

THERMOACCLIMATORY VARIATIONS IN THE HAEMATOLOGY OF THE COMMON CARP, *CYPRINUS CARPIO*

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(Received 12 January 1968)

INTRODUCTION

The effect of temperature upon the oxidative metabolism of freshwater teleosts has been abundantly demonstrated (Winberg, 1956; Fry, 1957; Brett, 1964; Beamish, 1964). The mechanisms underlying the satisfaction of temperature-induced variations in oxygen demand are, however, imperfectly understood at present. Nevertheless, a recent series of theoretical studies has focused attention upon four probable sites of cardiovascular-respiratory adjustment under circumstances prompting changes in oxygen uptake: ventilatory flow, cardiac output, branchial exchange area and blood oxygen-carrying capacity (Hughes, 1964; Rahn, 1966; Randall, Holeyton & Stevens, 1967; Taylor, Houston & Horgan, 1967). Under conditions of forced activity, which may elevate oxygen consumption by several hundred per cent (Brett, 1964), all four types of response are apparently invoked. Stevens & Randall (1967), for example, have demonstrated four- to fivefold increases in the branchial ventilation and cardiac output of moderately active rainbow trout. Black, Connor, Lam & Chiu (1962) report that a marked and sustained elevation in blood haemoglobin level is typically encountered in vigorously exercised trout. Direct evaluation of the magnitude of branchiovascular adjustments has not as yet been possible. However, recent simulation studies (Taylor *et al.* 1967) suggest the occurrence of substantial increases in effective surface area (or a reduction in the translamellar diffusion pathlength).

Comparable studies have yet to be carried out upon teleosts acclimated to different temperature conditions. Consideration of the consequences of modifying one at a time each of the variables mentioned suggests that elevation of blood oxygen capacity would probably impose fewer restrictions upon the system as a whole than would increases in ventilation, gill perfusion and exchange area (DeWilde & Houston, 1967).

The results of investigations upon thermoadaptive modifications in the haematology of freshwater fishes have, however, been surprisingly inconsistent. Quite different patterns of response have, for example, been described for a single species, the goldfish, held under apparently similar conditions in different laboratories (Spoor, 1951; Anthony, 1961; Falkner & Houston, 1966). Nevertheless, recent studies upon the rainbow trout, an active yet relatively steno-thermal species customarily found in cold, well-oxygenated waters of low carbon dioxide tension, suggest that increases in blood oxygen-carrying capacity may be of some importance at high temperatures (DeWilde & Houston, 1967). No comparable information appears to be available

for more temperature-tolerant species. The present study was accordingly undertaken as a component of a broader investigation of the systemic features of the thermo-acclimatory process in order to provide comparable data for the common carp, *Cyprinus carpio*. This species, though less metabolically active than the salmonid (Beamish, 1964), is decidedly more eurythermal, and is typically encountered in warm waters of relatively low oxygen content and high carbon dioxide tension. The respiratory adjustments of the carp are, moreover, particularly interesting in view of the fact that it can apparently effect facultative transition to a state of anaerobiosis given the appropriate environmental conditions (Blazka, 1958).

The general protocol of the study involved acclimation of groups of animals to selected temperatures (2°, 4°, 7°, 17°, 27°, 33° C.) spanning the near-low to near-high lethal zone of this species for periods of not less than 3 weeks. This interval appears sufficient for completion of the process of thermal acclimation in cyprinid fishes (Brett, 1946; Falkner & Houston, 1966; Heinicke & Houston, 1965). Three series of fishes, representing summer, autumn and winter populations, were used. Upon completion of acclimation, each animal was sacrificed and determinations of erythrocyte concentration, packed cell volume and blood haemoglobin were carried out. From these primary values a number of other haematological indices (mean erythrocytic volume, mean erythrocytic haemoglobin, mean haemoglobin/unit cell volume) were calculated.

Blood oxygen capacity is, of course, dependent upon the nature of the dissociation relationship as well as upon the amount of oxygen carrier available. Nevertheless, the work of Black (1940) and Itazawa (1957) upon this species suggests that virtual saturation is achieved at oxygen tensions similar to those obtaining in the present study. Consequently, it appeared probable that assessment of blood oxygen capacity would be possible in terms of the parameters noted, without consideration of the dissociation curve.

