DIURNAL RHYTHM IN THE RATES OF OXYGEN CONSUMPTION, LOCOMOTOR AND FEEDING ACTIVITY OF YEARLING ATLANTIC SALMON (SALMO SALAR) UNDER VARIOUS LIGHT CONDITIONS

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INTRODUCTION

Diurnal rhythm in the rate of oxygen consumption of fish has been studied by many workers and their results have been ably reviewed by Winberg (1960) and Fry (1957). Unfortunately, most of these experiments lasted only a day or two hence their results are not very useful. Locomotor and feeding activities show diurnal rhythms (Kuchin, 1960; Musinic, 1931; Sushkino, 1939; Oliphant, 1951; Hirata and Kobayashi, 1956; Hirata, 1957; Swift, 1962, 1964). There exists a close relationship between locomotor activity and oxygen consumption on the one hand and between locomotor activity and feeding on the other. In other words, the more active the fish, the more oxygen it consumes and therefore the more it must feed. There are no studies which deal with these three aspects in a single species. The present investigation was undertaken as a first step in the study of rhythms in the rates of oxygen consumption, locomotor and feeding activity in relation to light as an exogenous factor, using simple techniques. Based on the results of this investigation, further, more elaborate studies will be conducted with species varying in habits and habitats.

The Atlantic salmon was used since it has been the experimental object in my work on the retina (Ali, 1961). Further, some information is available about diurnal variations in its oxygen consumption (Power, 1959) and feeding (Hoar, 1942).

MATERIAL AND METHODS

Material

Atlantic salmon yearlings kindly supplied by the Margaree Fish Culture Station of the Canada Department of Fisheries were used. The body weight
of these fish ranged from 7 to 13 gm. They were held in refrigerated, running water (5° C.) when not used in the experiments. They were fed on a diet of raw ground beef liver once daily.

Since the fish room had several windows the light intensity and duration in it varied according to the conditions obtaining outside. The maximum intensity of light recorded at the surface of the water was 117 ft.-c. (with a Photovolt Model 200-M photometer).

Methods

Experimental conditions.—The five conditions under which the consumption of oxygen, activity and feeding were studied were: control (duration and intensity of light varying according to natural conditions), continuous light, continuous darkness, light and darkness alternating every 12 hours and light and darkness alternating every 6 hours.

Continuous light condition was created by covering a tank with a specially made light proof cover equipped with a fluorescent lamp. When this lamp was lit the intensity of light at the surface was 20 ft.-c. Continuous dark condition was created by simply covering the tank with the light proof cover without turning the light on during the experiment. The alternating light-dark conditions were created by turning the fluorescent lamp on or off manually. The switch was located about 5 metres from the tank, near the door. It was noted that with a flow of 95 ml./min. the water temperature was not elevated by the fluorescent lamp.

Oxygen consumption.—A schematic view of the apparatus is given in Fig. 1. Aspirator bottles (1.05 litres) were used as respiratory chambers (A) and as excretion filters (B). Water from the inlet (E) passed through the chamber and the filter into the BOD bottle (C). Water diverted from

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**Fig. 1.** Apparatus for measuring oxygen consumption; see text for explanation. Aspi-A, rator bottle used as respiratory chamber; B, Excretion filter; E, Inlet; C, B.O.D. bottle for sampling outlet water; D, B.O.D. water for sampling inlet water; I, outlet; L, water level.
the inlet was collected by another BOD bottle (D). The rate of the water flow was kept constant at 95 ml./min. The sand in the excretion filter was changed daily.

Three respiratory chambers, each with one fish, were placed in the experimental tank (90×45×30 cm.) with running water. Water samples of both inlet (E) and outlet (I) were taken simultaneously every six hours (2, 8, 14 and 20 hours). Oxygen content was determined using the Winkler technique. The difference in the oxygen content between the inlet and outlet yielded the quantity consumed (ml./l.) by the fish. This is expressed in ml./kg./hr. and was obtained by using the following formula:

\[
\text{Oxygen consumption (ml./kg./hr.)} = \frac{\text{Inlet } O_2 \text{ content} - \text{Outlet } O_2 \text{ content } \times \text{Rate of flow (ml./hr.)}}{\text{Body weight (kg.)}}
\]

Prior to its entry into the inlet the water was well aerated in order to keep it maximally saturated with oxygen. The temperature of the water in the respiratory chambers was 4.5 ± 0.5° C.

