

Diurnal Rhythm of Metabolic and Activity Rates in Juvenile Atlantic Salmon, *Salmo salar* LINNAEUS

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Introduction

It is reasonable to assume that all organisms have a diurnal, tidal, seasonal, or annual rhythm in their physiological and ecological aspects. Considering only the case of diurnal rhythm, herein, the study of the rhythm is a very important fundamental field in order to control animals and plants.

For instance, since we have found out that time of fertilization of rice and wheat occurs about two hours before sunrise, the techniques of cross-breeding have been making rapid progress. Recently, we have recognized how important this research could be to the human being. Studying of the diurnal rhythm of our body temperature, pulse, blood pressure, metabolism and kidney mechanisms contributes to the early diagnosis of disease (REINBERG and GHATA, 1957).

There has been recently a revival of interest in the study of diurnal rhythm amongst zoologists, physiologists, psychologists and botanists. Most interesting points among biologists who have been working in the field of diurnal rhythm of animal are : 1) what is the rhythm pattern (viz. diurnal, nocturnal, twilight or non-rhythm)? 2) Is the rhythm shown by the animal exogenous or endogenous? and 3) what is the mechanism of the rhythm? (HARKER, 1958). Additionally, in 1960, HIRATA has expressed a formula of the rhythm strength and emphasized that rate of the strength is also one of the most important phenomena for studying of rhythm in life.

There are only two reports on the diurnal rhythm of Atlantic salmon. However, they did not deal with all the problems mentioned above. In 1942, HOAR observed the feeding activity at several times of day and reported that the fish feed more actively during the day than at night. Recently, ALI (1961) has studied the position of retinal cones and pigment under constant conditions of light and dark, and stated that while in light neither the cones nor pigment show a rhythm, the later shows 24 hour rhythm in dark.

In this study the metabolism, activity and feeding rates of yearling Atlantic salmon under various conditions have been studied. The purpose has been to find out whether any of these show an inherent rhythm or are influenced by light alone. An attempt will be made to correlate results from the various conditions.

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Review of literature

1. General

The general aspects of diurnal rhythm in the animal kingdom have been reviewed by HARKER (1958). She divided 24-hour rhythm into two types; (a) exogenous : those which are under the immediate control of the environments ; (b) endogenous : those which persist in any constant environmental conditions due to inherent factors.

MORI (1947) discussed the diurnal rhythm of physiological aspects in invertebrate animals. These are the daily, tidal, monthly and annual cycles. He termed these phenomena "rireki gen-sho" (Biological clocks). Later, such phenomena were recognized by KOBAYASHI and WATABE (1958) in pearl oyster. Rhythms covering periods longer than 24 hours have also been investigated by BROWN (1957, 1959), who has described accurate tidal and lunar rhythms in a number of animals.

From the view point of applied biology, REINBERG and GHATA (1957) reviewed the diurnal rhythm and cycle of medical aspects in human beings. They pointed out that studying of diurnal rhythm of body temperature, pulse, blood pressure, standard metabolism and kidney mechanism contributes to the early diagnosis of disease.

2. Diurnal rhythm of oxygen consumption in fish

There are a few reports on the diurnal rhythm of oxygen consumption in fish. TAMURA (1935) designed the open system chamber for measurement of fish respiration. Later (1937, 1939, 1940, 1944, 1956) he studied the relation between metabolic rate and environmental factors in many fish, and pointed out that metabolism of the fish showed a diurnal rhythm. However, he has not observed the rate of metabolism in any Salmonoid. CLAUSEN (1936) studied oxygen consumption in eight species of fish and determined that in the species which inhabit relatively quiet water (*Huro salmoides*, *Ameiurus melas*, *Pomoxis annularis*, *Notemigonus crysoleucas*, and *Erimysan succetta*) the fluctuations tend to fall into a rhythm that is characteristic for each species. No definite rhythm was found in the oxygen consumption of fish (*Camptostoma anomalum*, *Lusilus cornutus* and *Ethoeostoma blennioides*) which inhabit rapid flowing water. Before beginning the experiment, he acclimatized the fish for 12 hours to a respiratory chamber. His observations lasted for only a day. As pointed out by TAMURA (1935), KAWAMOTO (1957) and HIRATA (1960b), it is necessary that an experiment must last longer than a day in order to provide reliable results.

3. Feeding

The feeding activity of animals is one of the most important factors to maintain their life. So, many reports have been published on this problem. However, all of them are based on qualitative analysis of the stomach content without considering the time factor. From this point of view, HOAR (1942) studied on diurnal variations in feeding activity of young Atlantic salmon and trout (*Salmo salar* and *Salvelinus fontinalis*), and reported that they always feed somewhat less avidly from 1 to 2 hours after sunset until 2 to 3 hours after sunrise. In addition, feeding activity is frequently depressed during the middle of the day. The nightly depression in feeding is not entirely due to temperature. Also, salmon and trout are not entirely dependent on light receptors

to locate their food, as shown by the fact that they take food readily if fed in darkness during the day time. Rather it seems that salmon and trout are habituated to sleep during the hours of darkness when food is less likely to be available, and that feeding gradually resumed during the morning hours. The slackening in feeding at mid-day is due primarily to rising temperature, although strong light is a possible associated factor.

Another investigation of quantitative and persistent analysis of fish feeding has been carried out recently by KOBAYASHI and his associates (KOBAYASHI and HIRATA, 1957 a, b; HIRATA, 1957, 1958, 1960 a, b; HIRATA and KOBAYASHI, 1959; TERA0, 1959; NISHIYAMA, 1960). Their experiments were conducted with the aid of an automatic feeding recorder (KOBAYASHI, YUKI and HIRATA, 1956).

Among their investigations, the idea of the inflection point, d^2T/dt^2 , of water temperature was applied to an analysis of the correlation between the feeding activity of goldfish and the changing rate of temperature (HIRATA, 1960 a). In both autumn and spring months, the maximum feeding was often found to occur just at the time of the inflection point of temperature. Especially, in late autumn as the temperature fell, the peak of the feeding frequency was found to correspond clearly with the time of the inflection point. In summer months, however, such a tendency was found to be very rare. From these results he concluded that when the fish were acclimatized to a relatively high temperature, the effect of change of temperature on fish feeding was rather inconspicuous, but when the fish were adapted to low temperature such as in late autumn, they became more sensitive even to a slight change in the water temperature.

HIRATA (1960 b) also studied the diurnal rhythm of some species of fish, and expressed the formula of coefficient of rhythm (CR)* in order to determine the strength of rhythm pattern. The results showed that the migratory fish (*Oncorhynchus* and *Salmo*) show lower coefficients and non-migratory fish (*Carpio* and *Carassius*) show higher coefficients. On the other hand, he examined the rhythm strength of some subspecies (Funa, Funao, Wakin, Ryukin and Demekin) of *Carassius auratus*, and found that the rhythm strength is closely related to "rate of wildness". That is, wild fish show low coefficient of the rhythm strength and fish of a higher rate of wildness have a high coefficient value.