MATERIALS AND METHODS

The specimens used were obtained by seining and trapping from local populations of the Milwaukee River system. Following transportation to the University the animals were initially housed for a 2-week period in 2000 gal. holding tanks. During this time all groups were treated for fungus, and similar surface infections. Acclimation was carried out in 200 gal. self-flushing, fibreglass aquaria housed in a walk-in freezer room, and supplied from a dechlorinating column. Tank temperatures were regulated by resistance heaters whose action was controlled by thermistor-activated electronic relays. Water temperatures were continuously monitored in each tank, and normally ranged between $\pm 0.2^\circ$ C. of set-point. Oxygen levels were close to, or somewhat in excess of saturation values at all times, and well above the tensions which prompt haematological response in the goldfish (Prosser, Barr, Pine & Lauer, 1957). The input water supply was devoid of free chlorine, while pH levels in the various tanks ranged between 7.2 and 7.6, with a mean of 7.4.

The maintenance of poikilotherms upon equivalent nutritional planes under varying temperature conditions constitutes an experimental problem of some difficulty (Prosser, 1962). In the present study all groups were fed once daily, *ad libitum*, in the early morning upon a pelleted ration (Purina Fish Chow). Under this regime they

Table 1

Acclimation temperature (°C.)	Sample size		Sample weight		Sampling date(s)	
	Winter	Summer	Winter	Summer	Winter	Summer
2	.	19	.	398.6 ± 115.1*	.	20, 21 July
4	.	15	.	.	.	4 Nov.
7	23	15	434.4 ± 129.4	364.9 ± 102.5	25 Feb., 4, 19, 21 Mar.	21, 22 July
17	.	17
27	10	13	410.3 ± 148.7	.	25 Feb., 3 Mar.	10 Oct. 4 Oct.
33	.	15	.	.	.	8 Nov.

* Mean ± 1 standard deviation.

remained active and in apparently healthy condition. There can be little doubt that the various groups differed in nutritional state, and the effect of this cannot as yet be ascertained. However, the recent work of Kamra (1966) upon the cod suggests that the primary haematological parameters are affected only by extreme variations in dietary planes.

Following transfer to acclimation aquaria all groups were held for 1 week at input water temperatures (10–14° C.). Subsequently, tank temperatures were adjusted by approximately 1° C. per day until the desired acclimation level was reached. Details relative to acclimation temperatures, sample numbers and weights, and sampling dates are recorded in Table 1 for the several series of fish used.

Sampling. Individual animals were anaesthetized before sampling in aqueous tricaine methane sulphonate (MS. 222, Sandoz), the concentration of which was adjusted as required to produce narcosis in 3–4 min. All samples were drawn by cardiac puncture, the needle of the sampling syringe being passed through the posterior wall of the branchial chamber directly into the lumen of the bulbus arteriosus. The ammonium salt of heparin, used in combination with ethylenediaminetetraacetate (di-sodium EDTA) was found adequate to prevent coagulation.

Haematological procedures. Erythrocyte counts were carried out in quadruplicate as described by Hesser (1960). Duplicate microhematocrit estimates were made upon samples drawn into 1.5 × 75 mm. tubes, sealed with Seal-Ease (Clay-Adams) and centrifuged at 15,000 rev./min. for 4 min. No correction for trapped plasma was applied. All samples in which the plasma showed visible signs of haemolysis or in which cell clumping was observed during erythrocyte enumeration were discarded.

The pyridine haemochromagen method was employed for estimation of haemoglobin levels, the procedure used being essentially that described by Anthony (1961). This method, though superior to the various haematin-derivative techniques (Larsen & Sniezsko, 1960; Anthony, 1961; Henry, 1964) which have been used in haematological studies on fish (e.g. Schiffman & Fromm, 1959; Black *et al.* 1962), includes a number of inactive derivatives of haemoglobin (e.g. sulphaemoglobin) as well as the oxygen carriers. Accordingly, the values reported are to be considered as being somewhat higher than the actual functional concentrations.

Values for mean erythrocytic volume and mean erythrocytic haemoglobin were estimated in accordance with the methods described by Anthony (1961).

Data analysis. Statistical analysis of the data was carried out by the University Computing Center. For each acclimation group, values for the mean, range, standard deviation, and standard error were calculated for each variant. Correlation coefficients relating each variant to all others were provided by the program used, as was an evaluation of the frequency distribution of the data. Group variant comparisons were based upon application of *t*- and *F*-tests. Seasonal series were compared at all common temperatures (i.e. 2°, 4°, 7°, 27° C.), while the values within each series were compared for all temperature combinations. Significance was assigned to comparisons differing at the 0.05 level or better. The results of these analyses are summarized in Figs. 1 and 2.

