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THE EFFECT OF TRAINING ON THE SWIMMING MUSCLES OF THE GOLDFISH (*CARASSIUS AURATUS*)

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SUMMARY

Goldfish (*Carassius auratus*) were exercised continuously for periods of 28 days at swimming speeds of 1·5, 3·0 and 4·5 body lengths per second and their rates of growth were determined. Changes in muscle fibre size were examined, as were changes which occurred in the concentrations of the major chemical constituents of these cells.

These fish, typical of the carp family in that they are found only in still or slowly moving water, did not adapt well to the flowing water environment at any swimming speed. They often grew less than the controls, although consuming much more food. Changes in the composition of the muscle fibres indicated that excess food was not being stored, and also indicated that the major fuel for swimming at all speeds was glycogen. The fish survived well at high speeds and it was suggested that this was due to the ability of the species to metabolize glycogen anaerobically without the production of lactic acid.

INTRODUCTION

The study of exercise in fish can broadly be divided into three categories: sprint, sustained swimming, and training. Much work has been carried out on sprint exercise, with notable work by Bainbridge (1958, 1962), Blaxter & Dickson (1959) and Wardle (1975). These authors have shown that short-duration sprints of very high speed can be achieved but that the fish quickly become exhausted. By far the greatest amount of work has been carried out on changes occurring in fish subjected to sustained swimming. Much of this work has been reviewed by Bone (1966), Love (1970) and Bilinski (1974).

Probably the least studied of the three exercies categories is that of training animals for a period of time in order to observe adaptive changes. Most of this work has been carried out on members of the salmon family for several reasons, the major one being that these fish are normally found in flowing water (Davidson, 1949; Hammond & Hickman, 1966). In a recent publication (Davison & Goldspink, 1977) we have shown that as long as the water speed was not too great, trout in moving water grew much more rapidly than those in still water, and utilized food much more efficiently. The present study examines the effects of the same type of training upon the goldfish, which, like most fellow members of the carp family, is found only in still or slowly moving water.

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MATERIALS AND METHODS

Goldfish (*Carassius auratus*) 8–10 cm in length were obtained from a local supplier and maintained in an aquarium at 12 °C and with a 10/14 h light/dark photoperiod. They were kept in still water tanks and fed daily on a diet of commercial fish-food pellets.

The fish were exercised continuously in a flume, the design of which has been described previously (Davison & Goldspink, 1977). Three sets of experiments were carried out: at swimming speeds of 1·5, 3·0 and 4·5 body lengths per second (bl s^{-1}). All experiments lasted 28 days and were initiated by placing the fish in groups of ten into the flume at a low water speed (< 1 bl s^{-1}) for 2 days to acclimate them to their new environment. The water speed was then steadily increased until the desired speed was attained. The lowest speed was reached after 1 h; the intermediate speed after 36 h; and the highest speed after 7 days. The fish were fed to satiation every morning by introducing pellet food into the top of the flume and allowing the fish to pick it up on its journey downstream. Feeding was stopped when all food was being rejected.

At the end of each 28-day period the fish were individually removed from the flume and sacrificed by a blow to the head. After quickly measuring and weighing each fish, blocks of muscle containing red, pink and white fibres (Johnston, Ward & Goldspink, 1975) were dissected out from a point on the lateral line immediately below the dorsal fin. These blocks were mounted on steel chucks and rapidly frozen. The rest of the fish was then rapidly plunged into liquid nitrogen to prevent any post-mortem changes in the composition of the muscles.

The frozen muscle blocks were cut in a cryostat at -20 °C and the sections, 10 μm thick, were stored below -10 °C until required. The sections were stained for myofibrillar ATPase (Padykula & Herman, 1955; Guth & Samaha, 1970), succinic dehydrogenase (Nachlas *et al.* 1957) and lipid (Pearse, 1960). The total number of red fibres in the sections was determined for each animal and also the mean diameters of red, pink and white muscle fibres (mean of 50 fibres per muscle type per animal).

