Development of homeothermy in the American coot (*Fulica americana*): thermoregulatory patterns in air and water.

by

Glenn C. Sutter

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

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Abstract

The precision and metabolic costs of temperature regulation in air and water were investigated in juvenile American coots (Fulica americana). Developmental changes in cloacal temperature (Tcl), resting metabolic rate (RMR), thermogenic capacity and plumage insulation were documented for hatchling through fledgling stages. Thermogenic response to cold air increased between 0-1 d and 1-5 d posthatch, when capacity to elevate RMR rose from 40% to 140%. Yet Tcl of 0-5 d coots was strongly dependent upon ambient temperature. Mean cooling rates at this stage varied from 0.05°C·min^{-1} in 25°C air, to 0.49°C·min^{-1} in 25°C water. In both media, coots were homeothermic by 8-15 d posthatch when body weight was 30-35 g. Achievement of homeothermy in this species appeared to depend on the attainment of adequate insulation and thermal inertia. A thermoneutral range of air temperatures (29-33°C) first appeared at 40-48 d posthatch. Fledglings displayed a lower critical temperature of 19-21°C in both air and water.

Throughout development, minimum RMR and whole-body conductance (Cmin) averaged 1.2-2.5 times higher in water than in air. Though fledgling Cmin was elevated by 60% in ice-water and in a -10°C atmosphere of helium-oxygen, these

birds displayed an impressive resistance to cooling in both media. Fledgling Tcl remained stable at 40-41°C during 4 h exposure to 5°C water. This thermoregulatory prowess is attributed to the insulation and exceptional buoyancy provided by the dense, water-repellent plumage of fledgling birds. Aquatic Cmin was further reduced by peripheral cooling in submerged appendages.

In coots >40 d of age, intramuscular injection of the ß-agonist, isoproterenol, increased thermoneutral metabolic rate by 30-50%. Younger birds did not respond to ß-adrenergic stimulation. The metabolic response of fledglings to isoproterenol was dose-dependent, and was effectively blocked by concurrent treatment with the ß-antagonist, propranolol. Though coots appeared to lack thermogenic brown fat, the ß-adrenergic sensitivity of birds >40 d old implies the existence of nonshivering thermogenesis in F. americana.

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Symbols and Abbreviations used in the Text

A	asymptotic body weight
BAT	brown adipose tissue
C	whole-body thermal conductance
Cmin	minimum whole-body thermal conductance
EHL	evaporative heat loss
EHLC	evaporative heat loss capacity
EMG	electromyography
Ff	final fractional oxygen content of chamber air
Fi	initial fractional oxygen content of chamber air
IP	internal pipping
K	posthatch growth rate constant
LCT	lower critical ambient temperature
M	growth-fitting parameter
MR	metabolic rate
NST	nonshivering thermogenesis
RCAG	relative core-ambient gradient
RHP	relative heat production
RMR	resting metabolic rate
RQ	respiratory quotient
SDA	specific dynamic action
T	age posthatch
t	elapsed time
Ta	air temperature
Tcl	cloacal temperature
TI	age of most rapid posthatch growth
TNZ	thermoneutral zone

Tw	water temperature
UCT	upper critical ambient temperature
V	volume of chamber air - mean egg volume
$\dot{V}o_2$	rate of oxygen consumption
WT	body weight

General Introduction

With a thermal conductivity nearly 25 times that of air, water presents almost zero resistance to heat flow in aquatic endotherms (Stahel and Nicol 1982). Exposure to cold water is particularly challenging to small-bodied birds and mammals, in which problems of heat loss are often amplified by limited insulation and low thermal inertia. Consequently, even brief immersion of such animals is frequently accompanied by deep body cooling (Stahel and Nicol 1982; Williams 1986; MacArthur 1984,1988). Compared to our knowledge of aquatic mammals (see Irving 1973; MacArthur 1988), almost nothing is known of aquatic thermoregulation in small water birds (Stahel and Nicol 1982; Epply 1984). To date, most avian studies of aquatic temperature control have focused on penguin species weighing >2 kg (see Kooyman et al. 1976; Barré and Roussel 1986). Thermoregulatory performance in water has apparently been examined in only one small-bodied adult bird, the little penguin, Eudyptula minor (Stahel and Nicol 1982). The ontogeny of avian temperature regulation in water has also been studied only in the Xantus' murrelet, Synthlioboramphus hypoleucus (Epply 1984).

This paucity of physiological data is remarkable, considering that many aquatic birds encounter cold water as precocial neonates, and that the adult body size of many species is <1 kg (Nice 1962; Koskimies and Lahti 1964). In

either instance, the problem of maintaining thermal balance in water should be acute--especially for birds that breed and overwinter at high latitudes. One such species is the American coot (Fulica americana, F. Rallidae), which breeds as far north as the central Yukon (Godfrey 1986). Despite body weights of only 19-20 g, chicks of F. americana accompany their parents on aquatic foraging excursions within days of hatching (Gullion 1954; Alisaukas 1986). Adult birds have been reported north of 50° latitude during late November, with records of coots overwintering in these areas (Gardner 1981; Robbins et al. 1983). F. americana thus presents an excellent avian model for examining aquatic thermoregulatory abilities at different stages of development. Compared to a wealth of ecological information (see Gullion 1954; Alisaukas and Ankney 1985; Desrochers and Ankney 1986), little is known about any aspect of rail physiology (Brent et al. 1984,1985; Bucher 1986).

This thesis describes the thermoregulatory performance of F. americana from three perspectives. Part I details the ontogeny of thermoregulation in air and water, with special attention given to developmental changes in body temperature control, metabolic rate and plumage insulation. Part II focuses on the fledgling stage, with particular emphasis on thermoregulatory abilities in extreme cold. Temperature regulation of fledgling coots is evaluated during exposure to a helium-oxygen gas mixture of low thermal resistance,

and during long-term immersion in ice water. Finally, Part III examines the potential for nonshivering thermogenesis in juvenile coots, as revealed from pharmacological studies of ß-adrenergic sensitivity.

PART I

Ontogeny of thermoregulation in a precocial aquatic bird, the American coot (*Fulica americana*).

Introduction

Exposure to cold water poses unique thermoregulatory problems for many aquatic endotherms--especially small-bodied forms with low thermal inertia and limited potential for improving insulation. In such animals, even brief immersion may seriously tax thermogenic abilities, and thus is often accompanied by deep body cooling (Stahel and Nicol 1982; Dawson and Fanning 1981; MacArthur 1988). Maintenance of thermal balance in water should be especially challenging to precocial chicks of aquatic birds--particularly young that are reared at high latitudes. Chicks of many of these species encounter cool water within days of hatching, when body mass is often <50 g (Nice 1962; Koskimies and Lahti 1964). Moreover, some neonates remain in water for extended periods, as they forage independently or are fed by attending parents (Nice 1962; Gullion 1954). Yet essentially nothing is known about aquatic temperature regulation in these amphibious chicks. Previous thermoregulatory studies of precocial young, including those of aquatic species, have focused on cold-tolerance in air (Koskimies and Lahti 1964; Untergasser and Hayward 1972; Matthew 1983). To my knowledge, natal temperature control in water has been examined only in the Xantus' murrelet, Synthliboramphus hypoleucus (Epply 1984); no information is available concerning aquatic homeothermy in the young of any waterfowl species.

In response to this need, I investigated the ontogeny of aquatic homeothermy in a common marsh rail, the American coot (Fulica americana, O.Gruiformes). A highly precocial species, F. americana may enter water within hours of hatching, when body-weight is only 19-20 g (Alisaukas 1986). Neonate coots <5 d old may remain in water for most of the day, as they forage independently or follow their parents (Gullion 1954). Since the breeding range of F. americana extends as far north as 60° latitude (Godfrey 1986), foraging chicks of this species probably encounter water temperatures <25°C on a regular basis. Thus, F. americana presents an ideal model for studying the ontogeny of homeothermy in aquatic birds. Despite a wealth of ecological information (see Gullion 1954; Alisaukas 1986; Desrochers and Ankney 1986), the development of thermoregulation in this, or any other Gruiforme is poorly understood (Bucher 1986).

This study examines the ontogeny of homeothermy in F. americana over a range of air and water temperatures which this species would likely encounter in nature. Precision of cloacal temperature (Tcl) regulation, as well as the relative contributions of thermogenesis and insulation were examined during different stages of development. Measurements of metabolic rate (MR) and Tcl were combined with studies of plumage morphology, shivering and evaporative heat loss. In addition to providing insight

into the thermoregulatory performance of neonate aquatic birds, this research also establishes the position of a representative Gruiforme within the avian hierarchy of natal temperature control (Koskimies and Lahti 1964; Epply 1984).

Materials and Methods

Animals

A total of 79 hand-reared coots was used in this study. Eggs were collected from marshes near Winnipeg and Neepawa, Manitoba during June, 1985, 1986. The eggs were incubated in a commercial incubator and the hatchlings reared on a diet of mealworms, chopped earthworms, moistened dogfood, poultry crumbles and turkey starter (Feed-Rite Mills). Poultry crumbles consisted of 21% protein, 5% fibre and 2% fat; turkey starter consisted of 29% protein, 5% fibre and 2.5% fat. The crumbles and starter were medicated with chlortetracycline and oxytetracycline, respectively. Food was provided ad libitum, and hatchlings were able to feed independently by 1-2 wk of age. Prior to this age, birds were individually fed by hand. Egg survival was 88% in 1985, and 96% in 1986. Chick mortality was highest (17-30%) during the first two weeks following hatch, declining thereafter to 0-5% in both years. All birds were reared at room temperature (23°C) with a 12L:12D photoperiod. Each individual was marked with a numbered tag which was secured to the tarsus and periodically replaced as the bird grew. Coots were kept in groups of 4-6 and were housed in progressively larger holding pens as the birds matured. For the first month, birds were held in 50 x 60 x 40 cm wire cages, each provided with a pan of standing water (3-6 cm depth) and access to an infrared brood lamp. Once coots

reached 30 d of age, brood lamps were removed and the birds were installed in fibre glass tanks (92 x 97 x 55 cm, or 210 x 60 x 55 cm). Each tank contained standing water to a maximum depth of 20 cm, and a wire mesh screen mounted above water level at one end of the tank provided birds with a dry resting site. Coots reached fledgling age by 9 wk, at which stage they were maintained on a diet of medicated crumbles, turkey starter and dogfood. Mean fledgling body weight was 386 g (range= 207-548 g, n=175), or 67% of the mean adult weight reported for wild coots (Alisaukas 1986). Upon completion of the study, birds were sacrificed by CO_2 inhalation.

To facilitate analyses of developmental changes in thermoregulatory ability, 6 age-dependent cohorts were established: 0-1, 1-5, 8-15, 21-28, 40-48 and 60+ d, respectively. The first cohort (0-1 d) included all birds <24 h old, while the last (60+ d) represented the fledgling stage.

Growth and development

The relationship between body mass and age was examined with three- and four-parameter growth models using the approach adopted by Sugden et al. (1981). Accordingly, a derivation of Richards's (1959) four-parameter model was adopted, which took the form:

$$[1] \qquad WT=A[1-(1-M)EXP[-K(T-TI)/M^{M/(1-M)}]]^{1/(1-M)}$$

(Sugden et al. 1981), where WT=body weight (g) at time T (wk), A=asymptotic body weight (g), TI=age (wk) of maximum growth, and K=overall growth rate in log units·wk^{-1}. Variable M is a growth-fitting parameter (see Causton 1969). When M=1, equation [1] becomes the Gompertz model: WT=A·EXP[EXP[-eK(T-TI)]]. When M=2, equation [1] is equivalent to the logistic model: WT=A·[1+EXP[-K(T-TI)]]$^{-1}$, where K is given in log units·d^{-1} (Ricklefs 1979).

Growth of the tarsus, bill and plumage were evaluated from carcass measurements. Bill depth and width, and culmen length were measured with dial calipers to the nearest 0.05 cm. Natal plumage was divided into three neossoptile classes on the basis of size and appearance (see Results), and juvenal feathers were classified as down, semiplume and contour (Stettenheim 1972). Ten feathers from each class were plucked from ventral, pectoral and dorsal sites of known-age carcasses, and measured ($\pm$0.05 cm). Five measurements of plumage depth ($\pm$0.1 cm) and follicle density were also taken at each of these sites. All examinations of juvenal plumage were confined to local feather tracts (see Results).

Respirometry

Prenatal MR was measured using closed-system respirometry. Pre-weighed eggs were installed in darkened, 200- to 250-mL glass chambers fitted with rubber stoppers. Eggs were held in these chambers at 35°C for a period of 15-30 min, following which 50 mL of chamber gas was withdrawn via a sampling port in the stopper. Chamber air was thoroughly mixed prior to withdrawing a sample of gas for analysis. The fractional oxygen content of each sample was measured by first passing the gas through water and CO_2 absorbents (Drierite and Ascarite, respectively), and then through a Beckman OM-14 oxygen analyzer. The duration of metabolic runs was adjusted for age, to ensure that the oxygen concentration of chamber air never fell below 17.00%.

Prenatal rate of oxygen consumption ($\dot{V}o_2$) was calculated from the equation:

$$[2] \qquad \dot{V}o_2 = \frac{V(Fi-Ff)}{(1-Ff)t}$$

(Vleck et al. 1979), where V=volume of air available to the egg (mL), Fi=initial fractional oxygen content of chamber air (0.209), Ff=final fractional oxygen content of chamber air, and t=elapsed time (h). V was derived by subtracting the mean volume of coot eggs (24.75 mL) from the chamber volume.

To measure $\dot{V}o_2$ of hatchlings in air, birds were placed in darkened, 2.4-9.2 L glass desiccators fitted with internal wire mesh platforms. Chambers were installed in a constant-temperature cabinet that could be regulated to within $\pm$0.5 C of the desired air temperature (Ta). Chamber Ta was monitored with a thermocouple probe mounted on the inside wall of the metabolism chamber and coupled to a Bailey BAT-12 thermocouple thermometer. Air entered the metabolic chamber through an inlet port positioned near the floor, and exited via an exhaust port mounted in the ceiling of the chamber. Incurrent and exhaust air streams were routed through Drierite and soda lime to remove water vapour and CO_2, respectively. Incurrent flow rates were adjusted for chamber size, and were maintained between 0.17 and 1.73 $L \cdot min^{-1}$ using a Fisher Lab-Crest rotameter calibrated according to the bubble flowmeter technique (Levy 1964). Fractional O_2 content of the excurrent gas was continuously monitored by routing a dry, CO_2-free sample of the gas through an Applied Electrochemistry S-3A oxygen analyzer connected to a chart recorder (Fisher Recordall 5000).

To measure $\dot{V}o_2$ of 1-28 d neonates in water, birds were placed in a partially water-filled metabolism chamber constructed from a 14-L glass aquarium. Water temperature (Tw) was regulated to within $\pm$0.1°C by immersing the chamber in a 35-L circulating water bath. The chamber was fitted with an air-tight Plexiglas lid equipped with ports for

incurrent and exhaust air streams, as well as a port for insertion of a YSI temperature probe. Water depth and floor height in the chamber were adjusted so that the free-air space above the water was 2.5 L for neonates, and 5.0 L for 21-28 d birds. To ensure that the gas-analysis system equilibrated rapidly, incurrent air flow was maintained at 0.95-1.25 $L \cdot min^{-1}$ using a calibrated Gilmont rotameter. Water within the chamber was always deep enough that birds were forced to float, though they could always touch bottom. Throughout most runs, coots floated quietly within the metabolism chamber; trials involving persistent activity were discarded.

Aquatic trials involving fledglings were performed in a large rectangular tank (210.5 x 121.5 x 60 cm) filled with water to a depth of 30 cm and housed in a controlled-environment room (see MacArthur 1984). Room temperature was held within 5-10°C of Tw in order to minimize Tw fluctuations during recordings. Tw remained within $\pm$0.1°C of the desired level (4-35°C) throughout most runs. The metabolic chamber consisted of a 3.5-L open-bottomed Plexiglas dome secured to a baseboard mounted just below water level at one end of the tank. An opening in the baseboard exactly matched that of the dome. Though coots could dive at will in this set-up, they usually floated quietly within the chamber during the entire run. Dry, CO_2-free air entered the chamber at a rate of 4.5 $L \cdot min^{-1}$,

and the fractional O_2 content of exhaust gas was continuously monitored by passing a dry, CO_2-free sample of the gas through a Beckman F-3 oxygen analyzer connected to a chart recorder (Fisher Recordall 5000).

To avoid jeopardizing the survival or nutritional well-being of these animals, birds were not fasted prior to metabolic testing. All runs were conducted between 0800 h and 2200 h, and each bird was tested at only one ambient temperature on any given day. A minimum of 72 h elapsed between consecutive runs on the same individual. Trials in air normally involved a 60-min equilibration period followed by a 30- to 60- min recording interval. For older birds, equilibration time was reduced to 45 min for trials at Ta=40°C, in order to reduce thermal stress. Only 10-15 min was allocated for equilibration in aquatic trials, which ranged from 20 to 25 min duration. Previous testing of the fledgling set-up at an identical flow rate revealed that the time required for the gas analysis system to achieve 95% equilibration was only 2.7-3.0 min (MacArthur 1986). For each trial in air or water, resting metabolic rate (RMR) was derived from three estimates of minimum $\dot{V}o_2$, each of at least 3-5 min duration. $\dot{V}o_2$ was calculated according to the method of Depocas and Hart (1957) and corrected to STP. All estimates of prenatal and posthatch $\dot{V}o_2$ were converted to units of heat production (W or $W \cdot kg^{-1}$) by assuming an RQ of 0.85 and a conversion factor of 0.0056 $W \cdot h \cdot mL\ O_2^{-1}$ (Brent et

al. 1985). Body weight (±0.1 g), Tcl, room temperature and barometric pressure were measured at the beginning and end of each run. Tcl was monitored with a fast-response, copper-constantan thermocouple (accuracy ±0.1°C) inserted 1-3 cm into the cloaca and connected to a Bailey BAT-12 thermocouple thermometer.

Evaporative heat loss

Evaporative water loss was determined gravimetrically from the increase in mass (±0.001 g) of a Drierite column inserted into the exhaust air stream for a precisely measured period. These data were converted to evaporative heat loss (EHL) using a value of 0.67 $W \cdot h \cdot g\ H_2O^{-1}$ for latent heat of vaporization (Murdock 1975). For runs involving measurement of EHL, sufficient mineral oil was placed beneath the mesh floor of the metabolic chamber to eliminate evaporation from excrement.

Shivering

To measure shivering in 0-48 d birds, electromyography (EMG) electrodes were fashioned from 34 Ga silver-plated copper wire (Cooner Sales Co.), and sewn directly into the left pectoralis muscle. Three such electrodes were fixed in an equidistant triangle (2 mm) with the ground electrode positioned near the sternum. For fledglings, an EMG assembly was constructed which consisted of an equidistant (3 mm) triangle of three stainless-steel needle electrodes

secured in a base of epoxy and dental acrylic. Skin sutures held this assembly in position and the electrode pins were embedded 4 mm into the left pectoralis muscle. In all experiments, electrode leads were fed craniad around the left wing and waxed or taped to the dorsal plumage. Hard-wire connections were made to an EMG amplifier on a Lafayette Model 76102 physiograph. Following electrode attachment, the bird was installed in a metabolic chamber and placed in a thermostatically-controlled cabinet. The cabinet was gradually cooled and rewarmed in a stepwise fashion, and the presence or absence of shivering noted at each successive Ta. Birds were tested over Ta ranges varying from 20 to 35°C for neonates, to -10 to 35°C for fledglings. Runs usually lasted 2-3 hr and the occurrence of shivering was inferred from visual inspection of the EMG tracing (Hohtola 1982; George 1984), as well as from direct observation of the instrumented birds.

Carcass conductance measurements

Cooling rates were derived for freshly-killed, and for rewarmed carcasses suspended from a metal frame for a period of 45 min in still, 23°C water. In all trials, the plumage was undisturbed and Tcl was monitored with a Bailey BAT-12 thermometer via a thermocouple probe inserted 2-5 cm into the cloaca. Pilot studies showed that cooling rates of freshly-killed birds were similar to those of rewarmed carcasses. For each trial, the carcass cooling constant was

calculated as the slope of the regression relating log (Tcl-Tw) to time (h). This constant was then multiplied by the specific heat of tissue (3.48 $kJ \cdot kg^{-1} \cdot °C^{-1}$) to derive values of carcass conductance (Hart 1951; Morrison and Tietz 1957).

Statistical analyses

Means were compared with a Student's t-test, or analysis of variance and Fisher's LSD test for multiple comparisons. In some instances, means were examined with paired t-tests. Regressions were derived by the method of least squares, and slopes were compared with analysis of covariance and the homogeneity-of-slopes model of the GLM procedure (Statistical Analysis System 1982, Cary, NC). All sigmoidal curves were fitted with a nonlinear regression procedure that employed an iterative algorithm to provide the best least squares estimate for each parameter in the model. In all cases, residuals were randomly distributed over time; hence the explained percentage of variation was used to assess goodness of fit (Zach et al. 1984). Means are presented with ± 1 SE, and in all comparisons significance was set at $P=0.05$.

Results

Growth rates of hand-reared coots

The majority of coots appeared healthy and vital at all stages of development, and there was no indication that growth was impaired by the hand-rearing procedure. Culmen and tarsal lengths of lab-raised birds (Appendix 1) were virtually identical to those of similar-aged wild coots (Alisaukas 1986). Moreover, the posthatch growth rate constant of the hand-reared birds was $0.131 \cdot d^{-1}$ (Appendix 2), which is only 0.016 log units less than the value predicted for precocial birds that attain an asymptotic mass of 370 g (Ricklefs 1979). Following the methods of Zach et al. (1984), three- and four- parameter growth models explained over 91 percent of the observed variation in the mass of these birds (Appendix 2). Posthatch growth was most rapid at 26 d, with 90 percent of the asymptotic mass attained by approximately 40 d posthatch (Fig. 1-1, Appendix 2).

Onset of homeothermy

Hatchlings were judged to be homeothermic if Tcl remained stable for a minimum period of 90 min in 20-35°C air, or for 15 min in 25-35°C water. Based on these criteria, homeothermic temperature control first appeared at 8-15 d posthatch, when most birds maintained a Tcl of 35-40°C at air temperatures of 15-35°C (Fig. 1-2). Evidence of aquatic

Figure 1-1. Body mass growth curve of hand-raised coots. The logistic equation was fitted by a nonlinear regression procedure (see text). Values are presented as means ± 1 SE; sample sizes are 28-68 for ages ≤ 30 d, and 1-20 for ages >30 d.