The fish were not fed for five days before each experiment. Since the temperature of the water was low (5° C.) it is reasonable to assume that starvation did not affect the fish seriously. Sampling started 3 hours after the fish were put in the respiratory chamber and lasted for 4 or 5 days. Care was taken not to disturb the fish during the experiment (Spoor, 1946). In order to do this, entry into the experimental room was kept at a minimum (i.e., 4 times daily) and water samples were taken about 2 metres away from the experimental tank.

![Diagram: Apparatus for recording the locomotor activity of fish. See text for explanation. C, Recording counter; P, Plastic pane; E, Electrical contact; O, Outlet; I, Inlet.]
Activity.—A diagrammatic view of the actograph used is shown in Fig. 2. An electric counter made by Hattori and Co., Tokyo, was used as the recorder (C). It makes one revolution every 24 hours and its maximum recording efficiency is 100 signals per min. A light plastic pane was attached to a rod, the middle of which was fixed. Agitation of the water caused by the swimming of the fish but not its opercular movement caused the movement of the pane thus closing a circuit. Each such movement of the pane was recorded by the counter as one vertical step. When 100 such counts were made the recording pen fell back to its former, lower level.

A glass aquarium (30 × 34 × 24 cm.) was used as the activity chamber. A small water flow was maintained through an inlet (I) at the bottom of the chamber. An outlet (O) maintained the water level constant. It has been shown (Spoor, 1946) that changes in water level cause variations in activity.

Three fish (8.0–10.4 gm.) were kept together in the activity chamber. Before the experiment commenced the fish were left in the activity chamber for 2 days so that they could acclimate themselves to the experimental conditions. The temperature in the activity chamber was 15° ± 0.5° C. to which the fish had been acclimated previously. A higher temperature was chosen for the activity and feeding experiments because at 5° C. the fish moved and fed very little. The experiment lasted 4 days and the fish were not fed during this time. The experimental room was entered into only once daily, in order to change the paper on the recording counter.

Feeding.—A diagrammatic view of the apparatus used for recording feeding activity is shown in Fig. 3. This is essentially similar to the one used by Hirata (1960). A bakelite lid (B) was attached to a rod (A) which was in turn connected to a recording lever by a fulcrum (C). The bait plate was

![Fig. 3. Feeding activity recorder. See text for explanation. B, Bakelite lid; A, Rod; C, Fulcrum; P, Pen; K, Kymograph.](image-url)
set in about the centre of the experimental tank (90 × 45 × 35 cm.). Feeding activity was recorded directly on a kymograph when the fish pecked at the bait (ground raw beef liver).

Every time the fish pecked at the bait the recording lever left a vertical trace on the smoked drum of the kymograph. Movements of the fish also caused fluctuations of the recorder but these traces did not exceed 2 mm. in amplitude. Therefore, only the traces longer than 2 mm. were counted. Amplitude of the traces (counted) was not taken into consideration because whether the fish just pecked at the bait or pressed the bait plate down after the attempt the trace was counted as only one.

Ten fish (9–11 gm.) were kept together in the experimental tank in running water. The bait was changed every 9 hours. The experiment lasted for 5 days. Care was taken to keep disturbances at a minimum by entering the room only when the bait or the kymograph drum had to be changed. The water temperature throughout these experiments remained at 15° ± 0.5° C. and the fish had been previously acclimated to it.

RESULTS

Oxygen Consumption

Control (Fig. 4 A).—The rate of oxygen consumption is higher during certain hours of the day than during the night. It is seen that on all days except on the third (December 14) day of the experiment, the peak in the rate of oxygen consumption was reached at 14 hours. The lowest rate of consumption was at 20 hours on all days of the experiment except the first (December 12) and the fourth (December 15) days of the experiment. The high value at 20 hours on December 12 may have been due to the fish having been handled three hours prior to measuring. Though the rate of consumption did not touch the lowest on the fourth day (December 15), the rhythm in the rate on that day is quite well marked with maximum consumption around noon and minimum around midnight. It is interesting to note that Power (1959) found that young salmon in the field consumed more oxygen at midnight and midday. His experiment lasted only 26.5 hours and the temperature of the water varied from 6° to 9° C. during his experiment. In view of this, it will not be in order to compare his results with those of the present investigation. However, it is interesting to compare his results with those obtained during the first 24 hours of the present investigation. When this is done, it is seen that they are somewhat similar. The fluctuations observed during the first day of the present experiment may be ascribed to the fish not yet being com-
pletely acclimated to the experimental conditions. This will apply equally to Power's results. That is, had he continued the experiment for three or four days he would have found a different rhythm on the second, third and fourth days. This indicates the desirability of continuing experiments of this nature for several days.

