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Group Effect on the Respiration of the Medaka *Oryzias latipes*

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Abstract Effects of grouping on respiration of the medaka *Oryzias latipes* were investigated using through-flow respirometers and sealed flasks. The oxygen tension of water was measured with polarographic oxygen electrodes. The potential changes accompanying respiratory movements were recorded with electrodes of platinum, active and indifferent, in order to determine the ventilatory frequency. The oxygen consumption and the ventilatory frequency of isolated fish in the respirometer tube did not change when the fish were placed in contact through vision alone with their own mirror images, or with others of the same species. The oxygen consumption of isolated fish in the sealed flask was also not affected by visual contact with others of its kind. The oxygen consumption of isolated fish in the sealed flasks slightly decreased when they were placed together in a group of formerly-isolated fish in another flask filled with water which was exactly the same as the total volume of water used for each isolated fish. However, the decrease was too slight to suggest that the metabolic rate of isolated fish is influenced by the changes in visual conditions and by direct contact with others of its kind.

Concerning the effect of aggregation and of isolation upon the physiological processes indicated by the rate of oxygen consumption, Bowen (1932) showed that catfish in groups of four have a greater rate of oxygen consumption per fish than do those in isolation, but the difference is not statistically significant, whereas Schuett (1933) found that the average oxygen consumption of each fish in a group of four goldfish placed in a given volume of water was less than the oxygen consumption of a solitary fish isolated in the same volume of water. He also showed that the decrease in oxygen tension of the medium and the accumulation of carbon dioxide or other metabolites was probably not responsible for the difference in oxygen consumption. However, Schuett (1934) stated that the unmodified Winkler method for determining oxygen tension, as used in his previous experiments, was at fault and found no significant difference in oxygen consumption between goldfish in isolation and in groups of four.

Schuett (1934), and Escober, Minahan and Shaw (1936) demonstrated increased locomotor activity of the goldfish when isolated rather than when in a group of four. Shlaifer (1938) also found that an isolated goldfish consumed more oxygen and had a higher rate of locomotor activity than did each fish in a group of two or four. According to Shlaifer (1939), the effect of numbers upon the oxygen consumption of grouped and isolated goldfish, manifested by a decreased rate of oxygen use in the group, is lost when the fish are placed in the dark or are blinded. In a recent paper by Uematsu (1971) more detailed attention has been given to the relationship which exists between grouping and both oxygen consumption and locomotor activity of the guppy. It is found that there are a higher rate of feeding but smaller rates of both oxygen consumption and locomotor activity if the fish are aggregated than if they are isolated. Itazawa et al. (1978), having studied the group effects of oxygen consumption on rainbow trout and medaka, stated that grouped fish or a fish in a large group reduce their oxygen consumption, while an isolated fish or a fish in a small group exhibit a higher rate of oxygen consumption.

It was the purpose of the present study to repeat the analysis of group effect upon consumption of oxygen in the medaka using a polarographic oxygen electrodes for determining oxygen tension.

Material and methods

Medaka, *Oryzias latipes*, weighing between 120 and 420 mg were used. The fish were collected from ponds and transferred into glass basins supplied with well-aerated tap water. About ten individual medaka were kept together with unicellular algae and microscopic freshwater invertebrates throughout the experiment.

Two methods were used to determine the respiration of the fish. In the first, a fish was kept in a cylindrical tube of about 5 mm diameter through which well aerated water passed continuously. The fish usually remained quiet and breathed regularly for prolonged periods under these conditions. However, in order to avoid the effect of handling, a delay of at least thirty minutes after introduction was required in this experiment before the measurements were taken. The oxygen tension of the water, before and after passing the fish in the respirometer tube, and its ventilatory frequency were measured successively using a method similar to that of Umezawa and Watanabe (1973).

The second method was designed to measure the amount of oxygen consumed by the fish placed in a sealed container filled with a known oxygen tension of water. After a definite time-lapse water in the container was put into a syringe and its oxygen tension was determined with an oxygen electrode similar to that used by Umezawa and Watanabe (1973).