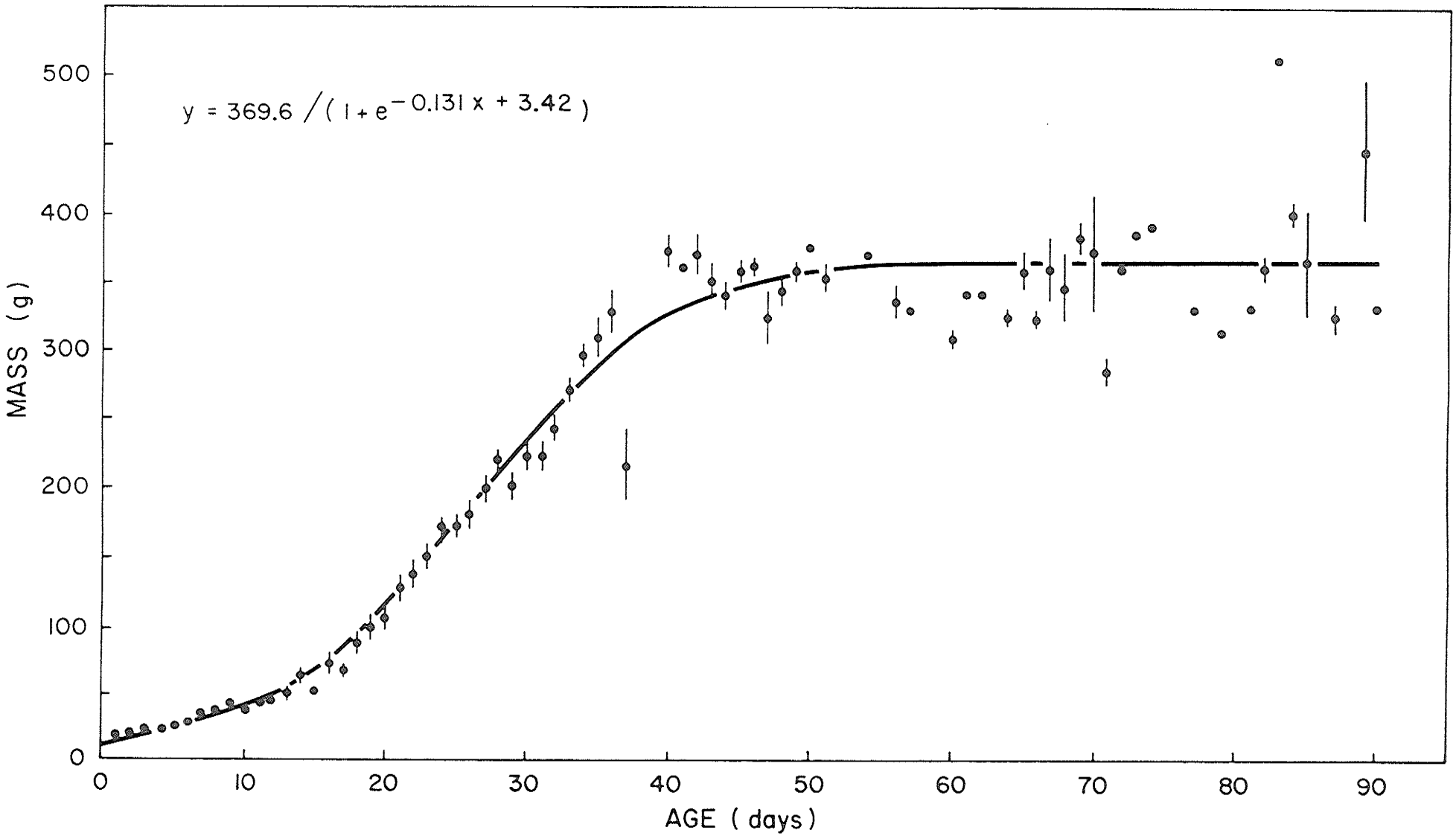
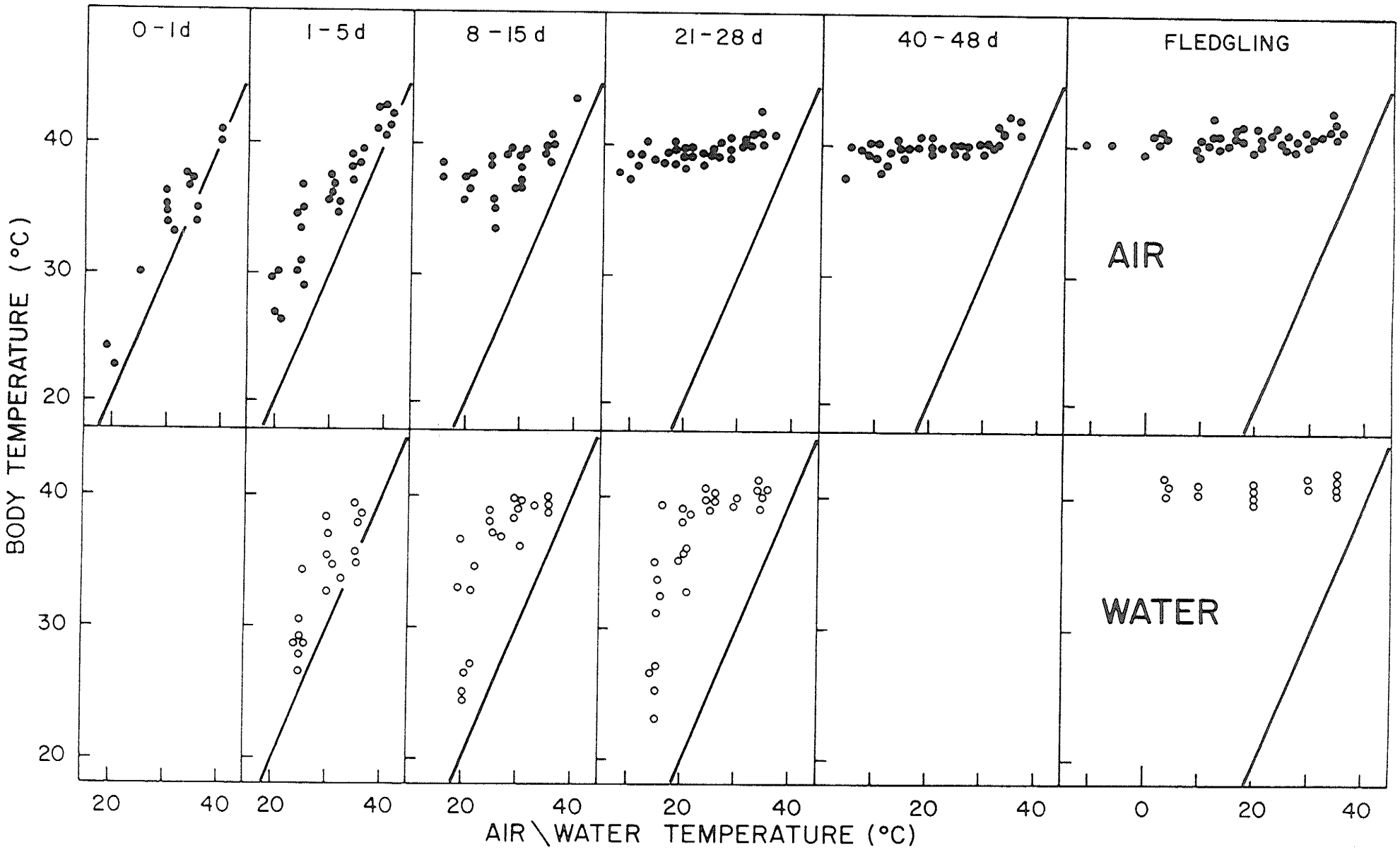

y = 369.6 / (1 + e^−0.131 x + 3.42)
MASS (g)
AGE (days)
0
100
200
300
400
500
0
10
20
30
40
50
60
70
80
90

Figure 1-2. Cloacal temperatures of immature coots following exposure to different air (closed circles) and water (open circles) temperatures. Measurements recorded during some trials in 40°C air were excluded, since these were <75-90 min duration. Slopes denote equality between cloacal and ambient temperatures.


0 - 1 d
1 - 5 d
8 - 15 d
21 - 28 d
40 - 48 d
FLEDGLING
AIR
WATER
BODY TEMPERATURE (°C)
AIR \ WATER TEMPERATURE (°C)
20
30
40
0
20
40

homeothermy also appeared at 8-15 d, though at this age, birds could regulate Tcl only in water at 25-35°C. In contrast, fledglings displayed an impressive tolerance to cold air and water (Fig. 1-2). As described in Part II, fledglings were capable of maintaining a stable Tcl for at least 4 h in 5°C water.

Based on measurements recorded prior to the start of metabolic trials, mean Tcl remained stable at 38.0-38.4°C during the first 5 d posthatch, but thereafter rose and remained within 2°C of the fledgling mean Tcl of 41.7°C (Table 1-1, $P<0.05$). Assuming that any stress-related effects of handling on Tcl were consistent across age groups (see Part II), my data suggest that Tcl was regulated below fledgling levels for at least 2 weeks following hatching.

Ontogenetic changes in thermoregulatory ability were also assessed from body cooling rates of birds exposed to 25°C air and 25°C water (Tables 1-1 and 1-2). Fisher's LSD test revealed that temperature control in cold air improved steadily from 0 to 15 d posthatch ($P<0.05$), while the first significant improvement in aquatic thermoregulatory performance appeared at 2-3 weeks of age. Tcl of 1-15 d coots fell 9.8-16 times faster during immersion in 25°C water, than during exposure to 25°C air (*t*-test, $P<0.05$).

Table 1-1. Thermoregulatory characteristics of juvenile coots in air.

Age group (d)	Mass (g)	Tcl (°C)	Cooling rate (°C·min^{-1})	LCT (°C)	UCT (°C)	Cmin (W·kg^{-1}·°C^{-1})	RMR (W·kg^{-1})		
							Max.	Min.	Max·Min^{-1}
0-1	19.3±0.2 (68)	^{a}38.0±2.2 (22)	^{c}-0.10±0.01 (7)	-	-	^{e}1.26±0.15 (7)	^{a}7.44±0.73 (7)	^{b}5.42±0.40 (7)	1.4
1-5	22.1±0.3 (282)	^{a}38.4±1.1 (40)	^{b}-0.05±0.01 (8)	34.8	34.8	^{e}1.26±0.06 (11)	^{b}13.85±0.68 (3)	^{b}5.82±0.51 (10)	2.4
8-15	46.0±1.5 (319)	^{b}39.7±0.7 (41)	^{a}-0.02±0.01 (8)	34.6	34.6	^{d}1.09±0.05 (19)	^{c}16.99±1.19 (7)	^{c}9.30±0.77 (9)	1.8
21-28	169.5±4.3 (316)	^{c}40.5±0.6 (50)	-	30.8	30.8	^{c}0.60±0.02 (35)	^{c}19.59±0.62 (6)	^{b,c}6.76±0.37 (8)	2.9
40-48	353.1±8.7 (28)	^{d}40.9±0.7 (62)	-	28.8	32.5	^{b}0.43±0.01 (47)	*13.36±0.99 (8)	^{b}5.97±0.25 (7)	*2.2
60+	386.4±5.6 (175)	^{e}41.7±0.7 (46)	-	21.1	-	^{a}0.23±0.01 (33)	*11.57±0.76 (10)	^{a,b}5.15±0.27 (19)	*2.3

Note: Tcl=initial measurements recorded at start of metabolic tests. UCT=upper critical temperature. Mean cooling rate is derived from initial and final Tcl's of coots exposed to 25°C air for 90 min. Values are presented as means ±1 SE; numbers in parentheses denote sample sizes. Within each column, means sharing the same letter are not statistically different (Fisher's LSD test, P>0.05).

*may be low estimates.

Table 1-2. Thermoregulatory characteristics of juvenile coots in water.

Age group (d)	Cooling rate ($^{o}C \cdot min^{-1}$)	Cmin ($W \cdot kg^{-1} \cdot {}^{o}C^{-1}$)	Carcass C ($W \cdot kg^{-1} \cdot {}^{o}C^{-1}$)	RMR ($W \cdot kg^{-1}$)		
				Max.	Min.	Max·Min^{-1}
1-5	^{b}-0.49±0.05 (8)	^{c}3.16±0.57 (6)	^{c}11.20±0.73 (9)	^{a}13.07±0.75 (6)	^{b,c}12.83±1.22 (6)	1.0
8-15	^{b}-0.32±0.14 (7)	^{b}1.43±0.06 (11)	-	^{b}20.14±0.61 (4)	^{c}15.12±0.73 (8)	1.3
21-28	^{a}-0.04±0.01 (5)	^{b}1.31±0.15 (24)	^{b}3.12±0.19 (4)	^{b}18.35±1.19 (5)	^{b}11.10±0.76 (7)	1.7
60+	-	^{a}0.36±0.03 (13)	^{a}1.89±0.08 (8)	*14.67±2.20 (4)	^{a}6.17±0.36 (9)	*2.2

Note: Mean cooling rate is derived from initial and final Tcl's of coots immersed in 25°C water for 15 min. Values are presented as means ±1 SE; numbers in parentheses denote sample sizes. Within each column, means sharing the same letter are not statistically different (Fisher's LSD test, $P>0.05$).

*may be low estimates.

To provide an index of natal homeothermy, the relative core-ambient gradient (RCAG) was derived according to the equation:

$$[3] \qquad RCAG = \frac{Tcl-Ta}{40.5-Ta}$$

(O'Connor 1975a), where Tcl is the average Tcl recorded during a 90-min trial in 30°C air, and 40.5 represents mean fledgling Tcl. As recommended by O'Connor (1975a), Tcl-Ta gradients of less than 5°C were omitted from these calculations to reduce statistical error. RCAG increased exponentially with age (Fig. 1-3A), rising from a mean value of 0.53 ± 0.04 (N=6) at 0-1 d, to 0.87 ± 0.02 (N=10) at 8-15 d, when homeothermy appeared to be established. Plotted against mass, RCAG increased in two distinct linear phases, separated by an abrupt inflection point at 30-35 g (Fig. 1-4A).

Metabolic development

Measurements of mass-dependent MR imply that embryonic growth was completed 3-4 d prior to hatching (Fig. 1-5). The MR of unpipped eggs stabilized during this period, and was age-independent from 2 to 5 d prior to hatching (Fisher's LSD test, $P>0.05$). A clearly exponential relation existed between prenatal MR and age ($r^2=0.92$), and this

Figure 1-3. Relative core-ambient gradient (A) and relative heat production (B) of juvenile coots in relation to age (see text, pgs. 24,30). Five age groups are represented: 0-1 d (closed circles), 1-5 d (open circles), 8-15 d (closed triangles), 21-28 d (open triangles) and 40-48 d posthatch (closed squares). Regression curve in (A) was fitted to semi-log transformed data by the method of least squares. The logistic equation in (B) was fitted by a nonlinear regression procedure (see text).

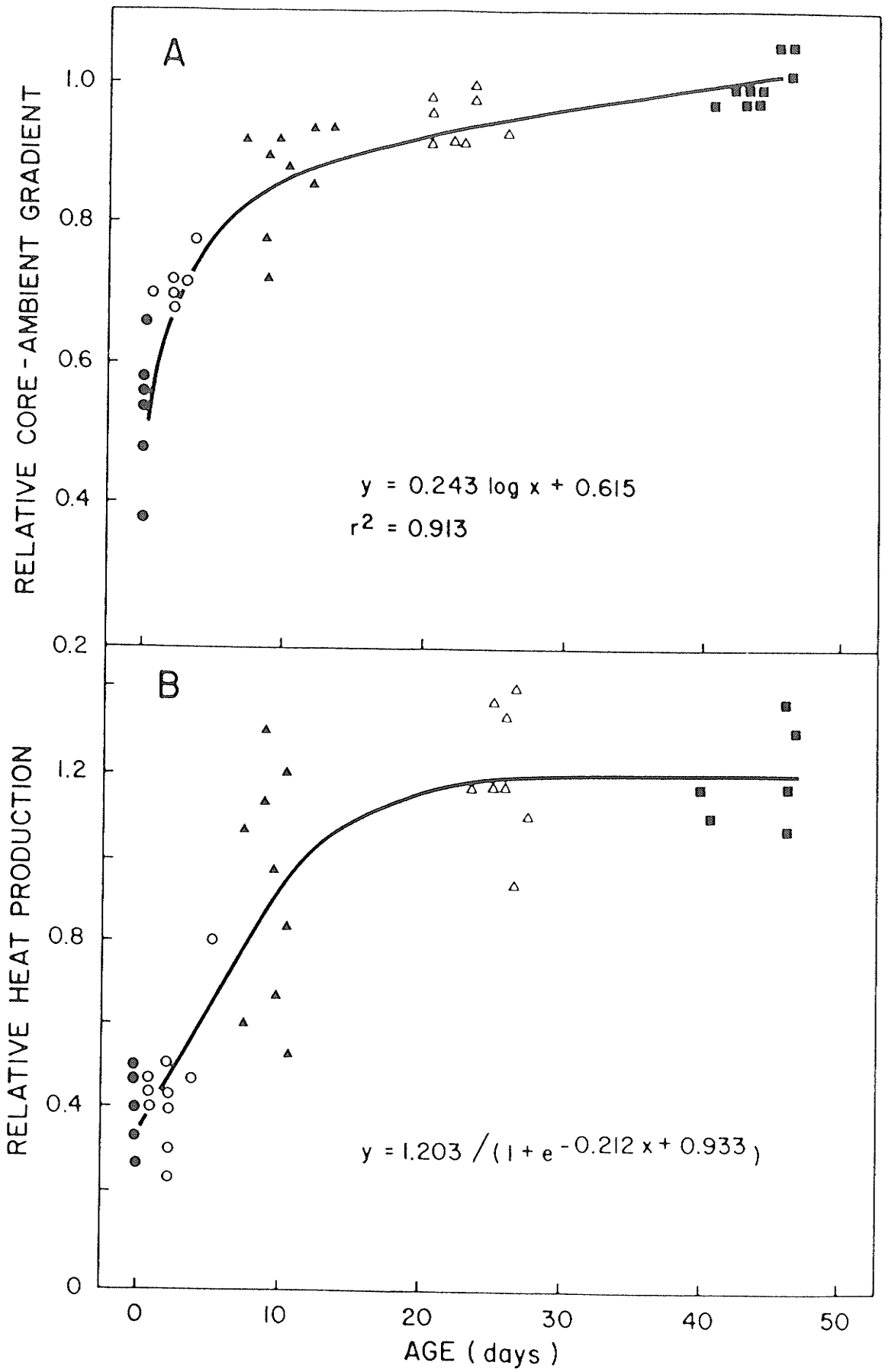

A
B
RELATIVE CORE-AMBIENT GRADIENT
RELATIVE HEAT PRODUCTION
AGE (days)
y = 0.243 log x + 0.615
r2 = 0.913
y = 1.203 / (1 + e - 0.212 x + 0.933)
0.2
0.4
0.6
0.8
1.0
0
0.4
0.8
1.2
0
10
20
30
40
50

Figure 1-4. Relative core-ambient gradient (A) and relative heat production (B) of juvenile coots in relation to body mass (see text, pgs. 24,30). Symbols as in Fig. 1-3. Regression lines were fitted by the method of least squares (see text).

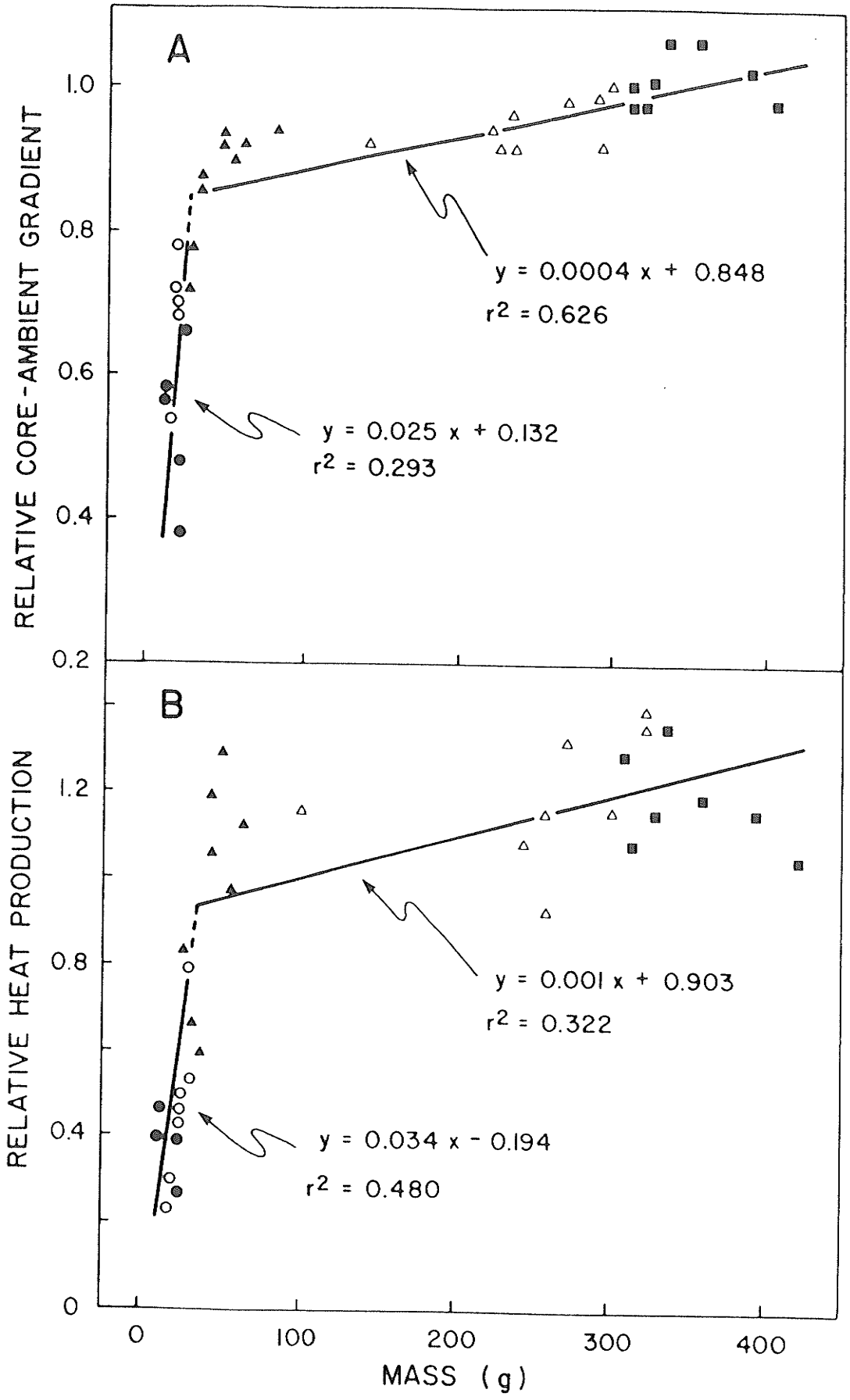
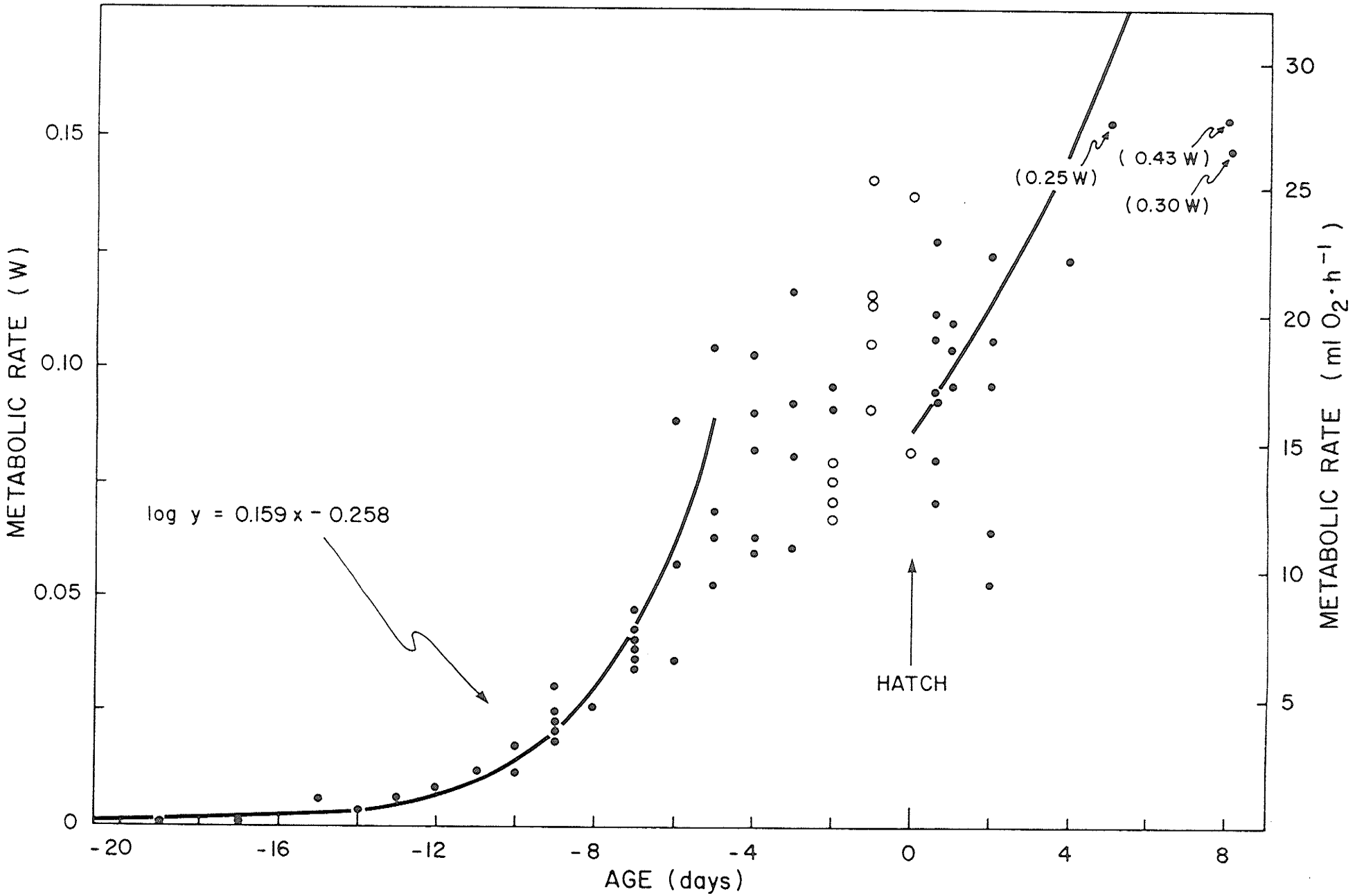

A
B
RELATIVE CORE-AMBIENT GRADIENT
RELATIVE HEAT PRODUCTION
MASS (g)
y = 0.0004 x + 0.848
r2 = 0.626
y = 0.025 x + 0.132
r2 = 0.293
y = 0.001 x + 0.903
r2 = 0.322
y = 0.034 x - 0.194
r2 = 0.480
0
100
200
300
400
0.2
0.4
0.6
0.8
1.0
1.2

Figure 1-5. Mass-dependent metabolic rates of embryonic and neonate coots. Regression curves were fitted to semi-log transformed data by the method of least squares (excluding ages 0-4 d prehatch, and >15 d posthatch). Posthatch data yielded the regression equation: log $y=0.062x-1.065$ ($r^2=0.71$). Open circles denote pipped eggs.