**Fig. 4.** Rate of oxygen consumption under various conditions. The vertical bars on most of the points represent standard error. Where no bar is given standard error was negligible. A, Control; B, Continuous light; C, Continuous dark; D, Light and dark alternating every 12 hours; E, Light and dark alternating every 6 hours. The vertical broken lines represent midnight. The dark horizontal bars in D and E represent periods of darkness.
Diurnal Rhythm in Oxygen Consumption, Locomotor & Feeding Activity

Continuous light (Fig. 4 B).—The rate of oxygen consumption decreases for the first 36 hours. After this, it remains virtually unaltered for a day and on the fourth day (December 22) shows some fluctuation with minimum occurring at 8 hours and maximum around midnight. On the fifth day (December 23) the maximum consumption occurs at 8 hours and the minimum around midnight. It appears reasonable to assume that continuous light has upset the normal rhythm in the rate of oxygen consumption of the fish.

Continuous dark (Fig. 4 C).—In continuous dark there appears to be a rhythm in the rate of oxygen consumption of the fish. Maximum consumption occurred at 14 hours and the minimum around midnight. The increased rate of consumption at 2 hours on January 10 may be attributed to the handling of the fish to place it in the respiratory chamber three hours prior to that hour. On the second day (January 11) there was no evidence of a rhythm but on the third day, greater consumption of oxygen occurred at 14 hours while it was lower and essentially the same at all other times of the day. The last measurements of the experiment, made at 14 hours on January 13, were high indicating that, had the experiment continued, there might have been a rhythm on this day. It appears that the rhythm persists for a day in continuous darkness, as a "physiological memory". An indication of a similar persistence of a 24-hour rhythm in continuous darkness was seen in the case of the retinal epithelial pigment (Ali, 1961). This 24-hour rhythm in the case of oxygen consumption is followed by a day or two when the fish's rhythm is upset by the continuous darkness. Subsequently, variation in the rate of oxygen consumption occurs which may either be due to the darkness being a condition of stress to the fish or due to the animal having re-established its normal rhythm. This re-establishment seems to be feasible in continuous darkness, unlike in continuous light, because while the latter is a stimulus the former is the absence of it. Experiments lasting longer than four days will have to be conducted in order to obtain a better idea of the influence of continuous light and darkness.

Light 12 hours: dark 12 hours (Fig. 4 D).—In the experiments wherein light and darkness were altered every 12 hours no relationship between the light-dark periods and rate of oxygen consumption was apparent except on the fourth day (February 8) when the rate of consumption went up during the light period and decreased during the dark period. This trend appeared to continue until the end of the experiment.

If we ignore the artificially induced light conditions and examine the data it is seen that on the first and second days there is a rhythm, oxygen
consumption being greater at 8 and 14 hours with the minimum around midnight. On the third day, while there is an indication of a rhythm, it is much less pronounced and is even less on the fourth day (February 8). Although the light-dark periods were kept approximately similar to the natural periods, they have nevertheless affected the normal rhythm of the animal. After about three to four days the fish appears to begin to align itself to the artificially arranged light-dark periods. In this case also experiments of much longer duration will have to be carried out to ascertain if this suggestion were true or not.

Light 6 hours : dark 6 hours (Fig. 4 E).—When light and darkness are alternated every six hours, the rate of oxygen consumption keeps decreasing without any reference to the light-dark periods during the first 18 hours. After this it remains unaltered for 12 hours. At this period it appears to become aligned to the artificial light regime and right to the end of the experiment a nice correlation between light-dark periods and oxygen consumption is seen. The latter is correspondingly higher when the lights are on and lower when they are off. It is obvious that light plays an important role in this case. It is interesting to point out that there is a general fluctuation in the rate of oxygen consumption throughout the duration of the experiment. The rate decreases on the whole during the first three days of the experiment and subsequently seems to increase.

If the rates of oxygen consumption are analysed from the point of view of natural day and night periods it is seen that, in this experiment, there is no relationship between the day-night periods and the rates. On some days they are more elevated at night and on some others, during the day. It is tempting to suggest that light acts as a “Zeitgeber” but the minor nature of its role in the 12 hours experiment renders this suggestion less valid.

