a value of 6.38 ml/day. The value which Degen *et al.* report for song sparrows, 267.5 ml/kg•day, does not agree with the value reported by Stephenson, 557 ml/kg•day. The data presented in Table 18 are from the same species and studies utilized by Degen *et al.*, with corrected values entered for zebra finches and song sparrows, and with the addition of values from the present study, Weathers and Nagy (1984), and Williams and Nagy (1984a). By regression analysis of the data in Table 18, I derived the following equation to predict daily WTR's:

$$y = 0.589 \log x + \log 1.313$$

Table 18. TOH estimates of water flux in adult birds.

Species	W_b	n	Water Flux ml/day	 ml/kg•day	 ml/kg$^{0.589}$•day	Source
Black-rumped waxbill *Estrilda troglodytes*	6.7	9	1.95	291.0	37.19	Weathers and Nagy 1984
Zebra finch *Poephila guttata*	11.21	4	5.57	496.9	78.46	Present Study
Zebra finch *Poephila guttata*	13.4	2	6.4	477.6	81.16	Skadhauge and Bradshaw 1974
Savannah sparrow *Passerculus sandwichensis*	16.11	7	7.33	455.0	83.39	Williams and Nagy 1984a
Song sparrow *Melospiza melodia*	20.0	4	11.14	557.0	111.58	Stephenson 1974
Semipalmated sandpiper *Calidris pusillus*	23.4	8	12.98	554.7	118.52	Purdue and Haines 1977
Snowy plover *Charadrius alexandrinus*	33.0	6	15.1	458.2	112.61	Purdue and Haines 1977
Killdeer *Charadrius vociferus*	71.1	1	32.9	462.5	156.12	Purdue and Haines 1977
Coturnix quail *Coturnix c. japonica*	111.0	10	23.5	211.3	85.78	Chapman and McFarland 1971

table continued on next page

Table 18 continued

Table 18 continued

Species	W_b	n	Water Flux ml/day	ml/kg·day	$ml/kg^{0.589}\cdot day$	Source
Burrowing owl *Speotyto cunicularia hypogaea*	140.0	1	10.2	72.9	32.47	Chapman and McFarland 1971
Roadrunner *Geococcyx califorianus*	297.4	6	41.8	140.6	85.38	Ohmart *et al*. 1970
Petz conure *Aratinga canicularis eburnirostrum*	312.5	1	17.8	57.0	35.31	Chapman and McFarland 1971
Chukar partridge *Alectoris chukar*	371.5	5	29.9	80.5	53.58	Degen *et al*. 1982
Glaucous-winged gull *Larus glaucescens*	763.0	7	48.8	64.0	57.23	Walter and Hughes 1978
Vulturine fish eagle *Gypohierax angolensis*	1590.0	1	133.0	83.7	101.21	Chapman and McFarland 1971
Pekin duck *Anas platyrhynchos*	1918.8	14	773.5	248.3	526.93	Thomas and Phillips 1975
Domestic chicken *Gallus domesticus*	4045.8	22	268.1	66.3	117.71	Chapman and Black 1967 Chapman and Mihai 1972

where y is the WTR in ml/day and x is the body weight in grams ($n=16$, $r=0.923$). The data for pekin ducks were omitted from the calculations for this equation because the researchers, Thomas and Phillips (1975), feel that the high daily WTR's may be the result of excessive drinking or percutaneous water flux in their study animals.

Degen et al. (1982) used weight-independent WTR's ($ml/kg^{0.6} \cdot day$) to compare species from different habitats. Degen et al. report that zebra finches and song sparrows have low weight-independent WTR's (61.2 and 56.5 $ml/kg^{0.6} \cdot day$, respectively) which they feel are indications that these two species are well suited to a desert environment. But, when the errors in reporting of Skadhauge and Bradshaw's and Stephenson's data are corrected and the addition of the data from the present study and those of Weathers and Nagy (1984) are considered, the weight-independent WTR's for zebra finches and song sparrows (Table 18) no longer are as low as values for other desert species (for example, black-rumped waxbills and chukars). The weight-independent WTR's for zebra finches support the natural history data which suggest that free-living zebra finches are dependent on open water.

The data available on TOH estimates of WTR's in passerine species are limited. Degen et al. note that physiological differences have been found between passerine and non-passerine species (see Lasiewski and Dawson 1967, Crawford and Lasiewski 1968), but feel that the WTR's in the two passerine species indicate physiological adjustment to desert environments and not differences between these two avian groupings. The data presented in Table 18 show that three of the passerine species listed have similar rates of water flux while one, black-rumped waxbills, has lower WTR's. Also, because the rates of water flux in these species are generally higher than rates in the non-passerine species, the data indicate that the higher metabolic rates in passerine species may contribute to large weight-specific WTR's. In general, the data presented in Table 18 support Degen *et al.*'s conclusion that WTR's and body size are correlated in birds, but as yet, there are insufficient TOH data to determine whether passerine and non-passerine species differ in their rates of water loss and whether desert-dwelling passerine species are more tolerant of high rates of water flux.

Previous studies that have examined WTR's in birds have shown that the amount of water lost is affected by body size, food selection, and habitat (see Davies 1982 for a recent review). The data presented in the present study show that age and nest environment also affect WTR's in birds. The effects of body size and physiological maturity appear to be most important in rapidly growing nestlings. As our knowledge of avian water relations and WTR's increases, we can begin to assess whether passerine and non-passerine birds differ in their water resource utilization and whether nestling WTR's vary between species and habitats.

WATER BUDGETS FOR NESTLING AND ADULT ZEBRA FINCHES

An animal's water requirements are affected by body size, age, physiological health, food selection, activity level, habitat, and climate (Bartholomew and Dawson 1953, Cade 1964, Hinds and Calder 1973, Davies 1982, Ricklefs and Williams 1984). Many of the studies on adult animals have shown that behavioral and physiological adjustments are made to compensate for short term changes in water requirements or availability. Also, on a broader scale, Davies (1982) has suggested that the breeding season of birds is timed to coincide with periods of high environmental water availability. Even so, Ricklefs and Hainsworth (1968) note that in cactus wrens (*Campylorhynchus bronneicapillus*) water is the limiting factor in nesting success. Our knowledge and understanding of how much water adult birds require for survival and successful breeding is increasing, but almost no information exists on the water requirements of nestling birds.