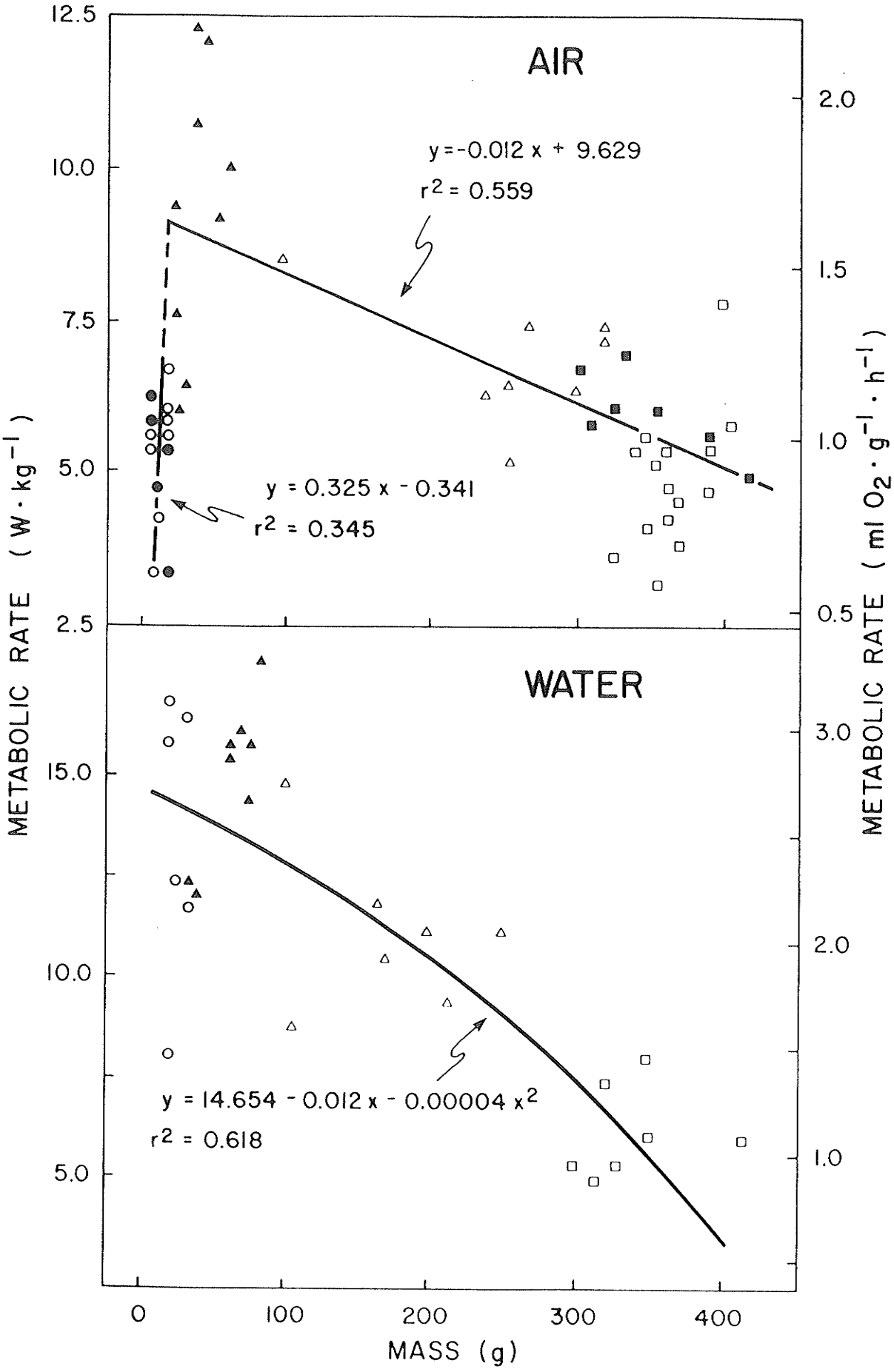

METABOLIC RATE (W)
0.15
0.10
0.05
0
log y = 0.159 x - 0.258
(0.25 W)
(0.43 W)
(0.30 W)
HATCH
-20
-16
-12
-8
-4
0
4
8
AGE (days)
30
25
20
15
10
5
METABOLIC RATE (ml $O_2 \cdot h^{-1}$)

relationship was strengthened (r^2=0.96) when the last 5 d of incubation were excluded from the analysis (Fig. 1-5). Not surprisingly, ontogenetic changes in hatchling mass-dependent MR closely tracked neonatal growth (Figs. 1-1 and 1-5).

In most birds, internal pipping (IP) occurs when incubation is 90% complete (Vleck et al. 1979), which in this case, would correspond to 2-3 d prior to hatching. The mean MR at 3-4 d prehatch (0.083±0.007 W, N=9) should thus provide a reasonable estimate of pre-IP MR in F. americana. This value only slightly exceeds the pre-IP MR of 0.073 W predicted from allometry (Hoyt and Rahn 1980), assuming an incubation period of 23 d (Gullion 1954) and a fresh egg mass of 28.8 g (Alisaukas 1986). Reflecting the energy demands of hatching, the mean MR of older pipped eggs (0-1 d prehatch, 0.113±0.008 W, N=7) was a full 36% higher than the estimated pre-IP MR of this species.

Following hatching, the weight-specific RMR of coots tested at thermoneutral air temperatures increased directly with body mass, until birds weighed approximately 30-35 g (Fig. 1-6). Beyond this size, which also corresponded to the stage when homeothermy first appeared, thermoneutral RMR varied inversely with body mass. In order to directly compare thermogenic responses of the different age groups, it was necessary to correct MR measurements for intraspecific size variation. This was accomplished

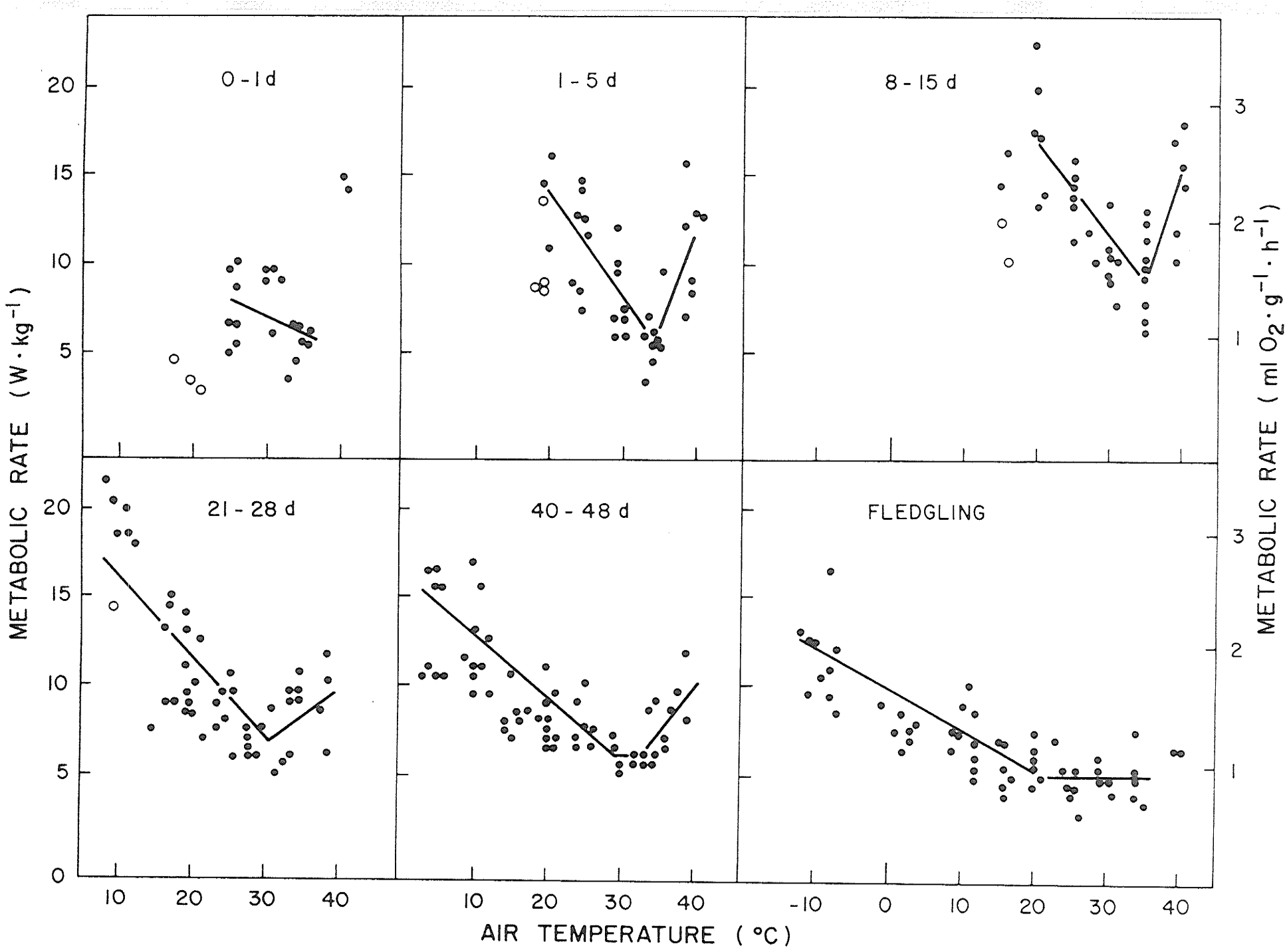
Figure 1-6. Resting metabolic rates of juvenile coots at thermoneutral air temperatures (upper panel) and in 35°C water (lower panel) in relation to body mass. Six age classes are represented: 0-1 d (closed circles), 1-5 d (open circles), 8-15 d (closed triangles), 21-28 d (open triangles), 40-48 d (closed squares) and 60+ d posthatch (open squares). Regression lines (air) were fitted by the method of least squares (see text). The exponential curve (water) was fitted by a nonlinear regression procedure and included data for fledgling birds in 30°C water.


AIR
y = -0.012 x + 9.629
r2 = 0.559
y = 0.325 x - 0.341
r2 = 0.345
WATER
y = 14.654 - 0.012 x - 0.00004 x2
r2 = 0.618
METABOLIC RATE (W · kg-1)
METABOLIC RATE (ml O2 · g-1 · h-1)
MASS (g)
12.5
10.0
7.5
5.0
2.5
15.0
10.0
5.0
2.0
1.5
1.0
0.5
3.0
2.0
1.0
0
100
200
300
400

by dividing RMR (mL $O_2 \cdot h^{-1}$) by body weight$^{0.67}$ (Heusner 1982; Bucher 1986). An index of relative heat production (RHP) was then derived, by dividing the weight-corrected RMR of juvenile coots by the mean weight-corrected thermoneutral RMR of fledgling birds (6.18 mL $O_2 \cdot g^{-0.67} \cdot h^{-1}$). As recommended by Bucher (1986), calculations of pre-IP RHP were based on the mean mass of newly-hatched chicks (19.3 g). It should be noted that this index differs slightly from earlier RHP indices, in which natal MR was divided by weight-predicted estimates of MR for similar-sized adults (Koskimies and Lahti 1964; Epply 1984). In the present study, RHP was 0.34 at the pre-IP stage, and averaged 0.41 ± 0.03 (N=7) at 0-1 d posthatch. This index increased logistically with age (Fig. 1-3B), attaining 90% of its asymptotic value (1.2) by 2 weeks posthatch. Reflecting the trend noted for RCAG and RMR, a sharp inflection in the relation between RHP and body mass occurred at 30-35 g (Fig. 1-4B).

Though hatchling coots <5 days old were unable to regulate Tcl (see above), they did respond metabolically to cold stress (Fig. 1-7). For 0-15 d following hatching, RMR rose at air temperatures above and below 35°C (Fig. 1-7). Lower critical temperatures (LCT) of 1-5, 8-15 and 21-28 d cohorts were calculated from the intersection of least squares regressions relating RMR to Ta. In these groups, linear regressions were generated for Ta's <30°C, and for

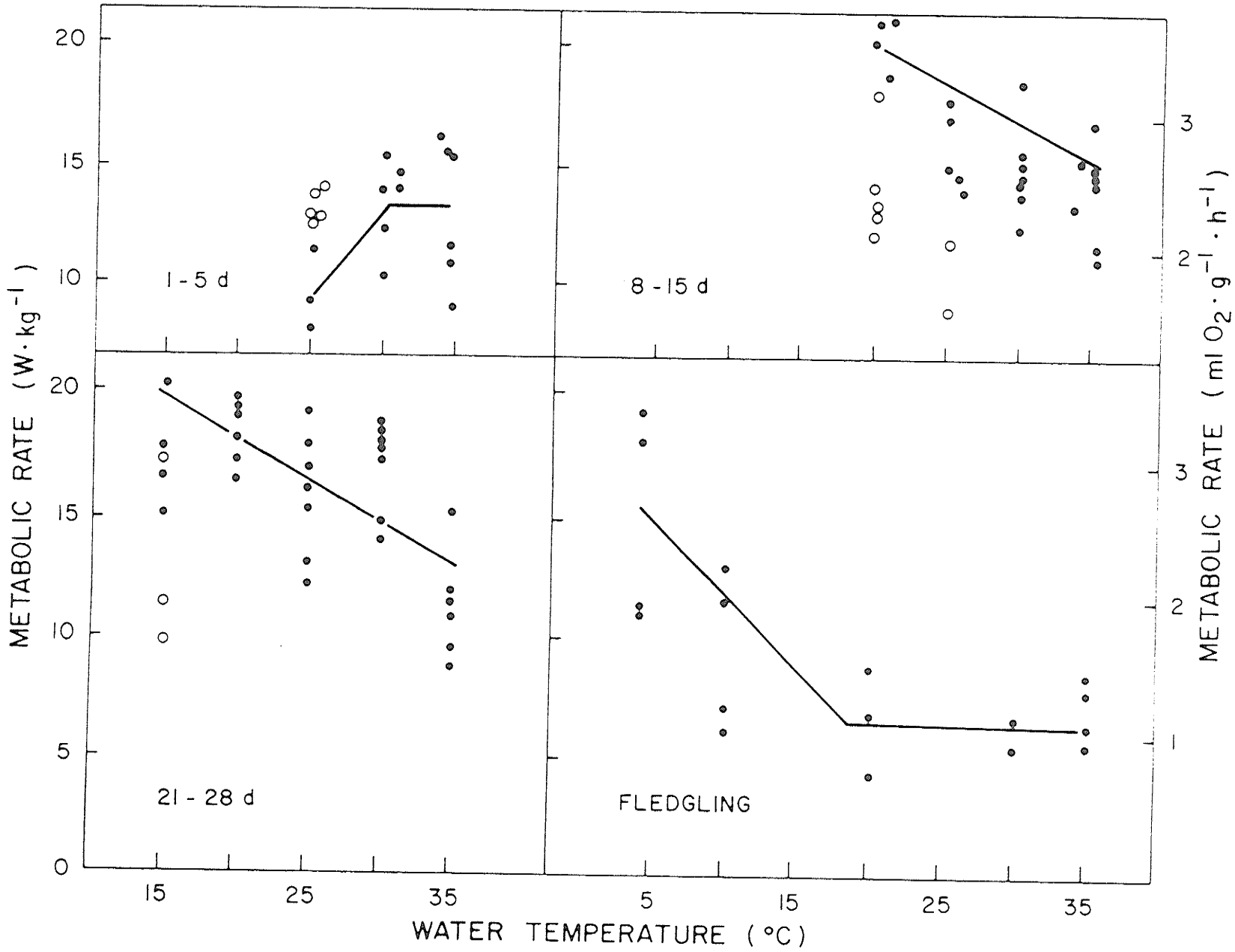
Figure 1-7. Resting metabolic rates (RMR) of juvenile coots at different air temperatures. Regression lines were fitted by the method of least squares, yielding the equations: $y=12.54-0.18x$ (0-1 d); $y=25.56-0.57x$ and $y= -30.98+1.06x$ (1-5 d); $y=26.82-0.52x$ and $y= -31.65+1.17x$ (8-15 d); $y=20.77-0.44x$ and $y= -1.58+0.29x$ (21-28 d); $y=14.50-0.30x$ and $y= -10.71+0.51x$ (40-48 d); $y=10.00-0.23x$ (fledgling). All regressions, except that for 0-1 d birds, were significant ($r^2=0.42-0.67$, $P<0.001$), Horizontal lines denote mean thermoneutral RMR; open circles indicate values recorded from hypothermic birds (see text).


0 - 1 d
1 - 5 d
8 - 15 d
21 - 28 d
40 - 48 d
FLEDGLING
METABOLIC RATE (W · kg⁻¹)
METABOLIC RATE (ml O2 · g⁻¹ · h⁻¹)
AIR TEMPERATURE (°C)
0
5
10
15
20
1
2
3
-10
0
10
20
30
40

Ta's between 35-40°C (Fig. 1-7). For coots >40 d of age, regression analyses excluded data within 3-5°C of obvious inflection points, and in these cases, limits of the TNZ were estimated from the points where minimum RMR intersected each linear regression (Fig. 1-7). Data from hypothermic animals (Tcl <30°C) were excluded from all regression analyses. The TNZ in air was extremely narrow for the first month of development, and even by 40-48 d, encompassed a Ta range of only 4°C (Fig. 1-7, Table 1-1). Estimated LCT of coots in air declined from 35°C at 1-5 d, to 21°C at 60+ d. There was no suggestion of an aquatic TNZ until 60+ d, when the LCT was remarkably similar for fledglings in air and water (Figs. 1-7 and 1-8). Immersion of 1-5 d coots induced maximal metabolic effort, even at Tw=35°C, while RMR of 8-28 d birds rose steadily at all Tw's <35°C (Fig. 1-8). The RMR of coots tested in 35°C water declined exponentially with increasing body mass (Fig. 1-6).

In air, the maximum RMR of cold-exposed, 0-1 d hatchlings averaged only 1.4 times thermoneutral RMR. This thermogenic capacity improved greatly by 1-5 d (Table 1-1), as revealed by increased maximum RMR and improved tolerance to low air temperatures. Yet despite the obvious increase in thermogenic capacity, 1-5 d birds displayed limited homeothermic abilities--both in air and water (Fig. 1-2). Beyond 1-5 d of age, maximum RMR of coots varied from 1.8 to

Figure 1-8. Resting metabolic rates (RMR) of juvenile coots at different water temperatures. Regression lines were fitted by the method of least squares, yielding the equations: $y=25.60-0.31x$ (8-15 d), $y=24.65-0.34x$ (21-28 d), and $y=17.27-0.60x$ (fledgling). Regressions were significant in all cases ($r^2=0.45-0.57$, $P<0.05$). Open circles denote values recorded from hypothermic birds. Mean RMR's of 1-5 d birds are joined by solid lines; mean thermoneutral RMR of fledglings is indicated by horizontal line.


1 - 5 d
8 - 15 d
21 - 28 d
FLEDGLING
METABOLIC RATE (W·kg⁻¹)
METABOLIC RATE (ml O2·g⁻¹·h⁻¹)
WATER TEMPERATURE (°C)
0
5
10
15
20
1
2
3
5
15
25
35

2.9 times thermoneutral RMR (Table 1-1). RMR in 35°C water generally exceeded that recorded during exposure to thermoneutral air temperatures (t-test, $P<0.05$). However, thermoneutral RMR of fledgling coots was similar in air and water ($P>0.05$).

