

THERMAL ACCLIMATION OF METABOLISM IN SALAMANDERS: FACT OR ARTEFACT?

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Abstract—1. The rate of O₂ consumption (\dot{V}_{O_2}) was measured in the salamanders *Desmognathus ochrophaeus*, *Plethodon cinereus* and *Plethodon jordani* to examine the interaction of repeated measurements, feeding, duration of equilibration to respirometers and duration of acclimation with thermal acclimation of metabolism.

2. Measurements of the \dot{V}_{O_2} of individual *D. ochrophaeus* for 8 consecutive days showed no consistent increase or decrease.

3. Feeding resulted in a prolonged elevation in \dot{V}_{O_2} . The \dot{V}_{O_2} of *P. jordani* increased by 77% on the day after feeding, did not begin to decline until 6 days after feeding, and still had not returned to initial levels after 11 days.

4. The post-feeding elevation in \dot{V}_{O_2} can mimic thermal acclimation of metabolism. *P. jordani* that were fed immediately before acclimation to 5°C had a higher \dot{V}_{O_2} than both unfed salamanders acclimated to 5°C and fed salamanders acclimated to 17.5°C. The \dot{V}_{O_2} of the latter groups were similar.

5. Acclimation to a warm temperature (17.5°C) required more than 1 week for completion. Significant metabolic acclimation occurred after 2-3 days.

6. Individual *D. ochrophaeus* varied in both \dot{V}_{O_2} and the rate of thermal acclimation of metabolism.

Key Word Index—Acclimation; amphibian; feeding; O₂ consumption; salamander; thermal acclimation.

INTRODUCTION

Thermal acclimation is only one of many factors that affect the metabolic rates of amphibians (Lagerspetz, 1977; Feder, 1982a, b). Others include activity, feeding, reproductive state, photoperiod, recovery from acute exposure to a new temperature etc. If these other factors alter metabolism during an acclimation experiment, they may suggest thermal acclimation where none exists or may mask actual thermal acclimation of metabolism (hereafter termed "metabolic acclimation").

Consider, for example, the possible consequences of feeding for an experiment in which amphibians are acclimated at cold and warm acclimation temperatures (ATs) and their metabolic rates determined subsequently at a warm experimental temperature (ET). If the animals are fed before being placed at their respective ATs, the cold AT may halt digestion of food in the gut while the warm AT may stimulate digestion. Upon measurement at a warm AT, the cold AT animals may resume digestion of food and show a pronounced specific dynamic action (Kleiber, 1961; Jobling and Davies, 1980; Jobling, 1981); whereas the warm AT animals, which would be post-absorptive, might have a lower metabolic rate on this basis alone. The resulting pattern would resemble positive metabolic acclimation [Precht (1958), types I-III], even if

no acclimation actually occurred. At a cold ET, digestion would still be depressed, yielding the appearance of no acclimation. This pattern, positive acclimation at warm ETs and no acclimation at cool ETs, is commonly reported in acclimation studies of salamanders and anurans (Feder, 1982a, b).

Similarly, when cold AT animals are measured at a warm ET, they undergo an abrupt shift in body temperature (BT) during experimentation. This change may affect pH regulation, heart rate and routine activity (Holeton, 1974; Withers, 1978; Moffitt and Crawshaw, 1983), and consequently can elevate metabolic rate long after the BT has equilibrated. Warm AT animals measured at a warm ET would not undergo nearly so dramatic a shift in BT, and might exhibit low metabolic rates for that reason alone.

The lack of information on the rate of metabolic acclimation poses a different sort of problem. In general, the kinetics of metabolic acclimation are poorly understood (White and Somero, 1982). Only a few studies have examined the rate of metabolic acclimation in anuran amphibians (Dunlap, 1969; Harri, 1973; Feder, 1982a, b), and no data are available for salamanders. Such data are obviously crucial in deciding whether the absence of metabolic acclimation in a species signifies lack of acclimatory ability or only that the time allowed for metabolic acclimation has been insufficient (Feder, 1982a, b).