4. Activity

SPENCER (1939) found a 24-hour rhythm of activity in goldfish which persists in continuous light. SPOOR (1948) and HIRATA (1957, 1958, 1960 a) also obtained similar result. However, in the case of diurnal rhythm of movement activity in Salmonoid, the reports are many and conflicting. SANO (1955), KOBAYASHI *et al.* (1956) and KOBAYASHI (1958) reported that *Oncorhynchus keta* fry show the twilight rhythm. That is, the fish are active at late evening and early morning. II *et al.* (1952) stated that *Salmo irideus* has a diurnal rhythm and is more active in the daytime. NEAVE (1942) reported that adult fish (*O. tshawytscha* and *O. kisutch*) migrated to the upstream mainly during daylight. No correlation was found between diurnal fluctuations in number of migrants and volume or temperature of the river water. Light seemed the determining factor. NISHIYAMA (1959) recognized that the rhythm patterns of Salmonoid show change according to their stage. He pointed out that their seaward migration occurs at nighttime and upstream

* : $CR = \frac{\sum_{i=1}^K \sum_{j=1}^n x_{ij}^2 - \frac{T^2}{kn}}{F_{0.05}[n, k(n-1)]}$

migration occurs at daytime. HIRATA (1955) found that *O. keta* fry has two behaviours of both schooling and milling. Schooling behaviour was found during the daytime in order to feed the bait and milling occurred at nighttime during seaward migration.

Material and Methods

1. Material

The experiments described herein were carried out in the Fish Research Laboratory of the Memorial University of Newfoundland during the period of September 1960 to February 1961. Yearling Atlantic salmon (*Salmo salar*) obtained from the Margaree Hatchery, Maritimes Area, Canada Department of Fisheries were used. They were fed on a diet of raw ground beef liver once a day.

Body weights of the fish ranged from 7 to 13 grams. Water temperature ranged from 4 to 16°C during the period this investigation lasted.

2. Methods

A. Oxygen consumption

Oxygen consumption of the fish was measured by a modified TAMURA'S method (TAMARA, 1956). A schematic view of the apparatus is presented in Fig. 1.

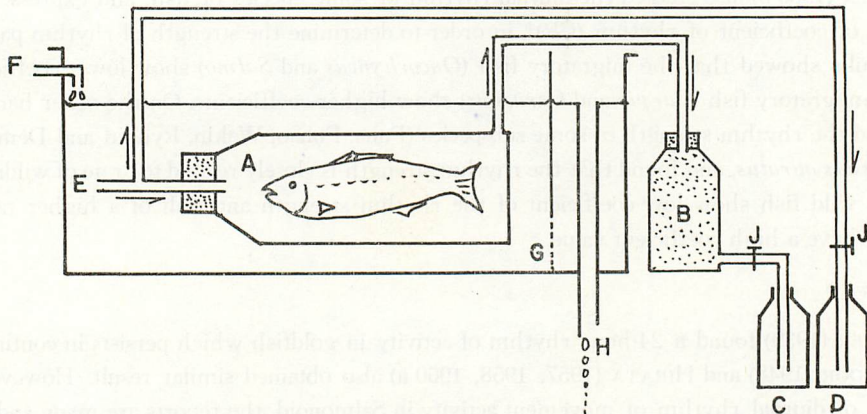


Fig. 1. Modified TAMURA'S method for measurement of oxygen consumption in fish.

- A : Respiratory chamber.
- B : Excretion sampler.
- C & D : BOD-bottle.
- E : Inlet of respiratory chamber.
- F : Inlet of the experimental tank.
- G : Nylon net.
- H : Water overflow pipe.
- I : Outlet of the respiratory chamber.
- J : Valve.

Aspirator bottles (1.05 litres) were used as respiratory chamber (A) and as excretion samplers (B). Water came from inlet (E) and passed through the chamber, and then the samples to be

tested were introduced into the BOD-bottle (C&D). The rate of the water flow was kept constant at approximately 95 ml/min. The sand in the excretion sampler was changed every day.

Three chambers, each containing 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 a. m., 8 a. m., 2 p. m., 8 p. m.). The oxygen content of the samples was measured by the WINKLER's method. The difference in the oxygen content, between the inlet and the outlet yielded the amount consumed by the fish.

For the sake of brevity, the experiments will be referred to as follows in this paper.

Ordinary condition in the hatchery“control”

Continuous light condition“light”

Continuous dark condition“dark”

Alternating light and dark every six hours“6-hour”

Alternating light and dark every twelve hours“12-hour”

Light intensities and temperature during the experiment and the body weights of fish used are shown in Table 1. In the case of “control”, the amount of light varied with the duration

Table 1. Experimental conditions of oxygen consumption, (three fish used in each condition).

Condition	Light intensity (ft-c)	Temperature (C°)	Body weight (g)
Control	20-100	4.1±0.2	13.0±0.2
Light	20	4.6±0.3	10.9±0.8
Dark	0	4.2±0.1	9.5±0.9
6-hour	20 or 0	4.1±0.1	10.1±0.5
12-hour	20 or 0	4.1±0.1	10.2±0.6

and amount of sunshine, and ranged from 20 to 100 ft-c. in the daytime. A 20 watt fluorescent lamp was used to produce “light” condition. The intensity on the surface of the water was 20 ft-c. In the case of continuous dark, the experimental tank was covered with a wooden plate and black cloth. Alternating light-dark (6- or 12-hour) conditions were produced by manually turning on and off a 60 watt lamp. In this case intensity of light at the water surface was about 20 ft-c.

Prior to beginning each experiment the fish were not fed for five days. Sampling started three hours after the fish were put in the respiratory chambers, and lasted for four or five days. Care was taken not to disturb the fish during water sampling and to keep water temperature constant (Table 1).

B. Activity

a. Feeding

The feeding activity of the fish was recorded with the simple feeding recorder (HIRATA, 1960 a). Fig. 2-A shows the recorder used. Vertical rod (A), bait plate (B), fulcrum (C), recording lever (D) and kymograph (E) are the main parts of the recorder. The bait plate of the recorder was set in about the center of the experimental tank (90×45×35 cm). Feeding was recorded directly on the kymograph when the fish pecked the bait. An example of the recorded is shown in fig. 2-B.

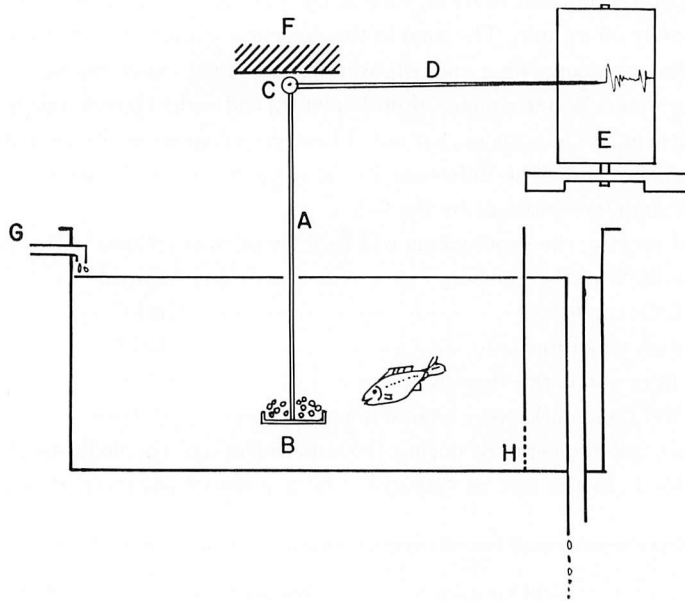


Fig. 2-A. Simple feeding recorder of fish.