Developmental changes in thermal conductance

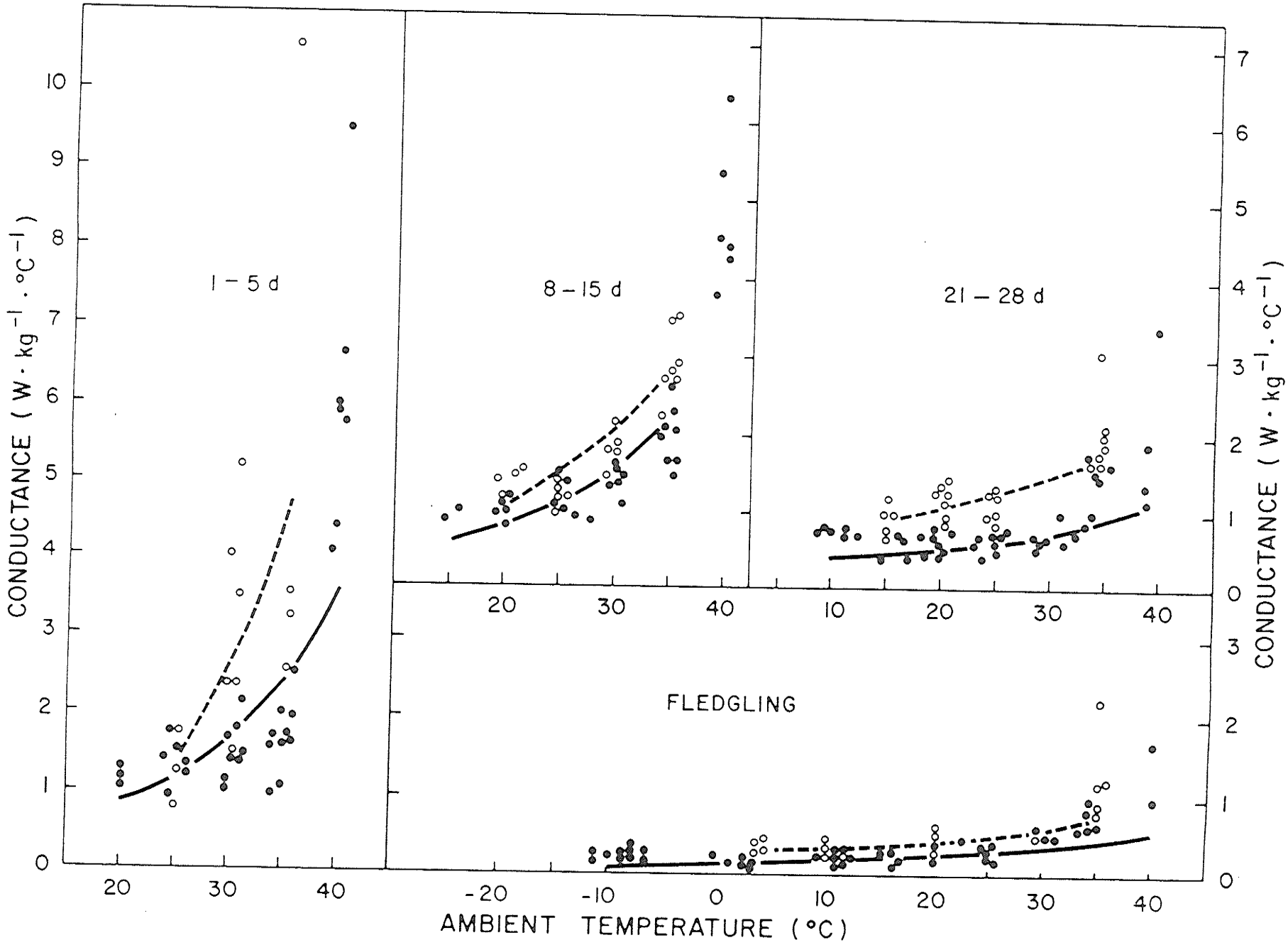
For each cohort, whole-body thermal conductance (C) in air and water was estimated from the equation:

$$[4] \qquad C = \frac{RMR}{Tc - \text{ambient temperature}}$$

(McNab 1980). Since in most cases C declined to minimal, and relatively stable values below an ambient temperature of 30°C (Fig. 1-9), C estimates below this temperature provided a reasonable approximation of minimum C (Cmin). Data from hypothermic birds were excluded from all calculations of Cmin.

In air, Cmin declined from 1.26 $W \cdot kg^{-1} \cdot °C^{-1}$ in 0-5 d birds, to 0.23 $W \cdot kg^{-1} \cdot °C^{-1}$ in fledglings (Table 1-1). The inverse relationship between Cmin and body mass is described by the exponential equation: Cmin=2.291-0.751(log WT) ($r^2=0.85$, $P<0.001$). Immersion induced a substantial rise in

Figure 1-9. Whole-body conductance of juvenile coots at different air (closed circles) and water (open circles) temperatures. Regression curves were fitted to semi-log transformed data by the method of least squares, and were significant in all cases (r^2=0.21-0.75, $P<0.05$).


1 – 5 d
8 – 15 d
21 – 28 d
FLEDGLING
CONDUCTANCE (W · kg⁻¹ · °C⁻¹)
CONDUCTANCE (W · kg⁻¹ · °C⁻¹)
AMBIENT TEMPERATURE (°C)
0
1
2
3
4
5
6
7
8
9
10
20
30
40
-20
-10
0
10

C in all age groups, though each cohort displayed an ability to modulate C in water (Fig. 1-9). Aquatic Cmin of 1-5 d coots was 2.5 times higher than Cmin in air. With exception of 21-28 d coots, immersion caused Cmin to rise by 1.3-1.6 times in the older age groups (Tables 1-1 and 1-2). The Cmin of 21-28 d coots was increased 2.2-fold during exposure to cold water. As expected, totally-submerged carcasses lost heat at a much higher rate than live birds floating quietly in water (Table 1-2). C of submerged carcasses averaged 3.7 times the aquatic Cmin of live birds, with the greatest difference (5.3-fold) documented in fledgling birds.

At no age did F. americana conform to the linearized model of heat transfer established for endotherms (McNab 1980). According to this model, the regression of RMR on sub-thermoneutral ambient temperature should extrapolate to body temperature when RMR=0. Departure from this model occurs frequently in avian studies, when regressions relating RMR and Ta often fail to intercept the X-axis at Tcl (Calder and King 1973; Stahel and Nicol 1982). Under these conditions, regression slopes may not reflect Cmin (Calder and King 1973; McNab 1980). Hence it is not surprising that C-slopes for coots (Fig. 1-7) underestimated Cmin (Table 1-1) by 27-52 percent. It should be noted that C-slopes were calculated for homeothermic birds only.

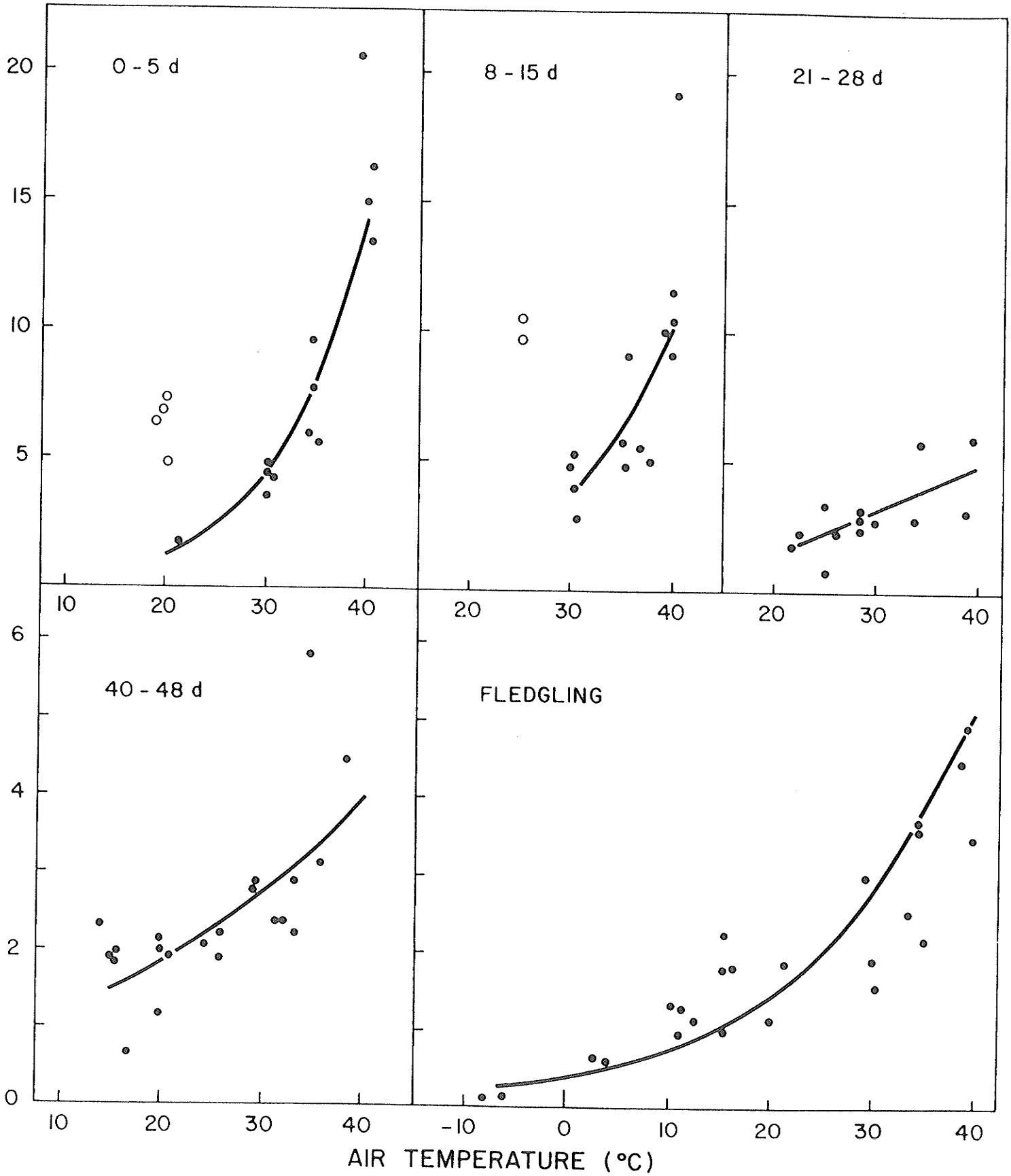
Onset of shivering

As newly-hatched coots were exposed to progressively lower Ta's, shivering was usually initiated at a Ta near 29°C (range 27.8-29.5°C) and appeared as continuous EMG activity. Hence in neonates, the onset of shivering seemed to occur at a Ta approximately 6°C below the LCT. At 60+ d, shivering was initiated as discrete EMG bursts in mild cold (18-21°C), and appeared as continuous EMG activity at all cooler temperatures. Thus onset of shivering coincided with the LCT in fledglings (Table 1-1). Attempts to integrate, and thereby quantify the EMG signals were unsuccessful.

Developmental changes in evaporative heat loss ability

Hatchlings responded to heat stress with an impressive increase in EHL, accompanied by mild hyperthermia (Tcl=40-41°C). There was little evidence of thermal discomfort in heat-stressed chicks--even at Ta's of 35-40°C. Maxmimum EHL approached 20 $W \cdot kg^{-1}$ at 0-5 d, compared to only 5 $W \cdot kg^{-1}$ at 60+ d (Fig. 1-10). Predictably, fledgling coots were most susceptible to heat stress, and in two cases, brief (60 min) exposure to 40°C air proved fatal to these older birds. A significant decline ($P<0.05$) in mean maxmimum EHL was first detected between 8-15 d and 21-28 d posthatch. It was also informative to examine the EHL capacity (EHLC), in which EHL is presented as a fraction of RMR (Brent et al. 1985). At Ta's >35°C, the EHLC of 0-5 d

Figure 1-10. Relation of evaporative heat loss to air temperature in juvenile coots. Regressions were fitted by the method of least squares, and were significant in all cases (r^2=0.47-0.91, $P<0.01$). Open circles denote values recorded from hypothermic birds (see text).


0 - 5 d
8 - 15 d
21 - 28 d
40 - 48 d
FLEDGLING
AIR TEMPERATURE (°C)

and 8-15 d coots often exceeded 1.0, implying that evaporative cooling plays a major role in heat dissipation at high ambient temperatures. Beyond 21-28 d, maximum EHLC of F. americana never exceeded unity (Fig. 1-11).

Plumage development

Based on size and appearance, natal down was classified into three categories (Table 1-3). The smallest feathers at each site were assigned to class C, while larger down was placed in either class A or B. The barbs of class A feathers were tightly intertwined and held in this position by orange or yellow plumes. Some of the feathers in class B had white plumes, but the barbs of these feathers were completely independent of one another. Except for the colored plumes, neossoptiles ranged from dark grey to black, and all had a minute rachis. If the plume of a class A neossoptile was removed, the barbs were easily separated to reveal a typical downy feather. In 0-5 d birds, all three neossoptile classes were indentified in the pectoral and dorsal plumages. Class A feathers were infrequent at the ventral site, though B and C feathers were abundant here. By 7-13 d, all neossoptile classes were confined to the dorsal plumage (Table 1-3). It is noteworthy that the apparent rarity of class C down in older chicks may reflect enhanced feather growth in these birds. ANOVA ($P<0.01$) revealed that dorsal class C down lengthened between

Figure 1-11. Relation of evaporative heat loss capacity to air temperature in juvenile coots. Regressions were fitted by the method of least squares, and were significant in all cases (r^2=0.33-0.82, $P<0.05$).

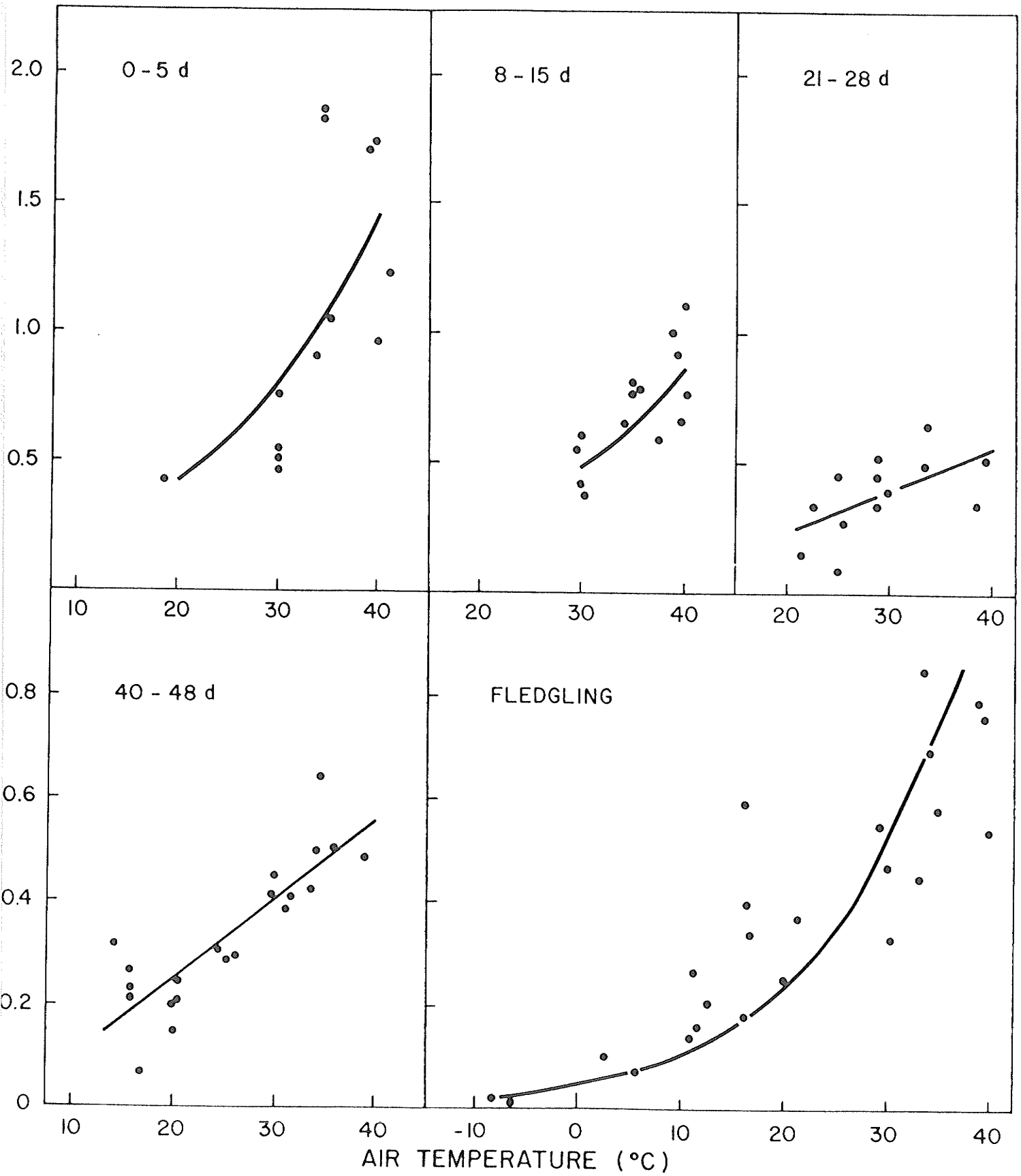

0 - 5 d
8 - 15 d
21 - 28 d
40 - 48 d
FLEDGLING
2.0
1.5
1.0
0.5
0.8
0.6
0.4
0.2
0
10
20
30
40
-10
0
AIR TEMPERATURE (°C)

Table 1-3. Mean length of natal down in the lateral, ventral and dorsal plumages of juvenile coots.

Site	Age group (d)	Natal Down (cm)		
		Class A	Class B	Class C
LATERAL	0-5	1.5±0.1 (6)	1.3±0.1 (6)	0.3±0.0 (4)
		1.2-1.6	1.0-1.6	0.2-0.4
	7-13	1.6±0.1 (2)	1.3±0.3 (2)	-
		1.5-1.7	1.0-1.6	
VENTRAL	0-5	1.0	1.1±0.0 (6)	0.4±0.1 (3)
			0.9-1.2	0.2-0.6
	7-13	-	1.2±0.2 (2)	0.3
			1.0-1.3	
DORSAL	0-5	1.4±0.0 (6)	0.7±0.1 (6)	0.3±0.0 (6)
		1.2-1.5	0.6-0.9	0.2-0.3
	7-13	1.5±0.1 (2)	0.9±0.2 (2)	0.5±0.1 (2)
		1.4-1.5	0.7-1.1	0.4-0.5

Note: Values are presented as means ±1 SE, range; numbers in parentheses denote sample sizes, where each value is itself a mean of 10 measurements.

0-5 d and 7-13 d. It is feasible, therefore, that class C feathers at ventral and lateral sites may have grown, transforming into class B feathers. Class B feathers were always significantly longer at lateral and ventral sites (paired t-tests, $P<0.05$)--an observation corroborated by measurements of plumage depth (Table 1-4).

Unfeathered regions (apteria) were most obvious on cranial and spinal regions of newly-hatched coots. Even in the wingpit, neossoptiles appeared to cover most of the available area. By 28 d of age, the cranial apterium was completely feathered, and body feather tracts were clearly delineated (Stettenheim 1972). Plumage depth increased and follicle density declined with age at pectoral, ventral and dorsal sites (ANOVA, $P<0.10$). Paired t-tests ($P<0.05$) revealed that the density and depth of natal down was greatest in ventral and lateral regions (Table 1-4). Juvenal plumage, which first appeared in coots at 1 month of age, was more uniform than natal down in length and density (Table 1-4). It is noteworthy that during the period in which coots maintained a natal plumage (from 0 to 15 d posthatch), Cmin declined by only 13% (Table 1-1). By comparison, the transition to juvenal plumage was coupled to a 45% reduction in Cmin (Table 1-1). Juvenal down was extremely rare in 28 d-old coots, but after 74 d, a full juvenal plumage was evident at all trunk locations. Though juvenal feathers tended to lengthen over time (Table 1-5), these changes were not significant (ANOVA, $P>0.05$).

Table 1-4. Depth and density of lateral, ventral and dorsal plumages in juvenile coots.

Site	Age group (d)	Plumage Depth (cm)	Follicle Density (per cm^2)
LATERAL	0-5	0.8±0.0 (6)	120±8 (6)
		0.7-0.9	100-150
	7-13	1.1±0.2 (2)	-
		0.9-1.2	
	28	1.5±0.1 (2)	47±6 (2)
		1.4-1.6	41-52
	74+	1.4±0.1 (3)	45±10 (3)
		1.3-1.5	30-64
VENTRAL	0-5	0.7±0.0 (6)	95±3 (5)
		0.6-0.8	82-101
	7-13	0.9±0.1 (2)	95±3 (3)
		0.8-0.9	89-99
	28	0.8±0.2 (2)	38±8 (2)
		0.6-0.9	30-40
	74+	1.3±0.9 (3)	46±3 (3)
		1.2-1.5	40-50
DORSAL	0-5	0.4±0.1 (5)	72±4 (5)
		0.3-0.6	59-84
	7-13	0.6±0.1 (2)	-
		0.5-0.6	
	28	0.7±0.0 (2)	30±1 (2)
		0.7	29-30
	74+	0.8±0.3 (3)	36±10 (3)
		0.3-1.4	24-57

Note: Values are presented as means ±1 SE, range; numbers in parentheses denote sample sizes, where each value is itself a mean of 5 measurements.

Table 1-5. Mean length of juvenal feathers in the lateral, ventral and dorsal plumages of immature coots.

Site	Age group (d)	Juvenal Feathers (cm)		
		Down	Semiplume	Contour
LATERAL	28	0.3	1.0±0.2 (2)	3.4±0.2 (2)
			0.8-1.1	3.2-3.6
	74+	0.4±0.1 (3)	1.7±0.4 (3)	4.5±0.4 (3)
		0.3-0.5	1.2-2.4	3.9-4.5
VENTRAL	28	0.2	1.2±0.2 (2)	2.3±0.6 (2)
			1.0-1.4	1.7-2.9
	74+	0.5±0.2 (3)	1.4±0.0 (2)	3.6±0.7 (3)
		0.2-0.8	1.3-1.4	2.2-4.5
DORSAL	28	-	0.6	3.4±0.8 (2)
				2.6-4.1
	74+	0.5±0.1 (3)	1.5±0.2 (2)	3.9±0.6 (3)
		0.4-0.7	1.3-1.7	2.9-4.9

Note: Values are presented as means ±1 SE, range; numbers in parentheses denote sample sizes, where each value is itself a mean of 10 measurements.

Discussion

Ontogeny of thermoregulation

Newly-hatched birds range from altricial forms that are blind, helpless and naked, to precocial chicks that are covered in down and independent at an early age (Nice 1962; Ricklefs 1979). Neonate coots are decidedly precocial in terms of behavior and physical appearance. These chicks hatch with opened eyes and well-developed plumage, and they begin to forage as soon as they are dry (Gullion 1954). Certain physiological traits of F. americana also imply a precocial pattern of development. Embryonic RHP of this species (0.34) was above that of most altricial birds (0.17-0.29), but well within the range of 0.20-0.50 reported for precocial and semi-precocial avian embryos (Bucher 1986). Hatchling RHP (0.41) also exceeded values reported for most altricial chicks (0.19-0.33), but fell within the range (0.38-0.99) characteristic of precocial and semi-precocial development (Bucher 1986). Precociality was also suggested by the late-incubation plateau in prenatal MR (Fig. 1-5), which is thought to reflect the prenatal establishment of functional organ systems (Bucher 1983; Vleck et al. 1979). As well, the posthatch growth rate constant of young coots ($0.131 \cdot d^{-1}$) was slightly below that predicted for birds of a similar asymptotic mass--a trend usually observed in precocial species (Ricklefs 1979).

These traits notwithstanding, F. americana appears to deviate from the precocial model on several counts. For example, proportions of water and lipid in freshly-laid coot eggs reflect values typical of semiprecocial species, and newly-hatched coots are relatively lean compared to most precocial chicks (Alisaukas 1986). Also, the 'early' appearance of homeothermic temperature control is normally included as a criterion of precociality (Ricklefs 1979). Yet results of this study suggest that coot chicks are imprecise thermoregulators for at least 8 d following hatching (Fig. 1-2). Though 0-5 d birds were capable of augmenting thermogenesis in the cold, Tcl was strongly dependent on ambient temperature at this age (Fig. 1-2)--an observation corroborated by low RCAG values (Fig. 1-3). In contrast, most ducklings are accomplished homeotherms within a day of hatching, especially those of diving Anatids (Koskimies and Lahti 1964). Several petrel species acquire homeothermic Tcl control as rapidly as ducklings, and may be left unattended in cold air within a few days of hatching (Ricklefs and Roby 1983). Newly-hatched ring-billed and laughing gulls (28-35 g) also appear to be more competent homeotherms than 0-5 d coots during exposure to cold air (Dawson et al. 1972,1976).