During the breeding season, the number of individuals within a population, and as a result, the demand for water placed on the environment, increases. The dependency of altricial nestlings on their parents for food and water places additional demands on the adults. The amount of water required by nestlings to meet growth and homeostatic demands is unknown. The limited data available on nestling water demands (water losses due to evaporation or increases in body-water volume associated with growth) suggest that as nestlings mature the amount of water required increases (see: Hudson et al. 1974 and the present study for EWL data; Ricklefs and Hainsworth 1968, Blem 1975, and the present study for data on changes in TBWV with age). The data presented in these studies do not provide a complete picture of water resource allocations in nestlings of any one species of birds. In order to interpret the cost (percentage of total water resources) of each specific water loss or demand, it is necessary to determine overall water flux in the nestlings.

The purpose of this section of my study is to examine water resource allocation in nestling and adult zebra finches. The water budgets presented in this section were derived from the data collected in earlier portions of this study. The specific questions on zebra finch water budgets which will be addressed in this section are: What proportion of a bird's water intake or whole body-water turnover rate do water losses or demands represent; do water demands or losses change with age; does one avenue of water loss constitute the major route of water flux; how much water does a brood of nestlings require per day and to reach fledging age; do nestlings face water stress and at which age is there the greatest potential for water stress?

METHODS

The data used to compile nestling and adult water budgets were drawn from the previous sections of this study. Individual water budgets were calculated for each of the birds used in daily water turnover rate (WTR) determinations. These birds were used because the most complete sets of data were available on them. Water budgets were compiled for nestlings ranging in age from 4 to 16 days of age and for adults. Four birds were examined at each of the nominal ages (see Methods in Chapter II for details on selection and age of experimental birds).

Water flux via the following categories was quantified for the nestlings and adults: Total water intake (TWI), evaporative water loss (EWL), excretory water loss (ExWL), water allocated for growth, and whole body-water loss (WTR). Daily TWI, water allocated for growth, and the daily WTR were calculated from the data collected using tritiated water (TOH). Daily TWI was calculated using a formula presented by Nagy and Costa (1980) and Degen _et_ _al_. (1981):

$$H_2O \text{ Intake (ml/day)} = \text{WTR} + \frac{(W_t - W_o)}{t}$$

where W_t and W_o represent final and initial total body-water volume (TBWV), respectively. In addition, the quantity $(W_t - W_o)$ represents the change in TBWV associated with growth. Daily EWL from the nestlings was estimated using mean brood SEWL (Table 4). Adult EWL rates were estimated using the mean SEWL for adult finches exposed to 27.5 and 35°C (Table 3). The estimate for the amount of water lost per day via excretion was the only avenue of water flux which was not derived directly from experimental data. The calculation of daily ExWL was dependent on the assumption that all birds were in water balance; that is, water intake equaled water loss. I was able to quantify all other avenues of water flux using both the EWL and WTR data. Daily ExWL was calculated by subtracting daily EWL from the daily WTR for each bird. Although it is possible to measure ExWL directly using weight analysis of excretory samples, I decided that the potential for error was too great using this approach. In particular, I felt that accurate measurement of the defecation rate in individual nestlings was not possible due to the presence of other nestmates and because adult birds also defecated in the nest. Also, I was concerned that excretory samples collected following handling of the birds might contain abnormal amounts of water. Nestling zebra finches usually defecate when handled, and it is possible that the excretory material voided might contain excess water because reclamation of water from the excreta may not have been completed.

Statistical Analysis

Statistical comparisons were made between age groupings for each component of the water budget. One-way ANOVA's (Steel and Torrie 1960) and LSD multiple-way comparisons were used to determine if there were any significant treatment (age) effects and if any statistical differences existed among mean values for each age grouping. Percentage data were transformed to the arcsin $\sqrt{\%}$ if they met the criteria outlined by Steel and Torrie.

RESULTS

The water budgets calculated for nestling and adult zebra finches are presented in Table 19. Each component of the water budget is presented as a daily rate (ml) and as a percentage of TWI or W_b.

The rate of total water intake (TWI) and TWI as a percentage of body weight ($TWI\%W_b$) were variable among and between age groupings. Daily TWI ranged from 2.61 ml (4 day old) to 6.70 ml (13 day old). Four-, 7-, and 10-day-old nestlings had similar rates of TWI. Daily TWI was not significantly different in 7, 13, and 16 day olds and adults. Mean $TWI\%W_b$ decreased significantly from 96.52 ± 14.99% in 4 day olds to 49.85 ± 6.74% in 10 day olds. No differences were found in TWI%W_b in birds 10 days old or older. Three nestlings, two 4 day olds and one 7 day old, were found to have TWI%W_b exceeding 99%, and four birds, one 10 day old and three adults, had TWI%W_b's which were less than 50%.

The rate of daily EWL was not affected significantly by age (Appendix B3). Mean daily EWL ranged from 0.377 ± 0.090 ml (4 day olds) to 1.200 ± 0.137 ml (13 day olds). No statistical analyses were made on EWL as a percentage of W_b because EWL has been calculated simply as a proportion of weight:

$$\text{Daily EWL (ml/day)} = \text{SEWL} \cdot W_b \cdot 24$$

where SEWL is the mean brood weight-specific EWL (ml H_2O/g hr) and W_b is body weight (g). The amount of water lost via evaporation ranges from 10.47 ± 1.54% (4 day olds) to 22.56 ± 0.33% (adults) of the daily TWI in zebra finches. Daily evaporative water loss as a percentage of the daily TWI (EWL%TWI) increases significantly with age (Table 19 and Appendix B4). In 4 and 7 day olds EWL%TWI was similar; it increased significantly between 7 and 10 days of age. In birds age 10 days or older, EWL%TWI varied. Mean EWL%TWI was similar between 10 and 13 day olds and 13 day olds and adults. Mean EWL%TWI in 16 day old nestlings was significantly lower than the mean for other birds 10 days old or older.

