THE EFFECTS OF PHOTOPERIOD AND TEMPERATURE ON THE DAILY PATTERN OF LOCOMOTOR ACTIVITY IN JUVENILE SOCKEYE SALMON, *ONCORHYNCHUS NERKA* (WALBAUM).

by

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ABSTRACT

The endogenous and exogenous factors contributing to a diel rhythm of locomotor activity in juvenile sockeye salmon Oncorhynchus nerka (Walbaum) were examined in the laboratory. The basic measure employed in the investigation was spontaneous locomotor activity. High frequency sound (800 kHz) was used as a monitoring technique to collect continuous activity records.

Three major areas received attention. First, a description and analysis were provided for the entrained diel activity pattern under three different temperatures (5°, 10°, and 15°C) and three different photoperiods (8L 16D, 12L 12D, and 16L 8D). The combined effects of temperature and photoperiod upon the basic 24 hour response were recorded and analyzed.

Juvenile sockeye salmon were nocturnally active immediately after emergence from the gravel. A diurnal activity pattern was gradually acquired during the following 14 days and was maintained for 12 months. Photoperiod was the primary environmental synchronizer for either diurnal or nocturnal activity.

The endogenous component of the activity rhythm was examined in constant environmental conditions. Constant light (34.4 lux at 10°C) facilitated the free-running response while constant dark inhibited it. The spontaneous frequency in constant light was 23.30 hours but this could

be altered by light intensity or periodic feeding.

The final experiments focused upon the relationship between the environmental stimulus (photoperiod) and the physiological sensory mechanisms mediating the entrained response. The eyes were the primary photoreceptors mediating information about the light-dark environment. The entrained activity response disappeared when the retina was not illuminated.

When the pineal body was removed or shaded, juvenile sockeye responded with increased activity. Intraperitoneal injections of melatonin (N-acetyl-5-hydroxy-tryptamine) or serotonin (5-hydroxy-tryptamine) selectively altered the activity amplitude in either the light or dark respectively.

Juvenile sockeye salmon possess an endogenous circadian activity rhythm which is synchronized by the photoperiodic cycle. The fish are generally light active, except for the period immediately after emergence. However, interactions between daylength and temperature can result in temporary dark active responses. Mediation of the photoperiodic information occurs via the retina, but without transmission by optic nerve pathways. Chemical agents (melatonin and serotonin) produced by the retina and/or pineal might control the activity amplitude in light and dark, thus resulting in the characteristic entrained pattern.

ERRATA

Bunning should read Bünning

Danielevskii should read Danilevskii

p. 36, line 15, significant

p. 87, line 23, locations

p. 105, line 22, Baggerman, B. 1957

p. 106, line 24, principles

p. 107, line 1, rhythms

p. 112, line 30, division

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1. INTRODUCTION

The primary objective of this thesis is to provide an accurate description and analysis of those endogenous and exogenous factors contributing to the expression of a daily rhythm of locomotor activity by juvenile sockeye salmon, Oncorhynchus nerka (Walbaum).

The term "juvenile" refers to the interval in the life cycle which these fish spend as residents in fresh-water lakes before migrating to a marine environment. The duration of this period is usually one, frequently two, and occasionally three years, but in the more northern lakes of their geographical distribution the longer terms of residence are more common (Foerster, 1968).

It appears that biological rhythmicities are ubiquitous in the animal kingdom, and can have a profound effect upon a diversity of physiological and behavioural processes. The display of periodic phenomena at both the cellular and intact organism levels has been well documented, and endogenous rhythms are apparently at the base of numerous expressions of behaviour and development receiving attention in recent years (reviews: Cloudsley-Thompson, 1960, 1961; Danielevskii, 1965; Harker, 1964; Farner, 1965; Bunning, 1967). Circadian and lunar periodicities have been identified in single neurons (Strumwasser, 1965), and at the sub-cellular level the rhythmic production of nucleic acids has received limited attention (Eling, 1967; Jerusalem, 1967).

Daily periodicities have been investigated at the intact organism level in a number of species ranging from the protozoa to the higher vertebrates. The unicellular dinoflagellate *Gonyaulax polyedra* exhibits a diel rhythm in bioluminescence and cell division (Sweeney and Hastings, 1957, 1958), whereas the higher vertebrates including birds, rodents and man have been demonstrated to possess characteristic diel cycles which are synchronized by specific environmental stimuli (DeCoursey, 1960; Hauty and Adams, 1965; Menaker, 1965; Menaker and Eskin, 1967).

Photoperiodic mechanisms receive frequent consideration in those investigations pertaining to the fields of orientation and navigation. Here, an endogenous rhythmicity with a characteristic 24 hour period is suggested to play an important role in the direction finding of a variety of organisms, including fish (Schwassmann, 1960; Schwassmann and Braemar, 1961; Groot, 1965; Hasler, 1967).

A survey of the literature reveals that only limited attention has been focused upon photoperiodic mechanisms in teleosts. Most efforts have considered the adaptive significance of the response to daylength and temperature, and its relationship to the timing and onset of migrations or the control of reproductive behaviour (reviews: Baggerman, 1957, 1959, 1960a, b; Farner, 1961a, 1965; Harrington, 1959; Hoar, 1951, 1953, 1965).

Cyclic patterns of locomotor activity have been reported in a few teleosts, including salmonids (Northcote, 1962; Blahm, unpublished; Davis and Bardach, 1965; Groot, 1965; Thines, et al., 1965; Bohun and Winn, 1966; Lichtenheld, 1967; Verheijen and DeGroot, 1967; Chaston, 1968). The evidence available to date suggests that a rhythmic diel activity pattern is expressed by sockeye salmon during all stages of their life cycle (Hoar, 1958; Johnson, 1961; Groot, 1965).

In this investigation the locomotor activities of juvenile sockeye salmon were monitored without the usual visual and tactile stimuli that have been associated with former techniques for recording the movements of small fish or other aquatic organisms. Sonic transducers were introduced as sensing devices to detect fish movement. Supporting electronic instruments transformed the output from these units to an electrical impulse actuating pens on an event recorder.

The first experiments provide a description and analysis of entrained locomotor activity under three different photoperiods and three different temperatures. The design of the experiments enabled the investigator to record and analyze the combined effects of temperature and photoperiod upon the basic twenty-four hour cycle.

The second phase focuses upon an analysis of the endogenous component, as expressed by the free-running rhythm of fish held under constant environmental conditions.

This is considered necessary since it is this component which is entrained by the environmental stimuli. Entrainment results in the characteristic diel periodicity apparent under normal oscillating environmental conditions.

The third and final phase investigates some relationships between the environmental stimuli and the physiological sensory mechanisms mediating the entrained response. The most striking changes in motor activity during any twenty-four hour period occurred in relation to the onset and termination of light. Emphasis is focused upon the role of the eyes and/or pineal body as the principal receptor organs for detecting changes in light intensity. Both surgical and pharmacological techniques were utilized in this phase of the investigation.

The following quotation is applicable to the problems at hand.

> "If any attempt is to be made to control activities of fish through a manipulation of environmental conditions it will be necessary to understand the physiological basis of some of the behaviour. This is particularly true with respect to migratory phenomena which occur cyclically and are associated with regular changes in the endocrine glands. It may or may not be possible to exercise a control over migratory behaviour but, in any case, it is essential to know something of the inter-relationships of environmental and physiological cycles in order to predict the sequence of behaviour. A study of the physiological changes which are responsible for migration demands particular attention.
>
> In addition, physiology can make an important contribution to ethology by evaluating the limits of perception of the receptor mechanisms of fish of all ages. Prediction of many activities which depend upon changing light, temperature or water currents demands such information" (Hoar, 1958).

In summary, the main purpose of this investigation is to follow a course of basic research concerning the effects of photoperiod and temperature on the locomotor activity of juvenile sockeye salmon. The data presented are applicable to a variety of problems which consider or are dependent upon the daily activity cycles expressed by teleosts.

2. MATERIALS AND METHODS

The investigation was conducted under controlled environmental conditions. The laboratory was located in a concrete basement area of an isolated building, where periodic disturbances could be kept at a minimum. The operation of air pumps for aquaria created a constant noise level, confusing any incidental production of sound in other parts of the building.

It is recognized that certain pervasive environmental fluctuations which may have the capacity to entrain the subjects could not be manipulated. (For a review of such naturally oscillating factors as electromagnetic fields, cosmic ray bombardment, changes in the local barometric pressure and their effects on cyclic activity, see Brown, 1960, 1965). At the present time, the author does not consider it practical to attempt the control of these subtle factors.

2.1 Water Supply

Chlorinated city water was used for general maintenance activities and as a supply for temperature regulated water baths.

A system was designed to dechlorinate, purify and adjust the pH of the city water. This dechlorinator was maintained at full water level by installing a control valve delivering a regulated water flow. The unit was placed outside the laboratory, in a location insuring an adequate water pressure for a gravity flow system.

The delivery system to the aquaria and activity chambers was constructed of 1/2" (1.3 cm) inside diameter polyethylene pipe. No metal fittings were in contact with the water supply and the complete system was insulated with fiberglass to reduce variations in the temperature.