Because such problems can create serious difficulties in the interpretation of metabolic data in acclimation studies (Holeton, 1974), we have determined the magnitude of potential errors associated with the inadvertent interaction of metabolic accli-

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mation with feeding, activity and duration of acclimation. We present these measurements as a "worst case" scenario and as an aid to the design of future acclimation experiments.

MATERIALS AND METHODS

Plethodontid salamanders, *Desmognathus ochrophaeus* and *Plethodon jordani*, were collected in the vicinity of Highlands, N.C., U.S.A. *Plethodon cinereus* were collected near Valparaiso, Ind., U.S.A. Animals were maintained in the laboratory on a L:D 14:10 photoperiod centered at 1300 local time, and were fed flies and fly larvae except as noted below.

The rate of O₂ consumption (\dot{V}_{O_2}) was measured with a Gilson respirometer [see Feder (1976, 1983) for details of the measurement procedure]. In summary, experimental animals were placed in respirometer vessels along with moist paper towelling and a CO₂ absorbent, the respirometers were allowed to equilibrate for 3–4 h, and the \dot{V}_{O_2} was determined for 2–3 h thereafter. All measurements were taken between 1230 and 1630, and are expressed in $\mu\text{l O}_2$ STPD.

Experiment 1

To determine whether repeated measurements of individual animals on consecutive days resulted in a decrease in \dot{V}_{O_2} (which might be interpreted as metabolic acclimation), 10 *D. ochrophaeus* were maintained unfed at 17.5°C AT for 2 weeks. The \dot{V}_{O_2} of each animal was then determined at 17.5°C ET for 8 consecutive days. Individual animals were housed separately to facilitate their identification.

Experiment 2

To quantify increases in \dot{V}_{O_2} associated with feeding, 5 *P. jordani* were maintained unfed for 2 weeks at 17.5°C AT. The \dot{V}_{O_2} of each animal was then determined at 17.5°C ET, after which animals were offered fly larvae *ad libitum*. All animals readily ate fly larvae. The \dot{V}_{O_2} of each individual was determined at 17.5°C ET on 8 of the 11 succeeding days.

Experiment 3

To determine whether increases in \dot{V}_{O_2} associated with feeding might be interpreted as metabolic acclimation in a typical experiment, 27 *P. jordani* were assigned randomly to three experimental treatments. All animals had been at 17.5°C AT for 2 weeks, and were fed fly larvae *ad libitum* at the end of this period. One group (A) was maintained at 17.5°C AT for an additional week and then at 5.0°C AT for another week; these animals were not fed during the 2 weeks. A second group (B) was also maintained at 17.5°C AT for an additional week, was then fed fly larvae and immediately transferred to and maintained at 5.0°C AT for another week. The third group (C) was maintained at 17.5°C AT for 1 week, fed and then maintained at 17.5°C AT for an additional week. The \dot{V}_{O_2} of each animal was then determined. Thus, all animals were unfed for 1 week before measurement of \dot{V}_{O_2} . Group A, however, was starved for an additional week before transfer to 5.0°C AT.

Experiment 4

To determine whether cold AT animals required more time to habituate to a warm respirometer than warm AT animals, two similar groups of 6 *P. jordani* each were acclimated at 5.0°C AT and 17.5°C AT, respectively, for 1 week. Animals were placed in respirometers, and determinations of \dot{V}_{O_2} were begun after only 20 min. The \dot{V}_{O_2} was then determined at regular intervals for 215–225 min for animals of both groups.

Experiment 5

To determine the rate of metabolic acclimation, salamanders of all three species were acclimated to cold ATs

(4.0°C for *D. ochrophaeus* and 5.0°C for the *Plethodon* species) for 2 weeks. Animals were previously at 17.5°C AT, and were unfed for at least 10 days before the start of cold acclimation. The \dot{V}_{O_2} of each animal was determined at 17.5°C AT. Thereafter, the animals were maintained at 17.5°C AT, and the \dot{V}_{O_2} repeatedly determined at 17.5°C ET at regular intervals.