Locomotor Activity

Control (Fig. 5 A).—Greater activity was recorded during the day than during the night. Peaks in activity were observed early in the morning on all days except on the third day (October 5). On all days minimum activity was recorded around midnight. The rhythm in activity is more marked during the first two days of the experiment than during the latter two days. On the last day of the experiment activity was more elevated than on the other days. The greatest activity observed was between midnight and 6 hours on the first day of the experiment. It is clear from these results that under ordinary conditions of the laboratory (control) the salmon yearlings
show a diurnal rhythm in their activity, that is, their active periods coincide with daytime.

Continuous light (Fig. 5 B).—While on the second day (March 20) activity is slightly greater at noon than at any other time, on the third and fourth days there is no variation in activity. In fact, activity is almost nil. However, on the last complete day (March 23) of the experiment, activity is not only high, reaching one of the highest values, but also shows a very clear diurnal
rhythm. It appears that during the first 24 or 30 hours of continuous illumination, the rhythm persists but further illumination acts as a stress resulting in the animal remaining quiet. However, periods of continuous light longer than three days bring about restlessness resulting in a great deal of activity. It would seem that continuous light has not altered the rhythm pattern but has only suppressed it for a couple of days.

Continuous dark (Fig. 5 C).—The results have proved to be quite surprising. On the first day (April 3) activity increased between 18 hours and midnight. On the second day it presented a rhythm pattern which was the reverse of what was expected. Greatest activity was observed between midnight and 6 hours and between 18 hours and midnight. Around noon there was no activity at all. On the third day (April 5) activity decreased between midnight and noon but increased between noon and midnight. On the third and fourth day between midnight and 6 hours, activity decreased but between 6 hours and 18 hours it was at a maximum and decreased thereafter. It would appear that continuous darkness upset the rhythm for a couple of days. It is difficult to explain the decrease in activity between midnight and 6 hours on the second day because from the commencement of the experiment at 18 hours (which corresponded fairly approximately to sundown) to 5 or 6 hours in the morning the condition was not abnormal. One possible explanation is that a great amount of activity normally occurs very early in the morning, say between 5 and 6 hours as the control shows, and since there was no light at this time during this experiment, unlike in the control and continuous light experiment, this activity failed to occur, thereby decreasing the amount of activity between midnight and 6 hours. There still can be no reasonable explanation for the increase in activity between 18 hours and midnight on the previous day (April 13) because handling the fish and other such factors were similar to those in the other experiments in which such a rise did not occur.

Light 12 hours: dark 12 hours (Fig. 5 D).—For the first 24 hours of the experiment there appeared to be not much variation in activity. After this for the next 12 hours (dark) activity increased and then decreased. For the next 30 hours there was practically no variation. Following this period, activity increased and when the lights were turned off, decreased. It increased again for 6 hours when the lights were turned on but decreased even when they were still on. In the last phase of darkness there was practically no variation.
Thus it appears that when light and darkness are alternated every twelve hours the inherent diurnal rhythm which may be present is changed. However, it is also clear that activity does not correspond to the light-dark periods.

**Light 6 hours: dark 6 hours** (Fig. 5 E).—In this experiment during the first five light-dark phases activity was lesser in darkness and greater in light. After this, however, there was no correlation between light-dark periods and activity. Indeed, for some time activity seemed to be greater in darkness and lesser in light. Towards the end of the experiment a relationship between the light-dark periods and activity appeared to re-emerge.

It is difficult to interpret these results. From the results of the 12-hour experiments it would seem that light plays no role in controlling activity. However, in this experiment it appears to do so to quite an extent. Further investigations alone can reveal the endogenous and/or exogenous factors involved.

**Feeding Activity**

**Control** (Fig. 6 A).—These results show clearly that feeding is greater during the day, usually from early morning to noon, than at night. This agrees well with what Hoar (1942) found in his study of the diurnal variations in the feeding activity of the salmon. His conclusions were based on the examination of stomach contents.

**Continuous light** (Fig. 6 B).—For the first 30 hours there seemed to be no rhythm in feeding activity. Subsequently, on March 21 and 23 there was more feeding in the forenoon than at other times. This was not as clear as in the control experiments (Fig. 6 A) and cannot be considered to represent a diurnal rhythm. It would be safe to say that in continuous light there is no rhythm in the feeding activity of the yearling salmon.