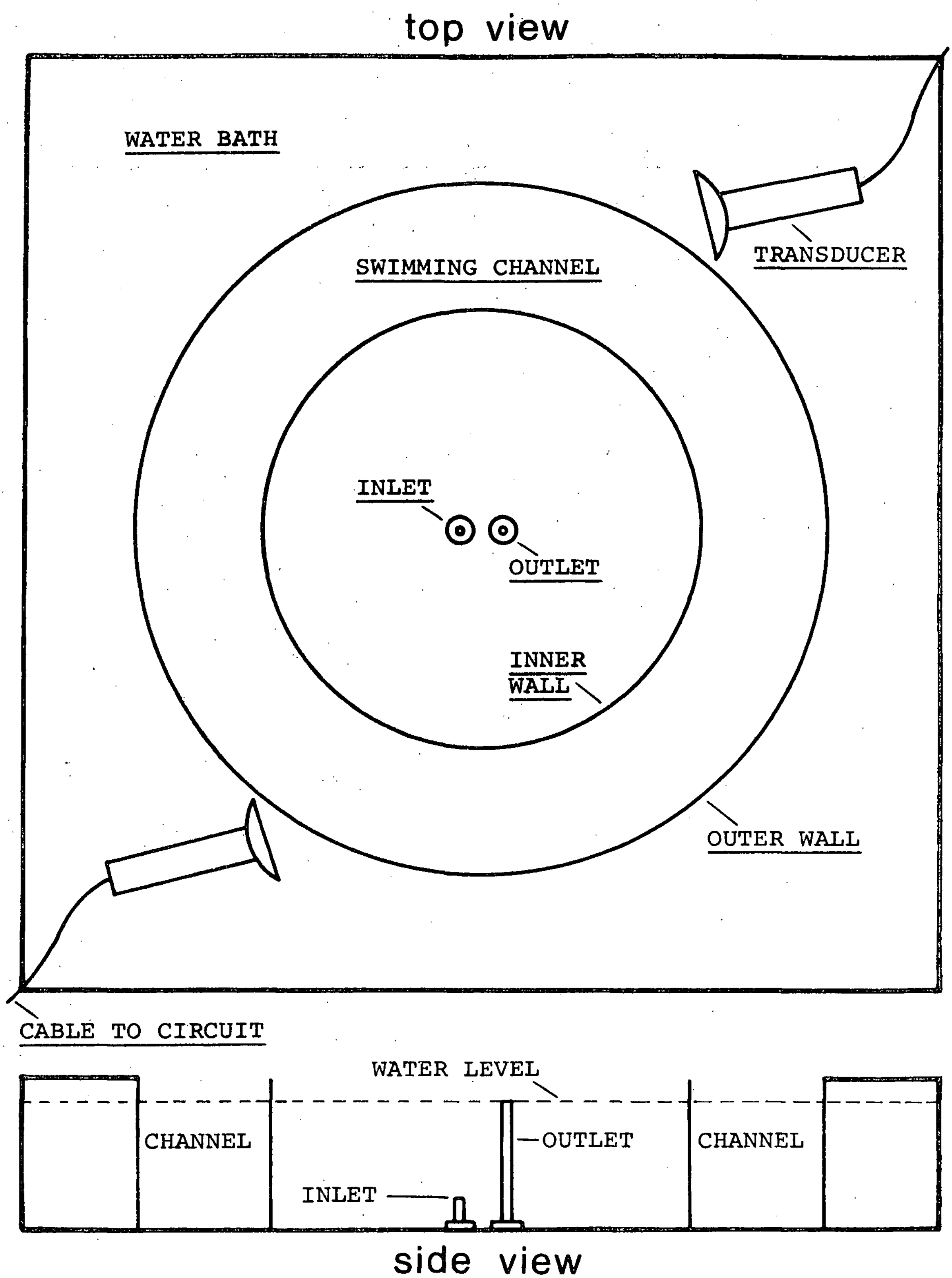
2.2 Activity Chambers

The external dimensions of each activity chamber were 48" x 48" x 8" (122 cm x 122 cm x 20.3 cm). The chambers were constructed of 1/2" (1.3 cm) plywood and the interiors were lined with fiberglass cloth, coated with Estrex #101 all purpose resin for waterproofing and added strength.

Each chamber contained a water bath and a circular swimming channel measuring 7" wide and 7" (17.8 cm x 17.8 cm) deep with a maximum diameter of 36" (91.44 cm) (Fig. 1). The swimming channel was formed by two rings of 1/16" x 8" (1.8 mm x 20.3 cm) plexiglass which were sealed to the bottom of the activity chamber with fiberglass resin. The walls were painted with non-toxic flat black rust-oleum paint (The Rust-Oleum Corporation, Evanston, Illinois) to reduce glare and to minimize the number of lateral cues available to the fish.

All dechlorinated water was contained within the maximum circumference of the swimming channel. The inner

Figure 1. Diagram illustrating top and side views of an activity chamber. All component sections are labelled.


top view
WATER BATH
TRANSDUCER
SWIMMING CHANNEL
INLET
OUTLET
INNER WALL
OUTER WALL
CABLE TO CIRCUIT
WATER LEVEL
CHANNEL
OUTLET
CHANNEL
INLET
side view

ring of plexiglass had sixteen 1/8" (3.6 mm) perforations to allow an exchange of water into and out of the channel. These holes also assisted in keeping detritus at a low level by acting as a "scouring system" when water was moving out of the swimming channel.

A total of nine activity chambers were constructed for the investigation. They were mounted in three vertical racks constructed of #225 steel dexion. Each rack contained three activity chambers and measured 49" x 49" x 84" (124 cm x 124 cm x 213 cm).

2.3 Temperature Controls

Water temperatures in the activity chambers were controlled by Kodak thermostatic mixing valves (Eastman Kodak Co., Rochester, N.Y.). These were connected to the hot and cold city water outlets and supplied the water baths surrounding the outer border of the swimming channels. The temperatures of these two bodies of water were always allowed to come into equilibrium before proceeding with an experiment.

Low water temperatures were maintained during the summer months by cooling the city water with a portable refrigerating unit.

A Honeywell thermograph was used to record the daily temperature fluctuations in the swimming channels during March, 1967. These data were compared with readings obtained by hand-held thermometers and the dial indicators on the thermostatic mixing valves.

After equilibrium was reached between the swimming channels and the water baths, the record indicated less than 0.3°C fluctuation in the environmental water temperature during a twenty-four hour period (Fig. 1, appendix). It was assumed that the temperature controls were adequate and the slight daily variation did not constitute a sufficient value to entrain the locomotor activity of juvenile sockeye salmon.

The use of the thermograph was discontinued after establishing that both the records obtained with the mixing valves and the hand-held thermometers were sufficient over the course of a seven day experimental period.

The environmental temperatures ranged from 5°C to 15°C, which are within the generally accepted limits of tolerance for juvenile sockeye salmon (Brett, 1952, 1956).

2.4 Photoperiodic Controls

Illumination was provided by incandescent lamps, except where specifically indicated. Indirect lighting was achieved with white plastic sheeting positioned 12" (30.5 cm) above the water surface. It was assumed the opaque sheeting also reduced the number of overhead cues available to the fish for orientation. Black plastic shielded each activity chamber to prevent interference with the environmental photoperiod by external light sources.

Sudden changes in the room light intensity were prevented by constructing a "false entry" to the laboratory and by maintaining a constant low illumination with a shielded 7 1/2 watt lamp.

The environmental photoperiods were regulated by model T-101 Intermatic time switches (Marr Electric Ltd., Cooksville, Ont.) which were synchronized with each other and connected to separate channels on the event recorder. This arrangement indicated the exact time of day that the lighting conditions were changed and provided a measure of the phase relationship between photoperiod and the onset or termination of activity.

An electronic dimming system introduced a twilight effect to the artificial environment. The circuitry had a 600 watt capacity and was adjusted to produce a gradual change in the environmental light intensity over a twenty minute period (Figs. 2 & 3, appendix). These "dimmers" eliminated the dramatic changes in light intensity associated with interval timers and avoided the initial "startle" response observed in juvenile sockeye when the lights were turned on or off.

Light intensity readings were taken at the water surface with a Brockway incident light meter. All readings were converted to "lux" where one lux equals 0.0929 candlepower.