Values of mass and \dot{V}_{O_2} were converted to their logarithms and the transformed values were analysed with BMDP2V, an analysis of covariance including repeated measures (Dixon, 1977). This program adjusts values of \dot{V}_{O_2} for differences in mass among and within experimental animals; the "adjusted mean \dot{V}_{O_2} " is presented in the figures.

RESULTS AND DISCUSSION

Experiment 1

D. ochrophaeus maintained at 17.5°C and measured daily on 8 consecutive days showed no significant variation ($P = 0.66$) in \dot{V}_{O_2} (Fig. 1). Variation in \dot{V}_{O_2} from day to day was at most 9% of the initial \dot{V}_{O_2} , and no consistent increase or decrease in \dot{V}_{O_2} was evident. Thus, \dot{V}_{O_2} did not undergo a decline as salamanders habituated to handling or to the respirometers.

Plethodontid salamanders are unusually sedentary animals that often inhabit burrows, crevices in rocks, or the spaces beneath rocks, logs or bark. Accordingly, small moist respirometer vessels are much better suited to plethodontids than to many other animals. The refractoriness of plethodontids to repeated measurement of their \dot{V}_{O_2} allows individual animals to be followed during an acclimation experiment, with each animal serving as its own control. Repeated measurements also permit application of an especially powerful statistical procedure, the repeated-measures analysis of covariance. Other animals are much more sensitive to handling and confinement (e.g. Holeton, 1974; Feder, 1981). A decline in \dot{V}_{O_2} with repeated measurements during an acclimation experiment with such animals may represent nothing more than habituation to the mea-

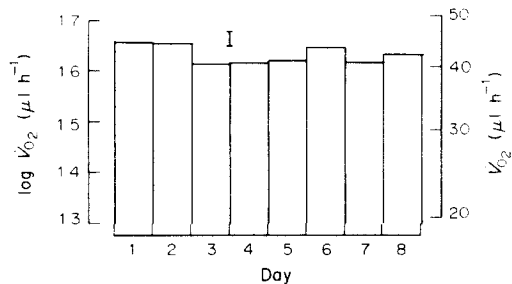


Fig. 1. The lack of effect of repeated measurement on \dot{V}_{O_2} of salamanders, *D. ochrophaeus*. Animals were acclimated to 17.5°C AT beforehand and measured daily on 8 consecutive days at 17.5°C. The height of the bars is equivalent to the mean $\log \dot{V}_{O_2}$ for each day adjusted for the log mass of salamanders. An arithmetic scale of \dot{V}_{O_2} has been added for illustration; computations are based on log-transformed values. The vertical bar indicates the honestly significant difference (Sokal and Rohlf, 1969); means separated by more than this distance on the graph differ significantly ($P < 0.05$). Adjusted means and the honestly significant difference were computed via a statistical package, BMDP2V (Dixon, 1977). The logarithmic mean body size of these salamanders was 1.6 g.

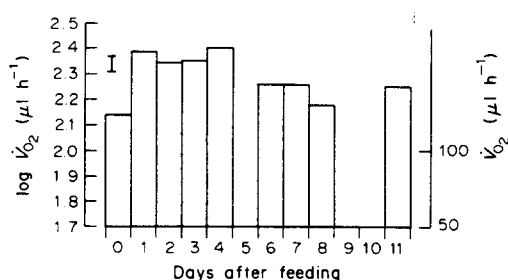


Fig. 2. Effect of feeding on the \dot{V}_{O_2} of *P. jordani* that were acclimated and measured at 17.5°C. Day 0 refers to animals immediately before feeding. Data and the honestly significant difference are plotted as in Fig. 1. The logarithmic mean body size of these salamanders was 4.3 g.

surement procedure, and thus necessitates careful controls.

