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CHANGES IN ACTIVITY AND VENTILATION IN RESPONSE TO HYPOXIA IN UNRESTRAINED, UNOPERATED DOGFISH (*SCYLIORHINUS CANICULA* L.)

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SUMMARY

Daily activity cycles, together with changes in activity and ventilation frequency in response to hypoxia (P_{O_2} about 8 kPa), have been measured in unrestrained, unoperated dogfish. Continuous recording of activity over 48 h reveals that dogfish are essentially nocturnal, being three to four times more active at night than during the day. In relatively inactive, diurnal dogfish, rapid reduction of environmental P_{O_2} does not cause any significant increase in swimming activity, whereas prolonged hypoxia actually appears to suppress activity. In more active, nocturnal dogfish, rapid reduction of environmental P_{O_2} causes an immediate reduction in activity which remains suppressed throughout the hypoxic period. It is concluded therefore that increases in circulating catecholamines in response to hypoxia are the result of hypoxia alone, rather than of any increase in locomotory activity.

In resting diurnal dogfish, ventilation frequency is lower than has previously been reported for this species and, contrary to previous reports, increases markedly by 49% in response to hypoxia. It appears that in previous studies on confined dogfish, respiratory frequency, and probably ventilation volume, may have been elevated to near maximum levels even in 'resting' normoxic fish. This may have profound effects on so-called resting values for oxygen transfer in this species.

INTRODUCTION

Frequently, studies of the cardio-respiratory responses of fish to induced environmental hypoxia require both confinement of the experimental animal and invasive techniques for recording physiological variables (Holeton & Randall, 1967*a*,*b*; Butler & Taylor, 1971, 1975; Short, Taylor & Butler, 1979). These procedures probably subject the fish to some degree of stress, even during so-called 'resting' conditions, while also preventing any behavioural responses to the hypoxic stimulus.

Hypoxia is reported to cause an increase in activity in resting teleost and elasmobranch fish (Randall, 1970) and a decrease in activity in swimming teleost fish (Davis, Foster & Doudorokk, 1963; Kutty, 1968), so it appears that during hypoxia

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there may be conflict between the need to escape from the hypoxic environment and the necessity to reduce the demand for oxygen by reducing activity. Both hypoxia (Butler, Taylor, Capra & Davison, 1978; Butler, Taylor & Davison, 1979) and induced struggling (Opdyke, Carroll & Keller, 1982) stimulate the release of adrenaline and noradrenaline into the bloodstream of elasmobranchs, and these catecholamines have been implicated as playing a role in the control of the cardiovascular systems of fishes (Randall, 1982; Butler & Metcalfe, 1983). Since an attempt to escape from the hypoxic environment may cause restrained fish to struggle, it becomes less clear whether the associated increase in circulating catecholamines is due to the hypoxic stimulus alone, or due to any increase in activity.

Changes in activity in response to hypoxia appear to depend on the state of activity prior to hypoxia (see above). Since many species of both freshwater and marine fish exhibit daily cycles of activity (Harder & Hempel, 1954; Wikgren, 1955; Kruuk, 1963) it is necessary to establish the daily activity pattern of any particular fish so as to be able to examine the effects of hypoxia in both the active and inactive state.

The present study is an attempt to quantify the daily activity cycle of the dogfish and to examine the changes in activity in response to hypoxia in both active and inactive fish. The fish were unrestrained and unoperated as the prime objective has been to study fish in as truly a resting state as laboratory conditions will permit. The experimental conditions also allowed the measurement of normoxic and hypoxic respiratory frequency in otherwise unstressed fish.

MATERIALS AND METHODS

Twenty-six dogfish of either sex were used in these experiments. These were obtained from the Plymouth laboratories of the Marine Biological Association of the U.K. and transported in oxygenated sea water to the aquaria in Birmingham where they were held in aerated, recirculating sea water, maintained at 15 °C for at least 2 weeks prior to the experiments. Fish were fed periodically on either sprats or whitebait obtained from a local fishmonger. Fish were not anaesthetized and so could not be weighed. However, since no weight-dependent variables were to be measured, this omission was not considered to be important. It was estimated that the fish weight was in the range 0·5–1·0 kg.