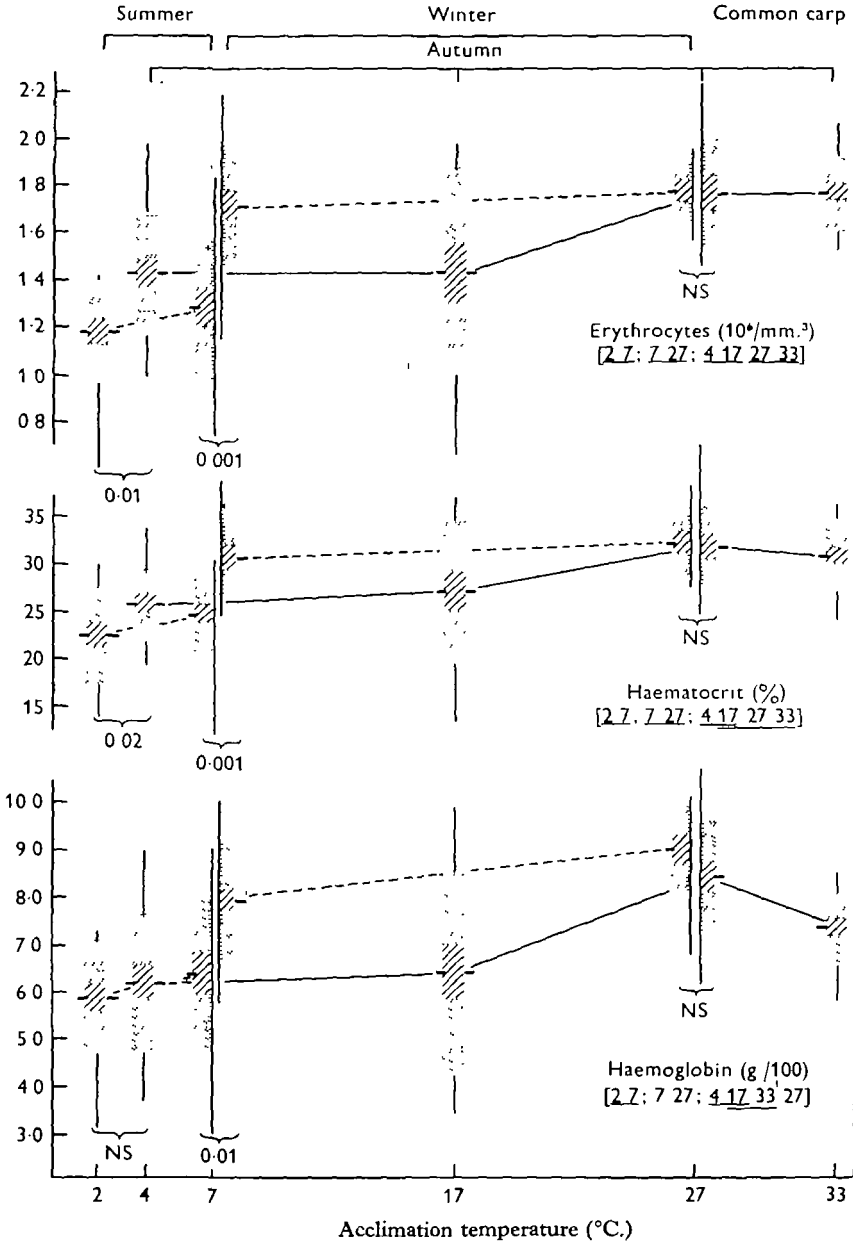


Fig. 1. Erythrocyte numbers (RBC), packed cell volumes (PCV) and haemoglobin (Hb) as a function of acclimation temperature in winter, summer and autumn populations of carp. Vertical line-range; horizontal line-mean; vertical solid bar-one standard error; vertical hatched bar-one standard deviation. Horizontal brackets-level of significance of difference between samples at common temperature. Square brackets, *t*-test summary; underlined groups show no significant difference at the 0.05 level.