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|---------------------|--------------------------------|
| A: Vertical rod. | F: Fixation of fulcrum. |
| B: Bait plate. | G: Inlet of experimental tank. |
| C: Fulcrum. | H: Nylon net. |
| D: Recording lever. | I: Water overflow pipe. |
| E: Kymograph. | |

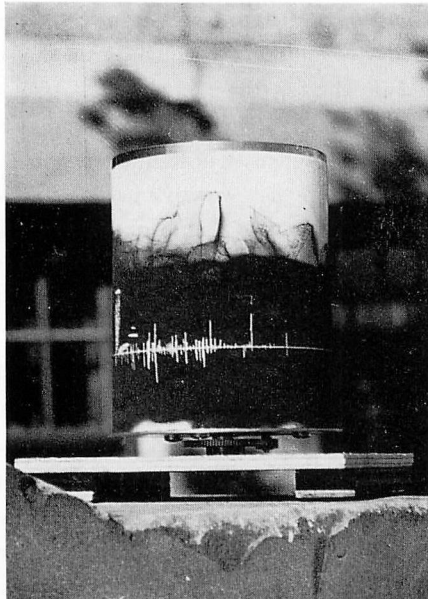


Fig. 2-B. An example of feeding records.

Every time the fish pecked at the bait the pecking left a vertical trace on the smoked drum of kymograph. Small movements of the fish also caused fluctuations of the recorder but these recordings did not exceed 2 mm, therefore only the traces longer than 2 mm were counted. Each trace was counted as one attempt at feeding. For example, 3 vertical traces are seen in Fig. 2-B which shows the record of feeding on October 19 between 4 and 5 a. m. This is taken to indicate 3 attempts by the fish to feed. Although the magnitude of the trace was related also to the fact that whether the fish just pecked at the bait or pressed the bait down after pecking, the attempt was still considered to be one only.

Ten fish, approximately of the same size (9 to 11 gram), were selected and kept together in the tank of running water (Fig. 2-A). The bait of the recorder was changed once every nine hours. The experiment to record feeding rates was carried out under "control" condition and lasted for five days. Care was taken not to disturb the fish except for the changing of bait in the feeding recorder, and to keep water temperature constant as far as possible.

b. Movement

Fig. 3-A shows the actograph (HIRATA, 1960 b) used for the recording of the fish activity (movement). Electric counter (C) made by Hattori Co., Tokyo, was used as recorder. Its maximum recording efficiency is 100 frequencies per minute. As shown in the figure, the recorder was

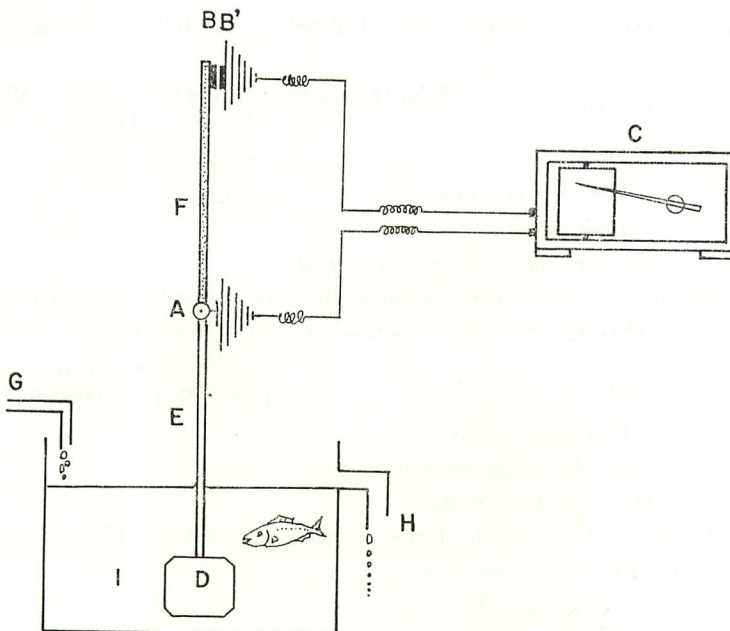


Fig. 3-A. Simple fish actograph.

- | | |
|-------------------------------|-------------------------|
| A: Fulcrum. | B&B': Electric contact. |
| C: Electric counter. | D: Plastic paddle. |
| E&F: Stalk of plastic paddle. | G: Inlet of aquarium. |
| H: Outlet of aquarium, | I: Activity chamber, |

connected with wire to fulcrum (A) and to the fixed side of the electric contact (B'). The fulcrum supports the stalk (E & F) of plastic paddle (D). Upper part of the stalk (E) was charged with electricity through the fulcrum. A glass aquarium (30×34×24 cm) was used as an activity chamber. A small water flow was maintained through an inlet (G) at the bottom of the chamber. An outlet maintained the water level constant. Movement of the fish caused the plastic paddle to swing like a pendulum. The activity was recorded by the closing of the circuit, B-B'.

Movements of the fish were recorded as steps on the recorder of the actograph. One closing of the circuit was recorded as one step on the recording paper. If fish was very active, the circuit closed often, and more steps were recorded. Therefore, the steps were recorded in proportion to rates of fish movements. The number of steps per hour is counted as the rate of movement activity.

Three fish were kept together in the activity chamber. Their weight ranged from 8.0 to 10.4 grams. Prior to beginning the recording, the fish were left in the actograph for two days so that they could acclimatize themselves to it. The recording was carried out in the hatchery under "control" condition and lasted for four days. The fish were not fed during the experiment. Every care was taken not to disturb the fish during the experiment and to keep the water temperature almost constant.

C. Calculations

a. Metabolic rates

The amount of oxygen consumption was calculated by the following expression (TAMURA, 1956) :

$$\text{Oxygen consumption (ml/kg/hr)} = \frac{(\text{Inlet O}_2\text{-content} - \text{Outlet O}_2\text{-content}) \times \text{Water flow}}{\text{Body weight}}$$

O₂-content.....ml/l
 Rate of water flowml/hr
 Body weight.....kg

b. Percentage of saturation of oxygen content

Percentage of saturation of oxygen content in the experimental tank was calculated by the following expression (Japanese Scientific Society of Oceanography, 1956) :

$$\text{Percentage of saturation of oxygen content (\%)} = \frac{(\text{amount of oxygen content in the tank}) \times 100}{10.291 - 0.2809T + 0.006009T^2 - 0.000063T^3}$$

Oxygen content.....ml/l
 T (water temperature).....C°

c. Coefficient of rhythm strength

In order to determine the strength of rhythm pattern, coefficient of rhythm strength (RS) was calculated by the following expression :

$$RS = \frac{\sum_{i=1}^K \sum_{j=1}^n x_{ij}^2 - \frac{T^2}{kn}}{F_{0.05}[n, k(n-1)]}$$

n, *k*(*n*-1).....degree of freedom
*T*².....each total of both day and night
*x*_{*ij*}..... each value

The coefficient was ordinarily calculated down to three decimals. If the coefficient is greater

than one, it is regarded as being statistically significant. Because $F_{0.05}(\phi_1, \phi_2)$ were obtained from F-table.