Experiments 2 and 3

Feeding had a dramatic and significant ($P < 0.0001$) effect on the \dot{V}_{O_2} of *P. jordani* (Fig. 2). The \dot{V}_{O_2} had increased 77% by 1 day after feeding, and did not begin to decline from these elevated levels until 6 days after feeding. By 11 days after feeding, the last day on which \dot{V}_{O_2} was measured, the \dot{V}_{O_2} still had not returned to initial levels.

In view of this substantial elevation of \dot{V}_{O_2} after feeding, might not acclimation of fed salamanders to cold temperatures "trap" food in the gut and thereby increase \dot{V}_{O_2} when animals are measured at warm temperatures? In Experiment 3, *P. jordani* that were fed immediately before acclimation to 5°C AT (Group B) had a 52% greater \dot{V}_{O_2} than did conspecifics (Group A) that were also acclimated to 5°C for 1 week but were unfed for 1 week before transfer to 5°C (Fig. 3). Group B animals had much greater \dot{V}_{O_2} than animals acclimated to 17.5°C (Group C), whereas Groups A and C had similar \dot{V}_{O_2} (Fig. 3). Differences among the groups were highly significant ($P = 0.0076$). Thus, compared to the results for Group C, the Group A data suggest no metabolic acclimation to cold temperatures and the Group B data suggest significant metabolic acclimation to cold temperatures, even though both Groups B and C were exposed to 5°C AT for identical times.

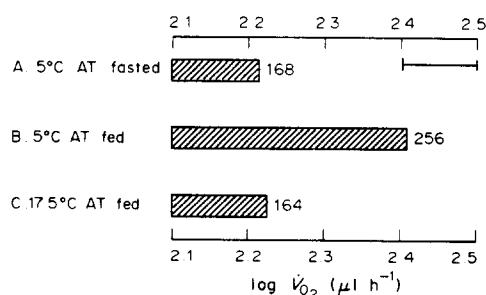


Fig. 3. Effect of pre-fasting and AT on the \dot{V}_{O_2} of *P. jordani* at 17.5°C. See Materials and Methods for a full description of the three experimental treatments. Data and the honestly significant difference are plotted as in Fig. 1, except that the arithmetic values of \dot{V}_{O_2} are indicated numerically. The logarithmic mean body size of these salamanders was 3.8 g.

Although specific dynamic action and the effect of cold temperature on digestion in ectotherms have been common knowledge for decades, surprisingly few studies of metabolic acclimation in amphibians have accommodated these factors in experimental design or even mentioned them. Fitzpatrick (e.g. 1973a, b) fasted salamanders 3–4 weeks before acclimation experiments, and noted (1973b) that "fasting effects on \dot{V}_{O_2} were not apparent during 10 day intervals". Lagerspetz and Skytta (1979) suggested that decreases in cutaneous Na transport after warm acclimation in fasted frogs might stem from depletion of energy stores at high temperatures, and performed control experiments to rule out this possibility. The magnitude and duration of specific dynamic action in ectotherms are correlated with the amount of food ingested (Jobling and Davies, 1980; Jobling, 1981). Accordingly, differences in ingestion rates associated with AT (e.g. Fitzpatrick, 1973a, b) may yield differences in \dot{V}_{O_2} at a common measurement temperature that are absent in unfed animals. In many cases experimental animals are simply fed regularly before and during acclimation experiments, presumably to promote the animals' health. Although this aim is laudatory, absence of a fasting period may yield the appearance of acclimation where none occurs. Our results suggest that amphibians should be fasted for at least 7 days before measurement of the standard metabolic rate, and that individuals to be acclimated at a cold AT be fasted at least 7 days beforehand.