Results

The effect of visual contact with mirror images. In the present experiment, measurements were made of oxygen consumption and ventilatory frequency in relation to the changes in visual environment which occurred when an isolated fish in the respirometer tube was alternately placed in contact with a mirror and without mirror. The rates of oxygen consumption and ventilatory rates in the front mirrored conditions were not significantly different ($P > 0.1$) from those under side mirrored conditions.

An example relating the side mirror-images to oxygen consumption and ventilatory frequency is given in Fig. 1. The response of the gills was a slight bradypnoea without the mirror, and the ventilatory rate fell and then maintained a steady level, whereas it was a tachypnoea when using a mirror, although an initial momentary decrease in ventilatory frequency was observed at the end of the

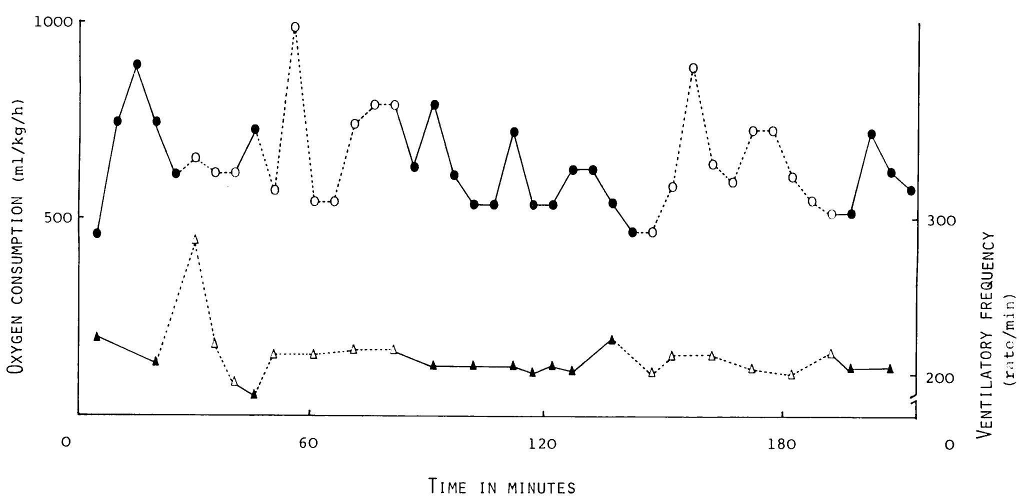

Fig. 1. Graphs showing the changes in oxygen consumption (○, ●) and ventilatory frequency (△, ▲), which accompany the changes in optical conditions of which an isolated fish in the respirometer tube was alternately in visual contact with a mirror (○, △) and without a mirror (●, ▲). (Medaka ♀ 224 mg, flow rate 216 ml/h, 25°C).

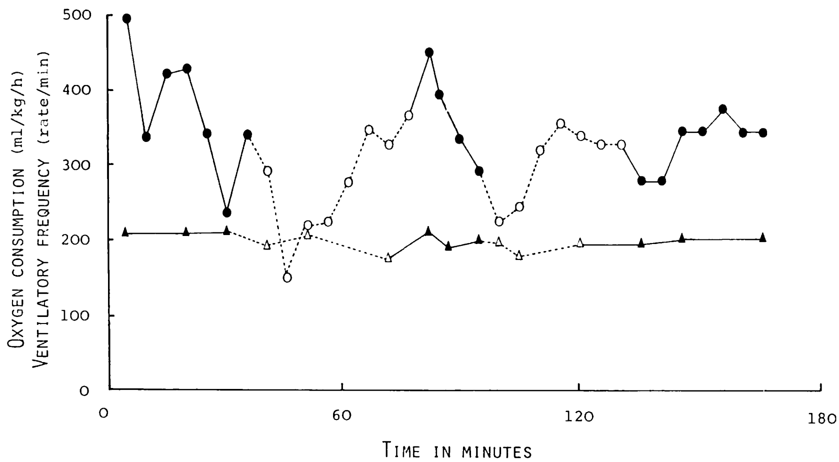

Fig. 2. Graphs showing the changes in oxygen consumption (○, ●) and ventilatory frequency (△, ▲), which accompany the changes in optical conditions of which an isolated fish in the respirometer tube was alternately placed in contact through vision alone with others (○, △) and without others of its kind (●, ▲). (Medaka ♂ 242 mg, flow rate 132 ml/h, 25°C).

experiment. The changes in the condition with and without the mirror were not effective stimuli producing changes in the oxygen consumption, although it showed a marked rise at the onset of both conditions.