The frozen fish were warmed to approximately -20 °C to allow sampling of red and white muscle. The samples were immediately frozen in liquid nitrogen, where they were kept until required. Samples were analysed for glycogen stores by the method of Carroll, Longley & Roe (1956) after initially extracting the carbohydrate from the tissues by the method of Seifter *et al.* (1949). Lipid was extracted using the Hanson & Olley (1965) modification of the Bligh & Dyer (1959) technique. This was then assayed using a Biochemica Test Kit (Boehringer Mannheim) no. 15991. Protein levels were estimated by the Biuret method of Gornall, Bardawill & David (1949) after initially digesting the samples in KOH. Water content was determined by drying to constant weight in a vacuum oven.

RESULTS

At the two lower swimming speeds all fish survived until the end of the experiments. At the highest speed an average of 20% had become exhausted by the time the desired speed had been reached, and this figure had risen to 50% by the end of the experiment.

Table 1. *Changes in length and weight of the goldfish after the 28-day experimental period*

Speed (bl s^{-1})			Initial	Final	Change (%)
1·5	Control	Length (cm)	8·54	8·63	+1·0
		Weight (g)	21·00	22·22	+5·8
	Exptl	Length (cm)	8·67	8·79	+1·4
		Weight (g)	21·99	22·09	+0·5
3·0	Control	Length (cm)	8·49	8·56	+0·8
		Weight (g)	22·05	22·23	+0·8
	Exptl	Length (cm)	8·65	8·57	−0·9
		Weight (g)	22·00	20·54	−6·6
4·5	Control	Length (cm)	8·34	8·46	+1·4
		Weight (g)	22·60	22·30	−1·4
	Exptl	Length (cm)	8·61	8·56	−0·6
		Weight (g)	22·90	18·80	−17·9

Table 1 shows the changes which occurred in the size of the fish. All of the control fish, kept in still water tanks, increased in length and weight, although the changes were quite small. At the lowest swimming speed the fish grew at a rate approximately equal to the controls; at the intermediate speed the weight fell by about 7 %; while at the highest speed a much greater fall of 18 % was recorded.

Exercise did not appear to have any marked effect on the size of the red muscle fibres, as the greatest change was an increase of only 4·5 % (Table 2). A slight decrease was noted at the lowest speed although this was associated with a 21 % increase in fibre number. Thus it would appear that the training produced an increase in red muscle mass at the lowest speed due to an increase in fibre number, but that at the two higher speeds much smaller increases occurred. These were due to increases in the size of existing fibres and not to an increase in fibre number. The size of the pink fibres did not change with training at any speed. White fibres increased in mean diameter at all speeds, this being related to the severity of the exercise.

The lack of body growth of the exercised fish was reflected in the chemical composition of the myotomal muscles (Table 3). At the lowest speed, glycogen levels in red muscle were 30 % lower than the controls, while the levels in white muscle were down by 5 %. Lipid had been built up in the red tissue, increasing by 40 %, while a 25 % drop in white muscle lipid was noted. Protein and water levels did not appreciably change. At 3·0 bl s^{-1} the loss of glycogen stores was 51 % in red tissue and 15 % in white. Lipid in red muscle was only 9 % greater than the controls although white muscle lipid had not changed. Again, there was no change in the water content of both muscle types, and no change in white muscle protein, although a 6 % increase was observed in the red. Glycogen levels fell at 4·5 bl s^{-1} also, this time by 80 % and 35 % in red and white muscle respectively. Lipid values fell by 42 % in red tissue and by 5 % in white, and there was an 11 % drop in the protein content of red muscle.

Table 2. *Changes in the number of red fibres in the myotomal muscle and changes in the mean diameter of the red, pink and white fibres*

Speed (bl s^{-1})		Red muscle: Fibre no.	Red muscle: Change (%)	Mean fibre diameter (μm): Red	Change (%)	Pink	Change (%)	White	Change (%)
1·5	Control	1960 ± 44	+21·5	28·13 ± 0·32	−1·5	31·96 ± 0·41	+2·5	46·59 ± 0·74	+4·0
	Exptl	1484 ± 63		27·71 ± 0·34		32·77 ± 0·49		48·52 ± 0·77	
3·0	Control	1960 ± 44	0	28·13 ± 0·32	+4·5	31·96 ± 0·41	+0·5	46·59 ± 0·74	+7·5
	Exptl	1968 ± 45		29·41 ± 0·38		32·15 ± 0·50		50·08 ± 0·78	
4·5	Control	1960 ± 44	0	28.13 ± 0·32	+3·7	31·96 ± 0·41	+0·75	46·59 ± 0·74	+22·4
	Exptl	1950 ± 39		31·98 ± 0·45		32·20 ± 0·59		57·02 ± 2·14	