Daily activity cycle experiments

Each of six dogfish was placed in an actograph which consisted of a large glass tank containing about 130 l of filtered, aerated, recirculating sea water maintained in a constant temperature room at 15 ± 1 °C. Movement of the fish within the tank activated a large glass recording plate (110 cm × 28 cm × 0·5 cm) which rested horizontally, about 1 cm above the base of the tank on four sponge rubber blocks situated at the corners of the plate. A strain gauge transducer (PYE Ether Ltd, UF1) situated vertically above one end of the tank was connected to the recording plate by a fine tungsten wire. Any whole body movement of the fish caused slight movements of the recording plate which were observed as changes in tension exerted upon the strain gauge *via* the tungsten wire. The output from the strain gauge was displayed on a pen recorder (George Washington Ltd) writing on curvilinear coordinates. This

method allowed continuous recording of activity in the absence of any disturbance of the fish by the experimenter. Only whole body movements (i.e. swimming) were detected and the recording device was not sensitive to small movements such as ventilation. A Perspex baffle was placed vertically at one end of the tank, between the main body of the water and the tungsten wire, to prevent the fish from becoming entangled in this wire. A thin layer of sand and stones was fixed to the upper surface of the recording plate with silicone rubber adhesive (Dow-Corning) since fish appeared to be reluctant to settle on a plain glass surface. Sea water circulated around the tank *via* a gas exchange column at a rate of about $3\,l\,min^{-1}$. The tank was illuminated from the rear by diffuse tungsten lighting, and all other sides of the tank were covered so that the fish received the minimum of visual disturbance from the experimenter. During the whole experiment fish were not fed, and were exposed to a light/dark regime of 14 h light/10 h dark. This was similar to the day and night lengths to which the fish had been exposed in the holding tanks prior to the experiment. Having been placed in the actograph, fish were allowed 3 days to acclimate to the experimental conditions and to recover from any trauma caused by handling. Activity was then recorded continuously over a period of 48 h.

Changes in activity and ventilation in response to hypoxia

Changes in activity in response to hypoxia were monitored in separate diurnal and nocturnal experimental series. The actograph used in these experiments was a modified form of that described earlier. The tungsten wire and strain gauge were replaced by a pressure transducer (Druck Ltd PDCR 75/2) connected to a blind-ended loop of water-filled rubber tube which was placed under one end of the recording plate. Movement of the recording plate caused by the fish swimming was observed as changes in pressure exerted on the pressure transducer, the output from which was displayed on a pen recorder (Ormed Ltd) writing on rectilinear coordinates. This modification of the actograph obviated the need for a tungsten wire passing through the water column. However both forms of the actograph were equally sensitive to swimming movements.

The actograph was connected, *via* a pair of three-way taps, to a reservoir which contained a volume of aerated sea water about twice that contained by the actograph. With the three-way taps in the 'open' configuration, water was drawn by a pump from the reservoir into the actograph at a rate of about $20\,l\,min^{-1}$, and then overflowed back to the reservoir. With the three-way taps in the 'closed' configuration, water was pumped at the above rate only around the actograph, thus isolating it from the reservoir.

In both diurnal and nocturnal experiments fish were again allowed 3 days to acclimate to the experimental conditions. Activity was then recorded continuously for 3 h in normoxic water followed by 1 h of rapidly induced hypoxia. For most of the normoxic recording period the actograph was 'open' to the reservoir. About 75 min before the onset of hypoxia the actograph was 'closed' from the reservoir without disturbing the fish. The water in the reservoir was then made hypoxic by bubbling nitrogen through it at an appropriate rate, without affecting the partial pressure of oxygen (P_{O_2}) of the water in the actograph.

Rapid hypoxia was initiated after 3 h of normoxic recording by 're-opening' the

actograph to the reservoir. Water samples for the measurement of P_{O_2} were taken in 5-ml syringes from the actograph at 0, 1, 5, 10, 15, 30 and 60 min during the 1 h of hypoxia. Partial pressures of oxygen were measured using an Acid-Base analyser (Radiometer, PMH 71 Mk 2) having its oxygen electrode housed in a glass cuvette maintained at the experimental temperature. In the diurnal experiments, respiratory frequency was recorded by eye just before, and at the end of, the 1 h of hypoxia by measuring the time taken for 100 ventilations.