The increase in TBWV associated with growth, as measured over the 24 hr experimental period, was similar for all age groupings (Table 19 and Appendix B5). The increase in TBWV due to growth when expressed in weight-relative terms (Growth%W_b) showed a significant decrease between 4 and 7 days of age. The Growth%W_b was similar in birds 7

Table 19. Water budgets for nestling and adult zebra finches. Data presented are values for individual birds and the mean (±SD) for each age grouping.

Age[a] (days)	W_b (g)	TWI[b] (ml/day)	TWI%W_b[b] (%)	EWL (ml/day)
4	4.75	5.524	116.30	0.47196
	2.61	2.607	99.89	0.25933
	3.45	2.889	83.74	0.34279
	3.99	3.437	86.14	0.39645
$\bar{x}$	3.70±0.90	3.614±1.319 A	96.52±14.99 C	0.37663
7	5.64	3.040	53.90	0.47782
	6.04	3.560	58.94	0.51171
	8.46	6.005	70.98	0.71673
	5.69	5.782	101.90	0.48206
$\bar{x}$	6.46±1.35	4.597±1.515 AB	71.43±21.54 B	0.54718
10	8.36	3.388	40.53	0.77648
	8.25	4.253	51.55	0.76626
	7.60	4.303	56.62	0.70589
	8.51	4.315	50.71	0.79041
$\bar{x}$	8.18±0.40	4.065±0.452 A	49.85± 6.74 A	0.75976
13	10.15	5.977	58.89	1.28864
	10.58	6.704	63.36	1.34324
	8.59	5.069	59.01	1.09059
	8.47	5.287	62.42	1.07535
$\bar{x}$	9.45±1.08	5.759±0.739 B	60.92± 2.31 AB	1.19946
16	8.36	6.002	69.55	0.78498
	7.80	5.165	66.22	0.70949
	11.58	6.417	55.41	1.05332
	11.13	5.801	52.12	1.01238
$\bar{x}$	9.79±1.85	5.846±0.522 B	60.83± 8.37 AB	0.89004
Adult	10.37	5.548	53.50	1.12743
	10.97	5.258	47.93	1.19266
	10.59	5.176	48.88	1.15135
	10.90	5.300	48.62	1.18505
$\bar{x}$	10.71±0.28	5.321±0.160 B	49.73±2.54 A	1.16412

Table continued on next page

[a] The first two nestlings listed in each age grouping are siblings from the same nest, and the second two listed are siblings from a different nest.

[b] In each column of means any two means followed by the same letter are not significantly different.

Table 19 continued.

Age	EWL%TWI (%)	Growth (ml/day)	Growth%W_b (%)	Growth%TWI (%)
4	8.54	1.065	22.42	19.28
	9.95	0.559	21.42	21.44
	11.87	0.371	10.75	12.84
	11.53	0.695	17.42	20.22
$\bar{x}$	10.47±1.54 D	0.673±0.294 A	18.00±5.29 B	18.45±3.84 B
7	15.72	0.408	7.23	13.42
	14.37	0.617	10.22	17.33
	11.94	0.636	7.52	10.59
	8.34	0.490	8.61	8.47
$\bar{x}$	12.59±3.24 CD	0.538±0.108 A	8.39±1.35 A	12.45±3.83 A
10	22.92	0.292	3.49	8.62
	18.02	0.676	8.19	15.89
	16.40	0.438	5.76	10.18
	18.32	0.440	5.17	10.20
$\bar{x}$	18.92±2.80 B	0.462±0.159 A	5.66±1.95 A	11.22±3.20 A
13	21.56	0.402	3.96	6.73
	20.04	0.873	8.25	13.02
	21.51	0.635	7.39	12.53
	20.34	0.702	8.29	13.28
$\bar{x}$	20.86±0.79 AB	0.653±0.195 A	6.53±2.27 A	11.20±3.12 A
16	13.08	0.630	7.30	10.50
	13.74	0.681	8.73	13.19
	16.41	0.670	5.79	10.44
	17.45	0.600	5.39	10.34
$\bar{x}$	15.17±2.09 C	0.645±0.037 A	6.80±1.53 A	11.12±1.38 A
Adult	22.97	--	--	--
	22.68	--	--	--
	22.24	--	--	--
	22.36	--	--	--
$\bar{x}$	22.56±0.33 A	--	--	--

Table continued on next page

Table 19 continued.

Age	ExWL (ml/day)	ExWL%W_b (%)	ExWL%TWI (%)	WTR (ml/day)
4	3.987	83.94	72.18	4.459
	1.789	68.58	68.62	2.048
	2.175	63.04	75.29	2.518
	2.346	58.80	68.26	2.742
$\bar{x}$	2.574±0.970 A	68.58±10.99 B	70.40±2.51 B	2.942±1.052 A
7	2.154	38.19	70.86	2.632
	2.576	42.65	72.36	3.088
	4.652	54.99	77.47	5.369
	4.810	84.53	83.19	5.292
$\bar{x}$	3.548±1.378 ABC	55.09±20.88 AB	75.97±5.58 A	4.095±1.439 A
10	2.320	27.75	68.48	3.096
	2.811	34.07	66.09	3.577
	3.159	41.57	73.41	3.865
	3.085	36.25	71.49	3.875
$\bar{x}$	2.844±0.380 AB	34.91±5.72 A	69.87±3.23 B	3.603±0.365 A
13	4.286	42.23	71.71	5.575
	4.488	42.42	72.79	5.831
	3.343	38.92	65.95	4.434
	3.510	41.41	66.39	4.585
$\bar{x}$	3.907±0.565 BC	41.25±1.61 A	69.21±3.54 B	5.106±0.700 B
16	4.623	53.57	77.02	5.372
	3.775	48.40	73.09	4.484
	4.694	40.54	73.15	5.747
	4.189	37.64	72.21	5.201
$\bar{x}$	4.320±0.427 C	45.03±7.28 A	73.87±2.15 AB	5.201±0.530 B
Adult	4.421	42.63	79.69	5.548
	4.065	37.06	77.31	5.258
	4.025	38.01	77.76	5.176
	4.115	37.75	77.64	5.300
$\bar{x}$	4.157±0.180 C	38.86±2.55 A	78.10±1.07 A	5.321±0.160 B

days old and older. In order for a neonate to meet the demands for water required by the production of new tissues, TWI must exceed the level necessary to fulfill the demands for simple maintenance. The amount of water allocated for growth represents 18.45 ± 3.84% of the daily TWI of 4-day-old nestlings. Growth water as a percentage of TWI decreased significantly between 4 and 7 days of age, but no significant differences were found among nestlings 7 days old or older. In general, older nestlings allocated about 11.5% of their TWI to growth.