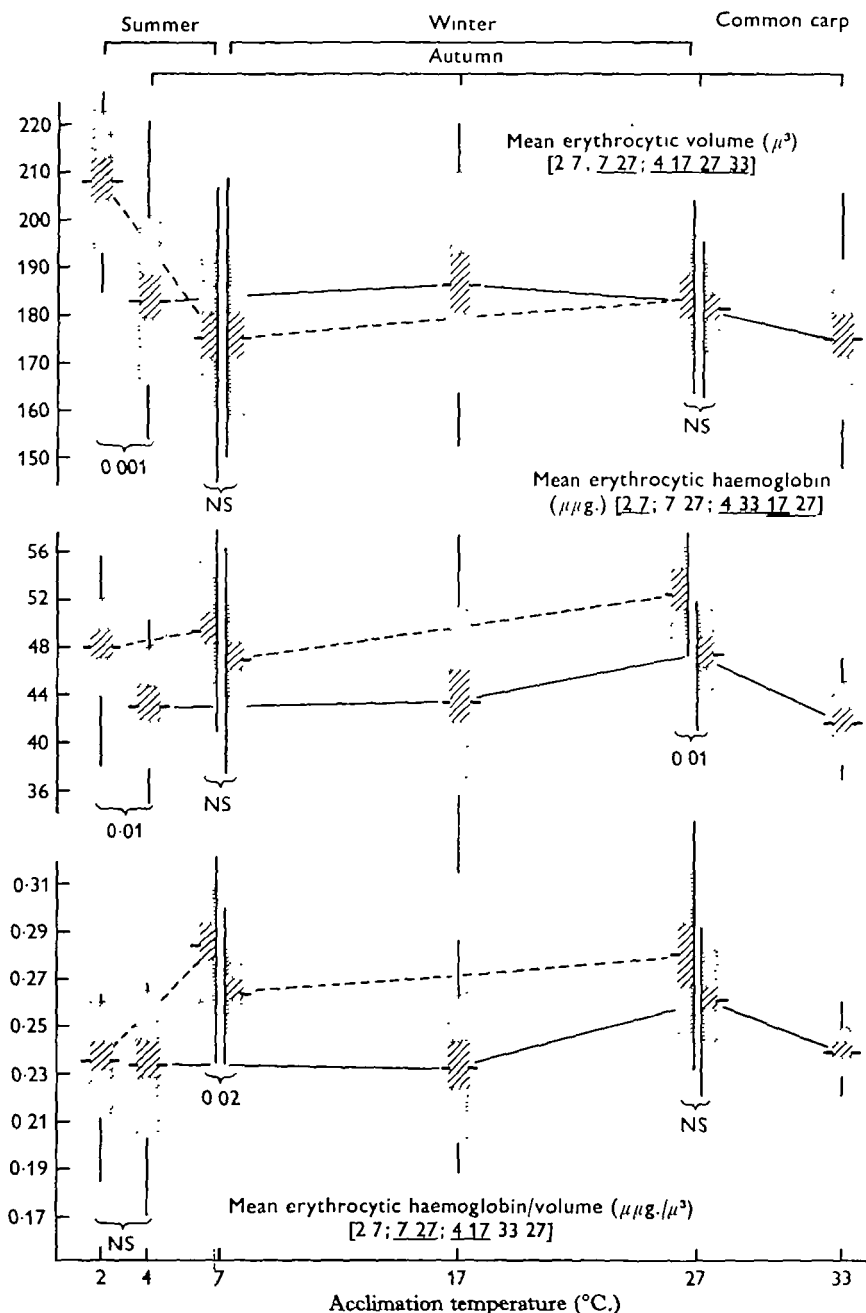


Fig. 2. Mean erythrocytic volume (MEV), mean erythrocytic haemoglobin (MEH) and mean haemoglobin per unit erythrocyte volume (MEH/MEV) as a function of acclimation temperature in winter, summer and autumn population of carp. Vertical line-range; horizontal line-mean; vertical solid bar-one standard error; vertical hatched bar-one standard deviation. Horizontal brackets, level of significance of difference between samples at common temperature. Square brackets, *t*-test summary; underlined groups show no significant difference at the 0.05 level.

RESULTS

Data obtained in the study are summarized in Figs. 1 and 2.