Experiment 4

In repeated measurements of \dot{V}_{O_2} at 17.5°C ET started soon after salamanders were placed in the respirometer, both groups of *P. jordani* exhibited significant variation ($P = 0.0003$) in \dot{V}_{O_2} (Fig. 4). Our expectation was that 5°C AT animals, having undergone a sudden 12.5°C increase in BT, would have a greater \dot{V}_{O_2} than 17.5°C AT animals, would be more active and would require more time to habituate to the respirometer. The \dot{V}_{O_2} indeed was greater in the 5°C AT animals than in the 17.5°C AT animals during the first two intervals (Fig. 4). Thereafter, contrary to our expectation, 5°C AT animals had lower and less variable \dot{V}_{O_2} than 17.5°C AT animals ($P = 0.02$). The overall time-course of the decline in \dot{V}_{O_2} after salamanders were placed in the respirom-

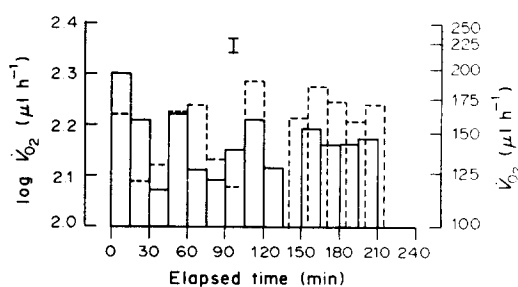


Fig. 4. The \dot{V}_{O_2} of *P. jordani* at 17.5°C as a function of time after being placed in the respirometer. Time 0 refers to 20 min after animals were placed in the respirometer. Solid lines indicate animals acclimated to 5°C; broken lines indicate animals acclimated to 17.5°C. Data and the honestly significant difference are plotted as in Fig. 1. The logarithmic mean body size of these salamanders was 3.4 g.

eters did not differ significantly between 5°C AT and 17.5°C AT animals (i.e. the interaction of time interval and acclimation temperature was not significant; $P = 0.073$). Nonetheless, the near significance of the result recommends that animals be allowed long times to habituate to respirometers before initiation of measurements (see also Holeton, 1974).

Experiment 5

All three species underwent significant ($P < 0.0001$ in each case) metabolic acclimation (Fig. 5). When first measured at 17.5°C ET, the \dot{V}_{O_2} of all three species, which had been acclimated at 4.0–5.0°C, was high. As the salamanders remained at 17.5°C AT, the \dot{V}_{O_2} gradually declined to approx. 80–85% (*D. ochrophaeus*), 65–75% (*P. cinereus*) and 75–85% (*P. jordani*) of initial values. This decline was not smooth; each species underwent substantial oscillations before the \dot{V}_{O_2} settled at a new steady state. Each species showed substantial metabolic acclimation after 2–3 days at the new AT.

The time-course of metabolic acclimation in the plethodontid species resembles that reported for an-

urans (Dunlap, 1969; Harri, 1973; Feder, 1982a). This rate of acclimation is fortuitous because most prior studies of metabolic acclimation in amphibians (Feder, 1982a) have assumed acclimation to be complete in 1–2 weeks without documenting its time-course. Nonetheless, the oscillations in \dot{V}_{O_2} may obscure metabolic acclimation if measurements are taken on a day when the \dot{V}_{O_2} happens to be elevated. This may explain some contradictions within the present study; e.g. no acclimation or inverse acclimation in Experiments 3 and 4 and clear positive acclimation in Experiment 5.

The rates of metabolic acclimation observed in plethodontids are best suited to compensate for weekly to seasonal variations in temperature. More rapid thermal variation is probably not encountered by plethodontid salamanders because they spend most of their time beneath rocks or logs or in burrows, where diel or day-to-day variation in temperature is usually minimal (Feder, 1982b). Yet, opportunities for behavioural thermoregulation are limited in such microhabitats and salamanders undergo seasonal variation in BT (Feder, 1982b). Ac-