The amount of water lost per day via excretion (ExWL) increased (Table 19 and Appendix B8) with age. Daily ExWL was significantly lower in 4 day olds than in 13 and 16 day olds and adults. Daily ExWL rates were similar among 7-, 10-, and 13-day-old nestlings. In general, larger nestlings within an age grouping had greater ExWL rates. Although there was a general trend for weight-relative ExWL ($ExWL\%W_b$) to decrease with age, only 4 day olds were significantly different from other age groupings. Four day olds lost a volume of water equivalent to 68.58 ± 10.99% of their body weight per day while birds ranging in age from 7 days through adulthood lost on average an amount of water equivalent to about 43% of their body weight per day. Excretory water loss represented a large proportion of the daily TWI in zebra finches. In 7-day-old nestlings and adults, ExWL comprised 75.97 ± 5.58% and 78.10 ± 1.07% of the daily TWI, respectively. The values calculated for 4, 10, and 13 day olds were significantly lower than the values calculated for 7 day old and adult birds.

The amount of water consumed by a brood of four nestlings at each of the nominal ages is presented in Table 20. These water demand estimates represent four times the mean TWI at each age. The amount of water consumed by a brood at each age ranges from 14.5 ml to 23.4 ml, in 4- and 16-day-old broods, respectively. In general, broods ranging in age from 4 to 10 days have similar water demands, and 13- and 16-day-old broods have a greater water demand. In order to estimate the amount of water consumed by a brood of four nestlings to reach fledging age (19 days), I made the following assumptions: First, I stipulated that all members of a brood were the same age; second, I assumed that the water demand calculated for each nominal age (Table 20) represented the mean water demand of a brood ± 1 day from the nominal age; third, I assumed that the $TWI\%W_b$ of 0- to 2-day-old broods would be the same as the mean $TWI\%W_b$ of 4-day-old nestlings (76.5%); and fourth, I assumed that the TWI of 17- to 19-day-old broods would be the same as estimated for 16-day-old broods. I estimated that 343 ml of water were consumed by a brood of four nestlings in my colony to reach fledging. A brood consumed, on the average, 18.1 ml H_2O/day. Similarly, a pair of adult birds consumed 14.1 ml H_2O/day. The total amount of water that a pair of adults would utilize in rearing a brood of four nestlings is 611.5 ml (an

Table 20. The volume of water consumed per day by a brood of four nestlings.

Age (days)	Volume of water (ml/day)
4	14.46
7	18.39
10	16.26
13	23.04
16	23.38

average of 32.18 ml H_2O/day). These data indicate that an average sized adult (13.5g) in my colony provided 2.4 ml H_2O per gram of body weight per day while there were young in the nest.

DISCUSSION

Animals gain water from the oxidation of foodstuffs (metabolic water) or by taking in preformed water (Hill 1976). Preformed water is available within the food or as drinking water. Because adult birds rarely have been observed to bring drinking water to their nestlings, it is assumed that most of the water which nestlings receive is provided to them in their food (metabolic and preformed water). Some nestlings obtain water in addition to the sources present in food. During feeding, nestlings may receive water from the saliva regurgitated with the food. Also, in some species, such as sand grouse (<u>Pterocles</u> <u>alchata</u> and <u>P</u>. <u>orientalis</u>), the adults bring water directly to their young (Thomas and Robin 1977). It is unknown how much water is taken in via food, saliva, and drinking water in nestling birds. The TWI measurements presented in this study incorporate water input via any avenue.

The TWI of young zebra finch nestlings, 4 to 10 days of age, was similar and except for two 7-day-old nestlings was lower than the rates for birds 13 days old and older. It is interesting to note that between 10 and 13 days of age TWI increased significantly (an increase of about 1.7 ml/day). As previously mentioned (see Chapter I), nestling zebra finches acquire the ability to maintain a stable body temperature within this age range. This increase in TWI suggests that the increased metabolic activity in thermoregulating nestlings is associated with an increase in metabolic water synthesis or an increase in water demand. Also, I reported that once a nestling was able to thermoregulate, its EWL and water turnover rates were similar to those of adult finches. Although TWI in birds 13 days old or older did not change significantly, 13 and 16 day olds took in a greater volume of water than did adults. If the amount of water allocated for growth is subtracted from the TWI of 13 and 16 day olds, the water demands of these nestlings becomes nearly equal to adult levels (TWI without growth water = 5.1 ml and 5.21 ml in 13 and 16 day olds, respectively, adult TWI = 5.32 ml). These data suggest that, for the birds in my colony, older nestlings and adult zebra finches have similar water intakes.