The actograph was illuminated as described earlier. In diurnal experiments the fish received continuous illumination and activity experiments were performed between 10.30 h and 15.30 h. In nocturnal experiments the fish received a light/dark cycle of 15 h light/9 h dark which was similar to the day and night lengths to which the fish had been exposed in the holding tanks. Nocturnal activity experiments were performed during the first 4·5 h of darkness, between 20.00 h and 00.30 h. No fish was used in both experimental series.

Activity during the first 15 min and final 30 min of hypoxia have been calculated as the total number of minutes of swimming summed for all fish. These values were compared using the d-test (Clarke, 1969) with the mean normoxic activity calculated as the mean of the total minutes of activity summed for all fish for successive 15- and 30-min periods during normoxia. Other mean values have been compared using the Student's t-test, the term 'significant' refers to the 95 % level of confidence ($P < 0{\cdot}05$) unless indicated otherwise. Mean values have been expressed ± S.E. of mean.

RESULTS

Daily activity cycles

The mean activity of six dogfish measured continuously over 48 h is presented graphically in Fig. 1. Activity has been expressed as the mean number of minutes active in each successive hour. It is apparent that during much of the day dogfish are not very active (about 7 min h^{-1}) (Fig. 1). However, during the night the activity increased three- to four-fold and reached a maximum of about 25 min h^{-1} towards the middle of the dark period.

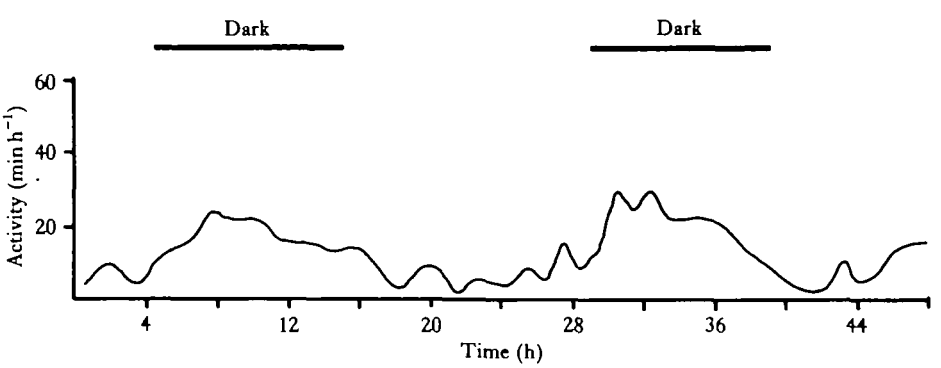

Fig. 1. A graphical representation of the mean hourly activity of six unrestrained dogfish in normoxic water over a 48-h period with a light/dark cycle of 14 h/10 h.

Changes in activity and ventilation in response to hypoxia

The activity of 12 diurnal and 8 nocturnal dogfish during 3 h in normoxic water (P_{O_2} about 21 kPa) followed by 1 h of rapidly induced hypoxia (P_{O_2} about 8 kPa) is presented in Fig. 2 which shows the total number of fish active together with the activity of each individual fish. Mean P_{O_2} of the water and mean normoxic and hypoxic ventilation frequencies are also shown.