The poor thermoregulatory performance of coot chicks may in part reflect inadequate thermogenesis. Though RHP rose from 0.34 at the pre-IP stage to 0.41 at 0-1 d posthatch,

this increase can be largely attributed to the normal physiological changes that accompany hatching (Untergasser and Hayward 1972). Other, more precocial species typically exhibit a higher RHP at the hatchling stage. Newly-hatched common teal (Anas crecca), for instance, are competent homeotherms, though they weigh 2.5 g less than 0-1 d coots (Koskimies and Lahti 1964). The superior thermoregulatory ability of hatchling A. crecca is reflected by a relatively high RHP of 0.59 (Bucher 1986). Similarly, chicks of the widgeon (Anas penelope) and the Xantus' murrelet are only 5-7 g heavier than 0-1 d coots at hatching (Koskimies and Lahti 1964; Epply 1984), yet, A. penelope and S. hypoleucus display hatchling RHP's of 0.73 and 0.48, respectively (Bucher 1986).

The limited metabolic abilities of young coots are also revealed by their relatively small thermogenic responses to cold air. Abilities to augment heat production developed rapidly in F. americana, with peak MR rising from 1.4 times minimum RMR at 0-1 d, to 2.4 times at 1-5 d (Table 1-1). Even the latter increment, however, was still inadequate for homeothermy (Fig. 1-2). More competent neonatal homeotherms such as A. crecca, A. penelope and S. hypoleucus display maximal metabolic efforts ranging from 3.3 to 4.7 times minimum RMR, and achieve peak RMR's of 29-45 $W \cdot kg^{-1}$ (Epply 1984). By contrast, maximum RMR of 1-5 d coots was only 13.85 $W \cdot kg^{-1}$. It should be stressed that in the present

study, homeothermy developed at 8-15 d without any corresponding improvement in thermogenic performance. In fact, the capacity to raise RMR in cold actually fell between 1-5 d and 8-15 d of age (Table 1-1).

Results of this study suggest that an increase in body size was pivotal to the establishment of endothermy. Attainment of a body mass of 30-35 g marked abrupt changes in RCAG, RHP and minimum RMR (Figs. 1-4 and 1-6). An obvious advantage of increased size is a reduction in available surface area for heat loss, and thus a greater ability to conserve body heat. Achievement of this weight may also reflect the deposition of subcutaneous fat and, therefore, improved tissue insulation (Alisaukas 1986). Interestingly, young coots tend to lose ventral body plumes between 0-5 d and 8-15 d posthatch (Gullion 1954). Loss of these plumes would probably allow class A neossoptiles to unravel, enhancing plumage insulation. In this context, it is noteworthy that smaller chicks of the closely related European coot (Fulica atra) tend to be more cold-sensitive than larger siblings (Visser 1974). The thermal advantages of a large body size may also contribute to the superior thermoregulatory abilities of ducklings and gull chicks (Koskimies and Lahti 1964; Dawson et al. 1972,1976).

Thermoregulatory performance in water

Evidence of aquatic homeothermy first appeared at 8-15 d posthatch (Fig. 1-2), when exposure of coots to water <30°C raised Cmin by an average of 31%. This was also the earliest stage at which RMR was observed to increase with declining Tw (Fig. 1-8). Prior to this age, immersion in even warm (35°C) water raised Cmin by 151%, and induced maximal RMR (12-13 $W \cdot kg^{-1}$). Exposure of 1-5 d birds to 25°C water caused Tcl to fall rapidly, at a mean rate of $0.5°C \cdot min^{-1}$ (Table 1-2). Though not tested, 0-1 d coots should have cooled even faster in 25°C water, since these hatchlings were unable to raise RMR in cold by more than 40% (Table 1-1). This prediction supports the view that newly-hatched coots 'lack sufficient strength' to remain afloat for more than 2-3 minutes (Gullion 1954). By comparison, 3-d-old chicks of S. hypoleucus display an impressive ability to generate heat in cold, and are able to maintain stable Tcl during 60-min exposure to 10°C water (Epply 1984).

Since thermogenesis in 1-5 d coots was clearly inadequate for sustaining thermal balance in water, heat-conserving strategies must be critical to aquatic endurance in these amphibious chicks. In this context, plumage insulation may be especially important to neonate coots. Ventral plumage in 0-13 d coots was denser than that on the dorsal trunk (Table 1-4), enabling submerged neossoptiles to entrap a

greater volume of insulating air. In all age groups, a large air volume in the plumage combined with a subcutaneous fat layer may have confered a high degree of buoyancy in water. The thermal significance of buoyancy is revealed by the observation that Cmin of live birds in water was only 0.19-0.42 times the C measured in totally-submerged carcasses of a similar size (Table 1-2). Obviously, dependence on plumage as a primary insulation in water requires that feathers be water-repellent. Coots chicks in nature are surprisingly prone to wetting, and parental birds must frequently oil, and thereby waterproof the plumage of their chicks (Fredrickson 1970). The oil-secreting uropygial gland is functional in this species by 28 d posthatch (Gullion 1954).

Conductance estimates imply that coots modulate body heat loss in accordance with prevailing air and water temperatures (Fig. 1-9), with maximum insulation achieved at ambient temperatures <30°C. To adjust C in water, coots may have cooled submerged extremities via countercurrent heat exchangers (see Part II). Hatchlings may also have reduced cutaneous circulation to the ventral trunk, thereby further enhancing tissue insulation. Chicks in the present study tolerated Tcl's as low as 30°C before motor coordination was obviously impaired, and Tcl measurements taken prior to metabolic tests imply the presence of low natal set-points for temperature control. Myhre and Steen (1979) suggest

that tolerance to hypothermia and adoption of a low thermoregulatory set-point are features that reduce heat loss in precocial young, especially during bouts of foraging. Both of these strategies for coping with cold-stress would appear highly adaptive to coot chicks that forage in water.

Temperature regulation in nature

To this point, I have discussed thermoregulatory performance of F. americana in the laboratory setting. These studies have shown that homeothermy is delayed in this species for at least 8 d posthatch (Fig. 1-2), presumably owing to the limited body mass and thermogenic potential of these younger birds. In nature, however, coots <8 d old may employ behavioral strategies that enable more precise regulation of Tcl than was observed in this study. For example, an important source of exogenous heat in nature is that provided by brooding parents. Chicks <7 d old are brooded frequently during the day, and nighttime brooding may continue until hatchlings reach 20 d of age (Gullion 1954). These field observations, together with my laboratory findings, support the view that the time required for chicks to achieve homeothermy is directly related to the length of the brooding period (Ricklefs and Roby 1983). Given that Tcl of hatchling coots appears to be poorly regulated, perhaps foraging chicks seek out brooding platforms once core temperature drops below a critical

threshold value. In support of this hypothesis, Pedersen and Steen (1979) noted that chicks of Willow ptarmigan (Lagopus lagopus) will usually return to the hen for brooding once Tcl has fallen to approximately 34°C.

Hatchling coots also depend, to a large extent, on food provided by their parents (Desrochers and Ankney 1986). In this task, adults rely on nearby food resources, supporting the hypothesis that parents of young that are poor thermoregulators, tend to forage closer to the nest (Ricklefs and Roby 1983). It is relevant to note that assimilation of food will directly increase thermogenesis through specific dynamic action, or SDA (Costa and Kooyman 1984). The potential to derive heat from SDA is relatively high in young coots, since natural diets may include an abundance of protein (Desrochers and Ankney 1986). As a supplementary source of endogenous heat, SDA may reduce the demand for shivering thermogenesis, which is a highly inefficient mode of heat production in water (MacArthur 1988). Conceivably, SDA may even benefit recently-fed coot chicks in their efforts to remain physically close to parents foraging on the water. SDA could provide a positive feedback mode of thermogenesis, since proximal chicks tend to receive the most food from foraging adults (Desrochers and Ankney 1986). Neonates begin to procure their own food within days of hatching, and are largely independent of parental feeding by 3 weeks of age (Gullion 1954). Even at

21-28 d, however, exposure to 15-20°C water was metabolically taxing (Fig. 1-8), and SDA would probably continue to defray thermoregulatory costs in water.

Another, and perhaps more vital source of supplemental heat is that supplied by solar radiation. Radiant heat gain may be most critical during early bouts of foraging, especially since chicks begin to dive for prey by 5 d of age (Gullion 1954). Several features suggest that neonate coots could benefit from solar heating. Under still-air conditions, dark feathers may absorb up to 40% more solar radiation than light plumage (Lustick et al. 1978), and a small body size ensures rapid thermal warming of body tissues. Moreover, sparse plumage on dorsal regions of the trunk and head may allow direct solar heating of skin in these regions--a feature that could accelerate post-dive rewarming.

The question also arises, as to why natal capacity for EHL was so well-developed in this species (Figs. 1-10 and 1-11). These findings are somewhat unexpected, since in nature, amphibious coot chicks can readily enter water to dissipate excess body heat. Perhaps the capacity for EHL in coot chicks is a natural consequence of the gaping response used in food-begging (Gullion 1954; O'Connor 1975b). It is noteworthy that EHLC data of 0-15 d coots compare favorably to data reported for certain gull species (Dawson et al. 1976; Chappell et al. 1984).

In summary, hatchling coots were unable to regulate Tcl for the first week of development, owing to low thermal resistance of body tissues and inadequate thermogenesis. Resistance to body cooling increased as insulation improved, and a mass of 30-35 g appeared to be critical for homeothermic temperature control. A low natal set-point combined with voluntary hypothermia, regional heterothermy, and parental brooding may all be implicated in reducing heat loss of coot chicks in nature. In addition, a high degree of buoyancy may be critical to restricting heat loss to the aquatic medium. Natal pterylosis in this species is conducive to promoting buoyancy in water, and also to maximizing radiant energy gain. Further research is required to examine whether SDA and solar basking are important components of thermoregulation in this species.

PART II

Thermoregulatory performance of fledgling American coots (*Fulica americana*) in air and water.

Introduction

Cold-water immersion presents a formidable challenge to the thermoregulatory abilities of small-bodied birds and mammals. For these animals, problems of heat loss to the aquatic medium may be exacerbated by the limited thermal inertia and insulative constraints imposed by a small body-size. Accordingly, many semiaquatic endotherms demonstrate immersion hypothermia during even brief exposure to low water temperatures (Stahel and Nicol 1982; MacArthur 1984; Williams 1986). Though several studies have examined thermoregulatory performance in aquatic mammals, (see Dawson and Fanning 1981; MacArthur 1986), surprisingly little is known of temperature regulation in adult aquatic birds. Studies of avian swimming energetics (see Butler and Woakes 1984) have provided limited metabolic and body temperature data for birds resting on water. Invariably, however, the water temperatures employed in these studies were relatively mild. Only in penguins (F.Spheniscidae) have cloacal temperatures (Tcl) and resting metabolic rates (RMR) been measured over a broad range of ambient water temperatures (Stahel and Nicol 1982; Barré and Roussel 1986). Given this paucity of avian literature, the present study was undertaken to document the thermoregulatory ability of a common marsh bird, the American coot (Fulica americana, F. Rallidae).

Abundant on inland and coastal waters, coots normally range from Alaska to Panama, though they have also been reported in Cuba and the Bahamas (Murdock 1975). A migratory species, F. americana has been reported at Oak Hammock Marsh, Manitoba (50°06'N,98°20'W) as early as 11 April, and has remained as late as 25 November (Gardner 1981). Coots have also been reported to overwinter at latitudes as high as 50° (Godfrey 1986). It is likely, therefore, that these birds regularly encounter cool (<20°C) water temperatures over much of their range--especially since their primary food resources are aquatic (Alisaukas and Ankney 1985; Desrochers and Ankney 1986). Thus F. americana offers an ideal avian model for studying the effects of cold-water exposure. Despite a wealth of ecological studies (Ryan and Dinsmore 1979; Alisaukas and Ankney 1985; Desrochers and Ankney 1986), little is known of the physiology of this, or any other Rallid (see Brent et al. 1985; Bucher 1986).

The aim of this study was to assess thermoregulatory abilities of F. americana at air and water temperatures which are normally encountered by these birds in nature. Metabolic performance and Tcl dynamics of coots in air and water were compared to those of birds cold-stressed with a Helium-oxygen (Helox) gas mixture. Helox has a thermal conductivity far exceeding that of air, and thus effectively reduces the insulating value of plumage or fur (Wang and

Peter 1975). This gas mixture is commonly used to evoke maximal, or near maximal MR in endotherms exposed to moderate cold (Rosenmann and Morrison 1974; Koteja 1986). Also investigated in this study were the metabolic and thermoregulatory responses of coots to short-term cold acclimation.

Materials and Methods

Animals

A total of 28 hand-reared fledgling coots were used in this study. Eggs were gathered near Winnipeg and Neepawa, Manitoba during June, 1985, 1986. Coots were hatched and raised in the laboratory as described in Part I. Individually-marked hatchlings were reared on a diet of mealworms, chopped earthworms, dogfood, medicated poultry crumbles and turkey starter (see Part I). Except for acclimation studies (see below), all birds were reared at room temperature (23°C) with a 12L:12D photoperiod. Coots were kept in groups of 4-6, and were housed in progressively larger holding pens as the birds matured. Fledglings were held in fibre glass tanks (92 x 97 x 55, or 210 x 60 x 55 cm) that provided standing water to a maximum depth of 20 cm. A hardware cloth screen mounted above water level at one end of the tank provided birds with a dry resting site. Coots reached fledging age by 9 wk, at which point they were maintained on a diet of medicated crumbles, turkey starter, and dogfood provided ad libitum. At time of testing, fledglings varied in age from 9 to 30 wk and had a mean body weight of 386 g (range= 207-548 g, n=175), which was 67% of the mean adult weight reported for wild coots (Alisaukas 1986).

Respirometry

Rates of oxygen consumption ($\dot{V}o_2$) in air and Helox were measured in an open-flow system as described in Part I. For this purpose, fledglings were installed in a darkened, 9.2-L glass desiccator and placed in a constant-temperature cabinet. Ambient air temperature (Ta) was monitored with a thermocouple probe mounted on the inside wall of the metabolic chamber and coupled to a Bailey BAT-12 thermometer. Both incurrent and exhaust air streams were routed through Drierite and soda lime in order to remove water vapor and CO_2, respectively. Incurrent flow rate was maintained at 1.71-1.73 $L \cdot min^{-1}$ using a Fisher Lab-Crest rotameter calibrated according to the bubble flowmeter technique (Levy 1964). For Helox trials, a mixture containing either 19.60 or 20.40% O_2 and balance He (Linde Specialty Gases) was infused into the metabolic chamber. Fractional O_2 content of the exhaust gas was continuously monitored by routing a dry, CO_2-free sample of the gas through an Applied Electrochemistry S-3A oxygen analyzer connected to a chart recorder (Fisher Recordall 5000).

Aquatic trials were performed in a large rectangular tank, also described in Part I. Room Ta was adjusted to within 5-10°C of water temperature (Tw) in order to minimize fluctuations in Tw during recordings. Tw remained within $\pm$0.1°C of the desired level (4-35°C) throughout most runs. The metabolic chamber consisted of a 3.5-L Plexiglas dome

secured to a baseboard situated near water level at one end of the tank (see Part I). Though coots could dive at will in this set-up, they usually floated quietly within the chamber throughout the entire run. Dry, CO_2-free air entered the chamber at a rate of 4.5 $L \cdot min^{-1}$, and the fractional O_2 content of the exhaust gas was continuously monitored by passing a dry, CO_2-free sample of the gas through a Beckman F-3 O_2 analyzer connected to a chart recorder (Fisher Recordall 5000).

To avoid jeopardizing the health and vitality of these animals, birds were not fasted prior to metabolic testing. All runs were conducted between 0800 and 2200 h, and each bird was tested at only one ambient temperature on any given day. A minimum of 72 h elapsed between consecutive runs on the same individual. Trials in air or Helox normally involved a 60-min equilibration period followed by a 30-60 min recording interval. Equilibration time was limited to 45 min for runs at Ta=40°C, in order to reduce thermal stress on the birds. Only 15 min was allocated for equilibration in the aquatic trials owing to the short duration of these runs. However, previous testing of this aquatic set-up at an identical flow rate (MacArthur 1986) revealed that the time required for the gas analysis system to achieve 95% equilibration was only 2.7-3.0 min.

Methods for calculating $\dot{V}o_2$ are outlined in Part I. All $\dot{V}o_2$ data were converted to $W \cdot kg^{-1}$, assuming an RQ of 0.85

and a corresponding conversion factor of 0.0056 $W \cdot h \cdot mL\ O_2^{-1}$ (Brent et al. 1985). Body weight (±0.1 g), deep Tcl, room Ta and barometric pressure were measured at the beginning and end of each run. Techniques for measuring evaporative heat loss (EHL), as well as shivering and carcass conductance (C) are detailed in Part I. Briefly, EHL was determined from gravimetric measurements of water absorption by a Drierite column inserted into the exhaust air stream. Shivering was monitored in the left pectoralis muscle of fledglings via a tripolar electrode system patched to an electromyography (EMG) amplifier on a Lafayette Model 76102 physiograph. Carcass C was calculated from the cooling rates of freshly-killed birds or rewarmed carcasses held in still 23°C water.

Temperature measurements

Tcl was monitored with a fast-response thermocouple (accuracy ±0.1°C) inserted 3 cm into the cloaca and connected to a Bailey BAT-12 thermometer. For regional body temperature determinations, a Sensortek type BT-1 thermocouple probe (accuracy ±0.1° C) was used to measure surface temperatures on the skin and plumage. Skin temperatures were recorded from the ventral and dorsal trunk, and from a point midway along the left tarsus. Regional body temperature measurements were obtained only from birds that had been held for 45 min in 8°C air, or had floated for an equivalent period in 4°C water. All

recordings were made within 60 s of removing the bird from its respective holding pen or tank.

Acclimation trials

For acclimation studies, six coots ranging in age from 140 to 154 d were housed for a period of 30 d (11 November-11 December, 1986) in a controlled-environment room maintained at 8±1°C with a 9L:15D photoperiod. Throughout this period, birds had access to a shallow (8-cm depth) pool of 8±1°C water. Holding pens and diet were similar to those described earlier for fledglings acclimated to 23°C.

Statistical analyses

Means were compared with Student's t-test, or with analysis of variance and Fisher's LSD test for multiple comparisons. Regressions were derived by the method of least squares, and slopes compared with analysis of covariance (Statistical Analysis System 1982, Cary, NC). Means are presented with ±1 SE, and in all comparisons significance was set at P=0.05.

Results

Body temperature

Fledgling coots were capable of precisely regulating Tcl over a broad range of air and water temperatures (Table 2-1, Fig. 2-1.). In both 23°C- and 8°C-acclimated birds, Tcl was independent of Ta over an ambient temperature range extending from -10°C to 33°C. Above a Ta of 33°C, Tcl increased in the 23°C-acclimated group (Fig. 2-1). These birds revealed an impressive tolerance to cold-water immersion; there was no indication whatsoever of deep body cooling following 15-20 minutes in 4°C water (Table 2-1, Fig. 2-1). In an ancillary experiment, three birds maintained normal Tcl throughout a 4-h exposure to 5°C water. Combined exposure to Helox and cold similarly also failed to induce even mild hypothermia in all but one of the 11 birds tested (Fig. 2-1). In that exceptional individual, Tcl fell 4.6°C during a 92-min exposure to Helox at -10°C.

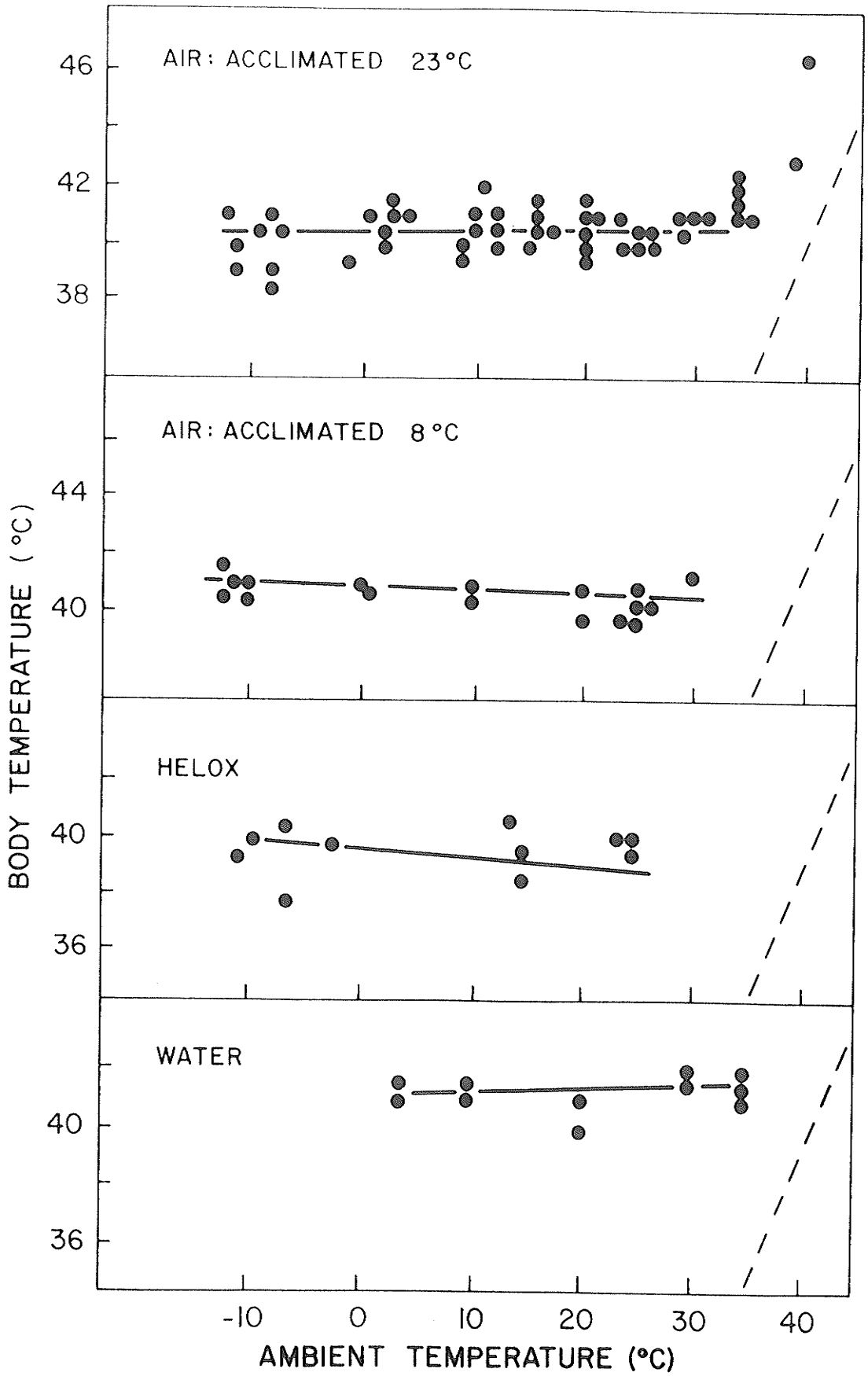
Resting Tcl did not appear to be affected by cold-acclimation (Table 2-1). Though mean initial Tcl was slightly greater for 8°C-acclimated, than of 23°C-acclimated birds (Fisher's LSD test, $P<0.05$), final Tcl recorded at the end of metabolic tests was similar for the two groups. The tendency for initial Tcl values to exceed final measurements probably reflects a higher state of excitement in birds

Table 2-1. Thermoregulatory characteristics of fledgling coots in air, water and Helox.