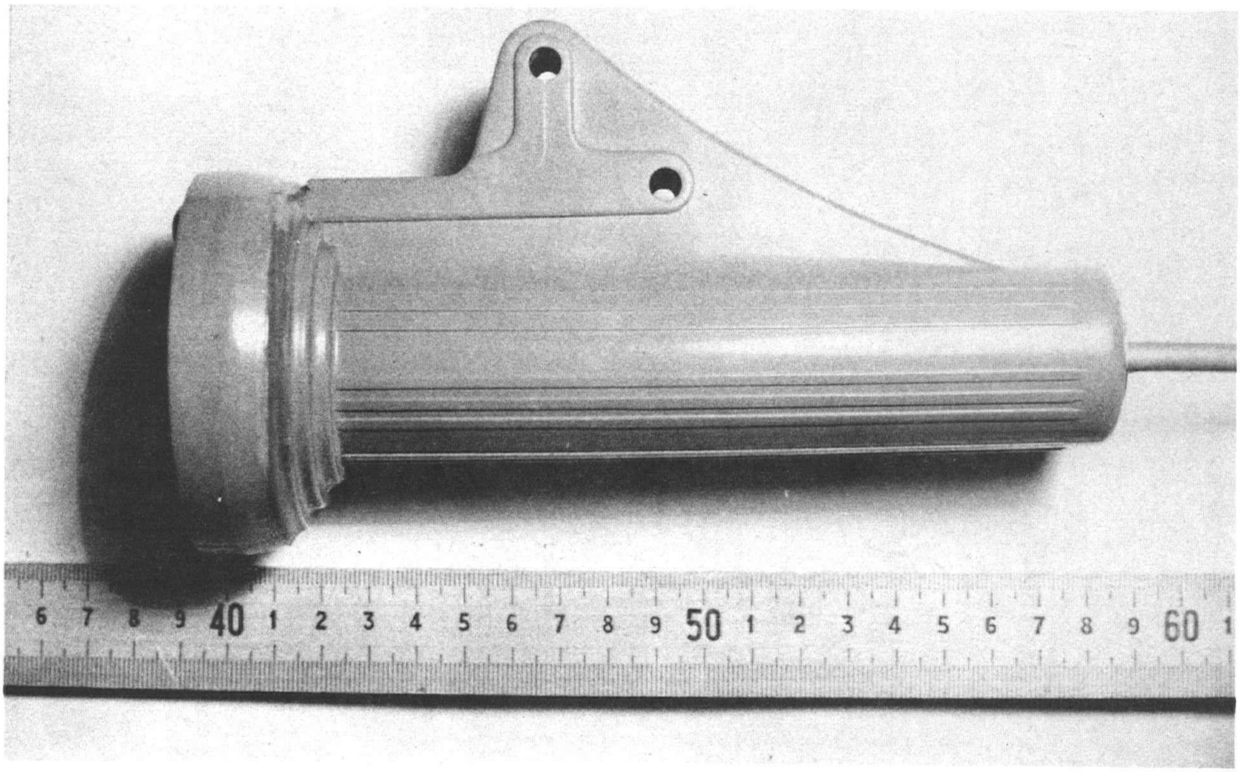
2.5 Activity Monitoring System

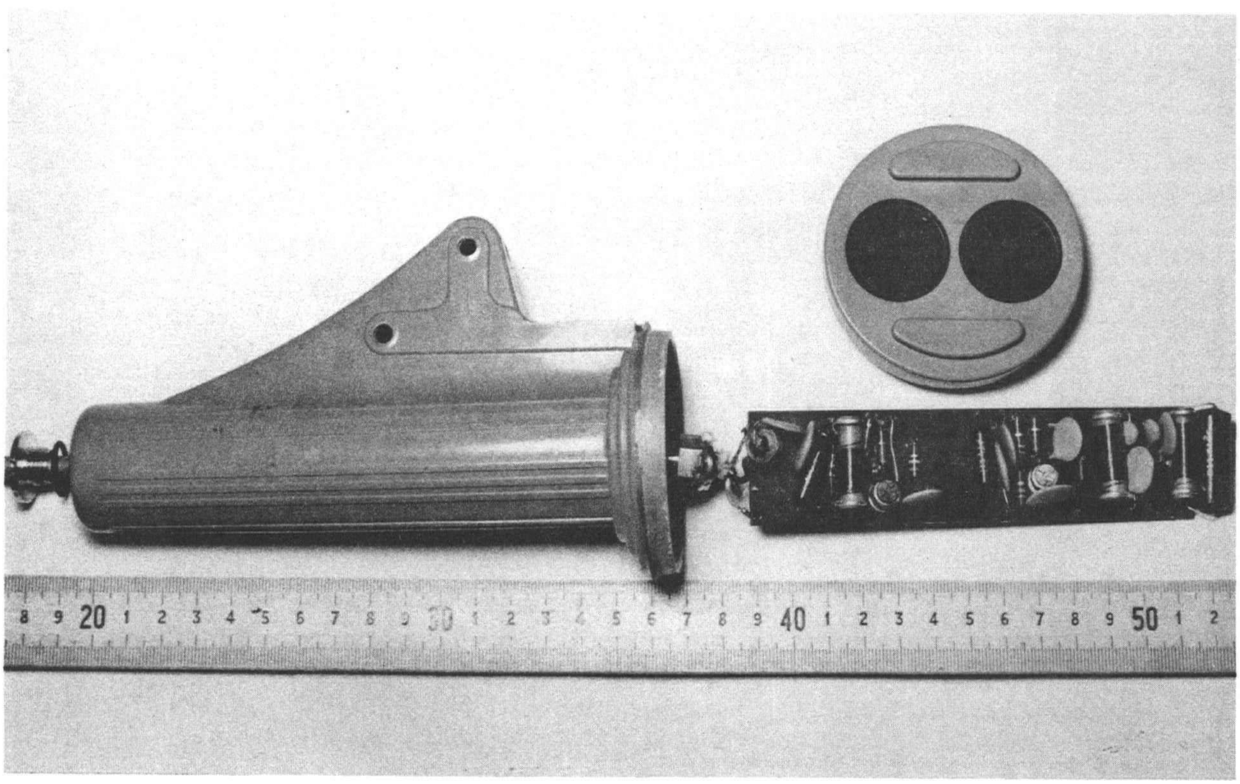
The electronic instruments for recording locomotor activity were built around a compact sonic transducer (Enterprise Manufacturing Co., Akron, Ohio) (Fig. 2). The plastic transducer head contained the circuitry in a sealed waterproof unit. All electronic components were sprayed with a rubberized compound for added resistance against shock and moisture.

Each transducer emitted a continuous wave signal of 800 kilohertz (1 hertz = 1 cycle/second) in a 4° cone. This frequency is well above the wavelengths heard by fish in general (Wodinski and Tavolga, 1963; Marshall, 1965; Protasov, 1967) and sockeye salmon in particular (Brett and Groot, 1963; Van der Walker, 1966). When the beam was transmitted through the water and came in contact with an object, the signal was "reflected" back to the transducer. The output of the instrument was the amplified frequency difference between the emitted and returning signals. If an object was travelling away from the source of sound emission, the wavelength of the "reflected" signal would be of a lower frequency than if the object were motionless. If the object was moving towards the transducer, the frequency of the returning wavelength would be greater. Therefore, a moving object in the "beam" resulted in a frequency change which could be amplified to operate an event recorder.

Two units were mounted in opposite corners of the activity chamber (see Fig. 1) in the area of the water bath. The beam penetrated the 1/16" (1.3 mm) outer

Figure 2. Photographs illustrating the transducer head and components. (From left to right, cable, cable securing screw, O-ring, waterproof body, end of cable, printed circuit board with rubberized compound removed to reveal the electronic components. Located above is the cap which houses both the sound emission and detecting units).


6 7 8 9 40 1 2 3 4 5 6 7 8 9 50 1 2 3 4 5 6 7 8 9 60 1


8 9 20 1 2 3 4 5 6 7 8 9 30 1 2 3 4 5 6 7 8 9 40 1 2 3 4 5 6 7 8 9 50 1 2

plexiglass wall and crossed the channel at a tangent. This arrangement eliminated any possible interference between the two units, and insured that an object passing through the field of sound would be recorded.

A cable from each transducer was coupled with supporting electronic circuitry. The design and construction of these instruments were commissioned to National Electrolab Associates, Vancouver, B.C. The circuit diagrams are presented in the last section of this thesis (Figs. 4 to 8, appendix).

The basic operation was as follows. Each activity chamber was associated with two transducers, a bistable logic circuit and a pen on a 20 channel Esterline-Angus event recorder (Fig. 3). The system was arranged so that fish movement detected by one transducer would cause the bistable logic circuit to assume a "set" state. Then, fish movement detected by the other transducer would alter the logic circuit to its "count" state. This action would release a pulse to the recorder. The fish movement had to be detected again by the first transducer and cause the bistable logic circuit to revert back to its "set" state before the next count could be released.

This circuit arrangement was necessary in order to insure that any count released was not the result of fish movement in the beam of only one transducer. This prevented recordings which might be interpreted as representing a high level of activity, but actually consisted of movement within

Figure 3. Block diagram illustrating the transducers and associated circuitry.

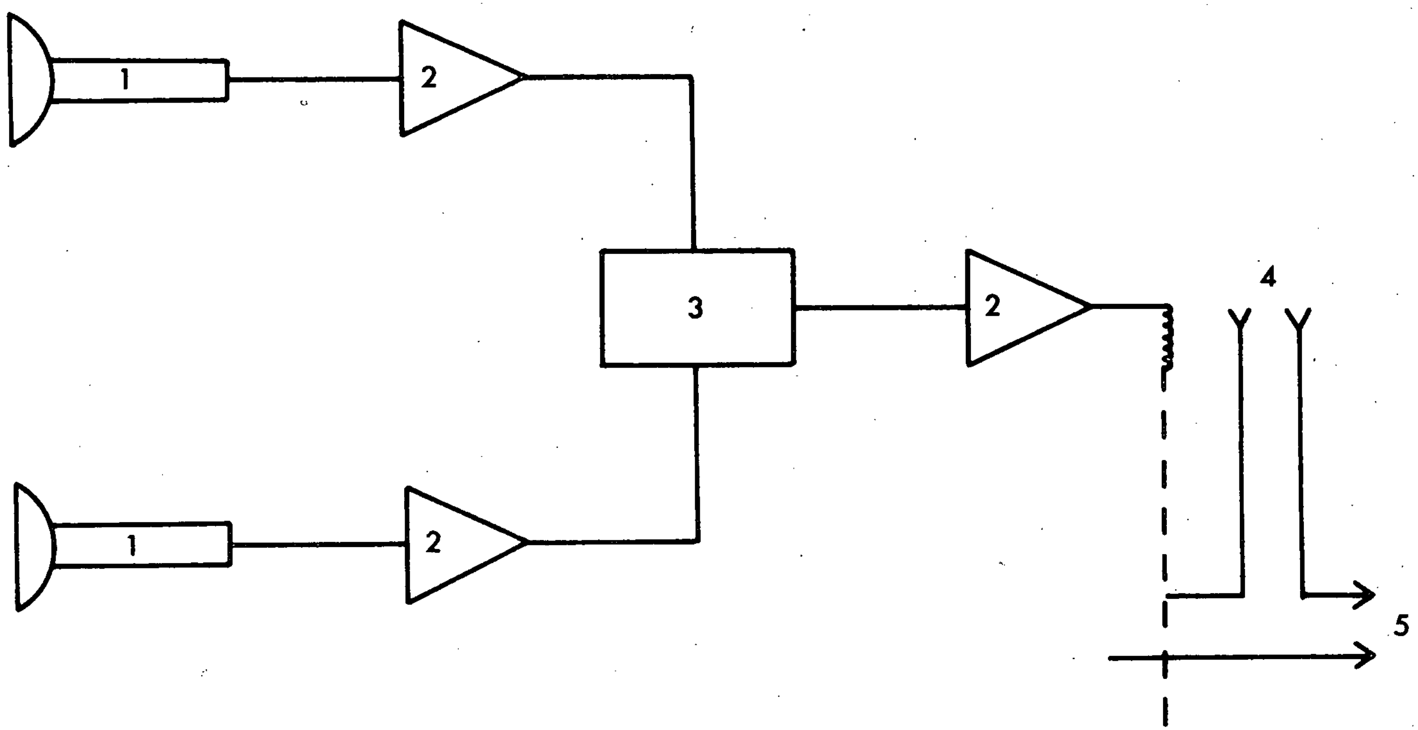
1 = Transducer

2 = Amplifier

3 = Bistable logic circuit

4 = 24 volts AC

5 = To Recorder



Block Diagram

a small area. The behaviour of pink and chum salmon fry is such that continued unidirectional movement occurs in a circular swimming channel (Hoar, 1956). The juvenile sockeye salmon used in these tests exhibited similar movement patterns. The result was a reliable measure of locomotor activity which, if needed, could also indicate the approximate distance covered during any interval.

No visual or tactile disturbance could be attributed to the monitoring devices. The transducers were located outside of the swimming channel, an arrangement permitting the investigation to proceed without the addition of another variable to the immediate environment. This instrument had definite advantages over the use of photocells and their ambient light intensity, or mechanical grids which interfered with fish movement (Davis and Bardach, 1965; Thines, et al., 1965; Bohun and Winn, 1966).

The transducers were tested for any interference with the activity of the experimental subjects. Ten fish were introduced singly into a swimming channel and their motor activities were recorded by visual means (aided by a hand tally meter). These measurements were conducted for a 10 minute interval before, during and after the instruments were set into operation. One mark was recorded for each revolution of the activity chamber. These data were subjected to analysis of variance, indicating that no significant activity changes resulted from the high frequency signal produced by the transducers (Table 1).

Table 1. A comparison of activity per 10 minute interval before, during and after instrument operation. (P<.01)

	Test Number										
	1	2	3	4	5	6	7	8	9	10	Total
Before "A"	6	1	4	3	2	5	4	1	1	7	34
During "B"	7	0	4	3	3	7	4	0	2	5	35
After "C"	6	1	3	1	5	6	5	0	1	4	32

Table 2. A comparison of visual and instrumental recording techniques. (P<.01)

	Test Number										
	1	2	3	4	5	6	7	8	9	10	Total
Tally	0	7	4	0	2	1	1	4	6	1	26
Inst.	0	7	4	0	2	1	1	4	7	1	27

A related series of tests compared visual and automatic recording methods to establish the accuracy of the instrumental recording system. Ten fish were introduced singly into an activity chamber and a simultaneous record was obtained with both methods.

A count was registered each time the subject passed a reference point which was situated in the beam of that transducer triggering the event recorder. This location facilitated the comparative aspect of the two methods. Each test lasted ten minutes and ten replications were produced for the series.

The instrumental technique of data collection gave records which were virtually identical to those obtained by visual observations (Table 2).

The instruments and circuitry appear to be well suited for recording the movements of small fish, and can be applied to any situation where it might be desirable to indicate a subject has passed a certain reference point in an activity chamber or aquarium.

2.6 Live Material

All specimens of juvenile sockeye salmon were obtained from the hatchery facilities at the Fisheries Research Board of Canada, Biological Station, Nanaimo, B.C. Most fish originated from Skully Creek at Lakelse Lake, B.C., but a small sample of Kamchatka Peninsula presmolts were also available for comparative purposes. Prior to all experiments, the fish were maintained on Clark's dry food (J.R. Clark, Salt Lake City, Utah).