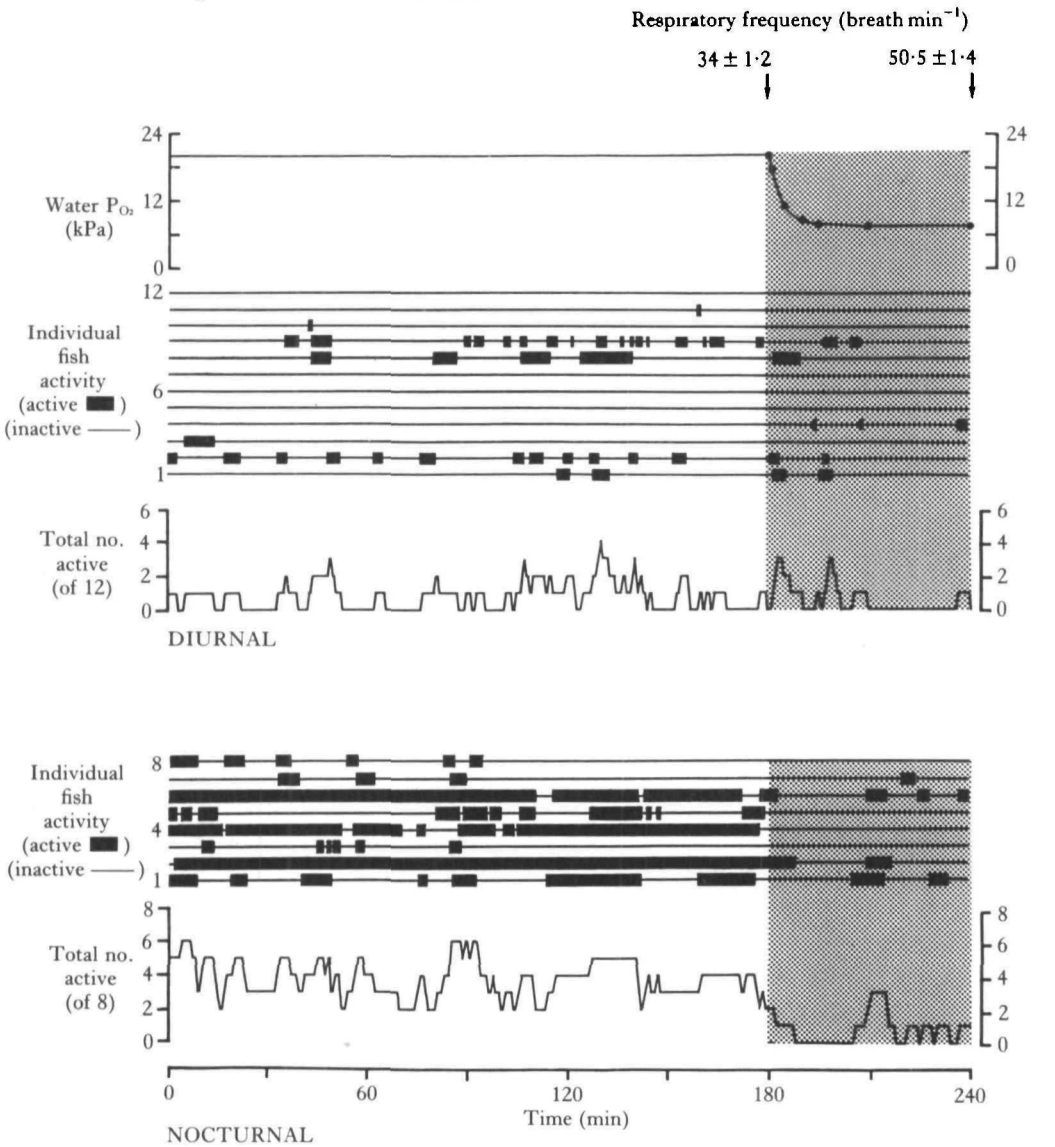

Fig. 2. A graphical representation of the activity of 12 diurnal and 8 nocturnal dogfish during 3 h in normoxic water (P_{O_2} = 21 kPa) followed by 1 h of rapidly induced hypoxia (P_{O_2} = 8 kPa), showing for each the minute-by-minute total number of fish active together with the activity of each individual fish. Mean water P_{O_2} and mean (±S.E. of mean) normoxic and hypoxic respiratory frequencies are also shown. The period of hypoxia is indicated by shading.

The activity of diurnal dogfish in normoxic water was very variable and of the 12 fish studied, 5 remained inactive for the entire 3 h of normoxia. During the 1 h of hypoxia, 7 fish remained completely inactive and only 3 fish swam at all during the first 15 min as the water P_{O_2} fell from 21 kPa to about 8 kPa. Total activity during the first 15 min of hypoxia (16 min) was not significantly different ($P > 0{\cdot}1$) from the mean activity during 15 min periods in normoxia ($11{\cdot}9 \pm 2{\cdot}12$ min). During the last 30 min of hypoxia, as the water P_{O_2} was maintained at about 7·7 kPa, only 1 fish was active and this only for 3 min. The total activity during this 30 min of hypoxia was 3 min and this is significantly lower than the mean normoxic activity during 30-min periods ($23{\cdot}8 \pm 4{\cdot}15$).

In normoxic water, mean respiratory frequency was $34 \pm 1{\cdot}2$ breaths min^{-1} and this increased significantly, in response to hypoxia, by 49 % to $50{\cdot}5 \pm 1{\cdot}4$ breaths min^{-1}. Although no measurement of ventilation volume was recorded, it was apparent that the vigour of the ventilatory movements was greatly increased in response to hypoxia.