Comparisons obtained between mirrored and non-mirrored states are given in Table 1, in which the values of oxygen consumption and ventilatory frequency for each fish are obtained on the average from successive measurements of at least thirty minute durations after the fish have been placed in contact with the mirror and without the mirror. This table indicates that with mirror images there is a rise in the mean of oxygen consumption but no change in the mean of ventilatory frequency. However, the values of t obtained from this table are 0.910 for the oxygen consumption and 0.120 for the ventilatory frequency. There were no statistically significant differences between the means in the former ($P>0.2$) and in the latter ($P>0.5$).

The effect of visual contact with others. Experiments were also carried out by means of the first method, by which an isolated fish in the respirometer tube was alternately placed in contact through vision alone with others (grouped) and without others (isolated), as was used by Umezawa et al. (in press).

Figure 2 shows a typical result obtained with an isolated fish. When the fish was placed in contact with others it showed signs of a momentary bradypnoea and simultaneously low rates of oxygen consumption. However, the oxygen consumption increased progressively, whereas the ventilatory frequency did not increase as much. When the fish did

Table 1. Oxygen consumption and ventilatory frequency measured for each fish in the respirometer tube, when placed in contact with a mirror (WM) and without a mirror (NM). (Flow rate 162～216 ml/h, 25°C). * Student's t-test.

Variation		1	2	3	4	5	6	7	8	9	10	Mean	P*
Oxygen consumption (ml/kg/h)	WM	614	700	629	294	984	685	379	319	292	240	513.6	>0.2
	NM	714	588	586	234	816	855	322	301	291	141	484.8	
Ventilatory frequency (rate/min)	WM	237	218	209	214	263	252	206	204	200	214	221.72	>0.5
	NM	192	211	197	228	255	240	240	216	228	201	220.8	

not find other fish capable of movement, it showed signs of a slight tachypnoea and simultaneously high rates of oxygen consumption, although decrease in oxygen consumption followed by subsequent increase was found in some cases.

Comparisons between isolated and grouped fish on oxygen consumption and ventilatory frequency are summarized in Table 2, in which the values of oxygen consumption and ventilatory frequency are obtained on the average from successive measurements in the same manner as described for Table 1. This indicates that the mean of oxygen consumption in groups, except ventilatory rate, is slightly higher than that in isolated fish. However, the values of t obtained from this table were 0.476 for oxygen consumption and 0.038 for ventilatory frequency. It indicates that the rate of oxygen consumption and the ventilatory rate of a solitary fish placed in the respirometer tube is not significantly different ($P>0.5$ in each case) from those of the same fish placed in contact through vision alone with others of its kind.

A series of experiments was made by means of the second method, in order to compare the amount of oxygen consumed by an isolated fish in a sealed flask filled with a definite amount of water, which was immersed in a basin (isolated), and that consumed by the same fish when it was placed in visual contact with others of its kind which were introduced into the basin (grouped). Each measurement was alternately performed with intermission for about one hour. The results (Table 3) showed that the amount of oxygen consumed by a fish when it was isolated differed somewhat from that consumed by the same fish when it was grouped. However, the values of t obtained from this table was 1.508. Thus, it was found that the statistical significance was not at all high ($P>0.1$). The trend obtained here coincides with that obtained in Table 2.

The effect of direct contact with others. Another series of experiments was also performed by means of the second method, in order to compare the amount of oxygen

Table 2. Oxygen consumption and ventilatory frequency measured for each fish in the respirometer tube, when placed in contact through vision alone without others (isolated) and with others of its kind (grouped). (Flow rate 178~240 ml/h, 25°C). * Student's t-test.