These fish were maintained at two sites which provided different photoperiodic and environmental conditions, semi-natural and artificial. The semi-natural conditions were obtained by placing fish in a large outdoor holding tank (3,633 gallons). This tank was subject to natural fluctuations in the photoperiod and also received an unmeasurable influence from artificial light sources in nearby buildings. The tank was divided by plastic screens into six equal compartments each of which contained individuals from one locality and parental stock.

The controlled artificial holding conditions were achieved with two 50 gallon aquaria. The photoperiod was regulated by an Intermatic time switch and incandescent lamps. These aquaria were supplied with airstones and a slow incoming rate of dechlorinated water. Temperature variations were kept at a minimum by insulating the water delivery system.

A separate group of sockeye eggs from Skully Creek were hatched and maintained in the laboratory under controlled environmental conditions. The fry were retained in 10 gallon aquaria for periodic testing to determine if developmental changes existed in the photoperiodic response.

2.7 Experimental Design

All tests, except where specifically indicated, were performed on single individuals. A fish was introduced to each activity chamber and allowed two days for acclimation to the new environment. The acclimation period was determined from the analysis of preliminary experiments. The actual data recording was focused upon the following six days of activity, but occasionally it was desirable to permit an experiment to continue for longer periods of time.

No fish was fed during an experiment except in those tests designed to study the effects of feeding upon the activity pattern.

The preliminary experiments examined the basic entrained response. The combined temperature and photoperiodic conditions used are listed in Table 3. Related tests in this series considered the relationship of age to the basic entrained response, and the synchronizing effects of photoperiod upon both individuals and groups of fish.

The experiments examining the endogenous component (Table 4) were conducted in constant light (LL), constant dark (DD) or constant light with feeding (LLF). Temperatures were maintained constant at 10°C and the light intensity was held constant at 34.4 lux (except where specifically indicated).

Table 3. Summary of temperature and photoperiod conditions.

(°C) TEMPERATURE	PHOTOPERIOD 8L 16D	12L 12D	16L 8D
5	+	+	+
10	+	+	+
15	+	+	+

Table 4. Summary of constant environmental conditions.

	ENVIRONMENTAL LIGHTING LL	DD	LL(F)
TEMPERATURE 10°C	+	+	+

Surgical techniques used in the final phase of the investigation are described in the appendix.

2.8 Analytical Procedure

Visual scanning of the event recorder charts indicated the general performance of each individual. The total number of events occurring in each sixty minute interval were recorded on data sheets and then transferred to IBM punch cards for analysis.

The daily activity pattern of each individual was plotted by comparing the hourly activity amplitude against time. This provided a survey of the overall performance for any individual or group during the experimental period. Mean hourly values were calculated to condense these data and to clarify the general patterns. Mean values were applied only to individuals or groups receiving the same treatment, such as the experiments examining the combined effects of photoperiod and temperature on the entrained rhythm. These data were subjected to analysis of variance and Bartlett's test.

Periodogram analysis (Enright, 1965a, b) was used to identify any major periodicity between 20.00 and 28.00 hours. This was a comparative procedure using the root mean square of the amplitude associated with any estimated period.

$$\text{Ap (root mean square amplitude)} = \sqrt{\frac{\Sigma\,(\text{avg.} - \text{grand avg.})^2}{\text{estimate of the period value}}}$$

The completed periodogram provided a comparison of the amplitudes for selected periods between 20.00 and 28.00 hours. It could indicate whether the assumption of a 24.0 hour period, or a 23.0 hour period etc., led to an unusually large amplitude, or whether the amplitudes were no greater than background "noise" (see Fig. 8b). The method did not assign any level of significance to the amplitude increase. It was difficult to assign an objective meaning to conventional statistics such as a T-test because the activity records were serially correlated measurements and did not represent random independent samples from the population.

At least five replications of a daily cycle were recorded before attempting analysis. This was in agreement with the generally accepted policy to register a minimum of five to seven periods before examining an endogenous rhythmicity (Aschoff, 1960).

Periodogram analysis provides a simple statistical approach to cyclic biological phenomena. The calculations are easily determined, but a computer facilitates the large number of estimates required.

3. RESULTS

3.1 Determination of a Basic Entrained Response

These experiments were focused upon entrainment to photoperiod as the primary environmental factor. Individuals and occasionally groups of fish were introduced to an activity chamber and a record of the motor activity was collected. The chart output was analyzed for the total number of events occurring during each sixty minute interval. The hourly "bits" of information served as the basic activity measure utilized in this phase of the investigation.

These tests were designed to provide answers to the following questions.

1. Do diel cycles of locomotor activity exist under the artificial laboratory conditions?
2. Is the environmental photoperiod a major synchronizer?
3. Are the organisms day active, night active or crepuscular in their activity patterns?
4. Does the organism need to adapt to the environmental chambers before expressing a consistent daily activity pattern?
5. Is the response stable or does it change during the course of development?
6. Is the entrained response expressed by one individual similar to that expressed by a group of individuals?

Nine Lakelse sockeye were introduced singly into the activity chambers in a controlled environment of 8L 16D (8 hours of light and 16 hours of darkness) at 5°C. A continuous six day record indicates that a single juvenile sockeye expressed a definite cyclic response to the photoperiod (Fig. 4). This record is considered to be representative of all individuals tested in this series. The apparent entrainment to the photoperiod would tend to omit the possibility of influence by unknown environmental factors functioning as the entraining agent, unless these factors were exactly 24.00 hours in their natural periodicity.

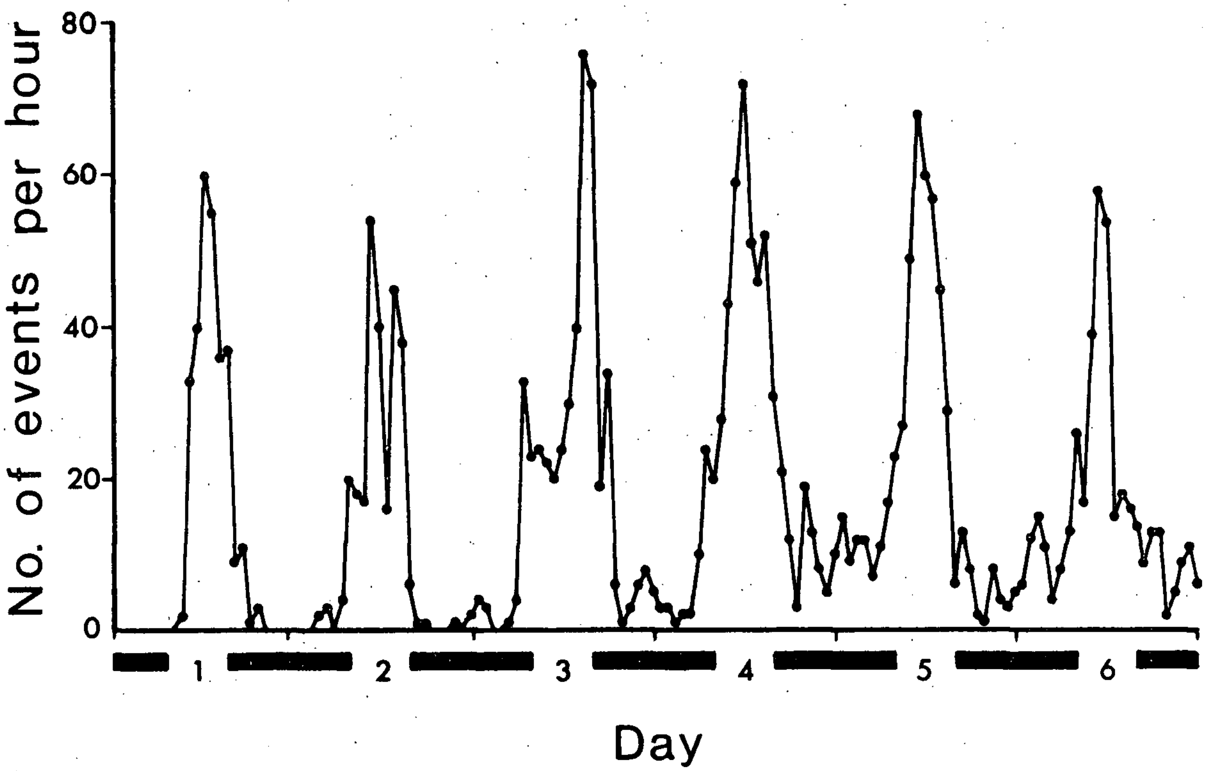
At the onset of light there was an increase in the activity amplitude. This increased activity level was generally maintained during the light phase, but some day to day variation in the hourly totals of events occurred. There was also an increase in the number of secondary peaks and associated troughs occurring during the dark phase in the fourth, fifth and sixth days of the test.

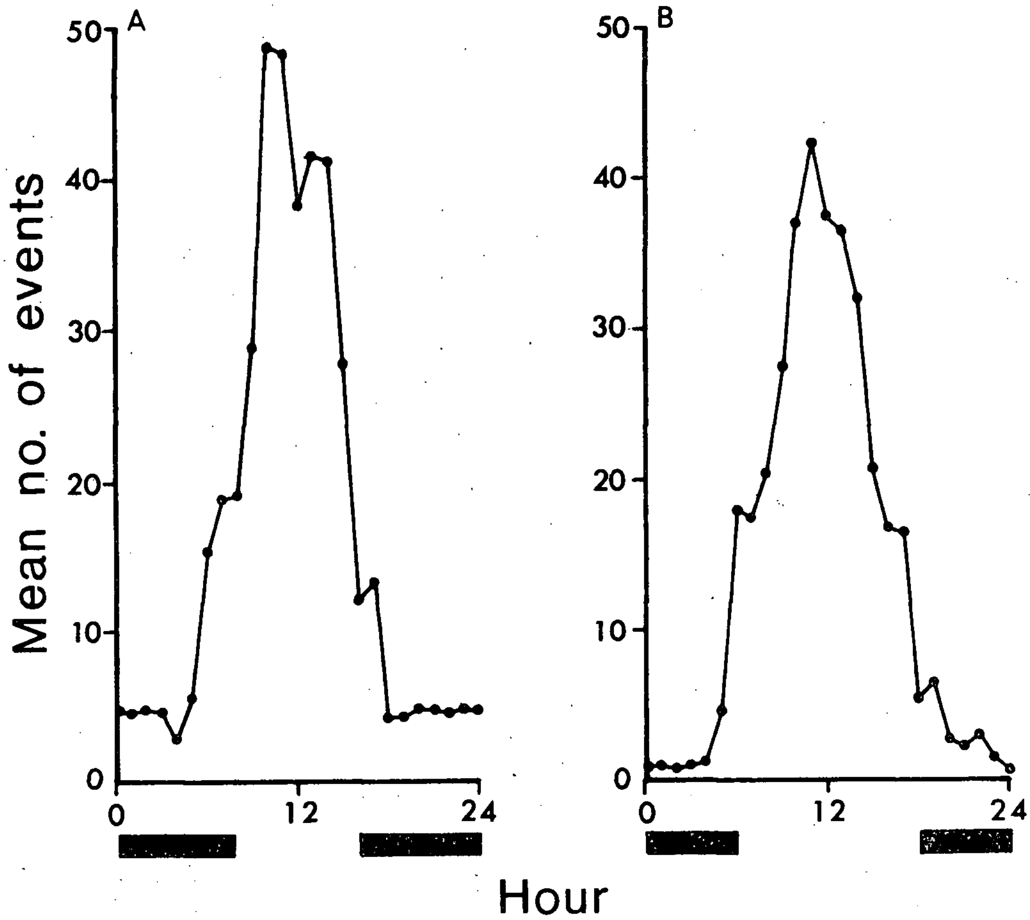
It is suggested that these secondary peaks and associated troughs occur randomly, since the calculation of mean values for the six day period does not indicate any consistent activity peak in the dark phase (Fig. 5a). There is however a positive phase relationship of activity to the light cycle (activity level increases before the onset of light). This is a general characteristic of

Figure 4. The entrained response of one individual to 8L 16D at 5°C. The amplitude is represented as the total number of events occurring each hour. The dark bars at the base represent the periods of darkness.