The $TWI\%W_b$ of nestling and adult zebra finches decreased as the birds increased in body size. Total water intake as a percentage of body weight ranged from 40 to 116% (Table 19). The highest values were recorded for 4 day olds, and in general, adults had the lowest values. The large variation in $TWI\%W_b$ may reflect the effects of the physiological demands of or behavioral responses to the nestlings. Ricklefs (1967b) showed that different tissue types have varying water contents. As a result, it would be expected that a nestling producing tissues with a high water content would have a greater demand for water. If zebra finches were similar to red-winged blackbirds and barn swallows in the water content of different tissue types, then it is possible that the higher weight-relative TWI of younger nestlings indicates that tissues with a high water content are being produced. Also, once the rapid growth phase of the nestlings has been completed (by 13 days in zebra finches, Figure 1), the per gram demand for water decreases. This weight-specific decrease in water intake suggests that growth is less costly in older nestlings. On the other hand, the high weight-relative TWI of young nestlings may be the result of the parental feeding rate. As nestlings are fed, they take in water. The begging response of young zebra finches is easily elicited, and on several occasions I noticed nestlings with very full crops begging. If nestlings beg in response to stimuli other than the need for food or water, it is possible for nestlings to take in an excess of these resources. Therefore, the high daily water intake of young nestlings may indicate how much or how often the nestlings were fed and not what the physiological demands for water were. If nestlings do take in water in excess of physiological demands, then TWI is not an accurate predictor of nestling water requirements. It was beyond the scope of this study to determine the minimal daily water requirements of zebra finches. Water budgets have been compiled for adult zebra finches by Calder (1964), Lee (1964), and Skadhauge and Bradshaw (1974). The data presented by Skadhauge and Bradshaw (1974) show that TWI can be reduced by approximately 68% in water stressed adults. If nestlings are similar to adults in their abilities to tolerate dehydration and to reduce water demands, then it is possible that TWI in nestlings could be reduced substantially.

The water taken in by nestlings can be allocated for growth or maintenance needs. While the amount of water allocated for growth does not change as nestlings mature, the percentage of the TWI utilized for growth decreases. Four-day-old nestlings direct 18.5% of their TWI towards growth. Nestlings, 7 days old or older, allocate about 11.5% of their TWI for growth. The only significant change in the percentage of TWI allocated for growth occurred between 4 and 7 days of age. The growth data for zebra finches (Table 1, Figure 1) indicate that the rapid growth phase begins between 4 and 7 days. I found that the percentage of TWI allocated for growth decreased during this early phase of rapid growth. During the later stages of the rapid growth phase, 7 through 13 days of age, the percentage of TWI allocated for growth did not change. These data suggest that the tissues produced during early growth require greater amounts of water than do the tissues produced at later stages.

The amount of water lost per day via evaporation was not significantly different at any of the ages studied. The daily EWL values calculated for each nestling in Table 19 were based on previous measurements (see Table 4) of brood EWL. No statistical differences were found in SEWL of broods of four nestlings among the experimental age groupings (Table 4). Although no significant differences were found in daily EWL among the age groupings studied, daily EWL varied with age. Interpretation of the daily EWL data is difficult, especially when it is considered that previously I showed that total EWL (mg H_2O/hr) increased significantly with age (Tables 2 and 3). It is possible that the absence of established differences in daily EWL is due to a sampling artifact. Daily EWL comprised 10.47 ± 1.54% of the daily TWI in 4-day-old nestlings and increased to 18.92 ± 2.80% by 10 days of age. This increase in EWL%TWI is surprising when it is considered that, statistically, daily EWL and daily TWI (Table 19) did not increase between 4 and 10 days of age. Inspection of the data in Table 19 shows that while the daily TWI for these two age groupings are similar the daily EWL in 10 day olds is twice the value reported for 4 day olds. The EWL%TWI data are difficult to interpret due to the non-significant variation in daily EWL rates.

The EWL data collected for nestling and adult finches are resting rates. Therefore, the daily EWL rates which are presented in Table 19 do not take into account any changes in EWL due to activity. Young nestlings, 4 to 10 days of age, are fairly inactive in the nest (personal observation); so, resting EWL rates should provide an accurate estimate of daily EWL. Older nestlings are more active. I observed some 16-day-old nestlings exercising their flight muscles within the nest. It is possible that the calculated daily EWL values for 13 day olds and, especially, 16 day olds are lower than the actual rates at these ages. The actual daily EWL rates for adult birds would surely be expected to be higher than the values presented in Table 19. The amount of water lost via evaporation in adult birds is affected by the duration and speed of flight (Torre-Bueno 1978). Also, if EWL and metabolic rate are correlated, then the increase in metabolic rate of active birds (see Williams and Nagy 1984b for a discussion of time-energy models in birds) would imply an increase in EWL. No data are available on the rate of change of EWL with different types of activity (other than flight and resting). If data on daily activities were available, then daily EWL could be calculated using time budget analysis. At the present time, the best estimate of daily EWL for adult zebra finches has been provided by Skadhauge and Bradshaw (1974). Their EWL estimates are derived from TOH measurements of daily WTR's. These EWL estimates incorporate changes due to activity, but provide no information concerning the effects of specific activities.

The amount of water lost via excretion increased with age in nestling zebra finches. Excretory water loss was similar in nestlings ranging in age from 4 to 10 days, from 7 through 13 days, and in 7, 13, and 16 day olds (Table 19). These data indicate that while ExWL increases with age, the increase is gradual. Some of the differences among age groupings were removed by expressing ExWL in weight-relative terms. Excretory water loss as a percentage of body weight ($ExWL\%W_b$) was similar among 4 and 7 day olds, but $ExWL\%W_b$ in birds 10 days old or older was significantly lower than in 4 day olds. In general, birds 7 days old and older excreted an amount of water equal to 44.06 ± 12.73% of their body weight per day.

The expression of ExWL as a percentage of the daily TWI shows that a majority of the water taken in per day is lost via excretory pathways in nestling zebra finches. In 4, 10, and 13 day olds, ExWL comprised a similar percentage, about 70%, of the daily TWI. The mean ExWL%TWI's in 7 day olds and adults were significantly greater than mean values at the other experimental ages, except for 16 day olds. Cade *et al*. (1965) and Lee and Schmidt-Nielsen (1971) have stated that EWL was the major avenue of water loss in adult zebra finches. My data do not support these findings. Cade *et al*. did not measure ExWL in their study, so their conclusion concerning EWL in zebra finches may not be valid. Lee and Schmidt-Nielsen measured ExWL from adults which were placed in a special cage with a wire bottom. The authors do not report whether food and water were provided or what the duration of their sampling period was. It is possible that Lee and Schmidt-Nielsen's measurements underestimate ExWL because the birds were fasted, and thus were not producing urine and feces at a normal rate. Ricklefs and Hainsworth (1968) report that cactus wren nestlings lose an amount of water equivalent to 11.2% of their body weight per day. Their findings are very different from those reported in the present study. Although Ricklefs and Hainsworth did not use TOH to quantify ExWL, they did study free-living birds. The differences between their results and my own may reflect differences in experimental approach.