Table 3. *The chemical composition of goldfish red and white myotomal muscle before and after training*

Speed (bl s^{-1})	Compound	Control: Red	Control: White	Exptl: Red	Exptl: White	Change (%): Red	Change (%): White
1·5	Water (%)	77·4 ± 0·97	79·3 ± 0·1	77·8 ± 0·67 NS	79·5 ± 0·46 NS	—	—
	Protein nitrogen (%)	17·1 ± 0·6	14·6 ± 0·6	17·0 ± 0·5 NS	14·8 ± 0·4 NS	−1·0	+2·0
	Glycogen (mg per 100 g)	1670 ± 75	336 ± 30	1341 ± 76**	322 ± 28 NS	−30	−5
	Lipid (g per 100 g)	2·1 ± 0·15	1·1 ± 0·10	3·0 ± 0·15 NS	0·8 ± 0·05 NS	+40	−25
3·0	Water (%)	75·6 ± 0·88	79·1 ± 0·13	76·5 ± 0·96 NS	79·6 ± 0·31 NS	+1	—
	Protein nitrogen (%)	16·2 ± 0·5	12·2 ± 0·6	17·9 ± 0·4**	11·5 ± 0·4 NS	+6	—
	Glycogen (mg per 100 g)	1848 ± 60	459 ± 44	897 ± 47***	397 ± 22 NS	−52	−15
	Lipid (g per 100 g)	2·1 ± 0·13	0·9 ± 0·05	2·3 ± 0·15 NS	0·9 ± 0·05 NS	+9	−1
4·5	Water (%)	75·5 ± 0·80	77·9 ± 0·49	79·0 ± 0·95**	81·8 ± 1·00**	+4·5	+5
	Protein nitrogen (%)	14·8 ± 0·5	11·3 ± 0·2	13·2 ± 0·3*	11·5 ± 0·6 NS	−11	—
	Glycogen (mg per 100 g)	2122 ± 219	642 ± 130	442 ± 87***	418 ± 110*	−80	−35
	Lipid (g per 100 g)	2·9 ± 0·14	0·8 ± 0·03	1·7 ± 0·15*	0·7 ± 0·04 NS	−42	−5

NS, Not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

DISCUSSION

The goldfish used in this study all survived well over the experimental periods with no exhaustion at the lower speeds and only 50% at the highest. This is in marked contrast to the trout of the earlier study (Davison & Goldspink, 1977) where mortalities always occurred, and at the highest speed most of the animals had died by the fourteenth day. The reasons for the better survival of the goldfish are probably twofold. Firstly, goldfish are very hardy animals, able to withstand very adverse conditions. For example, they are able to withstand prolonged periods under frozen ponds, while their upper lethal limit is 41 °C (Varley, 1967). Secondly, the muscle metabolism of goldfish is probably more advantageous under the training conditions. At a swimming speed of 4·5 bl s^{-1} with the size of fish used in this study, the critical speed will have almost been reached. Critical speed, as introduced by Brett (1964), is the maximum speed which a fish can maintain for a set period of time without becoming fatigued. Near the critical speed, insufficient oxygen will be available in the blood to allow all of the energy requirements to be met by oxidation of fuel to CO_2 (Smit *et al.* 1971). In an animal such as the trout, adapted for aerobic conditions, low-speed swimming appears to be powered by lipid breakdown (Davison, 1976) while high-speed sprinting is supplied by anaerobic breakdown of glycogen to lactic acid (Black *et al.* 1962; Beamish, 1968). This type of animal can swim at high speed only until the concentration of lactate rises to a critical point. With the goldfish, however, evidence points to a different type of metabolism. Results from this present work and of Davison (1976), indicate that glycogen is the major fuel for swimming at all speeds. At low speeds, carbohydrate is probably completely oxidized. At higher speeds the fish must resort to increasing amounts of anaerobic respiration (Smit *et al.* 1971). Work on the anaerobic respiration of this fish (Wunder, 1936; Blazka, 1958; Johnston, 1975) has shown that an oxygen debt is not built up as would be expected if lactate was the end product of anaerobic glycolysis. Smit *et al.* (1971) have also reported that no oxygen debt is repaid after severe exercise. Johnston (1975) has suggested that this is because goldfish can function as facultative anaerobes, producing not lactate, but succinate and alanine as end-products, these being much less toxic than lactate. However, this has only been shown in red muscle, and Driedzic & Hochachka (1975) have put forward other suggestions including the transport of end products to the red muscle for disposal, a proposal much favoured by Wittenberger (1972, 1973).