Figure 5. (A) The mean daily activity pattern calculated from the data presented in Figure 4.

(B) The mean daily response of a single individual from the Kamchatka Peninsula.


No. of events per hour
80
60
40
20
0
1
2
3
4
5
6
Day


A
B
Mean no. of events
50
40
30
20
10
0
0
12
24
Hour

diurnally active organisms.

Unfortunately, grouping these data omits some individual daily characteristics in the activity cycle, but the general overall pattern being expressed is more clearly defined. The resulting performance curve tends to be smoother than the daily record.

It is not known if a similar response would be obtained from genetic stocks originating at a variety of geographical locations. However, these preliminary tests did reveal that sockeye obtained from the Kamchatka Peninsula expressed a similar diel response to the photoperiodic environment in this laboratory (Fig. 5b).

These data indicate that juvenile sockeye salmon will exhibit a cyclic activity pattern during a twenty four hour day under controlled environmental conditions. A general pattern of increased activity occurred during the light phase of the experimental period. This was accompanied by a corresponding decrease in the activity amplitude occurring at the onset of the dark phase. This general pattern is representative of the majority of individuals tested.

The photoperiodic environment constitutes a strong entraining agent, and at this temperature (5°C) results in the expression of a basically unimodal activity pattern. No gross differences in the pattern could be identified as characteristic of either Lakelse or Kamchatka stock tested.

Further analyses of the daily records indicated that a minimum two day repetition of the cycle was necessary to determine the characteristic pattern (i.e. whether any individual was either light active or dark active). In addition, a comparison of the total day to day activity indicated that a minimum two day adaptation period was necessary before a consistent daily activity output could be obtained. Therefore, after introducing a subject to an activity chamber, data analysis considered only day 3 plus the following six days.

3.11 The relationship of age to the basic diel pattern of activity. This series was conducted to determine if there were changes in the locomotor activity pattern at different stages of development. This information was valuable in determining whether or not the investigation was examining a consistent activity pattern in the pre-smolts under investigation.

Approximately 900 sockeye salmon eggs were hatched in two of the activity chambers. The fry were maintained in 10 gallon aquaria under controlled environmental conditions for one year. Testing was conducted under conditions of 12L 12D at 5°C, except days 1-14 which were at 9.5L 14.5D.

Visual observations indicated the newly hatched alevins with yolk sac attached were negatively phototactic. This response was gradually reversed after emergence from the gravel. There was a positive rheotactic response to the water current in the swimming channel (produced by an incoming supply of fresh water which was directed into the gravel) and the fry proceeded in a unidirectional column around the activity chamber. This directional movement was reversed when the direction of the water flow was changed by 180 degrees. All unidirectional movement by the group terminated when the water flow ceased. A sample of this stock was maintained in several laboratory aquaria for further testing.

After emergence from the gravel the daily pattern was changed from a predominantly nocturnal, bimodal configuration to a diurnal activity pattern (Fig. 6 a, b, c, d). This response then remained unchanged if individuals were subjected to 12L 12D, 5°C environmental conditions. All subjects maintained a synchrony with the photoperiodic cycle and expressed more than 50 percent of their total activity during the light phase from the third month onward.

3.12 <u>The synchronizing effect of the environmental light cycle at a constant temperature</u>. Nine groups of Lakelse pre-smolts were subjected to an oscillating photoperiodic environment. The tests were designed to determine whether or not the photoperiod could synchronize several individuals to the same activity cycle.

Group size ranged from five to ten individuals which were introduced to a 12L 12D regime at 5°C. Over a period

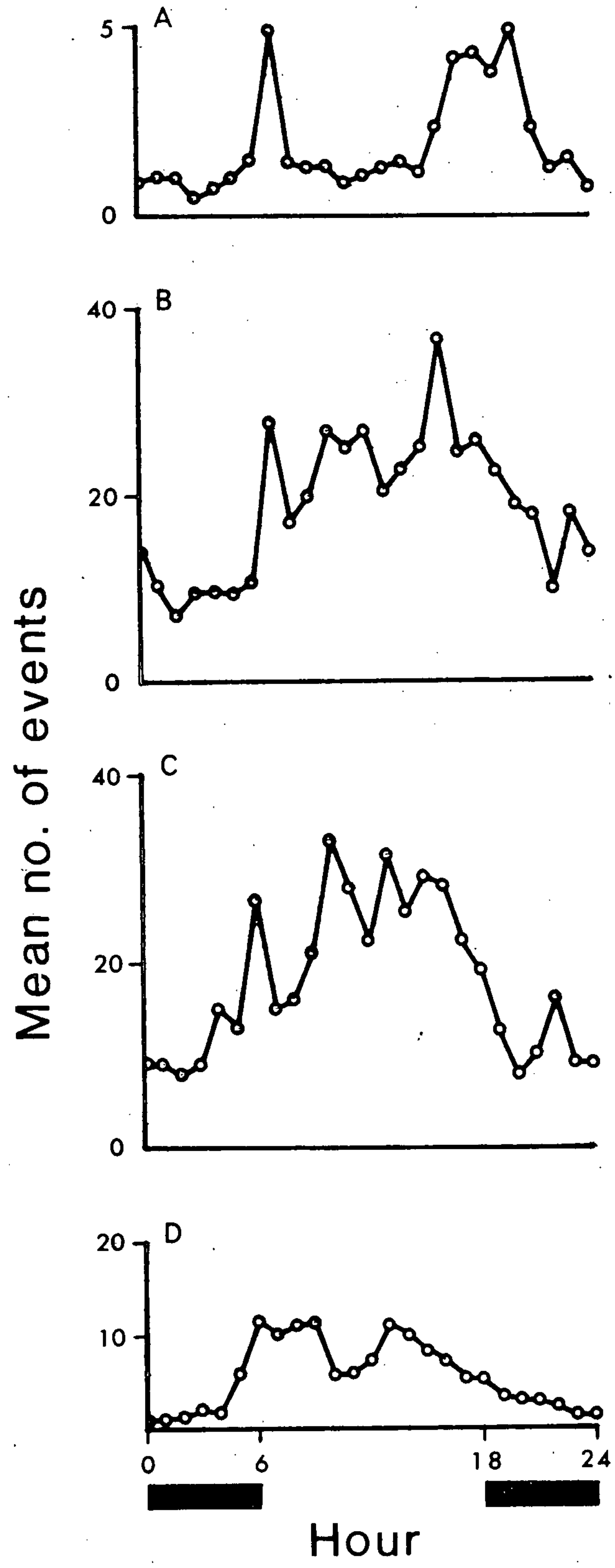
Figure 6 (a, b, c, d). The mean daily activity pattern at different stages of the life cycle.

A = The first 9 days after emergence.

B = Days 10 to 14.

C = 3 months after emergence.

D = 11 months after emergence.


A
B
C
D
5
0
40
20
0
40
20
0
20
10
0
6
18
24
Mean no. of events
Hour

of seven days this group of five fish exhibited a greater activity amplitude during the light period (Fig. 7a). A decrease in activity similar to that observed in solitary individuals occurred during the dark.

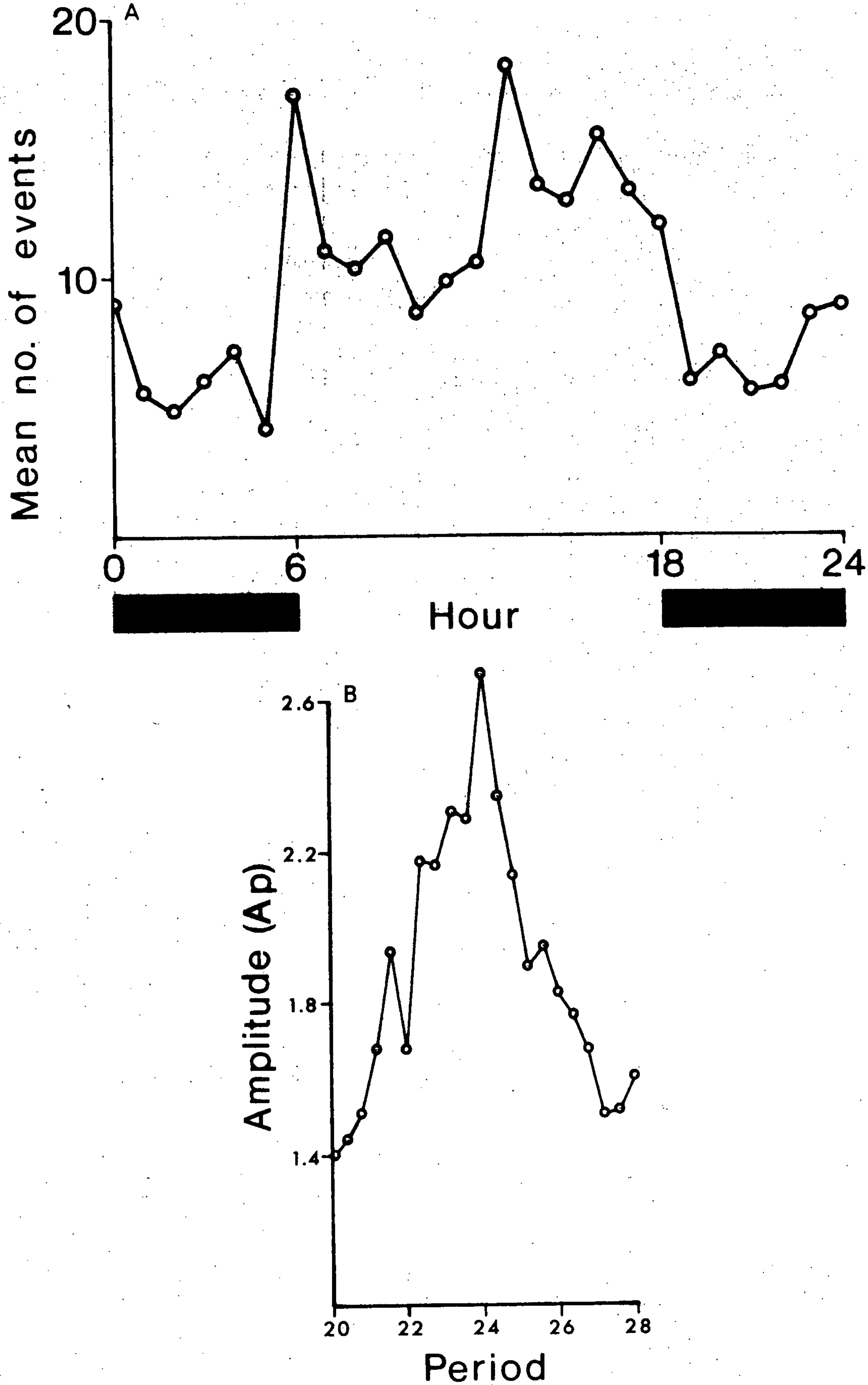
It is apparent from these grouped data that the activity pattern expressed is similar to the entrained response of one individual. Certain aspects of the behaviour will be altered due to the social effects between several individuals, but the basic pattern of a day active relationship to the photoperiodic cycle appears to be similar.