In zebra finches the most dramatic morphological and physiological changes occur between 7 and 16 days of age. Nestlings acquire the ability to thermoregulate, complete a majority of their growth, grow feathers, and begin to exhibit behaviors associated with flight in this age range. I have indicated previously that the ability to thermoregulate at low ambient temperatures, 27.5 and 20°C, develops between 10 and 13 days of age. The onset of the ability in altricial animals to thermoregulate has been used as a developmental landmark. Although recent studies have shown that neonates as a group can thermoregulate at an earlier age than individual neonates (Hill and Beaver 1982), the physiological changes that occur when an individual can thermoregulate may have an effect on water utilization. The TWI data show that 13 day olds take in a greater volume of water per day than do 10 day olds. These data suggest that 13-day-old nestlings may have a greater water demand than 10 day olds. I have shown previously that the rate of EWL in zebra finches is affected by the nestling's ability to thermoregulate. If the increase in TWI at 13 days is due to the onset of thermoregulatory abilities, then a corresponding increase in the rate of EWL should occur. Although no significant difference was found in the amount of water lost per day via evaporation at 10 and 13 days of age (Table 19 and Appendix B3), the daily EWL in 13 day olds was 63% greater than in 10 day olds. The data which I have collected on EWL from individual nestlings (Table 3) and on the onset of thermoregulatory abilities strongly suggest that daily EWL does increase between 10 and 13 days of age. The non-significant change in daily EWL indicates that there is an increase in physiological water demand between 10 and 13 days of age.

Daily TWI increased by 1.69 ml/day between 10 and 13 days of age. This increase in TWI was 2.5 times greater than the combined difference in the amount of water allocated for evaporation and growth at these two ages. Because the increase in TWI greatly exceeds the increase in daily requirements for evaporation and growth, it is possible that the nestlings in my colony are faced with the problem of voiding excess water. Sixty-nine to 78% of the water taken in per day by the birds examined in this study was voided via excretory pathways. This seemingly high rate of ExWL suggests that the birds in my colony took in excess water. Also, the ExWL data suggest that the zebra finches in my colony were not water limited and that excretion (urinary and fecal pathways) was used as the means of coping with excess water intake.

Mechanisms similar to those employed by adults to void excess water intake could be used by nestlings to reduce the amount of excretory water loss, as well. Although it is unknown whether nestlings can reduce ExWL in response to water stress, the ability of adults to modity ExWL implies that water regulatory mechanisms should be present in at least older nestlings. These mechanisms may be immature at hatching, but during the nestling period these systems should mature. Both Calder (1964) and Skadhauge and Bradshaw (1974) have shown that there is a reduction in ExWL in water-deprived adult zebra finches. They report that ExWL in dehydrated birds was 42.2% (Calder) and 33.3% (Skadhauge and Bradshaw) of the ExWL in hydrated birds. If the ability to reduce ExWL in response to water stress follows a maturation pattern similar to thermoregulation, then it would be expected that young nestlings would be unable to reduce ExWL while older nestlings would show modest control.

Periods of water stress (low environmental water availability) are often associated with high ambient temperatures. One method that can be used by birds to meet their water requirements for evaporative cooling is to increase body water reserves by reducing water losses via other pathways. In nestling zebra finches, the amount of water lost via excretion averages 4.82 times the rate of EWL (Tables 21 and 22). If all water lost via excretion could be used for evaporative cooling, then nestlings could increase EWL between 3.3 and 6.9 fold. Clearly, this is an unrealistic measure of the amount of water available for evaporative cooling, but the values in Table 21 suggest that the water lost via excretion represents a potential source of water during stress situations. The data in Table 21 also show that nestlings at some ages, 10 and 13 days, have significantly smaller excretory water reserves than 4- and 7-day-old nestlings. The nestlings at 10 and 13 days of age may face a greater danger of dehydration or death due to thermal stress. Mertens (1977a) has suggested that older great tit nestlings (Parus major) may be more susceptable to acute water stress while younger nestlings face a greater danger from chronic water stress. Although the findings which

Table 21. Water reserves contained within daily ExWL of nestling and adult zebra finches. Values listed are the mean number of times that ExWL exceeds EWL.

Age (days)	EWL Reserves[a]	
4	6.90±1.11	A
7	6.50±2.46	AB
10	3.76±0.62	C
13	3.25±0.13	C
16	4.95±0.80	BC
Adult	3.57±0.23	C

1) Any two means followed by the same letter are not significantly different.

Table 22. Results from the one-way ANOVA on excretory water reserves.

Source	df	SS	MS	F	p
Total	23	74.48			
Age	5	49.327	9.865	7.062	$p \leq 0.001$
Error	18	25.154	1.397		

Mertens and I present are not based on quantitative measurements, it is interesting to note that we both arrive at similar conclusions concerning the effects of water stress on differently aged nestlings.

The water budgets for nestling zebra finches show that the increases in TWI with age exceed the increases in water allocated for growth or evaporation at each age. These data indicate that the nestlings in my colony were not water limited and that rates of water intake were not governed by physiological demand. In most altricial nestlings, water resources are provided in the food items brought by the parents. Thus, it can be concluded that the rate of water intake in zebra finch nestlings is dependent on how often the nestlings are fed.

Even though I found that the amount of water allocated for growth or evaporation did not increase significantly with age, the nestling water budgets show that the cost, as a percentage of water resources, of meeting evaporative or growth demands varies with age. Younger nestlings allocate a greater percentage of their water resources to evaporation and growth than do older nestlings. The cost of meeting evaporative water demands and the WTR's or rates of TWI in older nestlings (10, 13 and 16 days old) were similar to those of adults. These findings suggest that the physiological processes that control water utilization become mature during the nestling period.