Group	Thermoneutral RMR ($W \cdot kg^{-1}$)	LCT (^oC)	Cmin ($W \cdot kg^{-1} \cdot {}^oC^{-1}$)	Tcl (^oC)	
				Initial	Final
Air: acclimated 23°C	^{a}5.15±0.27 (19)	21.1	^{a}0.23±0.01 (33)	^{a}41.7±0.7 (46)	^{b}40.5±0.1 (49)
Air: acclimated 8°C	^{a}5.32±0.29 (12)	16.9	^{a}0.21±0.01 (16)	^{b}42.2±0.1 (23)	^{b}40.7±0.1 (23)
Helox-exposed	-	-	^{b}0.36±0.02 (9)	^{a}41.7±0.3 (10)	^{a}40.1±0.2 (10)
Water-immersed	^{a}6.17±0.36 (9)	18.5	^{b}0.36±0.03 (13)	^{a}41.6±0.1 (15)	^{c}41.2±0.1 (16)

Note: Tcl data include measurements recorded before (=initial) and immediately following (=final) metabolic tests. Values are presented as means ±1 SE; numbers in parentheses denote sample sizes. Within each column, means sharing the same letter are not statistically different (Fisher's LSD test, $P>0.05$).

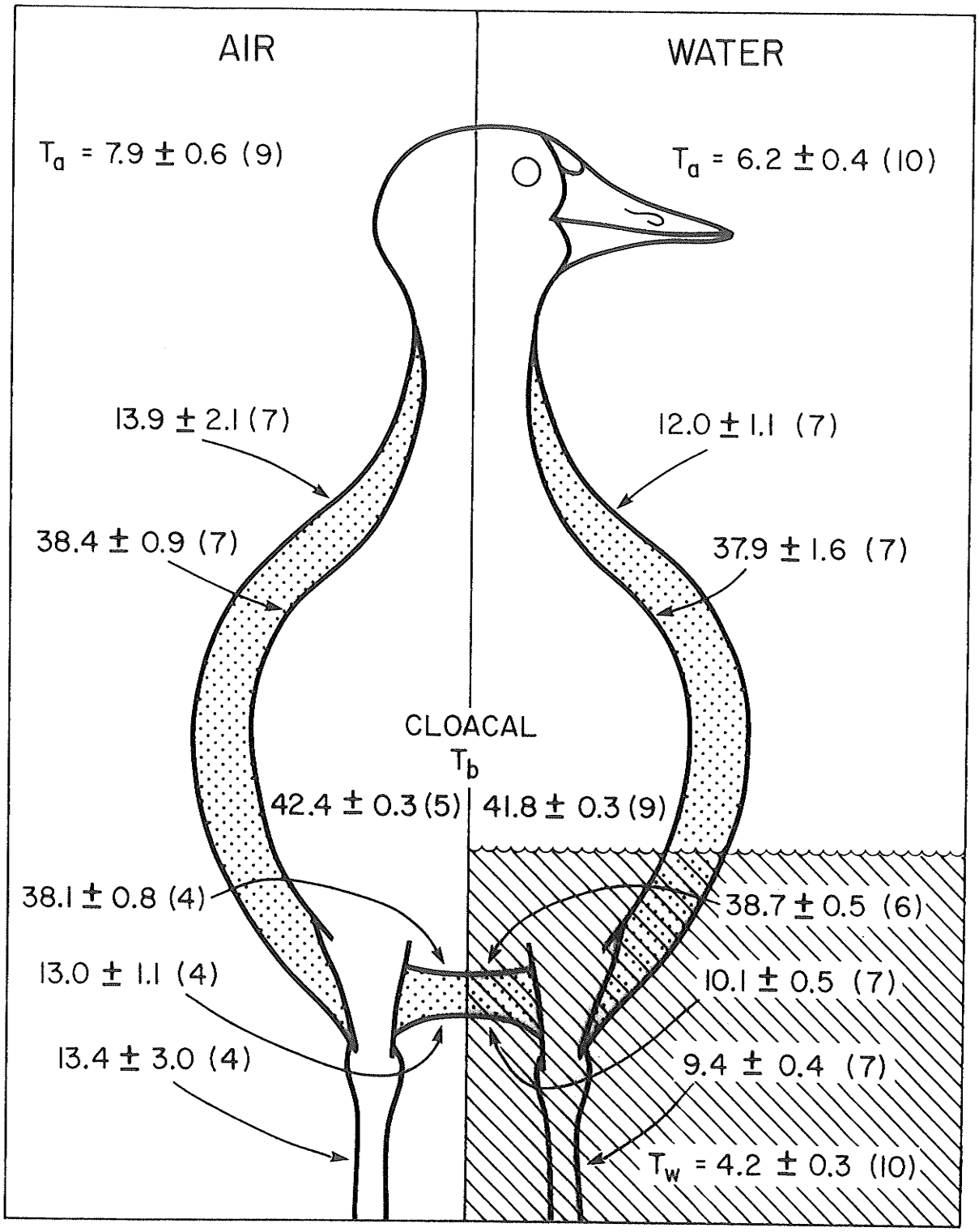
Figure 2-1. Mean cloacal temperature of fledgling coots at different air, water and Helox temperatures. Regression lines were fitted by the method of least squares (excluding air temperatures >33°C). Dashed lines denote equality between cloacal and ambient temperatures.


AIR: ACCLIMATED 23°C
AIR: ACCLIMATED 8°C
HELOX
WATER
46
42
38
44
40
40
36
40
36
BODY TEMPERATURE (°C)
-10
0
10
20
30
40
AMBIENT TEMPERATURE (°C)

at the start of metabolic trials (Drent and Stonehouse 1971). Upon removal from the chamber, coots were generally calm and only minimal handling was required to measure Tcl. Hence final recordings provide the best estimates of resting Tcl in this species. Mean final Tcl of 23°C-acclimated birds (40.5°C) is slightly greater than that (39.6°C) of F. atra (Brent et al. 1985), but is well within the normal range reported for adult birds generally (40.5±1.5°C, Calder and King 1973).

Mean regional body temperatures of coots following 45 min exposure to either 4°C water or to 8°C air are presented in Fig. 2-2. Skin temperature beneath the plumage averaged 3.1-4.3°C below Tcl in both air and water ($P<0.05$). In either medium, a thermal gradient of 24.5-28.6°C persisted between the skin and outer feather surface. Trunk skin temperature did not differ between ventral and dorsal aspects of the bird (paired t-test, $P>0.05$), nor was there evidence of ventral cooling following immersion. Not surprisingly, peripheral cooling was most pronounced in the tarsal region--especially in water (Fig. 2-2). In an ancillary experiment, subcutaneous tarsal temperature of one freely-swimming coot fell to 4.4°C during a 25-min exposure to ice-water. As in the metabolic trials, Tcl was similar ($P>0.05$) for birds in air and water (Fig. 2-2).

Figure 2-2. Regional body temperature profiles of fledgling coots following 45 min exposure to cold air and water, respectively. Values are presented as means ± 1 SE; numbers in parentheses denote sample sizes. Extent of immersion is indicated by cross-hatched area; stippled region denotes plumage.

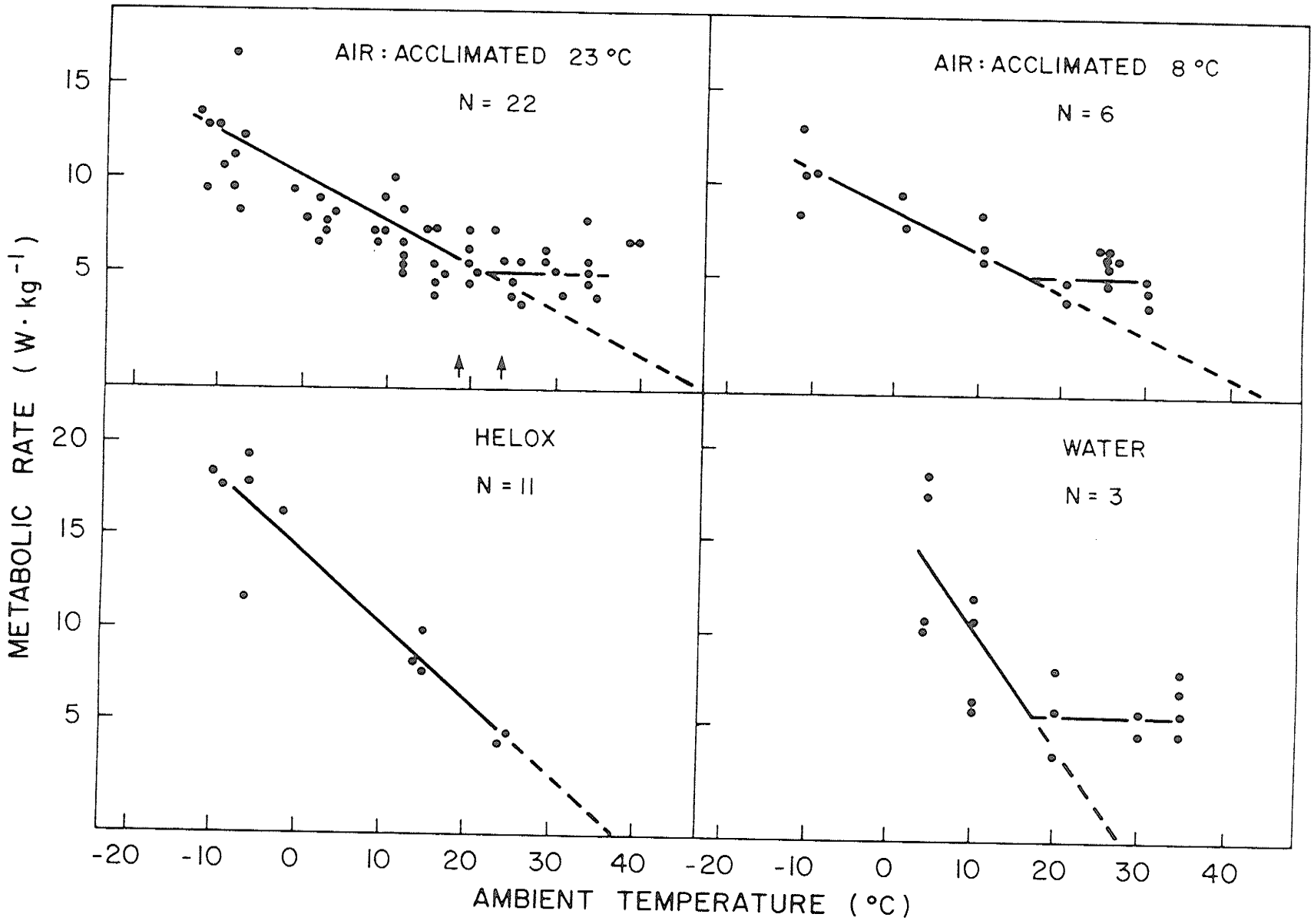

AIR
WATER
T_a = 7.9 ± 0.6 (9)
T_a = 6.2 ± 0.4 (10)
13.9 ± 2.1 (7)
12.0 ± 1.1 (7)
38.4 ± 0.9 (7)
37.9 ± 1.6 (7)
CLOACAL
T_b
42.4 ± 0.3 (5)
41.8 ± 0.3 (9)
38.1 ± 0.8 (4)
38.7 ± 0.5 (6)
13.0 ± 1.1 (4)
10.1 ± 0.5 (7)
13.4 ± 3.0 (4)
9.4 ± 0.4 (7)
T_w = 4.2 ± 0.3 (10)

Resting metabolic rate

The metabolic response of F. americana to varying Ta followed the classic endothermic pattern, with a clearly delineated thermoneutral zone (TNZ), and an essentially linear increase in metabolic heat production below this zone (Fig. 2-3). A remarkably similar metabolic pattern was evident in coots resting quietly in 3-35°C water (Fig. 2-3). In all cases, the lower critical temperature (LCT) was calculated as the point where mean thermoneutral RMR intersected the least squares regression relating RMR to sub-thermoneutral ambient temperature. Only temperatures that were clearly below (-10 to 15°C), or else well within (25-32°C) the TNZ were used to derive LCT. Calculated LCT's in air (16.9-21.1°C) were close to those (14-20°C) reported for F. atra (Gavrilov 1977; Brent et al. 1985), but well below the LCT of 30°C previously estimated for F.americana (Murdock 1975). In the present study, cold-acclimation appeared to lower LCT by 4.2°C (Table 2-1).

Mean thermoneutral RMR of 23°C-acclimated coots (5.15 $W \cdot kg^{-1}$) was 18.4% greater than Murdock's (1975) estimate for this species, but similar to the value reported for F. atra (Gavrilov 1977). I found no difference in minimum RMR at thermoneutrality between any of the groups tested (Table 2-1). Though not statistically significant, it is noteworthy that the mean thermoneutral RMR of 23°C-acclimated birds was 19.8% higher in water than in air

Figure 2-3. Resting metabolic rates (RMR) of fledgling coots at different air, water and Helox temperatures. Regression lines were fitted by the method of least squares, yielding the equations: $y=10.00-0.23x$ (air: acclimated 23°C), $y=8.53-0.19x$ (air: acclimated 8°C), $y=14.26-0.40x$ (Helox) and $y=17.27-0.60x$ (water). Regressions were significant in all cases ($r^2=0.57-.86$, $P<.001$). Horizontal lines denote mean thermoneutral RMR; arrows indicate air temperature range over which shivering first appeared in 23°C-acclimated birds.


AIR : ACCLIMATED 23 °C
N = 22
AIR : ACCLIMATED 8 °C
N = 6
HELOX
N = 11
WATER
N = 3
METABOLIC RATE (W · kg⁻¹)
AMBIENT TEMPERATURE (°C)
15
10
5
20
15
10
5
-20
-10
0
10
20
30
40

(Table 2-1). The mean RMR of coots floating in 4°C water was 15.53 $W \cdot kg^{-1}$, or 3.01 times the mean thermoneutral rate in air. By comparison, the mean RMR of birds exposed to Helox at -10°C was 15.63 $W \cdot kg^{-1}$. Maximum metabolic rate (19.53 $W \cdot kg^{-1}$) was recorded from the single individual that displayed mild hypothermia during Helox exposure. The latter measurement was 3.5 times the mean thermoneutral RMR of this bird, and probably offers the best estimate of maximum heat production in fledgling coots.

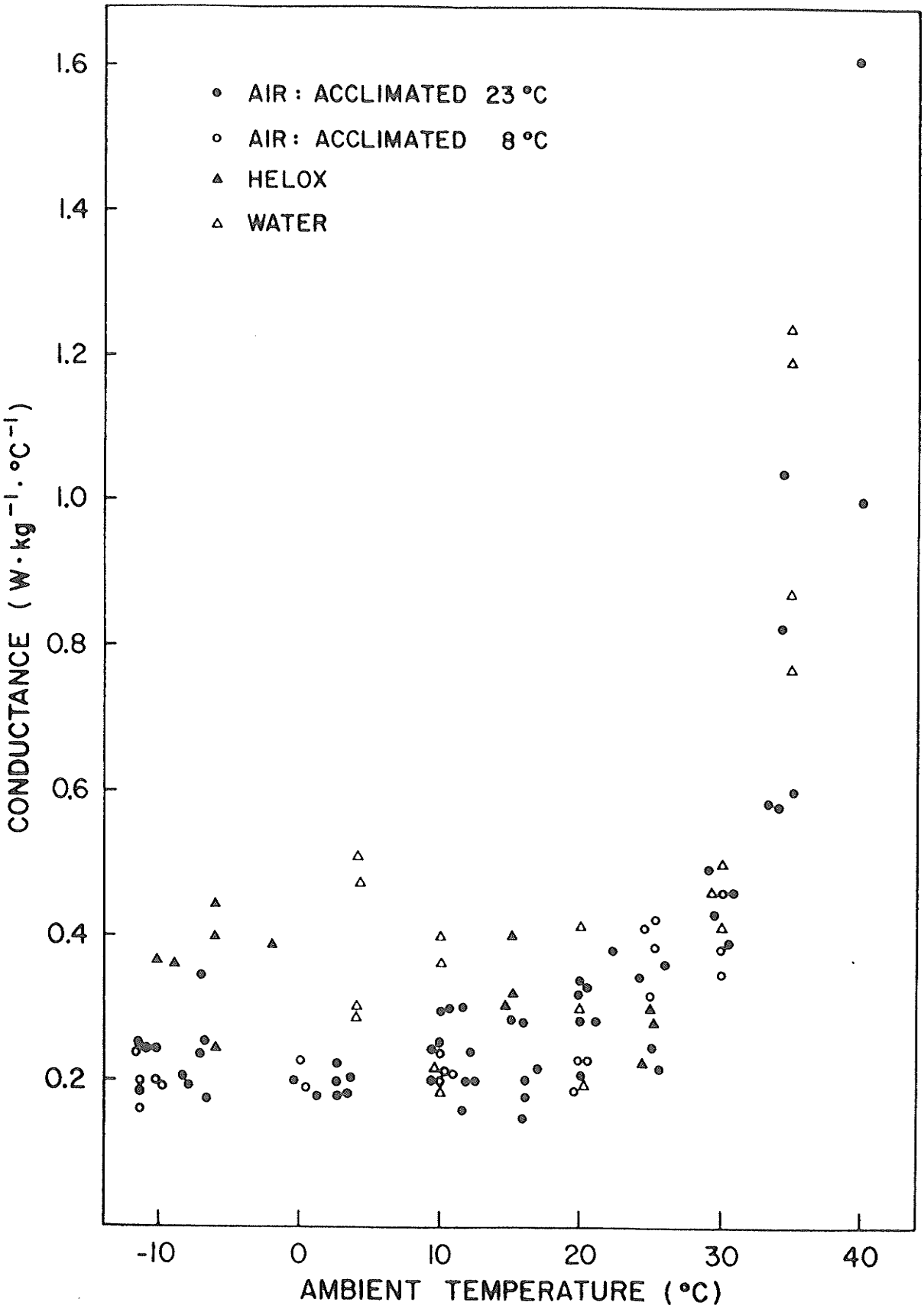
Whole-body conductance

Estimates of whole-body C were derived from the equation:

$$[1] \qquad C = \frac{RMR}{Tcl - \text{ambient temperature}}$$

(McNab 1980). In all treatment groups, C was relatively stable below an ambient temperature of 20°C, but rose in a curvilinear fashion at higher temperatures (Fig. 2-4). Based on Fig. 2-4, minimum C (Cmin) was calculated as the mean of all C measurements derived for ambient temperatures <20°C (Table 2-1). Fisher's LSD test indicated no difference in Cmin between 23°C-acclimated (0.23 $W \cdot kg^{-1} \cdot °C^{-1}$) and 8°C-acclimated (0.21 $W \cdot kg^{-1} \cdot °C^{-1}$) birds. However, the Cmin of coots resting on water (0.36

Figure 2-4. Whole-body conductance of fledgling coots at different air, water and Helox temperatures.


AIR : ACCLIMATED 23 °C
AIR : ACCLIMATED 8 °C
HELOX
WATER
CONDUCTANCE (W · kg⁻¹ · °C⁻¹)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
-10
0
10
20
30
40
AMBIENT TEMPERATURE (°C)

$W \cdot kg^{-1} \cdot °C^{-1}$) was 1.6-1.7 times that recorded for either group in air ($P<0.05$), yet was only 19% of the mean C measured in submerged carcasses (1.89 ± 0.08 $W \cdot kg^{-1} \cdot °C^{-1}$, N=8). Interestingly, the Cmin of Helox-exposed coots was identical to that of live birds resting on water (Table 2-1).

The slope of the regression relating RMR to sub-thermoneutral Ta is commonly used to estimate whole-body C (Calder and King 1973). In the present study, ANCOVA tests for differences in regression slope (Fig. 2-3) support the above trends in Cmin. However, as is often reported in avian studies (Drent and Stonehouse 1971; Calder and King 1973; Stahel and Nicol 1982), none of the regressions relating RMR to sub-thermoneutral temperature extrapolated to resting Tcl in F. americana. Since this represents a departure from the linearized model of heat transfer established for endotherms (McNab 1980), these regression slopes do not necessarily reflect Cmin.

Plumage characteristics

Down feathers ranged in length from 0.2 to 0.8 cm, while semiplumes varied from 1.2 to 2.4 cm. The length of contour feathers was more variable, ranging from 2.2 to 4.9 cm. Overall plumage depth generally varied from 1.2 to 1.5 cm, though one specimen exhibited a relatively thin (0.3 cm) dorsal plumage. Follicle density ranged from 24 to 64

follicles·cm^{-2}, and did not differ between ventral, lateral and dorsal sites (paired t-tests, $P<0.05$).

Shivering

As coots were exposed to progressively lower Ta's, shivering was usually initiated at a Ta near 20°C (range=18.9-23.6°C) Thus, the onset of shivering appeared to coincide with the LCT in fledgling coots (Fig. 2-3). Under mild cold-stress (18 to 21°C), shivering appeared only as discrete EMG bursts, but continuous EMG activity was documented at all cooler temperatures. Attempts to integrate, and thereby quantify the EMG signal were unsuccessful.