This group is expressing a 24.00 hour periodicity that is apparently synchronized by the light cycle (Fig. 7b). This is suggested because the photoperiod also has a 24 hour component and is the major oscillating environmental factor involved.

It is further suggested that the light-dark cycle is capable of synchronizing the individual members in a group to express similar activity patterns. This entrainment to a common stimulus results in a group expression of activity with a time period similar to that of the environmental cue.

Figure 7. (A) The mean daily activity pattern of five individuals monitored simultaneously for six days in the same activity chamber.

(B) Periodogram analysis of the six day record illustrated in Fig. 7A.


A
20
10
Mean no. of events
0
6
18
24
Hour
B
2.6
2.2
1.8
1.4
Amplitude (Ap)
20
22
24
26
28
Period

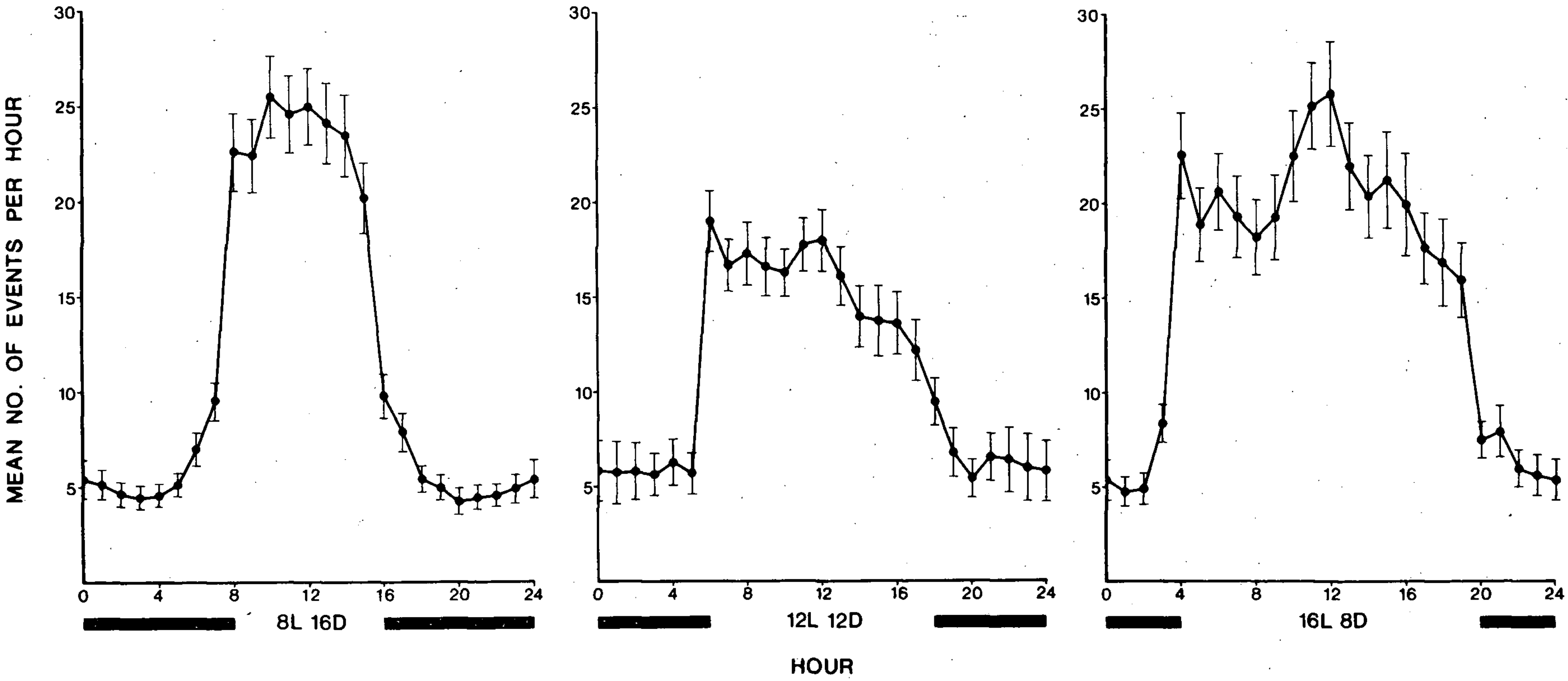
3.2 The Effects of Temperature and Photoperiod on the Daily Activity Pattern of Juvenile Sockeye Salmon.

These experiments were designed to provide a measure of the temperature effect on the entrained response to photoperiod. Only temperature and photoperiod were considered since they represented the major environmental variables affecting the basic activity pattern.

The tests were performed on single Lakelse pre-smolts previously held in the semi-natural photoperiodic conditions at an average temperature of 8°C. The activity patterns were recorded for a duration of six days, following a two day period of acclimation to the artificial environment. Nine individuals were to be examined at each temperature and photoperiod but several fatalities at the higher temperature values resulted in unequal sample sizes. A total of 512 daily activity cycles were examined and the mean daily patterns for each temperature and photoperiodic value are presented.

3.21 The entrained response at 5°C to three different photoperiods (8L 16D, 12L 12D, 16L 8D). The mean 24 hour activity patterns indicated that locomotor activity was greater during the light phase of the photoperiodic cycle (Fig. 8 a, b, c). At the onset of light there was a three-fold increase in the activity amplitude occurring during a sixty minute interval. This heightened amplitude was maintained throughout the light phase and was terminated

Figure 8 (a, b, c). Mean daily activity patterns representing 202 days of activity at 5°C. The standard errors are indicated by the vertical brackets at each value.


MEAN NO. OF EVENTS PER HOUR
30
25
20
15
10
5
0
4
8
12
16
20
24
8L 16D
12L 12D
16L 8D
HOUR

at the onset of dark by a corresponding decrease in activity.

A predawn increase in activity and a predusk decrease in activity are illustrated by these data, and indicate a positive phase relationship to the photoperiodic conditions (activity increased or decreased prior to the onset of the synchronizing stimulus). This phase relationship was circumstantial evidence for an endogenous rhythmicity having a characteristic period of less than 24 hours.

A comparison of the amplitude increases at the onset of light indicated a general upper activity limit for each photoperiod. This "dawn" peak was followed by a "midmorning" depression in activity that was influenced by daylength. The decrease in activity modified the basic unimodal pattern observed at 8L 16D to a bimodal pattern at 16L 8D referred to as the alternans[1] type (Aschoff, 1966).

There was an increase in the duration of heightened activity when the artificial day was lengthened from eight hours of light to twelve or sixteen hours. The limitation of these higher activity levels to the light phase indicated that at 5°C the length of the artificial day would not alter the basically diurnal pattern of the entrained response.

---

[1]Alternans describes a characteristic pattern where the major peak follows the minor peak.

3.22. <u>The entrained response at 10°C to three different photoperiods (8L 16D, 12L 12D, 16L 8D)</u>. The mean 24 hour activity patterns examined at this temperature support those data obtained at 5°C (Fig. 9 a, b, c).

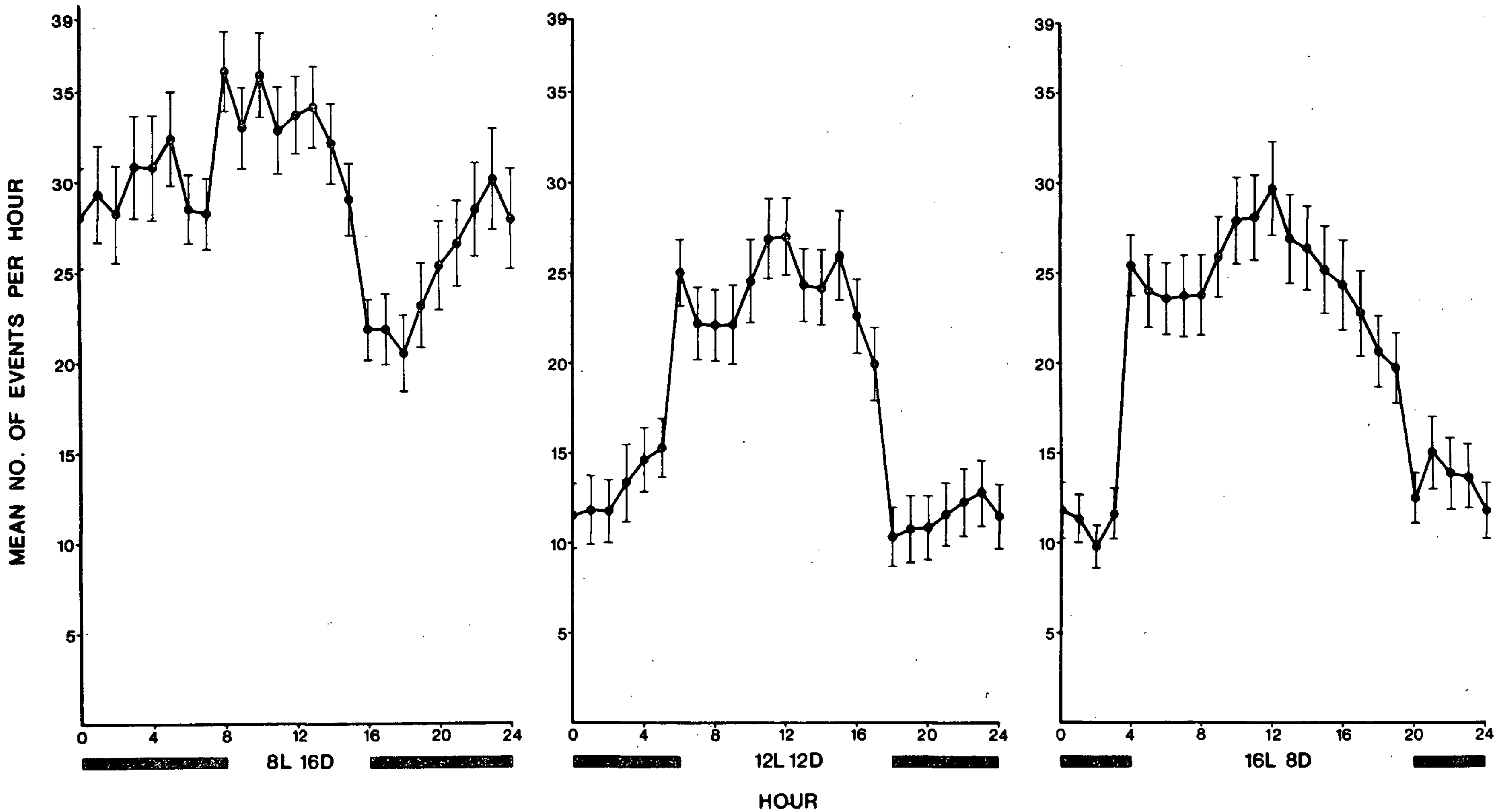
The major differences occurring in these tests include an apparent temperature-photoperiod interaction at 8L 16D, and a general increase in the activity amplitude resulting from the higher environmental temperatures.