Variation		1	2	3	4	5	6	7	8	Mean	P*
Oxygen consumption (ml/kg/h)	Isolated	462	334	493	383	368	619	680	331	458.8	>0.5
	Grouped	421	387	672	292	320	693	761	252	474.8	
Ventilatory frequency (rate/min)	Isolated	216	230	240	205	204	274	233	218	227.5	>0.5
	Grouped	216	216	236	214	212	265	230	230	227.4	

Table 3. Amount of oxygen consumed by each fish in the sealed flask, when placed in contact through vision alone without others (isolated) and with others of its kind (grouped), during a thirty minute duration at 25°C. * Student's t-test.

Variation		Fish 1	2	3	4	5	6	7	8	Mean	P*
Body weight of fish (mg)		230♂	237♂	291♂	298♂	209♀	212♀	281♀	205♂		
Volume of water in flask (ml)		57	59	57	57	62	62	59	57		
Amount of oxygen consumed (ml/kg/h)	Isolated	597	675	627	610	605	494	704	473	598	>0.1
	Grouped	424	578	464	612	593	400	588	645	538	

removed by fish in isolation (isolated) and the amount of oxygen removed by fish in aggregation (grouped). Ten fish were kept solitary in each sealed flask filled with a definite amount of water. The amount of oxygen removed by the solitary fish was measured for a definite period of time after the introduction of fish into the flask had stopped. When these fish were recovering fairly well from the experiments, they were all transferred together into another sealed flask filled with water of exactly the same amount as the total volume of water used for each isolated fish. Then, the amounts of oxygen removed by these fish were again determined.

An example relating isolated and grouped fish to oxygen consumption is given in Table 4. The mean amount of oxygen consumption of fish in isolation was somewhat larger than the oxygen consumption of the fish in a group of ten. In the comparison of four determinations between isolated and grouped fish on oxygen consumption (Table 5), there was a slightly statistically significant difference ($P \leq 0.02$). However, the statistical significance was assumed when $P < 0.001$, because there were only four determinations. It does not seem, therefore, that the fish in a group of ten have a smaller rate of oxygen consumption per fish than do those in isolation.

Table 4. Comparing the oxygen consumption of ten fish in isolation in definite amounts of water and that of the same ten fish in their total volume of water.

No. of fish isolated	Weight and sex of fish (mg)	Volume of water (ml)	Oxygen consumption (ml/kg/h)
1	215 ♂	57	520
2	246 ♂	62	413
3	235 ♀	57	439
4	272 ♀	59	336
5	420 ♀	59	490
6	256 ♂	57	460
7	248 ♀	61	450
8	240 ♂	60	633
9	180 ♀	58	661
10	236 ♀	57	505
Mean			491
Group of ten	2548	587	445

Table 5. Results of four experiments, showing the oxygen consumption of isolated and grouped fish (25°C). * Student's t-test.

	Experiment				Mean	P*
	1	2	3	4		
Isolated	491	536	450	510	496.8	≤ 0.02
Grouped	445	521	424	480	467.5	

Discussion

How to determine the amount of oxygen removed by fish is an important problem in studies where the effect of grouping on the respiration of the fish are examined. Determinations of oxygen concentration in the medium had been made by the Winkler method. There were some objections to this method, e.g. (1) the removal of iodine from samples, which was caused by the presence of slime or of excretion products of the fish; and also (2) the presence of nitrites produced by the fish, which led to an error in the analysis of oxygen dissolved. However, the defect in the first case could avoided by using experiments of a much shorter duration (Schuett, 1933). To eliminate errors due to nitrite contamination the permanganate modification of the Winkler method was corrected by Schuett (1934) and Shlaifer (1938).