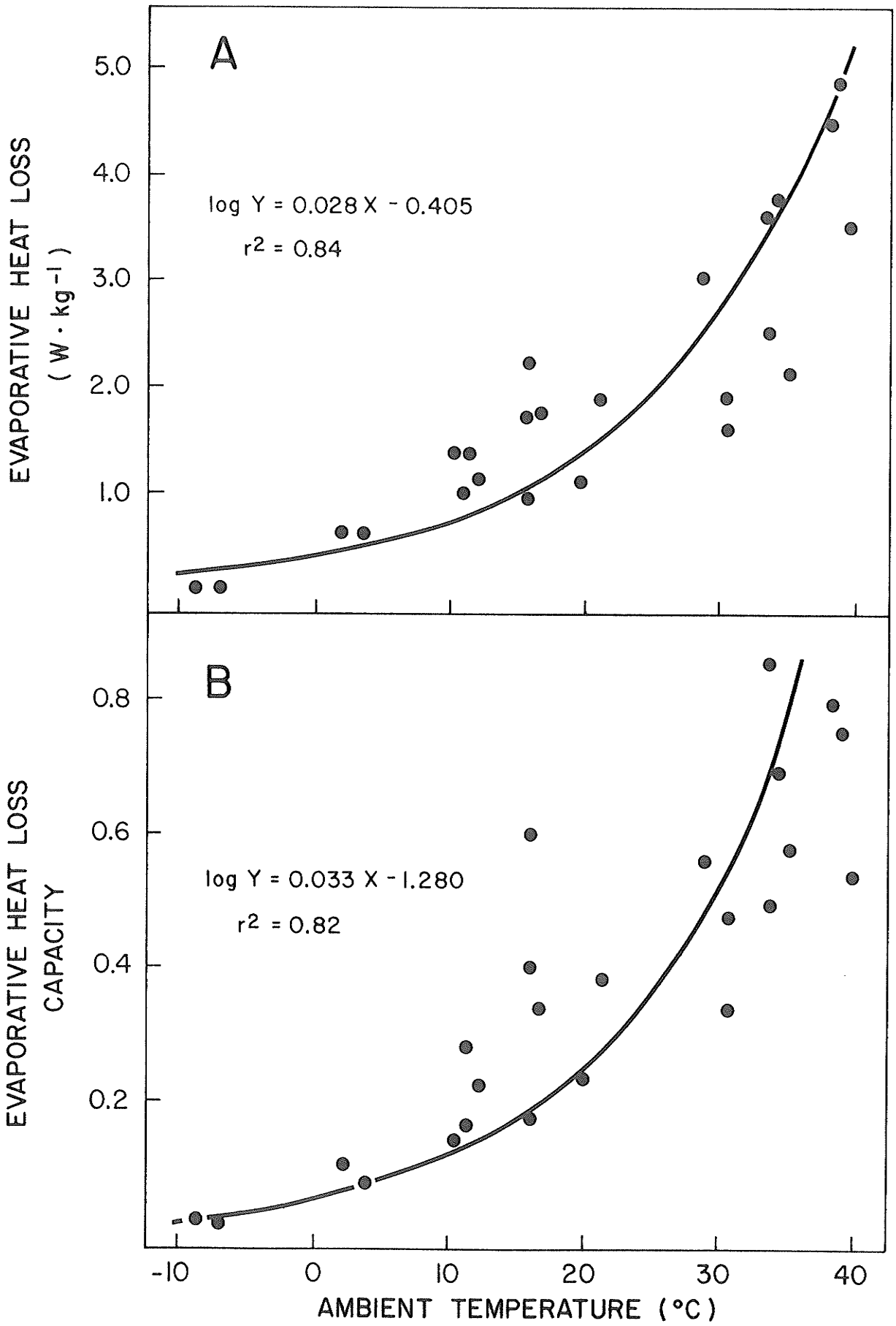
Evaporative heat loss

EHL by coots increased exponentially with Ta (Fig. 2-5A). The proportion of metabolic heat lost via evaporation is given by a dimensionless index termed the evaporative heat loss capacity (EHLC):

$$[2] \qquad EHLC = \frac{EHL}{RMR}$$

(Brent et al. 1985). EHLC ranged from 0.03-0.40 at sub-thermoneutral Ta's, to 0.74-0.85 at Ta's >35°C (Fig. 2-5B). In no case did EHLC exceed 1.00.

Figure 2-5. Relation of evaporative heat loss (A) and evaporative heat loss capacity (B) to ambient air temperature in 23°C-acclimated fledglings. Regression curves were fitted to semi-log transformed data by the method of least squares.


A
log Y = 0.028 X - 0.405
r2 = 0.84
EVAPORATIVE HEAT LOSS
(W · kg-1)
5.0
4.0
3.0
2.0
1.0
B
log Y = 0.033 X - 1.280
r2 = 0.82
EVAPORATIVE HEAT LOSS
CAPACITY
0.8
0.6
0.4
0.2
-10
0
10
20
30
40
AMBIENT TEMPERATURE (°C)

Discussion

Despite a body mass of only 0.4-0.5 kg, fledgling coots were highly competent homeotherms in all media tested (Fig. 2-1). The precision with which these birds regulated Tcl in Helox was surprising, considering that exposure to this gas mixture at subfreezing temperatures usually induces hypothermia in small passerines and rodents (Rosenmann and Morrison 1974; Wang and Peter 1975; Koteja 1986). Equally impressive was the outstanding resistance of fledgling coots to aquatic cooling. Coots floating in 5°C water were capable of precisely regulating Tcl for at least 4 h, and RMR increased only when birds were exposed to water <20°C (Fig. 2-3). To my knowledge, aquatic thermoregulation has been studied in only one other small water bird, the 0.9 kg little penguin, Eudyptula minor. This species cools at a rate of approximatley 0.4-0.5°C·h^{-1} during immersion in 5°C water (Stahel and Nicol 1982). Moreover, heat production in E. minor, as well as most small-bodied aquatic mammals, usually increases steadily with declining Tw below 30°C (Stahel and Nicol 1982; Dawson and Fanning 1981; MacArthur 1984). With exception of the platypus, Ornithorhynchus anatinus (Grant and Dawson 1978), most aquatic mammals under 3 kg cool in water <30°C, and thermoneutral Tw's have been documented only in larger marine forms (Irving 1973; Dawson and Fanning 1981; Gallivan et al. 1983).

The question then remains: why do fledgling coots exhibit such outstanding resistance to cooling--especially in cold water and at subfreezing temperatures in Helox? Any endotherm challenged by cold has two basic options which may, or may not be exercised concurrently: (1) it can implement mechanisms that conserve body heat, or (2) it can augment thermogenesis. The superior ability of this species to resist deep body cooling did not appear to involve an elevated basal rate of heat production. In fact, the mean resting thermoneutral rate of 23°C-acclimated birds (5.15 $W.kg^{-1}$) was slightly less than that (5.70 $W.kg^{-1}$) predicted for a 386 g nonpasserine during the active (α-phase) of its diel activity cycle (Aschoff and Pohl 1970). Results of this study thus confirm the recent view that aquatic endotherms are not endowed with an intrinsically elevated basal MR (Lavigne et al. 1986). It is noteworthy that the similar-sized wood duck (Aix sponsa) and partridge (Perdix perdix) each displays a thermoneutral RMR (Gavrilov 1977) that is within 4% of that obtained for F. americana. The LCT of F. americana in air (16.9-21.1°C) was also close to the LCT (18°C) reported for both A.sponsa and P.perdix (Gavrilov 1977). In the present study, thermoneutral RMR was increased only marginally in 8°C-acclimated birds, though this increase was probably sufficient to account for the slight (4.2°C) reduction in the LCT of these birds (Table 2-1).

Cold tolerance in coots also did not appear to involve an exceptional ability to increase thermogenesis at low ambient temperatures. Based on Helox exposure, maximum RMR in fledgling coots was only 3.5 times the mean thermoneutral rate, which agrees favorably with the 3.8-fold increase reported for F. atra (Brent et al. 1985). These values fall near the lower end of the range of metabolic increments associated with nonpasserine flight (Calder and King 1973), and are similar to the maximum metabolic efforts diplayed by other amphibious endotherms (Stahel and Nicol 1982; MacArthur 1984). Though the thermogenic abilities of F. americana appear unexceptional, the possibility exists that these birds may employ nonshivering thermogenesis in the cold. Occurrence of nonshivering thermogenesis is suggested by the intermittent bursts of EMG activity observed near the LCT (see George 1984), and by the metabolic sensitivity of these birds to ß-adrenergic stimulation (see Part III).

The thermoregulatory prowess of F. americana can be largely attributed to a superior ability to retain body heat in cold. This conclusion is supported by the observation that mean Cmin of 23°C-acclimated coots in air (0.23 $W \cdot kg^{-1} \cdot °C^{-1}$) was only 77% of that predicted for a 386 g nonpasserine during the α-phase of its activity cycle (Ashoff 1981). Based on thermal gradient analyses (Veghte 1964), body tissues accounted for roughly 12% of total insulation in fledgling coots, compared to 88% for plumage.

However, it should be noted that the tissue estimate may be inflated in lab-raised coots, owing to extensive deposition of subcutaneous fat in these birds. Obesity in 23°C-acclimated coots may partially explain why there was no apparent decrease in Cmin following 30 d acclimation to 8°C air (Table 2-1). Cold-acclimation in birds typically improves total insulation, thereby lowering C and LCT (Chaffe and Roberts 1971).

The high insulative capacity of the plumage of F. americana is also revealed by warm high skin temperatures of the ventral trunk--even after 45 min exposure to 5°C water (Fig. 2-2). In contrast, trunk skin temperature of E. minor, as well as many aquatic mammals, tends to decline during cold-water immersion (Stahel and Nicol 1982; Costa and Kooyman 1982; MacArthur 1986). The high ventral skin temperatures of immersed coots may have resulted, at least in part, from shivering and attendant blood flow in the pectoralis muscles of these birds (Calder and King 1973). The thermal significance of plumage insulation in F. americana is also inferred from the observation that peripheral cooling was confined to tarsal areas. Legs of fledgling coots were probably cooled by a vascular countercurrent heat exchanger similar to the rete tibiotarsale described for mallards (Midtgaard 1980).

The effective plumage insulation of fledgling coots was attributed to: (1) a substantial feather depth (1.2-1.5 cm),

(2) a high follicle density over the entire trunk (24-64 per cm^2), and (3) the presence of long contours (2.2-4.9 cm) overlapping layers of down and semiplume feathers. By comparison, Spheniscid plumage is relatively short (mean length=2.8 cm), and may be less effective in entrapping an insulating boundary of air next to the skin (Kooyman et al. 1976; Stahel and Nicol 1982). It is noteworthy that wild coots devote as much as 4% of daily activity to feather maintenance, in which the plumage is coated with oil secreted by uropygial glands (Fredrickson 1970; Murdock 1975).

Resistance to aquatic cooling in F. americana may also be related to an exceptional buoyancy arising from a large air volume entrapped by the ventral plumage. In support of this conjecture, totally-submerged carcasses lost heat 5.3 times faster than live birds floating quietly at the water's surface. A superior plumage insulation coupled with a high buoyancy best explains why Cmin rose, on average, by only 40% during immersion in 4-30°C water (Table 2-1). Most other small aquatic endotherms that have been studied are presumably less buoyant in water--especially since Cmin of these species usually increases more than 2-fold during immersion (Stahel and Nicol 1982; Dawson and Fanning 1981 MacArthur 1984). That buoyancy adjustments may contribute to heat conservation in water is suggested from studies of the sea otter (Enhydra lutris), which appears to modulate

buoyancy in accordance with prevailing Tw (Costa and Kooyman 1982). Whether F. americana is capable of similar buoyancy adjustments remains to be investigated.

Finally, the excellent plumage insulation of fledgling coots may partially explain why birds were highly susceptible to heat stress. EHLC never rose above 1 (Fig. 2-5), and labored panting ensued during exposure to air temperatures >35°C. Similar findings were reported for adult F.atra, which displayed limited panting abilities, and survived prolonged exposure to 40°C air only when the feet and legs were immersed in cool water (Brent et al. 1985). Limited capacities for evaporative cooling are frequently reported in aquatic endotherms (see Irving 1973; Brent et al. 1985), reflecting the ease with which excess heat loads are readily dissipated to surrounding water.

In summary, fledgling coots were capable homeotherms in all media tested, despite an unexceptional capacity to generate metabolic heat. Results from tests in Helox and water suggest that coots are able to withstand extreme cold. At least 88% of fledgling insulation was attributed to a complex, dense plumage which also promoted buoyancy in water. A high degree of buoyancy likely distinguishes F. americana from most small aquatic endotherms that have been previously studied, and may largely account for the exceptional resistance of this Rail to aquatic cooling. These findings should encourage further study of temperature control in aquatic birds.

PART III

Beta-adrenergic sensitivity in juvenile American coots (*Fulica americana*): evidence of nonshivering thermogenesis?

Introduction

The contribution of nonshivering thermogenesis (NST) to avian temperature regulation has generated much controversy in recent years (see Hissa et al. 1975; Barré et al. 1985). Whereas mammalian NST arises mainly in brown adipose tissue (BAT) in response to ß-adrenergic stimulation, this tissue is apparently lacking in most birds (Johnston 1971). However, several avian studies have shown positive thermogenic responses to exogenous catecholamines (Hissa et al. 1975; Barré and Rouanet 1983), and recent investigations have revealed depots of multilocular adipose tissue resembling BAT in black-capped chickadees, Parus atricapillus, and ruffed grouse, Bonasa umbellus (Oliphant 1983), as well as in cold-acclimated muscovy ducklings, Anas barbariae (Barré et al. 1986a).

Selection for avian NST should be especially strong in aquatic species, since shivering in cold water greatly amplifies convective heat loss to the surrounding medium. The need for aquatic NST would appear most critical in precocial aquatic chicks--especially those raised on high-latitude breeding grounds where neonates <50 g may encounter cool water within days of hatching (Nice 1962; Koskimies and Lahti 1964). Yet surprisingly little is known of the capacity for thermoregulatory NST in these amphibious chicks. Recent studies of NST in aquatic birds have focused

instead on month-old muscovy ducklings which may exceed 0.4 kg body weight (Barré et al. 1985,1986a,1987).

The aim of this study was to examine the possible occurrence and ontogenetic development of NST in a small-bodied water bird, the American coot (Fulica americana, O. Gruiformes). Despite body weights of only 19-20 g, neonate coots may enter water as soon as they are dry, and young <5 d old often remain in water for extended periods (Gullion 1954). Moreover, since F. americana breeds as far north as 60° latitude, and is known to overwinter at latitudes as high as 50° (Godfrey 1986), there is a strong probability that both adults and juveniles will encounter cool water (<20-25°C) over much of the species' range. Hence this bird is an excellent model for the study of avian NST. Though the ecology of F. americana has been extensively studied (see Gullion 1954; Alisaukas and Ankney 1985; Desrochers and Ankney 1986), few physiological data are available for this or any other Gruiforme (Brent et al. 1984,1985).

The specific objective of the present study was to investigate ß-adrenergic sensitivity of F. americana during different stages of development. Metabolic rate (MR) and cloacal temperature (Tcl) responses to the ß-agonist, isoproterenol were documented in birds ranging in age from 0-1 d to 60+ d posthatch. To minimize metabolic or Tcl responses to endogenous catecholamines or shivering thermogenesis, these experiments were performed at

thermoneutral temperatures. Responses of fledgling coots (60+ d) acclimated to room temperature were compared with those of cold-acclimated birds of similar age, and an isoproterenol dose-response relationship was established for birds >45 d old. Attempts were also made to abolish the thermogenic response to isoproterenol using the ß-adrenergic blocker, propranolol. As a further test for the possible involvement of ß-adrenergic pathways in the metabolic response to cold, propranolol was administered alone to fledgling coots exposed to mild cold stress. Carcasses of hatchling and fledgling coots were examined by gross dissection for the possible occurrence of BAT.

Materials and Methods

Animals

A total of 48 hand-reared coots was used in this study. Eggs were collected from local marshes during the first week of June, 1985, 1986. Birds were hatched and raised in the laboratory according to the protocol outlined in Part I. Individually-marked coots were reared on a diet of mealworms, earthworms, dogfood, medicated poultry crumbles and turkey starter (see Part I). Except for acclimation studies involving fledgling birds (see below), all coots were held at room temperature (23°C) with a 12L:12D photoperiod. Brood lamps were provided for 30 d following hatching, and chicks had access to standing water throughout development. Larger pens, each supplied with a 20 cm-deep pool of open water were provided once coots exceeded 30 d of age. Five age groups were studied: 0-5, 8-15, 21-28, 40-50 and 60+ d, respectively. Coots in the last age cohort (60+ d) were defined as fledglings. For cold-acclimation studies, six birds between 140 and 154 d of age were held for 30 d at an air temperature (Ta) of 8±1°C with a 9L:15D photoperiod. Further details of the acclimation procedure are given in Part II.

Drug treatments

To test for possible developmental changes in the thermogenic response to ß-adrenergic stimulation,

isoproterenol (± isoproterenol hydrochloride, Sigma Chemical Co.) was administered via intramuscular injection to each age class. Based on previous avian studies (Wekstein and Zolman 1968; Murrish and Guard 1974), an intramuscular dosage of 2 $mg \cdot kg^{-1}$ was initially tested on neonate coots. However, since this dosage failed to induce a thermogenic response in neonates (personal observation), isoproterenol was administered at 4 $mg \cdot kg^{-1}$ in all subsequent ontogenetic trials. A dose-response relationship was established for coots >45 d old, by treating them with isoproterenol dosages of 0.05, 0.5, 1, 2, 4 and 8 $mg \cdot kg^{-1}$, respectively. A dosage of 2 $mg \cdot kg^{-1}$ induced maximal thermogenesis in these tests (see Results); hence this dosage was selected for all trials involving cold-acclimated fledglings. Isoproterenol experiments were conducted at thermoneutral temperatures ranging from 35°C for 0-5 d birds, to 25°C for fledglings (see Part I). The latter Ta was also employed for tests in which fledglings received isoproterenol (2 $mg \cdot kg^{-1}$) in combination with the ß-adrenergic blocker, propranolol (DL-propranolol hydrochloride, 6 $mg \cdot kg^{-1}$). Finally, experiments were performed on fledglings treated with propranolol only (6 $mg \cdot kg^{-1}$), but exposed to a sub-thermoneutral Ta of 10°C. All drugs were dissolved in 0.85% saline, and isoproterenol was stabilized in solution by the addition of 1 $mg \cdot mL^{-1}$ ascorbic acid. In each series of experiments, a control group received an intramuscular injection of 0.85% saline only. Control groups were also run, in which coots >45 d of

age received no injection (NI). All drugs were injected into the left pectoralis muscle in volumes less than 1% body weight (Wekstein and Zolman 1968).

Respirometry

MR was measured with an open-flow system as described in Part I. Briefly, birds were installed in darkened, 2.4-9.2 L glass desiccators and placed in a thermostatically-controlled cabinet. Dry, CO_2-free air was infused into metabolic chambers at a controlled flow rate that was increased from 0.17 to 1.73 $L \cdot min^{-1}$ as the birds grew. Samples of exhaust gas were routed through soda lime and Drierite, and then through an Applied Electrochemistry S-3A oxygen analyzer connected to a strip-chart recorder (Fisher Recordall 5000). Each test on a given bird involved two consecutive metabolic runs, each of 75-90 min duration. The two runs were separated by a 10-min rest period, during which coots were held at room temperature (23°C) in a separate holding pen. Until coots were approximately 30 d of age, two birds were tested concurrently using duplicate metabolic chambers. In this case, the fractional O_2 content of exhaust gas was alternately measured for each of the two birds. In studies involving older coots, only one bird was involved in any given run. In all experiments, drugs or saline were administered at the end of the 10-min rest period, and birds were then immediately returned to the chamber for evaluation of the metabolic response. Animals

were not fasted prior to metabolic testing in order to avoid undue stress on these birds. A minimum of 72 h elapsed between consecutive trials on the same individual, and all experiments were performed between 0800 h and 2200 h.

Following a 10-min equilibration period, mean rates of oxygen consumption ($\dot{V}O_2$) were calculated from strip-chart records using a HIPAD digitizer (Houston Instruments Inc.) and microprocessor to integrate areas beneath the respective O_2 tracings. For ontogenetic trials, mean $\dot{V}O_2$ was calculated for the 12-min interval immediately following the equilibration period. Given the discontinous nature of the $\dot{V}O_2$ recordings for younger birds (see above), longer measurement intervals were not available in this phase of the study. However, in other experiments involving only single runs on birds >45 d of age, mean $\dot{V}O_2$ was determined for the 60-min period immediately following equilibration. $\dot{V}O_2$ was calculated after the method of Depocas and Hart (1957) and corrected to STP. $\dot{V}O_2$ data were converted to $W \cdot kg^{-1}$, assuming an RQ of 0.85 and a conversion factor of 0.0056 $W \cdot h \cdot mL\ O_2^{-1}$ (Brent et al. 1985). Body weight, Tcl, room Ta and barometric pressure were measured before and after each run. Tcl was monitored with a fast-response thermocouple probe (accuracy $\pm$0.1°C) inserted 1-3 cm into the cloaca, and connected to a Bailey BAT-12 thermocouple thermometer.

Statistical analyses

Means were compared with Student's *t*-test, or with analysis of variance and Fisher's LSD test for multiple comparisons (Statistical Analysis System 1982, Cary, NC). Means are presented with ± 1 SE, and in all comparisons significance was set at $P=0.05$.

Results

Coots <28 d of age showed no discernible metabolic response to intramuscular injections of isoproterenol (Fig. 3-1). Significant differences (t-test, $P<0.05$) between drug- and saline-injected groups were observed only in birds >40 d old. The metabolic responses of coots >40 d of age to isoproterenol were approximately 1.3-1.4 times greater than those recorded following saline treatment (Fig. 3-1). The dosage of isoproterenol used in ontogenetic studies (4.0 $mg \cdot kg^{-1}$) raised thermoneutral heat production of one 47-50 d individual to a level that was comparable to the MR of 40-48 d coots exposed to 0°C air (Fig. 3-2). Heat production of this bird increased steadily, peaking at approximately 13 $W \cdot kg^{-1}$ by 76 min post-injection. Treatment of this same bird with saline produced no apparent thermogenic response. Instead, MR declined almost to the thermoneutral level by 40 min post-injection (Fig. 3-2). In 7 other fledglings treated with 4 $mg \cdot kg^{-1}$ isoproterenol, MR averaged 8.88 ± 0.29 $W \cdot kg^{-1}$ between 12 and 76 min post-injection. Mean MR of 9 saline-injected fledglings during the same period (6.53 ± 0.45 $W \cdot kg^{-1}$) was significantly lower (t-test, $P<0.05$).

In coots >45 d old, the metabolic response to isoproterenol was clearly dose-dependent (Fig. 3-3). Though even a dosage of 0.05 $mg \cdot kg^{-1}$ induced a significant thermogenic response in these birds, mean increases in MR were markedly higher following dosages ≥ 2 $mg \cdot kg^{-1}$ (Fisher's

Figure 3-1. Mean metabolic responses of juvenile 23°C-acclimated coots following intramuscular injections of saline (open bars) and 4 $mg \cdot kg^{-1}$ isoproterenol (cross-hatched bars). Upper panel represents post-injection MR - pre-injection MR (Δ MR); lower panel represents post-injection MR / pre-injection MR. Vertical lines denote 1 SE; numbers indicate sample sizes. Asterisks identify means that are statistically different from saline controls (*t*-test, $P<0.05$).