Erythrocyte levels. As is evident from Fig. 1, a general trend toward higher red-cell numbers at elevated acclimation temperatures exists. Thus, the mean erythrocyte concentration at the highest temperature employed (33°C.), $1.77 \pm 0.04 \times 10^6$ cells/mm.³, is 51.2% higher than that typical of the lowest temperature group (2°C. , $1.17 \pm 0.05 \times 10^6$ cells/mm.³). No differences significant at the 0.05 level existed between the groups within the summer and winter series. Among the autumn animals, however, the concentrations at the two lower temperatures ($\text{RBC}_{4^{\circ}\text{C.}} = 1.42 \pm 0.07$; $\text{RBC}_{17^{\circ}\text{C.}} = 1.43 \pm 0.13 \times 10^6$ cells/mm.³) were significantly less ($P > 0.05-0.001$) than those at either 27°C. ($1.74 \pm 0.07 \times 10^6$ cell/mm.³) or 33°C. ($1.77 \pm 0.04 \times 10^6$ cells/mm.³). On a seasonal basis the data suggest increasing erythrocyte levels from summer to winter at the lower acclimation temperatures. At a higher temperature (27°C.), however, there was no significant difference between autumn ($1.74 \pm 0.07 \times 10^6$ cells/mm.³) and winter groups ($1.78 \pm 0.05 \times 10^6$ cells/mm.³).

Packed cell volume. As might be anticipated, packed cell volume varies in much the same fashion as erythrocyte concentration (Fig. 1). The difference between the lowest ($\text{PCV}_{2^{\circ}\text{C.}} = 24.5 \pm 1.0\%$) and highest haematocrits ($\text{PCV}_{27^{\circ}\text{C.}} = 31.7 \pm 0.9\%$) was 29.4%. Again, no differences significant at the 0.05 level of probability were found between the groups of the summer and winter series. Within the autumn series the haematocrit typical of 4°C. animals ($25.7 \pm 1.0\%$), while not greatly different from that of the 17°C. group ($27.1 \pm 2.0\%$), was significantly less than those at higher temperature ($\text{PCV}_{27^{\circ}\text{C.}} = 31.6 \pm 1.2\%$; $\text{PCV}_{33^{\circ}\text{C.}} = 30.9 \pm 0.9\%$). The 17° , 27° and 33°C. animals did not differ significantly. As was the case with erythrocyte number, packed cell volumes tend to rise significantly on a seasonal basis at lower temperatures. No similar variation is seen at higher temperatures, at least among the autumn and winter animals.

Haemoglobin. The results of the study upon haemoglobin levels are indicated in Fig. 1, where the trend recognized with respect to erythrocytes, packed cell volumes and temperature is again apparent. In this instance the highest haemoglobin level was that encountered at 27°C. in the winter series (8.8 ± 0.3 g./100 ml). This was some 51.2% higher than the lowest value recorded ($\text{Hb}_{2^{\circ}\text{C.}} = 5.8 \pm 0.3$ g./100 ml.). The two groups of summer fish showed no significant differences, although bare significance may be attached to difference between the 7° and 27°C. animals of the winter series ($\text{Hb}_{7^{\circ}\text{C.}} = 7.9 \pm 0.2$, $\text{Hb}_{27^{\circ}\text{C.}} = 8.8 \pm 0.4$ g./100 ml.). Among the autumn fish those at 27°C. had a higher haemoglobin content (8.2 ± 0.4 g./100 ml.) than did specimens acclimated to 4°C. (6.2 ± 0.4 g./100 ml.), 17°C. (6.4 ± 0.4 g./100 ml.) and 33°C. (7.3 ± 0.2 g./100 ml.). Of the latter groups only the comparison of the 4° versus 33°C. group revealed a significant difference. The conclusion to be drawn with respect to seasonal variations is essentially that alluded to with respect to erythrocyte number and packed cell volume; an increase in haemoglobin is seen in the progression from summer to winter animals at low, but not at higher, temperatures.