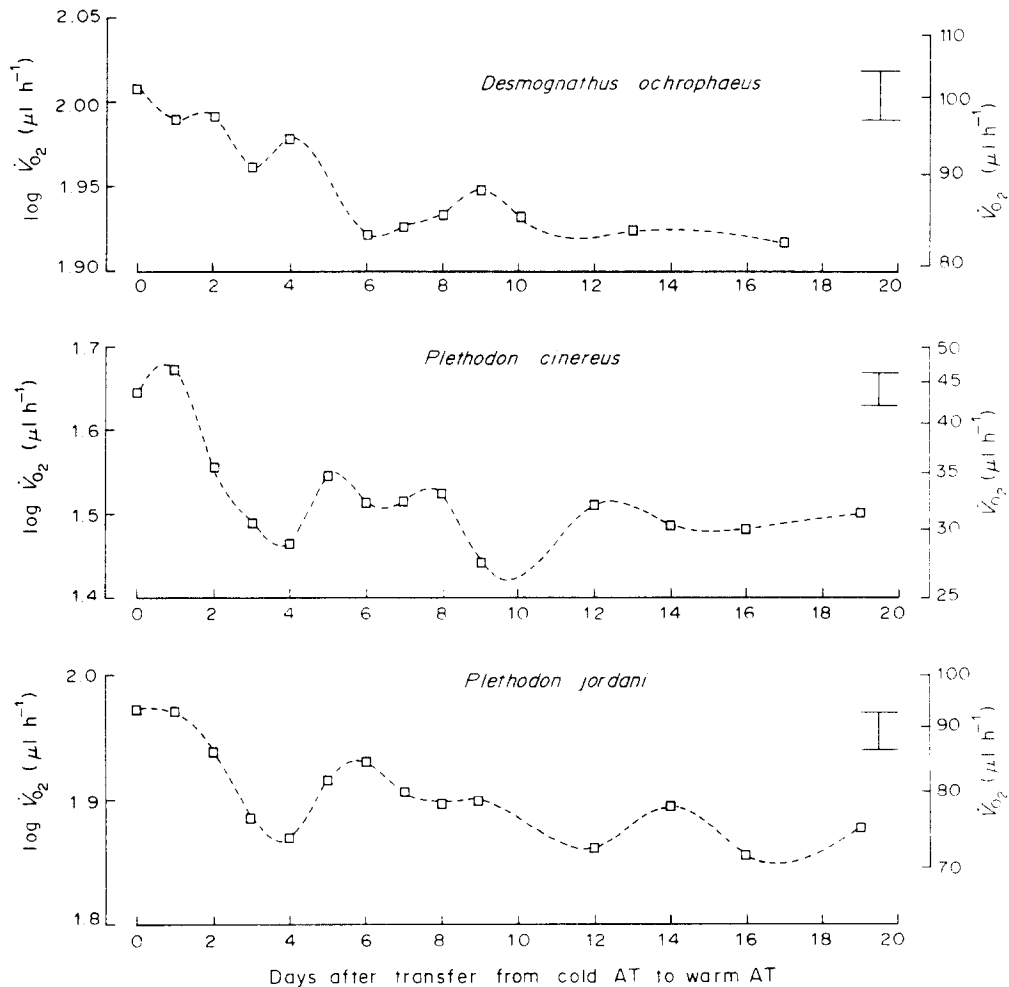


Fig. 5. The rate of thermal acclimation of metabolism in three species of plethodontid salamanders. Animals were acclimated at 4–5°C until the very first measurement at 17.5°C on Day 0. Thereafter animals were acclimated and measured at 17.5°C. Data and the honestly significant differences are plotted as in Fig. 1. The logarithmic mean body sizes of these salamanders were 2.5 g (*D. ochrophaeus*, $n = 8$), 0.9 g (*P. cinereus*, $n = 7$) and 2.2 g (*P. jordani*, $n = 7$).