At 8L 16D the increased activity amplitude was most apparent in the dark phase. This heightened nocturnal activity level decreased the magnitude of the difference between the diurnal and nocturnal phases of the activity pattern. However, these data still retained a cyclic relationship to the photoperiod and significent ($P < .01$) differences in activity level occurred between the light and dark phases of the activity cycle.

At 12L 12D and 16L 8D there was a definite day active relationship to the photoperiod. The magnitude of the activity increase at the onset of light was less than that observed at 5°C, and constituted a level twice that observed during the artificial night.

Predawn increases and predusk decreases in activity were indicated by these data, supporting the available evidence for an endogenous rhythmicity.

Figure 9 (a, b, c). Mean daily activity patterns representing 172 days of activity at 10°C.


MEAN NO. OF EVENTS PER HOUR
39
35
30
25
20
15
10
5
0
4
8
12
16
20
24
8L 16D
12L 12D
16L 8D
HOUR

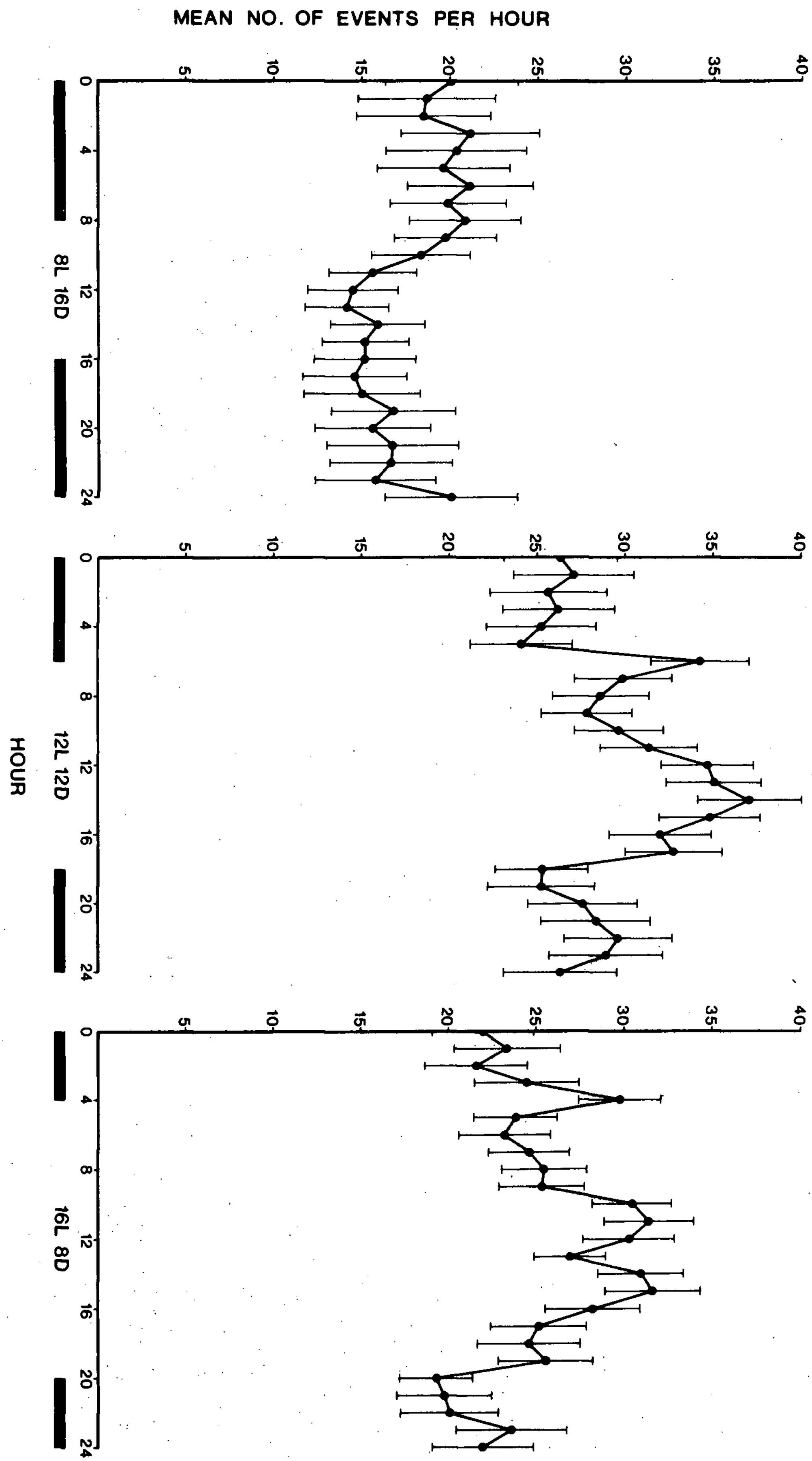
3.23 The entrained response at 15°C to three different photoperiods (8L 16D, 12L 12D, 16L 8D). The major differences between these tests at 15°C and those at 5°C and 10°C include a continued temperature-photoperiod interaction at 8L 16D and higher activity amplitudes occurring at twelve and sixteen hours of light (Fig. 10 a, b, c).

At 8L 16D there was an apparent decrease in the mean activity amplitude during all hours of the artificial day (Fig. 10a). The variations in these grouped data did not differ significantly (P <.1). However, this was due to the combination of both light active and dark active individuals (see Fig. 15) which tended to cancel each other.

There was still a light active relationship of activity to the photoperiod at twelve and sixteen hours of light. However, the magnitude of the increase at the onset of light was less than that observed at either 5°C or 10°C, and was followed by a significant midmorning (P<.01) depression of activity resulting in a bimodal pattern of the alternans type.

These data suggested that the locomotor activity pattern was influenced by both the environmental temperature and photoperiod. These extrinsic factors produced changes in both the amplitude of activity and the contour of the pattern expressed in a twenty four hour period.

Figure 10 (a, b, c). Mean daily activity patterns representing 138 days of activity at 15°C.



MEAN NO. OF EVENTS PER HOUR
HOUR
8L 16D
12L 12D
16L 8D

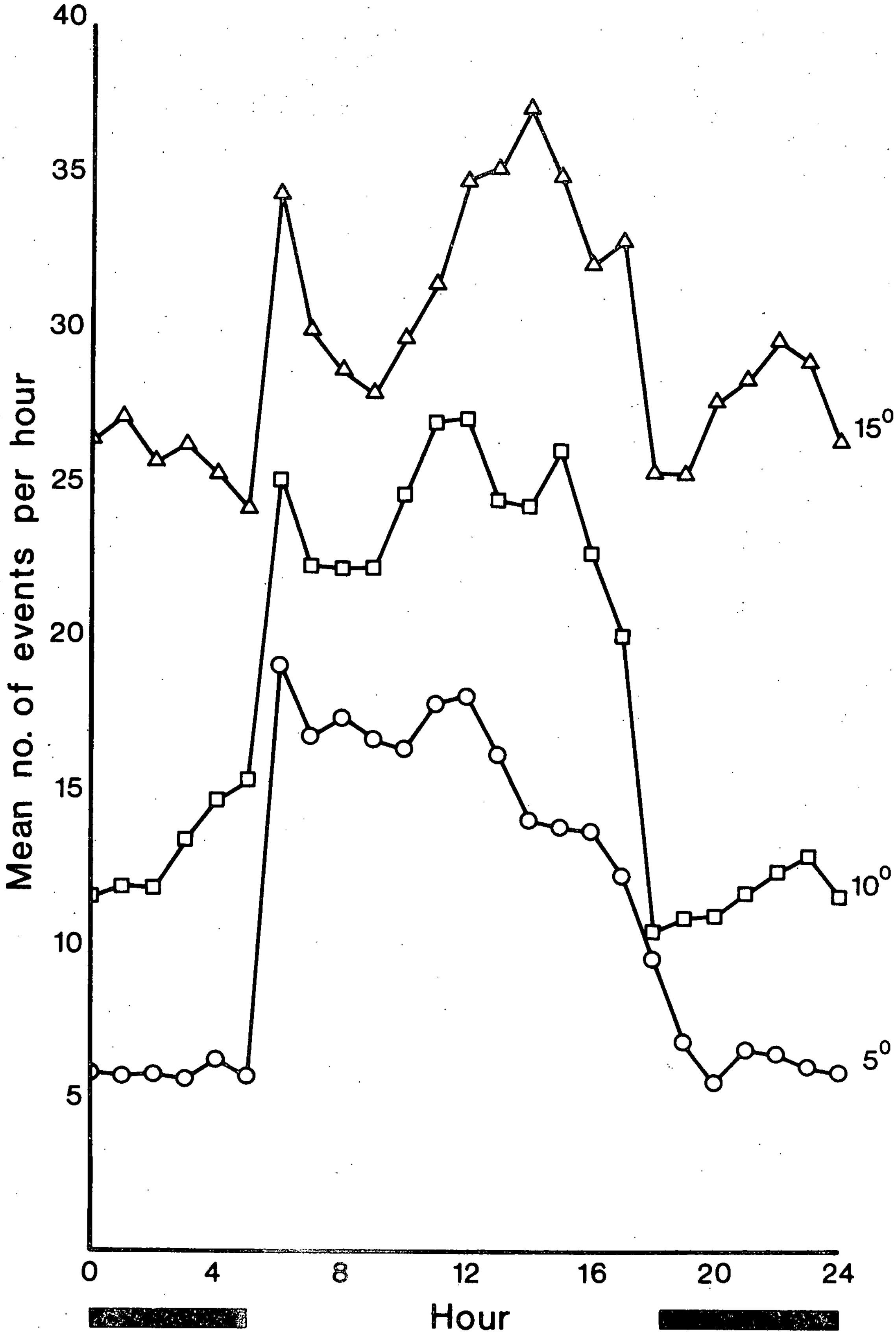
3.24 The effect of temperature on the entrained response. The mean hourly values recorded for 12L 12D at 5°C, 10°C and 15°C illustrated that temperature increased the activity amplitude in both light and dark (Fig. 11). These figures also indicated a development of the "midday" depression in activity which resulted in a bimodal activity pattern at the higher temperatures considered.