Mean erythrocyte volume. Values derived for mean erythrocyte volume are indicated in Fig. 2. Those for the 7° ($175 \pm 4 \mu^3$) and 27°C. ($183 \pm 5 \mu^3$) groups of the winter series did not differ significantly. Similarly, the values for the autumn animals, although

they tend to decline at higher acclimation temperatures, were not significantly different ($MEV_{4^{\circ}C.} = 183 \pm 5$; $MEV_{17^{\circ}C.} = 186 \pm 7$; $MEV_{27^{\circ}C.} = 183 \pm 5$; $MEV_{33^{\circ}C.} = 175 \pm 4 \mu^3$). However, the value at $2^{\circ}C.$ (summer series), $208 \pm 4 \mu^3$, was significantly higher ($P > 0.001$) than that at $7^{\circ}C.$ ($175 \pm 4 \mu^3$) in the same series. Thus, the data suggest that mean erythrocytic volume is little influenced by moderate to high acclimation temperatures or on a seasonal basis. At the lowest temperatures, however, there is some suggestion of an inverse relationship existing ($MEV_{2^{\circ}C.} = 208 \pm 4$; $MEV_{4^{\circ}C.} = 183 \pm 5$; $MEV_{7^{\circ}C. \text{ summer}} = 175 \pm 4 \mu^3$).

Mean erythrocytic haemoglobin. As indicated in Fig. 2, the mean erythrocytic haemoglobin is not greatly modified as a consequence of thermal acclimation. Thus, the values in the summer series ($MEH_{2^{\circ}C.} = 48 \pm 1$, $MEH_{7^{\circ}C.} = 49 \pm 1 \mu\mu g.$) do not differ significantly, while the differences in the winter series ($MEH_{7^{\circ}C.} = 47 \pm 1$; $MEH_{27^{\circ}C.} = 52 \pm 1 \mu\mu g.$) are only barely significant. Of the groups composing the autumn series those at $4^{\circ}C.$ ($43 \pm 1 \mu\mu g.$), $17^{\circ}C.$ ($44 \pm 2 \mu\mu g.$) and $33^{\circ}C.$ ($42 \pm 1 \mu\mu g.$) did not display significant differences at the 0.05 level. All but the $17^{\circ}C.$ fish were, however, significantly lower than the $27^{\circ}C.$ group ($47 \pm 1 \mu\mu g.$). Seasonally, the summer and winter animals can be regarded as essentially comparable, and characterized by higher mean erythrocytic haemoglobin levels than were typical of the autumn animals.

Mean haemoglobin per unit volume of erythrocyte. Finally, estimates of mean haemoglobin per unit red cell volume are indicated in Fig. 2. Of the three groups examined, the summer fish showed the greatest variation. Thus, the value typical of $2^{\circ}C.$ fish ($0.236 \pm 0.005 \mu\mu g./\mu^3$) is some 17.1% less than that of the corresponding $7^{\circ}C.$ group ($0.284 \pm 0.006 \mu\mu g./\mu^3$), a difference significant at the 0.001 level. The two values for the winter fish ($7^{\circ}C. = 0.264 \pm 0.004$; $27^{\circ}C. = 0.276 \pm 0.011 \mu\mu g./\mu^3$) were not significantly different. Again, the level typical of the $27^{\circ}C.$ group of the autumn series ($0.261 \pm 0.006 \mu\mu g./\mu^3$) was significantly higher ($P > 0.02-0.01$) than the values encountered at $4^{\circ}C.$ ($0.234 \pm 0.001 \mu\mu g./\mu^3$), $17^{\circ}C.$ ($0.234 \pm 0.001 \mu\mu g./\mu^3$) and $33^{\circ}C.$ ($0.239 \pm 0.003 \mu\mu g./\mu^3$). The latter, as is indicated in the *t*-test summary, did not vary significantly. On a seasonal basis there is some suggestion that the winter mean erythrocytic haemoglobin/volume of erythrocyte exceeds that of both summer and autumn fish at intermediate temperatures.

DISCUSSION

Relatively few haematological studies have apparently been carried out upon the carp. Of those known to the authors, that by Field, Elvehjem & Juday (1943) upon specimens held at approximately $5^{\circ}C.$ is the most complete. In this instance the mean erythrocyte level is given as 0.84×10^8 cells/mm³, haematocrit as 31.3%, haemoglobin as 10.5 g./100 ml., mean erythrocytic volume as $311 \mu^3$ and mean erythrocytic haemoglobin as $72 \mu\mu g.$ Thorson (1961) reports a mean haematocrit of 33% for a sample of seven specimens. The red cell count reported by Field *et al.* (1943) for the carp is low by comparison with the animals acclimated to nearby temperatures (2° , 4° , $7^{\circ}C.$) in the present study, while the values for packed cell volume are, with the exception of the $7^{\circ}C.$ winter carp, somewhat higher. Consequently, the mean value for red-cell volume is much above that reported herein. The

average haemoglobin level, 10.5 g./100 ml., is also considerably higher than was observed in the present investigation. In this instance the difference observed may stem from technical variations, since the unmodified haematin procedure yields values some 40% higher than those commonly obtained with the pyridine haemochromogen method (Anthony, 1961; Larsen & Snieszko, 1961).