Results

1. Oxygen consumption

A. Control

The mean rates of oxygen consumption of the three fish (Table 1) used in the experiment, at various times of day and night, are shown in Fig. 4. Vertical bars at each point represent the standard error. Where no bars are given, standard error was negligible. Temperature and oxygen content of the water during the period of this experiment lasted are also presented in (Fig. 4). It is observed that these factors do not show much variation except on the first day (temperature and oxygen content) and the third day (oxygen content only).

During the experiment, average rate of oxygen consumption was 94.2 ± 9.8 ml/kg/hr. Standard metabolism was seen in the night and the value was approximately 71 ml/kg/hr. The coefficient of the rhythm strength (RS) was 1.121.

The rates of oxygen consumption at various times show a variation that may be related to day and night. It is to be observed that the rate of consumption during the day is generally greater than that during the night. The peaks of the daily variation of the consumption occurred

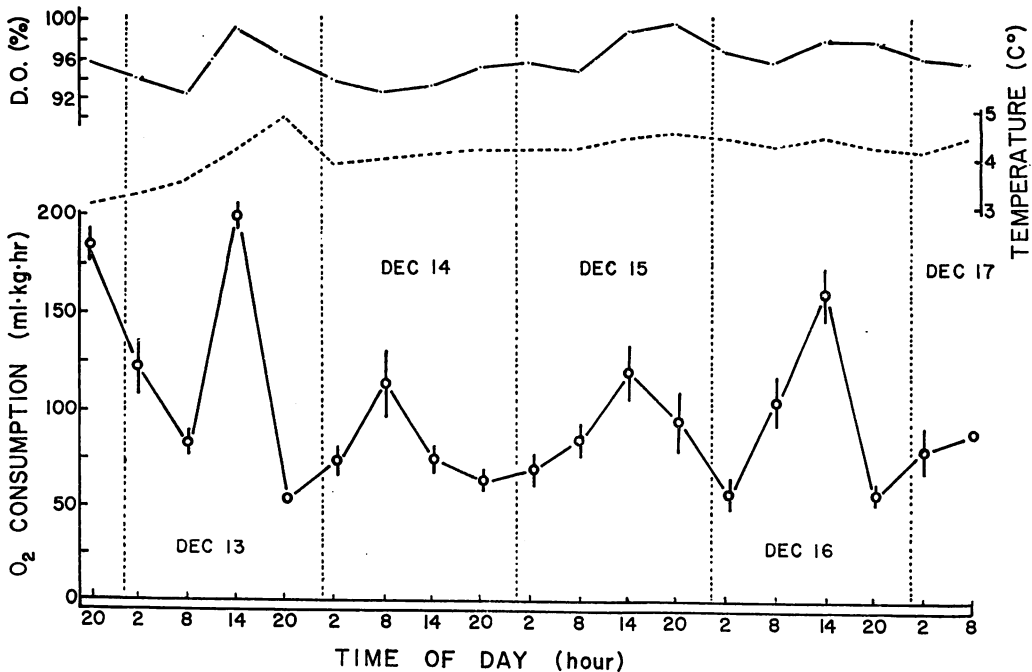


Fig. 4. Diurnal variation of oxygen consumption under the ordinary condition in the hatchery ('control'). Vertical bar at each point represents the standard error. Where no bars are given, standard error was negligible.

at 2 p. m. except in the case of December 14 in which the peak occurred at 8 a. m. Considerable fluctuation with significant difference among the means is observed during the first 24 hours of the experiment.

It is also surprising that the first value in the graph, viz. 8 p. m. of December 12, shows a high rate of oxygen consumption (195 ml/kg/hr). After the first 24 hours, however, the fluctuation becomes more orderly and indicates a regular diurnal rhythm in the rates of oxygen consumption (50 to 120 ml/kg/hr). It is also to be pointed out that samples taken at 8 p. m. and 2 a. m. of the 2nd, 3rd, and 4th days of the experiment show values that are lower than those of samples taken at 8 a. m. or 2 p. m. these days. An exception, however, is seen in the case of December 15 where the mean of the 2 p. m. is not significantly different from it. However, the trend is well marked.

B. Continuous light

The daily variations of the oxygen consumption are shown in Fig. 5 in the same way as in the case of control. The temperature and oxygen content show very little variation, and ranged 4.4 to 5.0 °C and 94 to 97%, respectively.

During this experiment, average rate of oxygen consumption was 80.7 ± 13.3 ml/kg/hr. The coefficient of rhythm strength (RS) was 0. Therefore, in general, there was no diurnal rhythm in the rate of oxygen consumption in continuous light.

Considerable fluctuation, with significant decrease in the means, is observed during the first 30

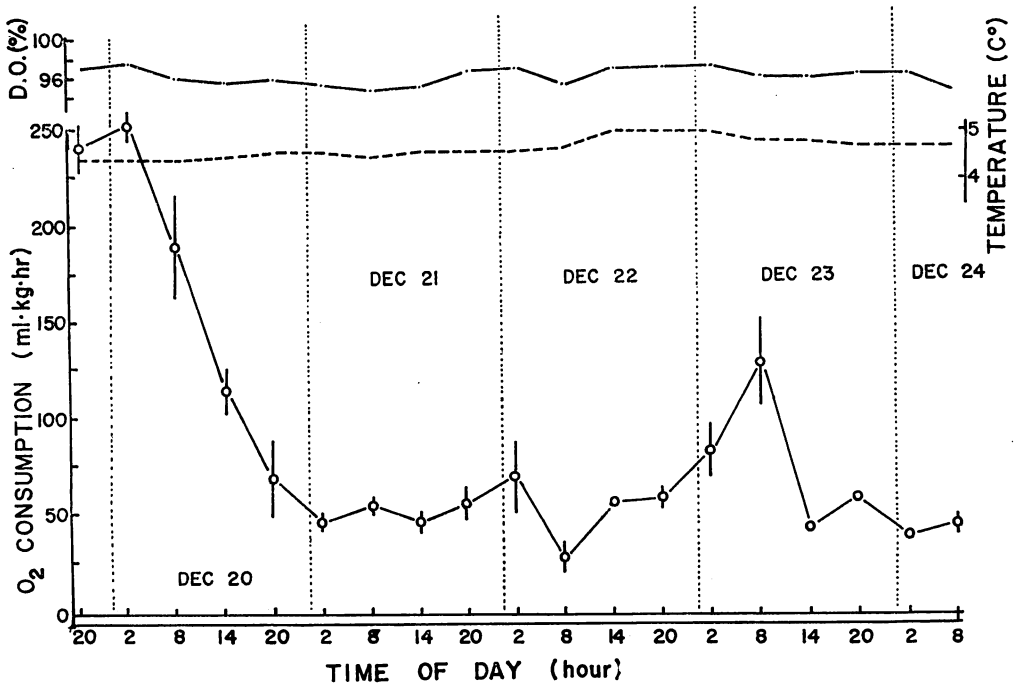


Fig. 5. Diurnal variation of rate of oxygen consumption under the continuous light condition ('light'). Vertical bar at each point represents the standard error. Where no bars are given, standard error was negligible.

hours of the experiment. The values of the first and second samples, viz. 8 p. m. of December 19 and 2 a. m. of December 20, show very high rates of oxygen consumption (approximately 250 ml/kg/hr), but decrease to 50 ml/kg/hr towards the end of this period. It is to be observed that the rate of consumption on the 2nd and 3rd days of the experiment shows almost no variation. Only peak of the rate of consumption during the period of this experiment is found at 8 a. m. of the 4th day (December 23), while the lowest rate of the consumption shows at 8 a. m. of the 3rd day. Since the fluctuation in the rates of consumption can not be related to day and night periods, it may be concluded that in continuous light the metabolic rate does not exhibit a diurnal rhythm.