The data collected on TWI can be used to estimate the amount of water supplied to a brood at each of the nominal ages and over the entire nesting period. Although I have argued that the amount of water taken in per day appears to be governed by feeding rate and not physiological demand for water, the TWI of a brood provides an indication of the water demand that a brood places on the parents. I estimated that 345 ml of water were consumed during a 19 day nestling period by a brood of four nestlings within my colony. If the water intake for a pair of adults is included, then 611 ml of water were utilized to rear a clutch. At this rate of water consumption, adults must acquire 2.4 ml H_2O/g body weight per day. The water budget data suggest that the nestlings within my colony received more water than was necessary for survival. Even though my calculations on the amount of water required by a brood appear to overestimate actual demands, they do show the degree to which adult water acquisition may increase during the breeding seasons.

Water budgets have not been compiled for any altricial nestlings, but Calder (1964), Lee (1964), and Skadhauge and Bradshaw (1974) have compiled water budgets for adult zebra finches. Calder and Lee measured water loss via specific pathways while Skadhauge and Bradshaw used TOH to quantify daily WTR's and rates of ExWL. The approach used to quantify daily water flux in the present study was similar to Skadhauge and Bradshaw's. The water budget data presented in Table 23

Table 23. Water budgets for adult zebra finches.

EWL (ml/day)	ExWL (ml/day)	WTR (ml/day)	source
1.922	0.182	2.191	Lee 1964
2.552	1.280	3.832	Calder 1964
3.980	2.400	6.380	Skadhauge and Bradshaw 1974
1.164	4.157	5.321	present study

were collected under similar experimental conditions, but reflect differences in the experimental approach used. For example, rates of EWL were measured under resting conditions by Calder, Lee, and myself while Skadhauge and Bradshaw estimated EWL from the difference between daily WTR and ExWL. Calder, Lee, and Skadhauge and Bradshaw directly measured ExWL using dry-weight analysis (Calder and Lee) or TOH activity (Skadhauge and Bradshaw). I estimated ExWL to be the difference between the daily WTR and daily EWL. The daily WTR's presented by Calder and Lee are the sums of the water lost via specific pathways. Skadhauge and Bradshaw and myself used TOH to measure WTR's. The differences in experimental approaches used in all four studies present some difficulties in interpreting the variation among water budgets.

The rates of energy consumption and water utilization are affected by activity levels in animals. Torre-Bueno (1978) has shown that EWL in active adult birds is much higher than in resting birds. The daily EWL values presented by Calder, Lee, and myself are resting rates and, therefore, must underestimate the daily EWL of an active, free-living bird. Skadhauge and Bradshaw calculated EWL from the difference between daily ExWL and daily WTR. Their estimate of EWL assumes that the bird is in water balance, and more importantly, their estimate incorporates changes in EWL due to activity. Because the daily WTR's presented by Calder and Lee are based on measurements from resting birds, their water budgets can not be applied to active birds. Their water budgets do provide an indication of the minimal (resting) daily water requirements for adult zebra finches. The daily WTR's which I calculated do incorporate changes in water flux due to activity, but because I used resting EWL rates to derive ExWL rates these two water budget categories are not representative of active adults. The daily EWL rates are underestimates and the daily ExWL rates are overestimates for active adults. I feel that Skadhauge and Bradshaw's water budget is more representative of normally active adult zebra finches than the other water budgets discussed. Skadhauge and Bradshaw's method for determining daily EWL provides the most realistic estimate of this avenue of water flux in active adult birds. Their daily ExWL values are greater than Calder's and Lee's and are indicative of how much water active birds void per day. The daily WTR values presented by Skadhauge and Bradshaw are greater than the values I report. If the daily WTR's from both studies are expressed in weight-specific (WSWTR) terms, then the differences between the two studies decrease, WSWTR = 0.476 ml H_2O/g•day and 0.470 ml H_2O/g•day in Skadhauge and Bradshaw's and my own study, respectively. The similarity in WTR's between our studies indicates that the data are reliable estimates for captive birds with ad lib. access to food and water.

Throughout this section, I have indicated that Skadhauge and Bradshaw's (1974) approach for compiling adult water budgets was superior to my own. Skadhauge and Bradshaw's method for partitioning water losses allowed them to include the effects of activity on EWL. Although their approach is superior for adult birds, it is not appropriate for nestlings. As indicated, Skadhauge and Bradshaw measured WTR's by analyzing the tritium content of fecal water. I felt that it was not possible to measure nestling fecal water volumes accurately. Zebra finch nestlings do not form a fecal sac, and they defecate in the nest. The combination of these two factors presents difficulties in collecting fresh fecal material. As soon as feces are voided, some of the water contained in the sample will be absorbed by the nest material. Also, zebra finch nestlings will defecate when handled. If the fecal material is voided prematurely, it is possible that excess water will be found in the sample. As a result of these factors (no fecal sac, defecation in the nest, and defecation when handled), it is difficult to obtain direct measures of excretory water loss in zebra finch nestlings.

The approach that I used to quantify water flux in nestlings minimized disturbance of the birds and, more importantly, allowed the adults to care for and feed the young during the experimental period. Any approach which requires that nestlings be removed from the nest for long periods of time risks the possibility of physiological changes due to an alteration of feeding patterns and the absence of parental and sibling interactions. As previously stated, my experimental approach results in an underestimate of EWL in active birds. But, nestlings are relatively inactive within the nest, and it is likely that resting EWL rates will closely approximate rates of water loss within the nest. My study shows that the experimental approaches used to measure water flux in adult birds may be inappropriate for the study of nestling water relations.

As we learn more about water relations in animals, it becomes clear that the acquisition and allocation of water resources constitute a complex system. The strategies that animals use to cope with the problems of water budgeting are varied. Although our understanding of how adult birds marshall their water resources has increased, our knowledge of water relations in neonatal birds is limited. The breeding season in birds has been depicted as a stressful period for the adults. Researchers have examined the energetic cost of establishing and defending a territory, courtship, incubation, and feeding the young; the risk due to predation associated with breeding; and the strategies used by adults to lessen the risk of predation to themselves and their young. Even though it is recognized that water is essential for life and that a successful breeding season is necessary for continued survival of a species, our knowledge of water relations in breeding birds is scanty. Few studies have assessed water relations in breeding adults, and only Ricklefs and Williams (1984) have measured water turnover rates in incubating adults. No studies have examined water relations in free-living, altricial nestlings.