Although it has undergone various improvements, there were some problems avoiding air contamination during the course of the experiment. Different methods of experimentation have been used by various investigators. Determinations of the oxygen concentration in the medium were made with a sealed container into which fish were placed (Schuett, 1933; Uematsu, 1971). Such a closed method may be criticized not only because of the increase in carbon dioxide and other products of metabolism, but also because of the decrease in oxygen dissolved in the medium. Another determination of the oxygen concentration in the medium was performed with a flask containing a fish, into which a layer of heavy mineral oil was poured through the neck (Bowen, 1932; Schuett, 1933). Samples were withdrawn for analysis by means of glass siphoning tubes kept in the flasks. In this method the same criticisms apply as in the first case, as has been pointed out by Schuett (1933). He also used in his studies apparatus which was somewhat modified from a set-up employed by Keys (1930). Determinations of the oxygen consumption were made on fish kept in a chamber through which a steady rate of freshwater passed continuously, by means of collecting water from time to time and by comparing it with control water. According to Schuett (1933), 'in this type of experiment the possibility of contamination by excretory products is at a minimum, but even here there is a chance for some effect from such a substance as carbon dioxide. The rate of flow must be slow enough to measure the amount of oxygen removed by the isolated fishes, and at the same time fast enough to maintain a supply of fresh water to the respirometer chambers, so that the diminished oxygen will not enter as a possible factor.'

In recent years oxygen electrodes have been used to determine the concentration of oxygen dissolved in water which passed over the fish at a constant flow rate (Hughes and Umezawa, 1968a, b; Umezawa and Watanabe, 1973). This is one of the most practical and approved methods, since it seems to have little harmful effect due to the presence of slime or excretion products, nitrite contamination, and the reduction in oxygen concentration in the medium.

Some of the results obtained in the present experiments, in which the oxygen consumption was measured by the constant flow method mentioned above, indicate that the respiration of isolated fish in the respirometer tube is not affected by contact through vision alone with its own mirror images, or with others of the same species. The oxygen consumption and the ventilatory rate of the isolated fish are not significantly different ($P>0.2$ and $P>0.5$, respectively) from those of the same fish in visual contact with a mirror self-image, or significantly different ($P>0.5$ in each case) from those in visual contact with other fish of its kind. However, oxygen consumption per unit weight was lower in grouped fish or fish in a larger group than that of an isolated fish or fish in a smaller group, and there were statistically significant differences between their means, according to Itazawa et al. (1978) by whom the measurements of the oxygen consumption in the medaka were made with the constant flow method.

Determination of oxygen consumption of the fish, by a method using a sealed flask which is similar to that used by Schuett (1933) for the guppy and others and by Uematsu (1971) for the guppy, give evidence that there is no statistically significant difference ($P>0.1$) between the oxygen consumption of isolated fish kept in the sealed flask and that of the fish which are placed in contact through vision alone with others of its kind.

According to Shlaifer (1939), 'the effect of numbers upon the oxygen consumption of grouped and isolated goldfish, manifested by a decreased rate of oxygen in the group, is lost when the animals are placed in the dark or are blinded.' It seems, however, that the metabolic rate of an isolated fish is not influenced by visual contact with its own mirror images or with others of the same species in the medaka.

Experiments performed by means of sealed flasks show that the values of oxygen consumption of isolated fish are larger than those in a group of ten of the same isolated fish and the comparison of four determinations indicates a statistically significant ($P\leq0.02$). However, statistical significance is assumed when $P<0.001$, since only four determinations are made in the present experiment. It is thus surmised that the oxygen consumption of the isolated fish does not decrease when they are placed in groups of ten.

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メダカの呼吸に及ぼす群効果

梅澤俊一・渡部英機

メダカの呼吸に及ぼす群効果を流水式及び密閉止水式呼吸室を用い調査した．酸素消費量は，ポーラログラフ酸素電極により測定した魚の呼吸前後の水の溶存酸素量の差から求め，また呼吸頻度は流水式の場合のみ鰓蓋の呼吸運動に伴う筋電位を白金電極により記録して調べた．呼吸室に入れられた単独個体のメダカは，鏡によってその鏡像を眺め，あるいは周囲に配した同種のメダカに接した場合に，酸素消費量及び呼吸頻度を変化することはなかった．他方，密閉止水式の場合，一定量の水における各単独個体の酸素消費量は，各個体をそれぞれ一定量の水に分離収容した場合より，それらの魚をまとめてそれぞれの水の総量に等しい水においた場合の方が僅かに少ないという傾向を示した．しかしその差は小さく，従ってメダカの呼吸は群効果をうけないものと考察した．

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