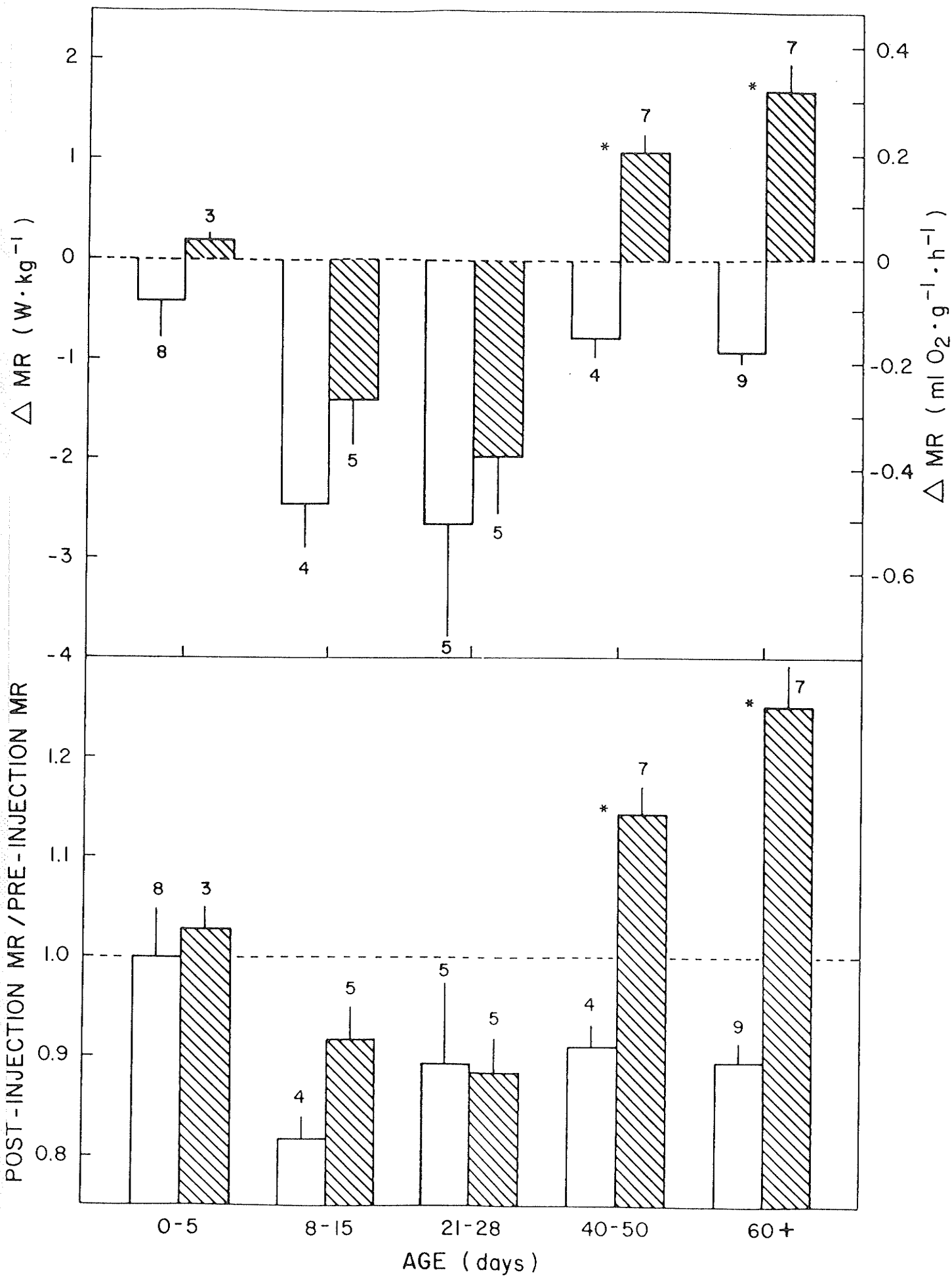

Δ MR (W · kg⁻¹)
Δ MR (ml O2 · g⁻¹ · h⁻¹)
POST-INJECTION MR / PRE-INJECTION MR
AGE (days)
0-5
8-15
21-28
40-50
60+

Figure 3-2. Metabolic responses of a single 47-50 d coot following intramuscular injections of saline (open circles) and 4 $mg \cdot kg^{-1}$ isoproterenol (closed circles). Vertical bar indicates range of mean metabolic rates of similar-aged birds exposed to 0-33°C air. Vertical lines denote ± 1 SE.

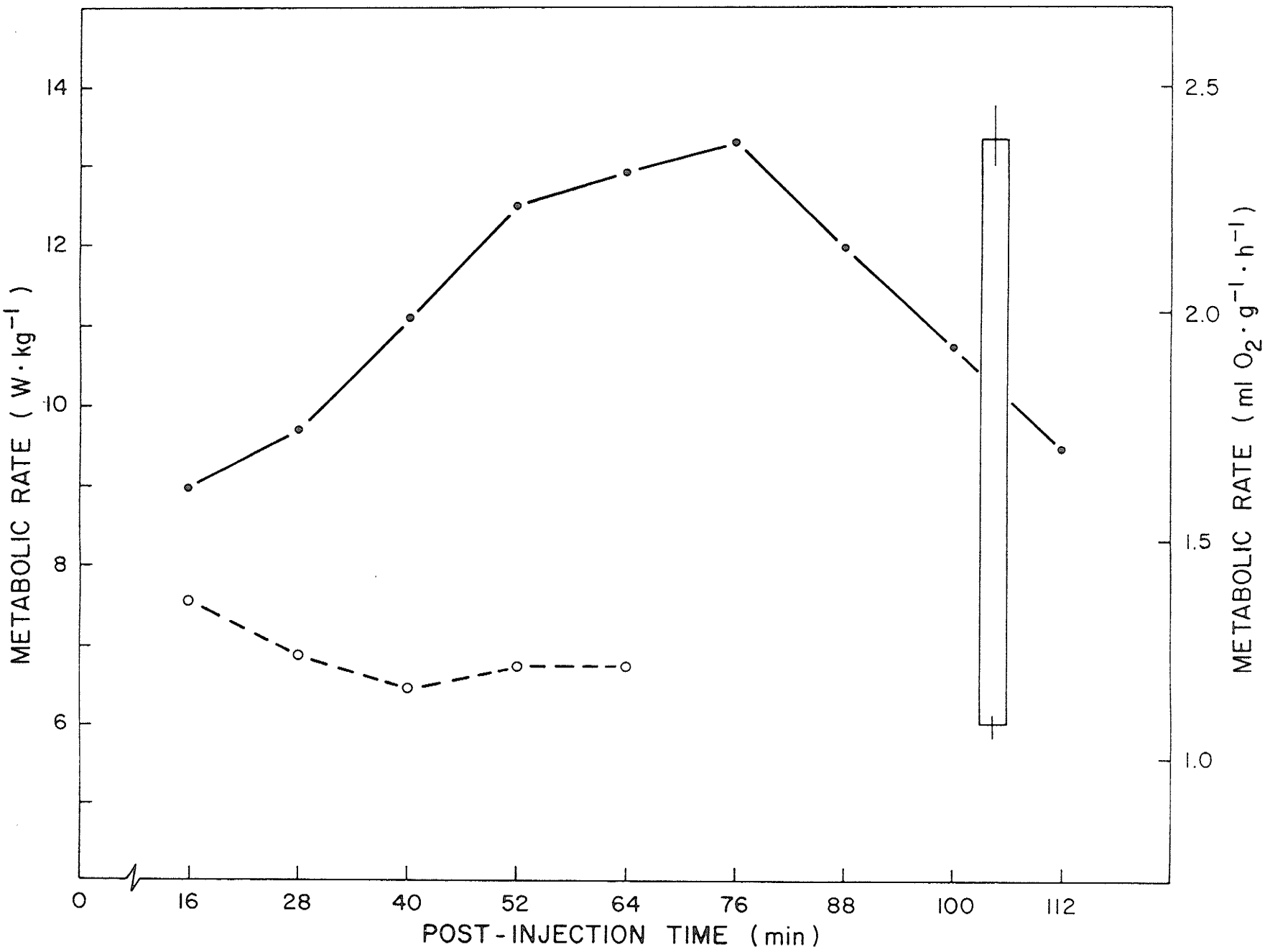
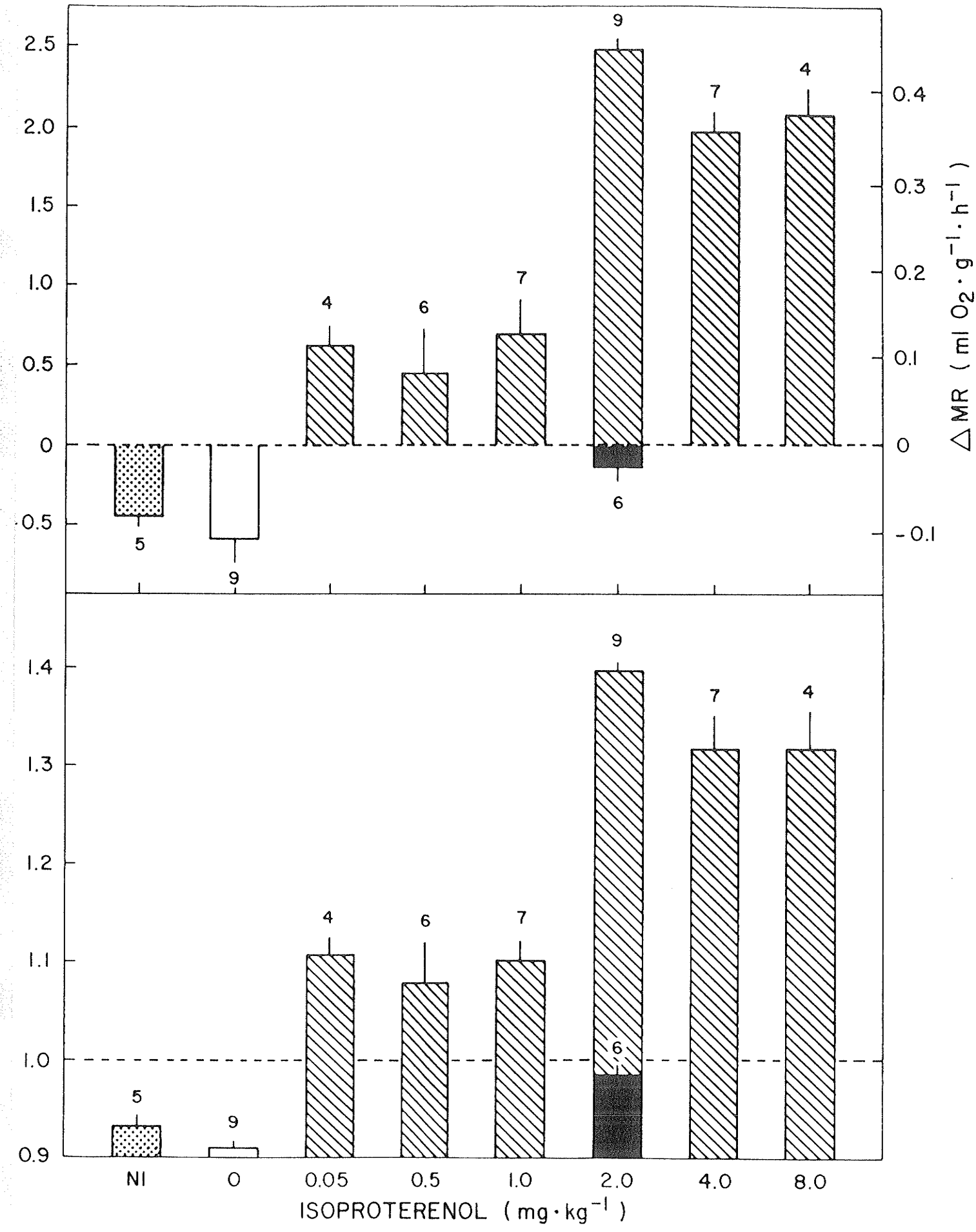

METABOLIC RATE (W · kg⁻¹)
METABOLIC RATE (ml O2 · g⁻¹ · h⁻¹)
POST-INJECTION TIME (min)
14
12
10
8
6
2.5
2.0
1.5
1.0
0
16
28
40
52
64
76
88
100
112

Figure 3-3. Mean metabolic responses of 23°C-acclimated coots >45 d old following intramuscular injections of saline (open bar), isoproterenol (cross-hatched bars) and isoproterenol plus 6 $mg \cdot kg^{-1}$ propranolol (solid bar). NI=no injection (stippled bar). Upper panel represents post-injection MR - pre-injection MR (Δ MR); lower panel represents post-injection MR / pre-injection MR. Vertical lines denote 1 SE; numbers indicate sample sizes.


2.5
2.0
1.5
1.0
0.5
0
-0.5
0.4
0.3
0.2
0.1
0
-0.1
ΔMR (ml O2 · g-1 · h-1)
1.4
1.3
1.2
1.1
1.0
0.9
5
9
4
6
7
9
6
7
4
NI
0
0.05
0.5
1.0
2.0
4.0
8.0
ISOPROTERENOL (mg · kg-1)

LSD test, $P<0.05$). At lower drug dosages (0.05-1.0 $mg \cdot kg^{-1}$), mean MR was increased by about 20%, or 1 $W \cdot kg^{-1}$ over control values. By contrast, injections of 2.0-8.0 $mg \cdot kg^{-1}$ isoproterenol evoked increases in MR that averaged close to 50%, or 2.7 $W \cdot kg^{-1}$ above control levels (Fig. 3-3). Metabolic responses to 2 $mg \cdot kg^{-1}$ isoproterenol were abolished by treatment with propranolol (6 $mg \cdot kg^{-1}$). Combined isoproterenol-propranolol injections induced mean changes in MR that compared favorably with measurements for NI and saline-injected birds (Fig. 3-3).

The mean post-injection MR of 5 propranolol-treated fledglings exposed to 10°C air (8.40 ± 1.08 $W \cdot kg^{-1}$) was not significantly different from that (8.55 ± 0.28 $W \cdot kg^{-1}$) of birds treated with saline (*t*-test, $P>0.05$). Following propranolol treatment, however, post-injection MR averaged 1.70 ± 0.43 $W \cdot kg^{-1}$ below pre-injection levels. This post-injection decline in MR was significantly greater than that (1.00 ± 0.34 $W \cdot kg^{-1}$) observed in saline-treated birds (*t*-test, $P<0.05$). There was no significant change in the ß-adrenergic sensitivity of 6 fledglings following 30 d acclimation to 8°C. It should be noted that these same 6 coots were previously tested as 23°C-acclimated birds. Following treatment with 2 $mg \cdot kg^{-1}$ isoproterenol, the thermoneutral MR of these cold-acclimated coots averaged 2.14 ± 0.61 $W \cdot kg^{-1}$ above that of saline-treated birds. This response compared favorably (*t*-test, $P>0.05$) to that of

23°C-acclimated birds following similar isoproterenol treatment (Fig. 3-3). Cold-acclimated coots responded to saline-injection with a slight decrease in MR (0.77±0.40 $W \cdot kg^{-1}$) that was similar ($P>0.05$) to the response of 23°C-acclimated birds (Fig. 3-3).

At no age was mean Tcl significantly elevated in response to isoproterenol. Nor was there any appreciable change in fledgling Tcl due to cold-acclimation, or treatment with propranolol during exposure to 10°C air (Appendix 3). Tcl appeared to be affected by initial handling, since Tcl usually declined by 1-1.5°C during the 75-90 min pre-injection phase of each experiment. However, this response was highly variable, and some birds from each age group displayed a relatively stable Tcl (±0.5°C) during the pre-injection run. Mean changes in Tcl during post-injection runs were generally small (±0.5°C), suggesting that birds were less excited during the second phase of these trials.

Discussion

Evidence of adrenergic sensitivity first appeared in coots at 40-50 d of age, when injection of isoproterenol (4 $mg \cdot kg^{-1}$) increased MR by an average of 27% over that of saline-treated birds (Fig. 3-1). Treatment of one coot with this dosage of isoproterenol raised thermoneutral heat production to a level attained by similar-aged birds exposed to 0°C air (Fig. 3-2). MR of isoproterenol-injected fledglings (2 $mg \cdot kg^{-1}$) averaged 56% greater than that of saline controls (Fig. 3-3), comparing favorably to metabolic responses of young laboratory rats following intramuscular injection of noradrenaline (Himms-Hagen 1967). Metabolic responsiveness to adrenergic stimulation has also been reported in adult pigeons (Columba livia), in which intramuscular injection of noradrenaline (3 $mg \cdot kg^{-1}$) caused thermoneutral MR to rise 10-15% above that of saline-treated birds (Hissa et al. 1975). Larger thermogenic effects appeared in king penguin chicks (Aptenodytes patagonicus), in which thermoneutral MR rose approximately 150% following a 20-min intravenous infusion of adrenaline at a dosage of 4 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (Barré and Rouanet 1983).

In the present study, some saline- and drug-injected birds showed a slight decline in mean MR during the post-injection period. This trend probably reflects reduced excitement levels and adaptation of birds to handling in the second phase of each trial. The injection procedure may

also be implicated here, though even NI birds showed a slight decline in MR during the second phase of each experiment (Fig. 3-3).

The sensitivity of older coots to isoproterenol clearly involved activation of ß-adrenergic pathways. In birds >45 d of age, responses to isoproterenol were dose-dependent, and the thermogenic effects of this drug were effectively blocked by the ß-antagonist, propranolol (Fig. 3-3). Since these trials were performed at thermoneutrality, suppression of the thermogenic response was probably not a reflection of propranolol's ability to limit shivering in birds (Hissa et al. 1980). It is possible, however, that reduced capacity for shivering may have accounted for the slightly depressed MR of propranolol-treated fledglings tested in 10°C air. Alternatively, the reduced thermogenic response to cold may have reflected impaired NST in the propranolol-treated birds.

Though results of this study implicate ß-adrenergic pathways in the thermogenic response of coots to cold, the site of this thermogenesis remains obscure. There was no evidence of BAT in coots--an observation that supports the general scarcity of this tissue in birds (Johnston 1971). However, observations presented here were based on gross dissection only; resolving whether or not coots possess thermogenic BAT must await closer, histological examination. Nevertheless, even if BAT proves to be lacking in F.

americana, the adrenergic sensitivity of fledgling birds strongly implies the existence of NST in this species. Though unaffected by cold-acclimation, the response of these birds to isoproterenol was certainly large enough to contribute to endothermic heat production in cold, and recent studies indicate that avian NST can operate in the absence of thermogenic BAT (Barré and Rouanet 1983; Barré et al. 1985,1987). Occurrence of NST in fledgling coots is also suggested by the discontinous nature of electromyographic activity in mild cold (see Part II).

It is possible that at least part of the metabolic response of fledgling coots to isoproterenol may have resulted from ß-adrenergic stimulation of glycolytic and lipolytic pathways (Innes and Nickerson 1970). Sympathomimetic drugs are known to activate carbohydrate metabolism in birds, targeting hepatic stores of glycogen (Freeman 1966). More recently, Barré et al. (1986b) have shown that mobilization of free fatty acids may be implicated in the muscle-based NST response of cold-acclimated muscovy ducklings. Though glucagon tends to be more potent than noradrenaline in stimulating avian lipolysis, this trend does not apply to all birds (Prigge and Grande 1971). It is conceivable, therefore, that treatment with isoproterenol may have promoted lipolysis in fledgling coots, potentiating a muscle-based NST similar to that observed in A. barbariae (Barré et al. 1985,1986b).

Finally, the question arises as to why ß-adrenergic stimulation failed to raise thermoneutral MR in coots until 40 d posthatch. This pattern is at variance to that of most small mammals, in which capacity for NST usually declines with age (Himms-Hagen 1967; Jansky 1973). In the present study, ß-receptors of younger birds (<40 d) may have occurred at too low a density in thermogenic tissues for isoproterenol treatment to have manifested a thermogenic response. Alternatively, glycogen and lipid fuel reserves may have become more abundant in these tissues during the later stages of development, when less energy was required for somatic growth and tissue maturation. This latter view is supported by the finding that ß-adrenergic sensitivity first appeared in coots that had attained fledgling body size (see Fig. 1-1, Part I). In any event, there is clearly more evidence of NST in older coots, implying that (1) aquatic endurance of neonates does not depend on catecholamine-based NST, and (2) older coots may potentially employ NST to thermoregulate in water. Further study is needed to resolve which specific mechanisms are activated by adrenergic stimulation in this species.

Summmary and Conclusions

Despite their seemingly precocial nature, hatchling coots <1 wk old displayed limited abilities to regulate Tcl. This observation was attributed to a low thermal inertia and to a limited capacity for natal endothermy. Development of adequate insulation was vital to achieving homeothermic temperature control in this species, as was the attainment of a critical body weight of 30-35 g. Factors that may limit heat loss of wild coot chicks include a low natal set-point, voluntary hypothermia and regional heterothermy, as well as parental brooding and preening. Sustaining thermal balance in water may also depend upon the high degree of buoyancy that arises from air entrapped by the ventral plumage. As well, natal pterylosis may promote the absorption of solar radiant energy.

Fledgling coots were highly competent homeotherms in air, water and Helox, displaying an impressive tolerance to cold in all three media. Despite this remarkable homeothermic ability, the thermogenic capacity of fledglings appeared unexceptional. A complex and dense plumage accounted for more than 80% of total insulation in fledgling coots, and undoubtedly enhanced buoyancy of these birds in water. A high buoyancy appears to separate F. americana from many other small aquatic endotherms that have been studied in the past, and may largely account for the exceptional resistance of fledgling coots to aquatic cooling.

Metabolic responsiveness to ß-adrenergic stimulation first appeared in coots >40 d of age, suggesting that older birds may utilize catecholamine-based NST. This hypothesis is supported by observations of intermittent shivering in fledgling coots exposed to mild cold. Given the apparent absence of BAT in coots, further study is required to identify the origin, effector pathways and thermoregulatory role of NST in F. americana.

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Appendices

Appendix 1. Growth of the bill and tarsus in juvenile coots.

Age group (d)	Tarsus (cm)	Bill (cm)		
		Culmen	Depth	Width
0-1	16.69±0.34 (8)	13.10±0.63 (8)	5.74±0.21 (8)	3.44±0.12 (8)
1-5	16.31±0.39 (8)	12.13±0.36 (8)	5.68±0.36 (8)	3.40±0.08 (8)
6-15	19.18±2.05 (6)	14.89±1.11 (6)	5.81±0.14 (5)	3.50±0.23 (6)
23-27	53.72±0.70 (6)	32.67±1.16 (7)	10.55±0.21 (7)	6.74±0.29 (7)
47-57	50.16±1.22 (5)	34.52±1.10 (5)	11.06±0.26 (5)	6.97±0.28 (5)
60+	51.98±0.74 (21)	39.20±0.65 (21)	11.85±0.65 (21)	7.45±0.15 (21)

Note: Values are presented as means ±1 SE; numbers in parentheses denote sample sizes.

Appendix 2. Characteristics of Richards, logistic and Gompertz growth models as applied to juvenile coots.

Model	Explained Percentage of variation (%)	Parameter			
		A (g)	K (wk^{-1})	TI (wk)	M
Richards	91.6	367.0	0.246	3.9	2.441
Logistic	91.5	369.6	0.230 0.131*	3.7 26.1*	-
Gompertz	91.1	380.7	0.074*	21.7*	-

Note: Each parameter is defined in the text.
*units = d^{-1} (K) and d (TI).

Appendix 3. Mean cloacal temperatures of juvenile coots in response to intramuscular injections of saline, isoproterenol and propranolol.

Age group (d)	Drug	Dosage ($mg \cdot kg^{-1}$)	Mean Tcl (^{o}C)	
			Pre-injection	Post-injection
0-5	S	-	39.2±0.3 (8)	39.2±0.2 (10)
	I	4	38.7±0.2 (8)	38.6±0.2 (9)
8-15	S	-	39.6±0.2 (5)	39.4±0.3 (5)
	I	4	40.1±0.3 (5)	39.9±0.3 (6)
21-28	S	-	40.2±0.1 (5)	39.8±0.3 (6)
	I	4	40.9±0.1 (6)	41.0±0.1 (6)
40-50	I	4	41.0±0.2 (5)	40.8±0.1 (5)
60+	NI	-	40.9±0.2 (4)	41.1±0.3 (5)
	S	-	41.0±0.1 (4)	41.0±0.2 (6)
	S*	-	41.4±0.1 (4)	40.6±0.2 (4)
	S***	-	41.4±0.2 (4)	40.9±0.2 (3)
	I	0.05	41.7±0.1 (4)	41.9±0.3 (3)
	I	0.5	41.2±0.2 (2)	41.1±0.2 (3)
	I	1	40.9±0.1 (3)	41.1±0.2 (3)
	I	2	41.4±0.2 (4)	41.7±0.3 (3)
	I*	2	41.5±0.1 (5)	40.9±0.1 (5)
	I,P**	2,6	41.1±0.2 (4)	41.1±0.2 (3)
	I	4	40.9±0.4 (3)	41.0±0.5 (3)
	P***	6	41.2±0.1 (3)	40.4±0.2 (3)
	I	8	41.0±0.4 (3)	41.1±0.4 (3)

Note: S=saline; I=isoproterenol; P=propranolol; NI=no injection. Values are presented as means ±1 SE; numbers in parentheses denote sample sizes.

* $8^{o}C$-acclimated birds.

** combined injection of 2 $mg \cdot kg^{-1}$ isoproterenol + 6 $mg \cdot kg^{-1}$ propranolol.

*** birds exposed to $10^{o}C$ air.