My study utilizing domesticated zebra finches was implemented to address specific questions on nestling water relations. The data collected on nestling zebra finches has shown that the ability of neonates to thermoregulate and the presence of nestmates affect rates of evaporative water loss. When EWL was estimated for individuals within a brood, no differences were found between differently aged broods. A similar trend was found for the amount of water allocated to growth. Nestlings ranging in age from 4 to 16 days allocate the same amount of water for the production of new tissues. These findings indicated that some nestling water requirements did not change with age, and as a result, there was a predictable demand placed on the adults and environment. Further, the water budgets showed that a majority of the daily water loss in water-satiated nestlings was via excretory pathways. Both the high rates of ExWL and the unchanging evaporative and growth water requirements indicated that the nestlings in my colony received excess water. The total water intake and loss per day varied among age groupings and between nests within an age grouping. This internest variation may be important in free-living birds because the nest's location and construction may affect nestling water requirements. The results from my study show that water relations in nestlings form a complex system. A nestling's environment may be as important in determining water requirements and availability as is its physiological maturity. In general, the results from my study suggest that patterns of water utilization in nestlings may be as diverse as in adults, and that examination of nestling responses to water limitation is necessary. The ExWL data indicate that the nestlings in my colony received excess water. As a result, it is not possible to determine whether water is a limiting factor in nestling survival. Free-living birds are exposed to fluctuations in water availability, and in order to fully understand nestling water relations we must determine what the tolerances of nestlings are to water restriction and what the effects of a reduced supply of water are on growth and survival.

APPENDICES

APPENDIX A

Appendix A. Mean body weight (± SD) and number of measurements for zebra finch growth curve (Figure 1).

Age (days)	$\bar{x}$ W_b ± SD (g)	n	Range
0	0.78 ± 0.10	55	0.49-0.97
1	1.15 ± 0.21	55	0.74-1.72
2	1.58 ± 0.31	55	0.86-2.09
3	2.10 ± 0.42	55	1.33-3.11
4	2.74 ± 0.60	55	1.66-4.35
5	3.11 ± 0.86	55	2.05-5.89
6	4.52 ± 0.97	55	2.98-6.38
7	5.57 ± 1.13	55	3.84-7.80
8	6.61 ± 1.28	55	4.04-9.01
9	7.62 ± 1.27	55	4.88-10.45
10	8.53 ± 1.25	55	5.47-11.58
11	9.38 ± 1.21	55	6.18-12.09
12	9.96 ± 1.07	55	7.18-12.64
13	10.30 ± 1.05	55	7.57-12.80
14	10.48 ± 0.99	55	7.85-12.92
15	10.53 ± 0.80	53	8.49-12.60
16	10.60 ± 0.75	51	8.92-12.73
17	10.67 ± 0.67	49	8.80-11.99
18	10.84 ± 0.68	38	9.10-12.45
19	10.88 ± 0.72	34	9.28-12.36
20	11.20 ± 0.73	26	9.47-12.61
21	11.40 ± 0.63	19	9.96-12.72
22	11.55 ± 0.54	14	10.04-12.39
23	11.90 ± 0.65	14	10.47-12.82
24	11.93 ± 0.65	14	10.22-12.92
25	12.28 ± 0.76	14	11.41-13.85
26	12.16 ± 0.55	14	10.76-12.66
27	12.29 ± 0.83	15	11.11-13.79
28	12.35 ± 0.54	17	11.16-13.23
30	12.31 ± 0.74	25	10.12-13.27
32	12.57 ± 0.73	28	10.75-13.64
34	12.74 ± 0.66	28	11.29-13.87
36	12.92 ± 0.65	28	11.36-14.18
38	12.97 ± 0.60	28	11.41-14.26
40	13.16 ± 0.62	28	11.31-14.50
42	13.25 ± 0.67	28	11.12-14.58
44	13.25 ± 0.68	28	11.48-14.82
46	13.33 ± 0.60	28	11.92-14.80
48	13.35 ± 0.59	28	12.55-14.59
Adult	13.47 ± 1.13	23	11.20-16.00

APPENDIX B

Appendix B. Analyses of variance tables for water budgets.

	Source	df	SS	MS	F	p
1) TWI	Total	23	34.559			
	Age	5	18.320	3.664	4.062	$0.01 \leq p \leq 0.025$
	Error	18	16.239	0.902		
2) TWI%W_b	Total	23	8442.221			
	Age	5	5852.248	1170.450	8.135	$p \leq 0.001$
	Error	18	2589.973	143.387		
3) EWL	Total	23	2.411			
	Age	5	0.582	0.116	1.141	$0.25 \leq p \leq 0.50$
	Error	18	1.829	0.102		
4) EWL%TWI	Total	23	535.668			
	Age	5	458.289	91.658	21.674	$p \leq 0.001$
	Error	18	77.379	4.299		
5) Growth	Total	19	0.619			
	Age	4	0.132	0.033	1.016	$0.15 \leq p \leq 0.50$
	Error	15	0.487	0.0325		
6) Growth%W_b	(arcsin $\sqrt{\%}$ transformation)					
	Total	19	427.286			
	Age	4	324.818	81.205	11.885	$p \leq 0.001$
	Error	15	102.468	6.831		
7) Growth%TWI (arcsin $\sqrt{\%}$ transformation)						
	Total	19	218.760			
	Age	4	103.008	25.752	3.337	$0.025 \leq p \leq 0.05$
	Error	15	115.752	7.717		
8) ExWL	Total	23	20.713			
	Age	5	10.156	2.031	3.460	$0.01 \leq p \leq 0.025$
	Error	18	10.557	0.587		
9) ExWI%W_b	Total	23	5073.760			
	Age	5	3120.028	624.056	2.841	$0.025 \leq p \leq 0.05$
	Error	18	3953.732	219.652		
10) ExWI%TWI	Total	23	466.432			
	Age	5	253.664	50.733	4.292	$0.005 \leq p \leq 0.010$
	Error	18	212.768	11.820		
11) WTR	Total	23	31.674			
	Age	5	19.356	3.871	5.660	$0.001 \leq p \leq 0.005$
	Error	18	12.318	0.684		

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