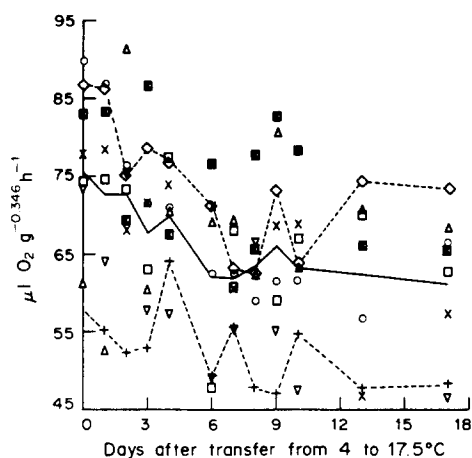


Fig. 6. Individual variation in the \dot{V}_{O_2} of *D. ochrophaeus* during acclimation to 17.5°C. Values have been corrected for the differing body sizes of individuals by multiplying each \dot{V}_{O_2} by $M^{-0.346}$, where M is the body mass and 0.346 is the pooled regression coefficient of $\log \dot{V}_{O_2}$ on $\log M$. Each type of symbol ($n = 8$) represents a different salamander. Records for two extreme salamanders (---) and the daily means for all salamanders (—) have been connected to emphasize the extent of individual variation in \dot{V}_{O_2} .

cordingly, metabolic acclimation may be especially important to salamanders in maintaining positive energy balance throughout the year. For example, Fitzpatrick and his co-workers (e.g. Fitzpatrick, 1973a, b; Fitzpatrick and Brown, 1975) have demonstrated that positive acclimation when animals are feeding maximizes energy allocation to secondary production, whereas inverse or no acclimation during cold seasons minimizes the depletion of energy stores in fasting salamanders. Without this pattern of compensation, growth and reproduction would suffer.

The time-course data for *D. ochrophaeus* were re-analysed to determine whether individual salamanders varied randomly with respect to the mean \dot{V}_{O_2} on each day, with each individual having greater than average \dot{V}_{O_2} on some days and less than average \dot{V}_{O_2} on other days, or whether individual salamanders consistently had higher or lower \dot{V}_{O_2} than average. This was done by multiplying each \dot{V}_{O_2} by $M^{-0.346}$, where M is the body mass and 0.346 is the pooled regression coefficient of $\log \dot{V}_{O_2}$ on $\log M$ (Fig. 6). The regression coefficient, 0.346, is low (i.e. not 0.6–0.8), but this is not surprising in view of the small range of body sizes of experimental animals. Individual salamanders differed consistently in \dot{V}_{O_2} ($P < 0.001$) during acclimation to 17.5°C AT (Fig. 6). Some individuals acclimated rapidly or usually had a greater than average \dot{V}_{O_2} ; other individuals acclimated slowly or often had a less than average \dot{V}_{O_2} . These individual differences in \dot{V}_{O_2} during acclimation have several important implications. First, as discussed above, individual variation in acclimation may be directly associated with differences in energy allocation, growth and reproduction. Hence study of this individual variation and its consequences is essential to an understanding of the role of physiological characters in natural selection (Arnold, 1983). Second, experimental designs that do not include repeated measures of \dot{V}_{O_2} may fail to detect significant

metabolic acclimation, or at least may require relatively large sample sizes or metabolic differences to do so.

CONCLUSION

Thermal acclimation experiments may inadvertently incorporate variables that lead to erroneous results and confound conclusions. As we have demonstrated for salamanders, such variables may include effects of feeding, fasting, prolonged equilibration to a new temperature, insufficient duration of acclimation and uncontrolled individual variation in \dot{V}_{O_2} . Others may include seasonal variation in \dot{V}_{O_2} and nutritional state (Lagerspetz, 1977). We do not feel that previous studies of metabolic acclimation are valueless nor can we identify any studies in which the potential problems discussed above have definitely led to erroneous results. Nonetheless, the present study suggests that extreme caution be exercised in the design and interpretation of future experiments on thermal acclimation of metabolism in amphibians.