C. Continuous dark

The daily variations of the oxygen consumption are shown in Fig. 6 with the water temperature and oxygen content in the experimental tank. Temperature and oxygen content of the water were almost constant, (4.1 to 4.3°C and 91 to 94.5%, respectively).

During the experiment, average rates of oxygen consumption was 50.6 ± 9.0 ml/kg/hr. A diurnal rhythm in the rate of oxygen consumption similar to that seen in the case of control is found only on the first day on the experiment (January 10). The lowest rate of the consumption (66 ml/kg/hr) occurred at 8 a. m., and the peak of the consumption occurred at 2 p. m. (150 ml/kg/hr) on that day. On the 2nd day, however, there was no rhythm in the rate of consumption. The lower rate of consumption continued on the next day (January 12). Small increase in con-

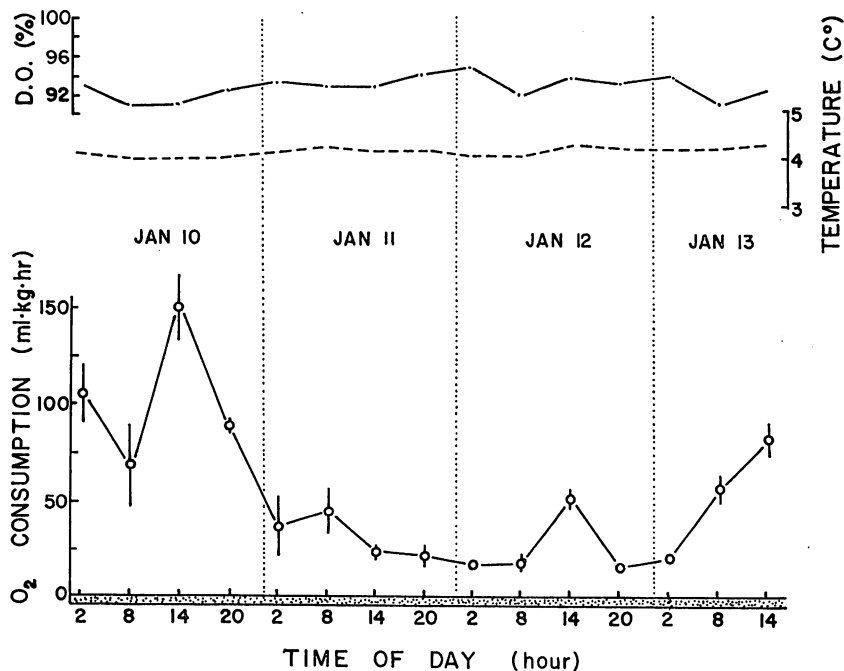


Fig. 6. Diurnal variation of rate of oxygen consumption under continuous dark condition ('dark'). Vertical bar at each point represents the standard error. Where no bars are given, standard error was negligible.

sumption (50 ml/kg/hr) occurred at 2 p. m. on the 3rd day. That small peak decreased again to 16 ml/kg/hr. Thereafter the consumption increased gradually to 80 ml/kg/hr on January 13, the 4th day of the experiment.

The amount of oxygen consumption was lower in this experiment than in the case of the control and light experiments. There is generally no evident rhythm in this condition. Coefficient of the rhythm strength was 0.643.

D. 12 hour alternating light and dark

The data obtained from the experiment in 12-hour alternating light and dark are presented in Fig. 7. The water temperature and the oxygen content in the experimental tank were almost constant throughout the experiment.

During the experiment, average rate of oxygen consumption was 97.3 ± 8.6 ml/kg/hr. The experiment began under light condition on February 5. The amount of oxygen consumed in that condition was 172 and 140 ml/kg/hr. When the light was turned off, the rate of consumption decreased to 79 ml/kg/hr at 2 a. m. on February 6. At 8 a. m., while the dark condition still continued, the rate of consumption increased to about 150 ml/kg/hr. Then, the light was turned on, the rate of consumption was 140 ml/kg/hr at 2 p. m., but was 50 ml/kg/hr at 8 p. m. In the second period of darkness (February 6-7), the consumption increased from 50 to 147 ml/kg/hr. On February 7, 6 hours after the light was turned on (2 p. m.), the rates of consumption decreased suddenly to 45 ml/kg/hr. After 12 hours of light (8 p. m.), the rate of consumption increased to

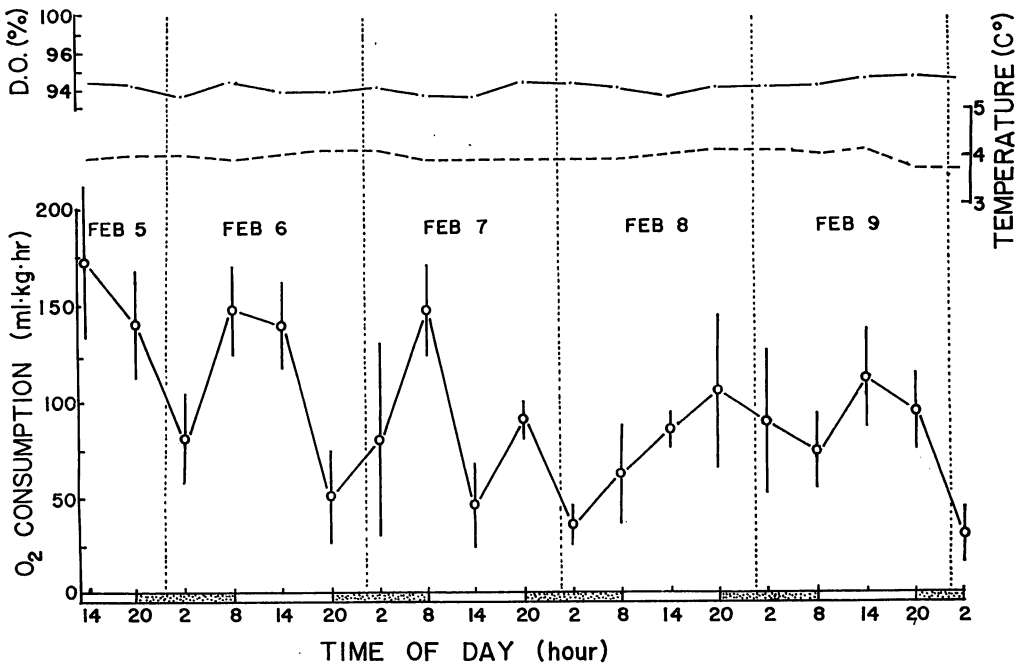


Fig. 7. Diurnal variation of rate of oxygen consumption under alternating light and dark every twelve hour ('12-hour'). Vertical bar at each point represents the standard error. Where no bars are given, standard error was negligible.