**Continuous dark** (Fig. 6 C).—On April 4, the first full day of the experiment maximum feeding occurred between noon and 18 hours. On the following days it was between 6 hours and noon. The degree of feeding activity is greater during the last two days of the experiment. From these results it is quite clear that even in continuous darkness a rhythm persists. However, it is not possible to explain the reason for the difference between the periods of maximum feeding in this experiment and those in the control experiment. It may be recalled that in the control experiment (Fig. 6 A) maximum feeding occurred between 6 hours and noon on the first and last days and between midnight to 6 hours on the second and third days.
Light 12 hours: dark 12 hours (Fig. 6 D).—No relationship between the light-dark periods and feeding activity was evident. On April 12 and 14 feeding was greater in the forenoon regardless of the light-dark periods. It would appear that light has no influence on the feeding rate.

![Figure 6](image-url)

**Fig. 6.** Feeding activity of ten fish under various experimental conditions. The vertical, broken lines indicate midnight. The thick, black, horizontal bars represent periods of darkness. A, Control; B, Continuous light; C, Continuous dark; D, Light and darkness alternating every 12 hours; E, Light and darkness alternating every 6 hours.

Light 6 hours: dark 6 hours (Fig. 6 E).—Here also as in the case of locomotor activity (Fig. 5 E) there appeared to be a relationship between the
light-dark periods and feeding. During the first 60 hours of the experiment, the feeding rate was greater when the lights were on than when they were off.

This relationship was upset during the next 18 hours but was re-established subsequently and continued till the end of the experiment.

From these experiments it appears that while feeding is greater earlier in the day under control conditions (Fig. 6 A), light is not a controlling factor. This may also be dependent on the type of food used in the experiment. If practically odourless food were to be employed it is likely that the feeding rate will be greater when the lights are on, that is, the fish will have to depend mainly on light to locate the food.

The relationship observed between the light-dark periods and oxygen consumption (Fig. 4 E), activity (Fig. 5 E) and feeding (Fig. 6 E) in the 6-hour experiments suggests the possibility that there is a complex interplay of some unknown endogenous factor, metabolism, light, activity and feeding.

**DISCUSSION**

The results of this preliminary study indicate that under control conditions the rates of oxygen consumption, activity and feeding show a rhythm. They are in general higher during the day than during the night. The rhythm shown by them corresponds approximately to the natural day and, therefore, may be considered to be a diurnal or circadian rhythm. Even though there is rhythm, the maxima of oxygen consumption, activity and feeding do not occur at the same time. The difference in time between maximum feeding and oxygen consumption may be ascribed to specific dynamic action.

The influence of light as an exogenous factor is not clear. On the basis of these results it appears safe to suggest that light plays only a very small role in the production of a rhythm. That it plays a role, however small, is shown by the results of the experiments wherein light and darkness were alternated every six hours (Figs. 4 E, 5 E, 6 E). It is seen from the results of these experiments that there is a relationship between the light-dark periods and oxygen consumption, activity and feeding. Had this been so in the experiments in which light and darkness were alternated every 12 hours it would have been possible to consider light as a principal factor underlying the rhythm. This has not been so. Therefore, it seems that there is or are some endogenous factors which have an inherent 12-hour rhythm. When the light-dark periods get aligned with this or these factors a 12-hour rhythm results. This is the reason that in the experiments wherein light and darkness
were each 12 hours long an increase and decrease in oxygen consumption, activity and feeding were seen during a 12-hour period when the light was either on or was off. In conclusion, it may be suggested that under control conditions a circadian rhythm in oxygen consumption, activity and feeding is brought about as a result of an interplay between the endogenous factors and the natural conditions to which the fish is adjusted. When these natural conditions are experimentally altered the endogenous factors become dominant and when the experimentally produced light durations become aligned with these factors a 12-hour rhythm is brought about.

It would be best to study oxygen consumption, activity and feeding simultaneously. An apparatus is being developed which would permit the simultaneous recording of oxygen consumption and activity. Using this apparatus it is hoped to conduct a detailed study of rhythms in fish belonging to various families and habitats.

**SUMMARY**

Under control conditions of the laboratory, the rates of oxygen consumption, locomotor activity and feeding show a diurnal rhythm. It appears that light plays only a minor role in the production of this rhythm in the juvenile Atlantic salmon.

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