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REFERENCES

- Arnold S. J. (1983) Morphology, performance and fitness. *Am. Zool.* **23**, 347–361.
- Dixon W. J. (1977) *BMDP-77 Biomedical Computer Programs P-Series*. Univ. of California Press, Los Angeles, Calif.
- Dunlap D. G. (1969) Influence of temperature and duration of acclimation, time of day, sex and body weight on metabolic rates in the hylid frog, *Acris crepitans*. *Comp. Biochem. Physiol.* **31**, 555–570.
- Feder M. E. (1976) Lunglessness, body size, and metabolic rate in salamanders. *Physiol. Zool.* **49**, 398–406.
- Feder M. E. (1981) Effect of body size, trophic state, time of day, and experimental stress on oxygen consumption of anuran larvae: an experimental assessment and evaluation of the literature. *Comp. Biochem. Physiol.* **70A**, 497–508.
- Feder M. E. (1982a) Environmental variability and thermal acclimation of metabolism in tropical anurans. *J. therm. Biol.* **7**, 23–28.
- Feder M. E. (1982b) Thermal ecology of neotropical lungless salamanders (Amphibia: Plethodontidae): environmental temperatures and behavioral responses. *Ecology* **63**, 1665–1674.
- Feder M. E. (1983) Biochemical and metabolic correlates of thermal acclimation in the rough-skinned newt, *Taricha granulosa*. *Physiol. Zool.* **56**, 513–522.
- Fitzpatrick L. C. (1973a) Energy allocation in the Allegheny Mountain salamander *Desmognathus ochrophaeus*. *Ecol. Monogr.* **43**, 43–58.
- Fitzpatrick L. C. (1973b) Influence of seasonal temperatures on the energy budget and metabolic rates of the northern two-lined salamander *Eurycea bislineata bislineata*. *Comp. Biochem. Physiol.* **45A**, 807–818.
- Fitzpatrick L. C. and Brown A. V. (1975) Metabolic compensation to temperature in the salamander *Desmognathus ochrophaeus* from a high elevation population. *Comp. Biochem. Physiol.* **50A**, 733–737.
- Harri M. N. (1973) The rate of metabolic temperature acclimation in the frog, *Rana temporaria*. *Physiol. Zool.* **46**, 148–156.

- Holeton G. F. (1974) Metabolic cold adaptation of polar fish: fact or artefact? *Physiol. Zool.* **47**, 137–152.
- Jobling M. (1981) The influences of feeding on the metabolic rate of fishes: a short review. *J. Fish Biol.* **18**, 385–400.
- Jobling M. and Davies P. S. (1980) Effects of feeding on metabolic rate, and the specific dynamic action in plaice, *Pleuronectes platessa* L. *J. Fish Biol.* **16**, 629–638.
- Kleiber M. (1961) *The Fire of Life*. Wiley, New York.
- Lagerspetz K. Y. H. (1977) Interactions of season and temperature acclimation in the control of metabolism in Amphibia. *J. therm. Biol.* **2**, 223–231.
- Lagerspetz K. Y. H. and Skytta M. (1979) Temperature compensation of sodium transport and ATPase activity in frog skin. *Acta physiol. scand.* **106**, 151–158.
- Moffitt B. P. and Crawshaw L. I. (1983) Effects of acute temperature changes on metabolism, heart rate, and ventilation frequency in carp *Cyprinus carpio* L. *Physiol. Zool.* **56**, 397–403.
- Precht H. (1958) Theory of temperature adaptation in cold-blooded animals. In *Physiological Adaptation* (Edited by Prosser C. L.), pp. 50–78. American Physiological Society, Washington, D.C.
- Sokal R. R. and Rohlf F. J. (1969) *Biometry*. Freeman, San Francisco, Calif.
- White F. N. and Somero G. (1982) Acid–base regulation and phospholipid adaptations to temperature: time courses and physiological significance of modifying the milieu for protein function. *Physiol. Rev.* **62**, 40–90.
- Withers P. C. (1978) Acid–base regulation as a function of body temperature in ectothermic toads, a heliothermic lizard, and a heterothermic mammal. *J. therm. Biol.* **3**, 163–171.