90 ml/kg/hr.

During the first two or three days, as mentioned above, the rates of oxygen consumption fluctuated independently of the light or darkness. Thereafter, the rate of the consumption decreased in dark and increased in light. For instance, at 8 a.m. on February 8, when light was turned on, the rate of oxygen consumption increased gradually from 62 to 85 or 104 ml/kg/hr. Then the light was turned off, the rate decreased gradually to 89 and 74 ml/kg/hr. At 2 p.m. on that day, six hours after the light was turned on, the consumption increased to 113 ml/kg/hr. Then, with the light off, the oxygen consumption decreased again from about 100 to 35 ml/kg/hr (February 10).

In 12-hour alternating light and dark, oxygen consumption shows a 24-hour rhythm. It is observed that the rhythm shown during this experimental period can be divided into two cases. The rhythm in the first case (February 5-7) does not depend upon light. The second is the latter half (February 7 to 10). During this period, the rhythm depends upon light. It is also observed that the amount of oxygen consumed during the experimental period is not so variable, and ranges from 50 to about 125 ml/kg/hr in each day. Coefficient of the rhythm strength was 1.265, and it is regarded as being statistically significant.

E. 6 hour alternating light and dark

The mean rates of oxygen consumption of the three fish (Table 1) used in the experiment, at various times of day and night are shown in Fig. 8, in the same way as in the case of control.

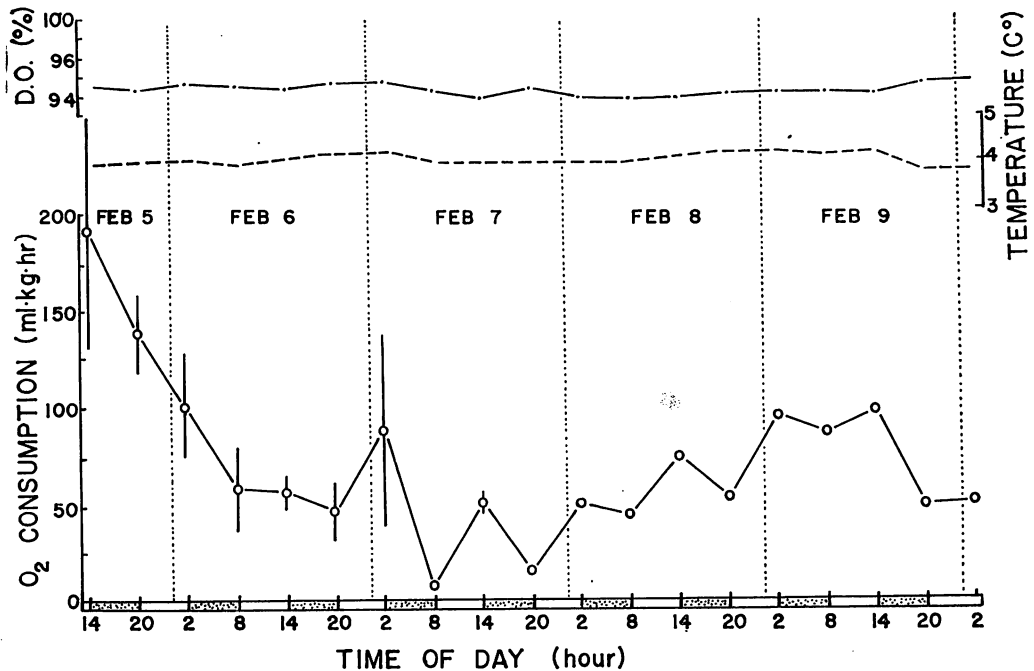


Fig. 8. Diurnal variation of rate of oxygen consumption under alternating light and dark every six hours ('6-hour'). Vertical bar at each point represents the standard error. Where no bars are given, standard error was negligible.

Experimental conditions such as water temperature and oxygen content in the tank are the same as in the previous experiment (Fig. 7, '12-hour'). These factors were almost constant throughout the experiment.

During the first two days, the amount of oxygen consumed decreased from 193 to 47 ml/kg/hr without showing any rhythmic pattern. Thereafter, however, the rate of consumption depended upon light. That is, the rate of oxygen consumption increased from 47 to 88 ml/kg/hr at 2 a. m. on February 7 (light-on). Then, when the light was turned off, rate of consumption decreased suddenly to a minimum of 8 ml/kg/hr (the lowest throughout this investigation). Again when the light was on, oxygen consumption increased to 99 ml/kg/hr at 2 p. m. on that day. The typical example of this event was found between 8 a. m. on February 8 to 2 a. m. on February 9. During this period, the rate of oxygen consumption increased in light and decreased in dark, and showed as 12-hour rhythm.

In the latter half of the experimental period, the amount of oxygen consumed increased gradually from 8 (8 a. m., February 7) to 97 ml/kg/hr (2 p. m., February 9). During the latter part of the experiment, the variation in the rate of oxygen consumption depended upon the presence or absence of light. In general, coefficient of the rhythm strength in this experiment was 0.846.

2. Activity

A. Feeding

The rates of the feeding activity of ten fish which were kept together in the same tank under 'control' condition are shown in Fig. 9. To analyse the pattern of rhythm, feeding frequency (per hour) of each day was divided into four parts (viz. midnight to 6 a. m., 6 a. m. to noon,

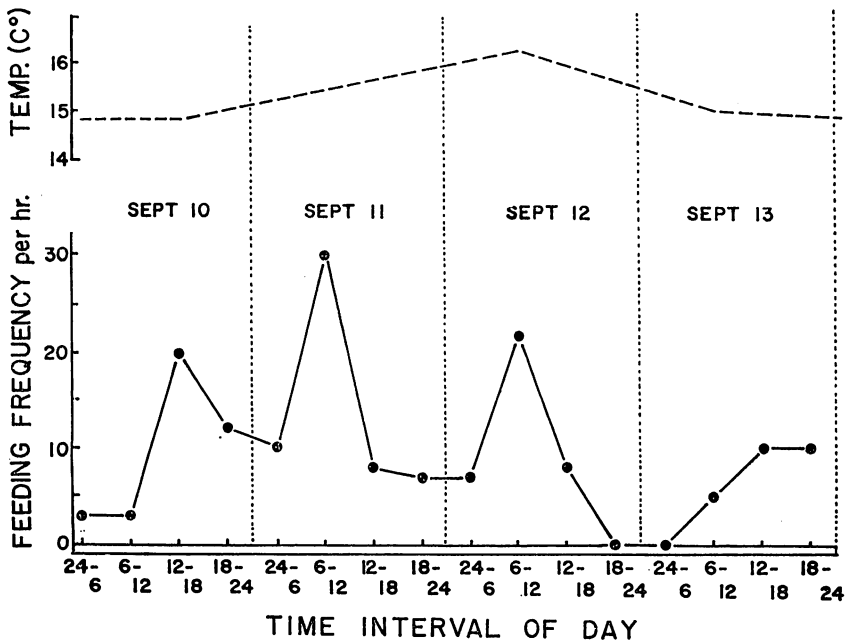


Fig. 9. Diurnal variation of rate of feeding activity under 'control' condition in the hatchery. Ten fish were used in the experiment

noon to 6 p. m., and 6 p. m. to midnight). The water temperature in the experimental tank ranged from 14.1 to 15.4 C° throughout this experiment (Fig. 9).