The occurrence of thermoadaptive changes in the haematology of the carp has received little attention, a situation true for most of the Cyprinidae. The common goldfish is, perhaps, the best-investigated species of this group. However, a series of studies upon the goldfish (Spoor, 1951; Anthony, 1961; Falkner & Houston, 1966) has given rise to a conflicting picture of thermal responses as expressed at the haematological level. For example, while Spoor (1951) reported a positive correlation between erythrocyte numbers and acclimation temperature, Falkner & Houston (1966) found no significant difference between specimens maintained at 20° and 30° C., and Anthony (1961) observed an inverse relationship between red-cell counts and temperature. No consistent variation in haemoglobin with temperature has been observed in this species (Anthony, 1961; Falkner & Houston, 1966), although Anthony (1961) suggests that the oxygen-carrying capacity of single cells is somewhat increased after warm-acclimation. This conclusion may be related to the incidence of an additional haemoglobin polymorph in goldfish maintained at temperatures above 10° C. (Falkner & Houston, 1966). With regard to the other parameters measured, Anthony (1961) and Falkner & Houston (1966) found little effect of thermal acclimation upon haematocrit values, a view also consistent with the findings of Haws & Goodnight (1962) upon the channel catfish and brown bullhead. Finally, while Anthony (1961) reported some increase in the mean erythrocytic volume of cold-acclimated goldfish (5°, 6° C.) relative to those held higher at temperatures (26°, 30° C.), no significant differences were observed in populations acclimated at 20° and 30° C. (Falkner & Houston, 1966).

In the present investigation significant increases in haemoglobin, packed cell volume and red-cell levels were observed. These may be correlated with known variations in the oxygen demand of this species. Beamish (1964), for example, found the oxygen demand of a standard '100 g. fish' to be 17.0 mg./kg./hr. at 10° C. whereas that at 35° C. was 117.3 mg./kg./hr. It will, however, be obvious that the rise in oxygen consumption over this range (690%) far exceeds the increase in oxygen-carrying capacity as represented by the total haemoglobin level. Although the shift to the right in the oxygen dissociation curve typically observed at higher temperatures (Black, 1940) might be expected to amplify oxygen flow through the system, it would seem probable that adjustments of ventilation, perfusion and branchial exchange area must play a large part in meeting temperature-induced variation in oxygen demand.

The carp, like the closely related goldfish (Winberg, 1956; Kanungo & Prosser, 1959), apparently displays the type IV_A form of capacity acclimation (Prosser, 1958). Present interpretation of this type of metabolic modification is not precise, but it has been suggested (Kanungo & Prosser, 1959; Prosser, 1962; Hochachka & Hayes, 1962) that variations in pathways for substrate oxidation, or alterations in enzyme activation energy or concentration may be involved. Whatever be the case, such changes, which must normally occur on a seasonal basis, enhance the oxygen demand of the cold-

acclimated animal. Thus, it is tempting to speculate that the observed progressive seasonal increases in erythrocyte number and haemoglobin levels represent responses which are involved in the normal acclimatory phenomena occurring in the animals.

SUMMARY

1. The haematology of winter, summer, and autumn populations of carp has been investigated as a function of acclimation temperature.

2. Red-cell number, packed-cell volume, and haemoglobin tend to vary directly with temperature. Mean erythrocyte volume is increased at very low temperatures but, like mean erythrocytic haemoglobin and mean haemoglobin/unit erythrocytic volume, tends to be stable at moderate to high temperatures.

3. Although the data suggest a temperature-correlated increase in blood oxygen capacity, it is apparent that the animals must invoke other forms of cardiovascular-respiratory adjustment as well.

4. On a seasonal basis increases in oxygen capacity are seen at low, but not high, acclimation temperatures. It is suggested that this form of response may be related to the pattern of capacity acclimation typical of cyprinid fishes.

The financial support of the National Science Foundation (grant GB-4212) is gratefully acknowledged, as is the co-operation of the Wisconsin State Conservation Department in providing the animals used in the study. Personnel of the Marquette University Computing Center assisted greatly in analysis of the data.

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