It is interesting that at the lowest swimming speed the white muscle appeared to be utilizing lipid. A similar observation was reported in the earlier paper on trout. It is probable that at this low speed, sufficient oxygen is reaching the white muscle to allow aerobic respiration, and that this muscle type is selecting lipid for energy production, even though it is classified as very anaerobic. This would be of great benefit to the fish as during normal cruising the white muscle would be used quite often for short periods. The use of lipid here would mean that the glycogen, at a relatively low concentration in this muscle type, would be saved until required for sprinting.

Although the goldfish showed high survival rates at all swimming speeds, the results indicate that these fish did not adapt to the new environment but merely tolerated it for the duration of the experiment. All fish, control and experimental, were given as much food as they would take every day. In all cases the exercised animals consumed

much more food than the controls in still water. However, the growth rates of the exercised fish never exceeded those of the controls and only equalled it at the slowest speed. In training studies on other species of fish, both control and experimental were given equal amounts of food (Hochachka, 1961; Hammond & Hickman, 1966; Greer Walker, 1971; Greer Walker & Pull, 1973). In these studies the growth of the exercised fish was equal to or greater than the controls, indicating a more efficient conversion of ingested food. Davison & Goldspink (1977) fed trained trout to satiation and found very significant increases in food conversion efficiencies except at very high swimming speeds. It would seem that with goldfish exercise does not result in increased conversion efficiencies.

The differences in food conversion efficiencies are probably due to hormonal influences. Studies upon exercise of mammals has shown that this produces an increase in growth hormone levels in plasma (Hunter, Foneska & Passmore, 1965; Keul, 1975). At present a reliable method of determining growth hormone concentrations in fish blood is not readily available, although many studies have shown that injection of growth hormone promotes rapid growth in teleosts (Pickford, 1954; Swift, 1954; Enomoto, 1964). Thus in an animal such as the trout it is probable that once the fish has become acclimated to the flowing water, elevated levels of growth hormone and associated hormones such as thyroxine produce greater growth. These hormones would also be expected to rise in the goldfish, though it is probable that this animal never acclimates to the flowing water and is constantly stressed. This would lead to elevated levels of the hormones associated with stress such as cortisol, adrenaline and noradrenaline. This may have the effect of altering the function of the hormone pool in the blood from anabolism to catabolism. It has been shown that stress produces marked elevation of adrenaline and especially noradrenaline in dogfish plasma (Davison, unpublished data) and work is in progress on a study of the effects of exercise on these catecholamines.

Study of the trout (Davison & Goldspink, 1977) and coalfish (Greer Walker, 1971; Greer Walker & Pull, 1973) has shown that exercise produces increases both in the size and number of myotomal muscle fibres, this being proportional to the swimming speed. In the present study a marked increase in fibre size was noted in the white muscle. As this was proportional to the swimming speed it probably represents increasing usage of this muscle type with increase in speed. A large increase in red fibre number was observed at the lowest swimming speed, although this was not seen at the other two speeds. However, there was very little hypertrophy of the red or the pink fibres at any speed. That the pink fibres did not increase in size is probably an indication of the failure of these fish to acclimate to the flowing water, particularly as these fibres have been shown to have an important function in carp at intermediate swimming speeds (Davison, Goldspink & Johnston, 1976; Johnston, Davison & Goldspink, 1977).

This study has indicated that the goldfish is not able to adapt to a flowing water environment despite being very hardy and able to withstand high water speeds quite well. It is probable that these findings also apply to animals in the wild and thus give an indication of the reasons why these fish are only found in still or very slowly moving water.

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