The rates of feeding activity of the fish show a variation that may be related to day and night. It is to be observed that rate of feeding during the day is generally greater than that during the night. Especially, the trend is well marked on the 2nd and 3rd days of the experiment. During these days, a diurnal rhythm is seen. The peaks of the feeding frequency occur at 8 a. m., and lowest rates of feeding occur in the night. On September 10 (1st day of the experiment), peak of the feeding activity occurs between noon and 6 p. m. An exception, however, is seen in the case of September 13, when only 10 frequencies per hour were recorded between noon and midnight. Coefficient of the rhythm strength in the fish feeding was 0.847 during the experiment.

B. Movement.

Diurnal variations in the rates of movement (activity) of three fish which were kept together in the activity chamber under control conditions are shown in Fig. 10, in the same way as in the case of feeding activity. The water temperature in the experimental tank ranged from 14.1 to 15.4 C°.

The movement activity showed a clear diurnal rhythm during the first two days: Higher rates of movement were found in the daytime (6 a. m. to 6 p. m.) than at night (6 p. m. to 6 a. m.), during those days. However, there appears to be no diurnal rhythm at the latter half of the experimental period. The movements increased gradually from 1 (between midnight to 6 a. m. on October 5) to 32 (between 6 a. m. to noon on October 6) frequencies per hour, and did not

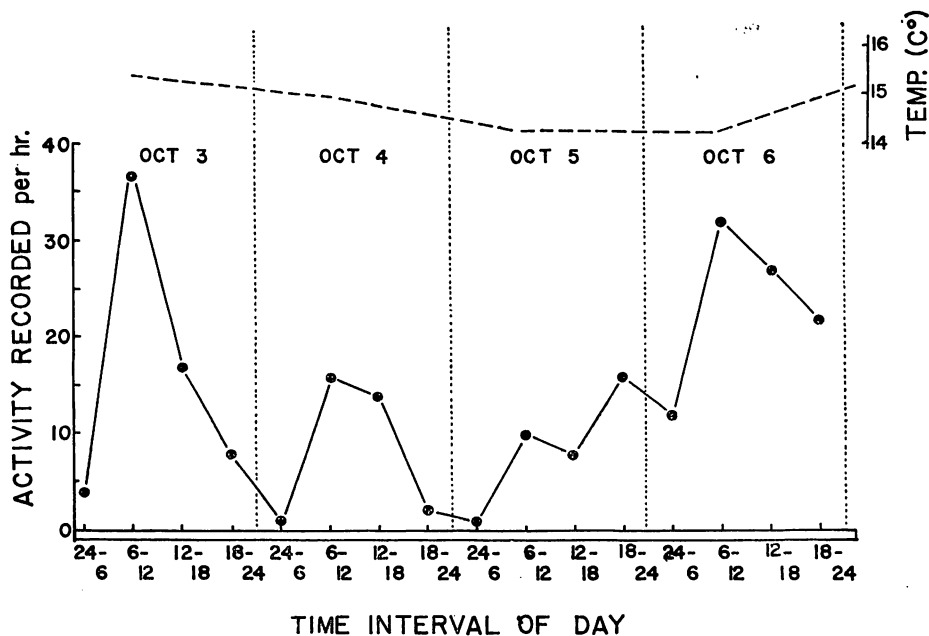


Fig. 10. Diurnal variation of rate of movement under ordinary condition in the hatchery ('control'). Three fish were used in the experiment.

show any diurnal rhythm. On October 6, much frequency (22 to 32 per hour) was found during both day-and night-time, but a peak in the frequency of movement occurred in the morning.

Discussion

1. Rhythm patterns.

A. Oxygen consumption in different condition

In each condition, the rates of oxygen consumption during the first one or two days are fluctuative (viz. 'control', '12-hour') and on the decrease (viz. 'light', 'dark', '6-hour'). Thereafter, the fluctuations become to be a regular ('control', '12-hour', '6-hour'), or no rhythm ('light', 'dark'), which depend upon the light. It is considered that this event is due to the fish adaptation to the experimental condition. Especially, in the cases of continuous light and dark, it is also presumed that the decrease or non-fluctuation of the rates of oxygen consumption is due to physiological fatigue (MORI, 1948) which was caused while constant condition lasted.

On the other hand, the interesting point in the study of rhythmic aspects is whether the fish show an exogenous or endogenous rhythm. In general, typical diurnal rhythm of oxygen consumption was found in the cases of 'control' and '12-hour' conditions. In these cases, the rates of consumption increased in the daytime or light-on condition, and decreased in the nighttime or light-off condition. The rate of oxygen consumption in '6-hour' condition showed a 12-hour rhythm (like twilight rhythm) according to the light-on or -off, but there was no diurnal rhythm. On the contrary, the diurnal variation of the oxygen consumption did not show any rhythm in the cases of 'light' and 'dark'. From the results, it is concluded that the rate of the oxygen consumption in fish shows an exogenous rhythm which occurs as immediate responses to light change.

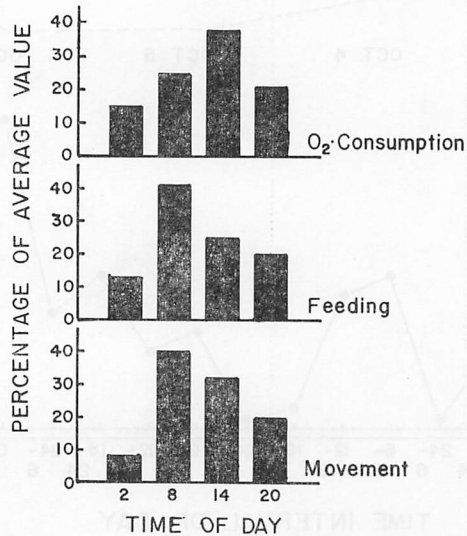


Fig. 11. Rhythm patterns of oxygen consumption, feeding and movement with percentage of the variations in average at various times of day.

B. *Relationship between the rates of oxygen consumption and of feeding and movement*

To facilitate comparison between rhythm patterns of oxygen consumption, feeding and movement, the histograms of average variation at various times in percentage are shown in Fig. 11. When considering the histograms, peaks of rhythm are different between oxygen consumption and both feeding and movement. That is, a clear peak of oxygen consumption occurs at 2 p. m., while the peaks of feeding and movement occur in the morning. It is to be considered that this event is originated from "biological hysteresis" (MORI, 1948, KOBAYASHI and WATABE, 1958) and due to the specific dynamic action SDA (SHIRAI, 1950) which is well known in the field of energy metabolism. That is, a lot of oxygen is consumed by the animals a few hours after feeding, in order to digest the food. In this connection, HIRATA (1960 b) has also observed in the relation between feeding and respiration in goldfish.