In contrast to diurnal dogfish, nocturnal dogfish were very active (Fig. 2). All fish swam at some time during the 3 h of normoxia and 3 fish were almost continuously active. At the onset of hypoxia, activity decreased dramatically with never more than 3, and rarely more than 1, fish active during the 1 h of hypoxia. During hypoxia the total activity was 8 min during the first 15 min and 32 min during the final 30 min. These values are significantly lower than the mean normoxic activity during 15- and 30-min periods ($56{\cdot}3 \pm 8{\cdot}48$ and $112{\cdot}6 \pm 4{\cdot}66$ min respectively).

DISCUSSION

In the present study the behavioural and ventilatory responses to hypoxia have been observed in unrestrained, unoperated dogfish in which no invasive recording techniques have been used. These factors, combined with the 3 days allowed for acclimation to the experimental conditions, have meant that the fish have been as near to a resting condition as laboratory conditions will allow.

From the continuous recording of daily activity cycles it appears that dogfish are essentially nocturnally active. This can also be clearly seen in Fig. 2, which shows the activity of individual fish during both the day and the night. Nocturnal activity has been observed in a number of fish species (Spencer, 1939; Carlander & Cleary, 1949) and may be related to feeding activity in the wild. However, fish were not fed during the experiments in the present study.

The observed activity responses to hypoxia in the present study do not support the statement that elasmobranchs 'become more active in hypoxic conditions and attempt to leave the oxygen depleted environment' (Randall, 1970). In relatively inactive diurnal dogfish 75 % of those studied remained inactive during the rapid reduction of the water P_{O_2} from 21 to 8 kPa. In relatively active nocturnal dogfish there was a significant reduction in activity during a similar decrease in water P_{O_2}. However, other fish species may indeed become more active in response to hypoxia. Congleton (1980) reports that some species of tidepool fish become more active in response to both natural nocturnal hypoxia, and to experimentally induced hypoxia. In both nocturnal and diurnal dogfish, prolonged hypoxia causes significant reductions in activity. This suppression of activity may represent an attempt to reduce the demand

for oxygen when it is scarce. The lack of any significant increase in activity in response to hypoxia suggests that the increase in the levels of circulating catecholamines observed in response to hypoxia (Butler *et al.* 1978, 1979) is the result of hypoxia alone rather than of any increase in locomotory activity (Opdyke *et al.* 1982).

In normoxic water the mean respiratory frequency was much lower than has previously been reported for this species at similar temperatures (Hughes & Umezawa, 1968; Butler & Taylor, 1971, 1975; Short *et al.* 1979). In the studies of these authors, the fish were restrained in their experimental apparatus and this may have stressed the fish causing an elevation in 'resting' respiratory frequency. In the present study mean respiratory frequency increased significantly by 49 % in response to hypoxia. This increase is contrary to previous reports of the response of *S. canicula* to hypoxia (Butler & Taylor, 1971, 1975; Short *et al.* 1979) in which respiratory frequency remained unchanged. This increase in respiratory frequency, together with the apparent increase in ventilatory amplitude, may represent a large increase in ventilation volume. Dogfish are easily stressed in experiments which involve their confinement. This causes respiratory frequency, and probably ventilation volume, to become elevated to near maximum levels even during so-called 'resting' conditions in normoxic water. This may have profound effects on the transfer of oxygen from the water to the blood across the gills of 'resting' dogfish.

Interlamellar water has been shown to contribute a considerable proportion of the resistance to oxygen diffusion across the gills of fish (Hills & Hughes, 1970; Scheid & Piiper, 1971) which is reduced by increases in the velocity of ventilatory water caused by increases in ventilation volume (Scheid & Piiper, 1971). Such decreases in the resistance to oxygen diffusion will result in increases in the effectiveness of oxygen transfer, calculated as $\dot{T}_{O_2}$ (Randall, Holeton & Stevens, 1967). The increase in normoxic ventilation brought about by the procedures required to measure $\dot{T}_{O_2}$ in dogfish (Short *et al.* 1979) may cause an elevation in normoxic $\dot{T}_{O_2}$ due to the reduction of the resistance to oxygen diffusion presented by interlamellar water. This may reduce the degree to which $\dot{T}_{O_2}$ may be increased in response to hypoxia and so partly explain the lack of any measured increase in $\dot{T}_{O_2}$ in response to hypoxia in this species (Short *et al.* 1979).

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