2. *Effect of light duration on the oxygen consumption*

Table 2 shows the influence of light duration upon the amount of oxygen consumption. In the control, average amount of oxygen consumption was 92.8 ml/kg/hr, and this value was the

Table 2. Rates of oxygen consumption under different light duration. Each value shows average rate (ml/kg/hr) of oxygen consumption in three fish.

time of day	'control'	'light'	'dark'	'12-hour'	'6-hour'
14	—	—	—	173.1	191.5
20	186.6	240.3	—	139.8	138.5
02	123.9	251.9	100.7	80.4	100.8
08	68.1	190.8	67.9	148.2	58.7
14	199.3	115.3	140.2	139.8	56.4
20	54.4	70.3	87.3	49.6	46.9
02	76.6	48.3	36.2	77.9	88.7
08	115.2	55.1	43.0	148.6	110.2
14	75.3	45.8	22.5	61.4	12.9
20	63.7	55.7	20.3	90.7	15.4
02	69.4	70.9	16.0	36.0	49.1
08	83.3	27.7	17.8	63.8	44.4
14	118.2	56.4	49.1	84.7	73.3
20	94.5	59.0	14.9	106.3	52.6
02	57.3	83.9	19.5	90.7	95.4
08	103.0	129.6	56.4	76.1	129.2
14	158.1	44.8	80.1	112.4	97.1
20	55.0	59.0	—	93.7	45.8
02	77.8	39.8	—	38.2	48.2
08	85.9	46.4	—	42.8	76.3
Total Av.	92.82	89.02	51.46	85.91	70.71
RS	1.176	0.003	0.254	1.242	0.331
Date	Dec. 12-17	Dec. 19-24	Jan. 10-13	Feb. 5-9	Feb. 5-9

greatest among five light conditions. The lowest rate of oxygen consumption was found in the case of continuous dark condition (50.6 ml/kg/hr).

Statistical analyses show that there is no significant difference between control and 12-hour at the 95% confidence level. Also when comparing the control-light, -dark and -6-hour, the first two groups show significant difference, (t : 0.01 and 0.05), while in the last group the difference from control and dark condition is highly significant.

From these results, it is found out that prolonged light or dark conditions cause injurious influence on the oxygen consumption. While the '12-hour' condition is similar to the control, the '6-hour' condition is considerable different.

On the other hand, daily amount of oxygen consumption is also influenced by different light

Table 3. Daily variation of the amount of oxygen consumed (ml/kg/hr) by three fish in each condition.

day after exp. start	control	12-hour	6-hour	dark	light
1	144.5	135.4	122.4	101.3	199.6
2	79.9	104.0	75.6	30.5	54.9
3	83.7	63.0	30.5	27.8	52.7
4	103.2	89.5	87.6	52.0	45.2
5	72.9	70.3	66.9	—	45.2
coef. of variation	0.269	0.281	0.389	0.558	0.670

duration. The daily variation of the amount in each condition is shown in Table 3. The lowest variation of 0.269 was found in the case of the control, while continuous light condition shows the highest variation of 0.67 (Table 3). To explain in more detail, one may classify the coefficient of variation into two categories from this table. That is, the first category contains control, 12-hour and 6-hour (0.269, 0.281 and 0.389). The second category contains dark and light (0.558 and 0.670). When comparing the decreasing rate of oxygen consumption for the first two days, the value of the first category shows a gradual decrease while that of the second category shows a sharper decrease than the first one.

Therefore, it is also said that control and alternating light or dark conditions are different from continuous conditions. That is, continuous conditions resulted in decreased oxygen consumption when compared with alternating conditions. Namely, it is concluded that considerable daily variations of environmental factors are very important for their respiration.

HIRATA (1960a) has emphasized that the inflection point of temperature changes, d^2T/dt^2 , (here, T : temperature and t : time) is one of the important factors for the goldfish feeding and growth as a stimulus. From the present investigation, it is also considered that d^2L/dt^2 (here, L : light and t : time) is an important factor for the salmon metabolism being similar to d^2T/dt^2 in the case of goldfish feeding. It is able to suggest that d^2T/dt^2 and d^2L/dt^2 are effective factors for both warmer (*viz.* *Carpio*) and colder (Salmonoid) fish.

Summary

1. The experiments on the diurnal rhythm in young Atlantic salmon were carried out in the hatchery during September to February. The oxygen consumptions of three fish were measured at several times (2 a. m., 8 a. m., 2 p. m., 8 p. m.) by modified TAMURA's method under the conditions of 'control', 'light', '12-hour' and '6-hour'. Rates of feeding (ten fish) and movement (three fish) were recorded with simple feeding recorder and actograph under just 'control' condition. Each experiment lasted for four or five days. Temperature and oxygen content in the water were kept almost constant during each experiment.

2. In the cases of oxygen consumption, considerable fluctuations ('control', '12-hour' '6-hour') or decreases ('light', 'dark') of the rates are observed during the first one or two days of each experiment. Later, the fluctuations or decreases become to be a regular ('control', '12-hour', '6-hour') or no rhythm ('light', 'dark'). From the events, it is considered that the fish take one or two days to adapt to the experimental condition due to the physiological fatigue.

3. The rate of oxygen consumption in the 'control' shows a variation that may be related to day and night. Higher rates of consumption show in the daytime, generally, and lower rates were seen during the nighttime. On the contrary, there is no diurnal rhythm in the cases of continuous light and dark conditions. After two or three days, the rates of oxygen consumption in the cases of alternating every 12-hour and 6-hour depend upon the light-on or -off, and show 24-hour-rhythm and 12-hour-rhythm, respectively. It is to be presumed from the results that the daily variation of the rate of oxygen consumption shows an exogenous rhythm from those which are under the control of light factor.

4. The rates of feeding and movement show a diurnal rhythm like a rate of oxygen consumption. Only one point of difference between oxygen consumption and both feeding and movement is generally found in the time of their peak occurrence. That is, the peak of rate of oxygen consumption occurs at 2 p. m. while the peaks of both feeding and movement occur in the morning on the whole. It is to be considered that the maximum rate of oxygen consumption is due to the specific dynamic action (SDA) which occurred a few hours after their feeding and caused by the food digestion.

5. Daily amount of oxygen consumed is influenced by different light duration. In the control, average amount of oxygen consumed was 92.8 ± 9.8 ml/kg/hr, and was the greatest among the conditions. The lowest rate of oxygen consumption was found in the case of continuous dark condition (51.5 ml/kg/hr). The coefficient of daily variation of the amount is divided into two categories. That is, the first category contains control, 12-hour and 6-hour. The second category contains dark and light conditions. When compared with the decreasing rate of oxygen consumption for the first two days in each experiment, the first category shows gradual decreases while the second category shows sharper decrease. Therefore, continuous light or dark condition caused more injurious influence on the oxygen consumption than under the alternating condition.

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