The mean activity values were plotted against temperature for each photoperiod. The values for 8L 16D at 15°C indicated a definite decrease in the mean number of events during light and dark (Fig. 12). However, the general trend from 5°C - 15°C was one of increasing activity amplitudes with increasing temperatures (see Fig. 12, 12L 12D, 16L 8D).

The decreased activity amplitude that was associated with 8L 16D above 10°C indicated that optimum values of photoperiod and temperature might determine the expression of diurnal or nocturnal activity. When the upper temperature "limit" for a certain daylength was exceeded, the organism reversed the photobehavioural response and became dark active.

3.25 The effect of photoperiod on the entrained response. Mean total activity was plotted against daylength at each temperature value (Fig. 13). At 5°C, dark activity showed a very slight increase between 8L and 16L, whereas

Figure 11. Mean daily activity patterns representing 184 days of activity at 12L 12D. Temperatures are in °C.



Mean no. of events per hour
40
35
30
25
20
15
10
5
0
4
8
12
16
20
24
Hour
15^0
10^0
5^0

Figure 12. Plots of activity on temperature at three different photoperiods.

D = Dark; L = Light

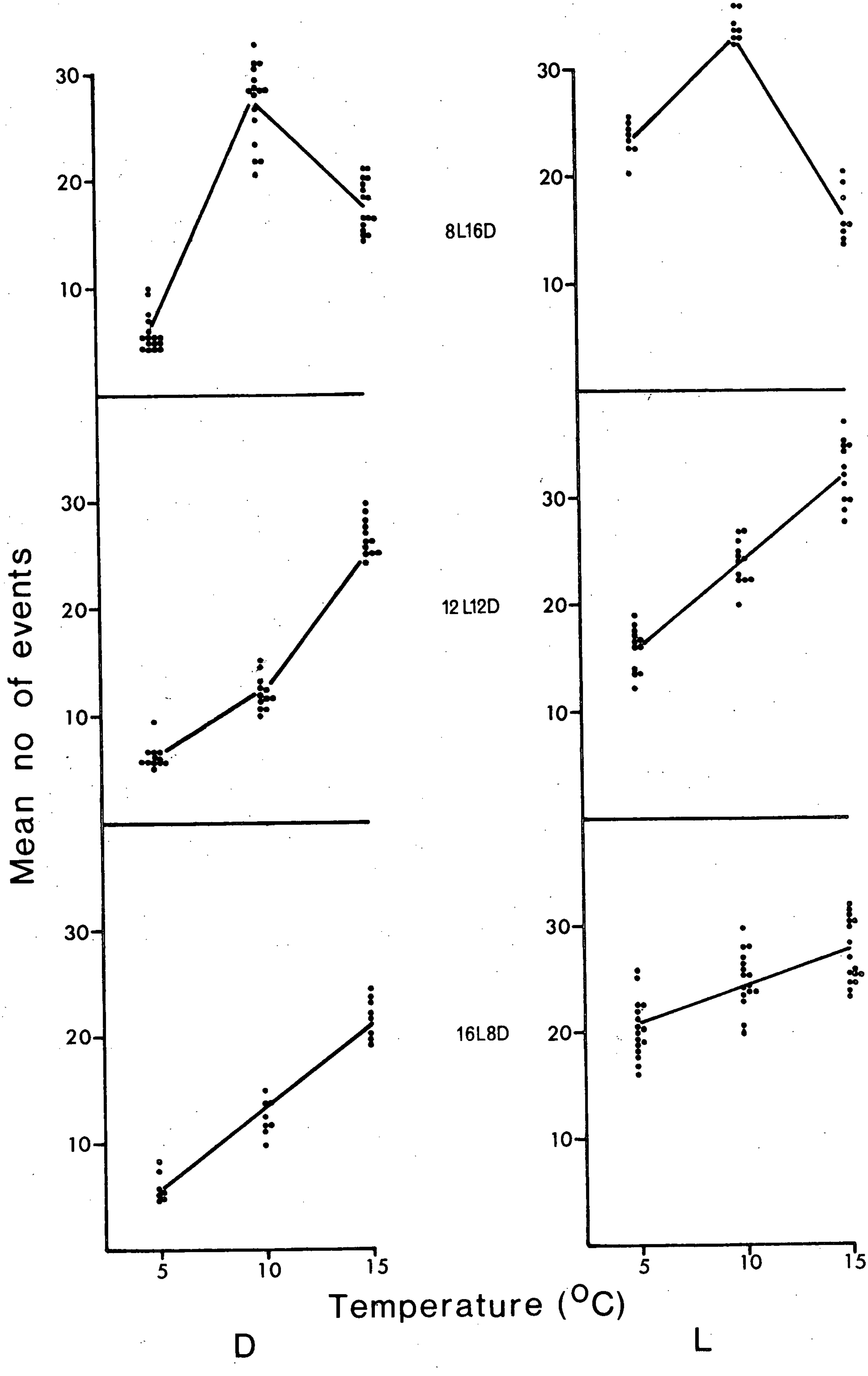
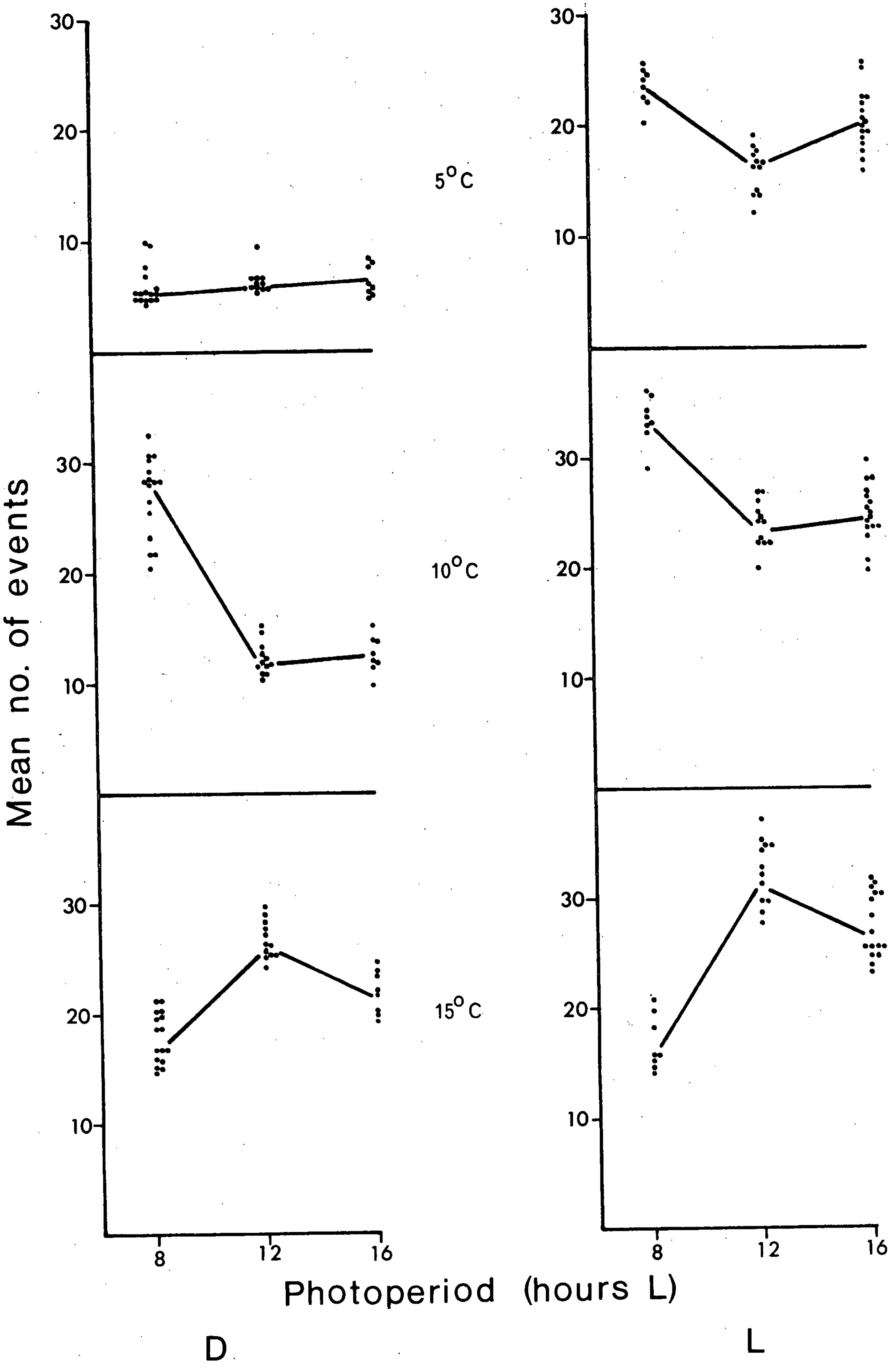

Mean no of events
8L16D
12 L12D
16L8D
Temperature (°C)
D
L
30
20
10
5
10
15

Figure 13. Plots of activity on photoperiod at three different temperatures.

D = Dark; L = Light



Mean no. of events
5°C
10°C
15°C
30
20
10
8
12
16
Photoperiod (hours L)
D
L

light activity indicated maximum amplitudes at 8L and 16L.

At 10°C both light and dark phases indicated maximum activity at 8L, whereas at 15°C minimum activity amplitudes were associated with 8L.

These data provide additional evidence for an interaction between daylength and temperature. It is suggested that at 8L 16D a temperature above 10°C will result in increased nocturnal activity. When more than 50% of the total activity occurs during the dark, this is interpreted as a reversal in the photobehavioural response and the organism is considered to be nocturnally active.

A three dimensional presentation of mean total activity plotted against temperature and photoperiod indicated the general trend of the photobehavioural response (Fig. 14). A rise in temperature resulted in increased mean total activity at most daylengths. However, when short days (8L) and high temperatures (15°C) were combined, decreasing activity occurred.

3.26 <u>The interaction of temperature and photoperiod</u>. The mean daily activity records for 8L 16D at 10°C and 15°C (see Figs. 9a and 10a) indicated an interaction between temperature and photoperiod. These records resulted from combining individual patterns which tended to cancel each other (Fig. 15), giving a mean daily pattern that did not differ significantly from a straight line ($P>.1$). Analysis of the previous sections (2.4 and 2.5) indicated a general

Figure 14. Three dimensional illustration of mean total activity amplitudes at each temperature and photoperiod. The broad lines indicate the surface contour.

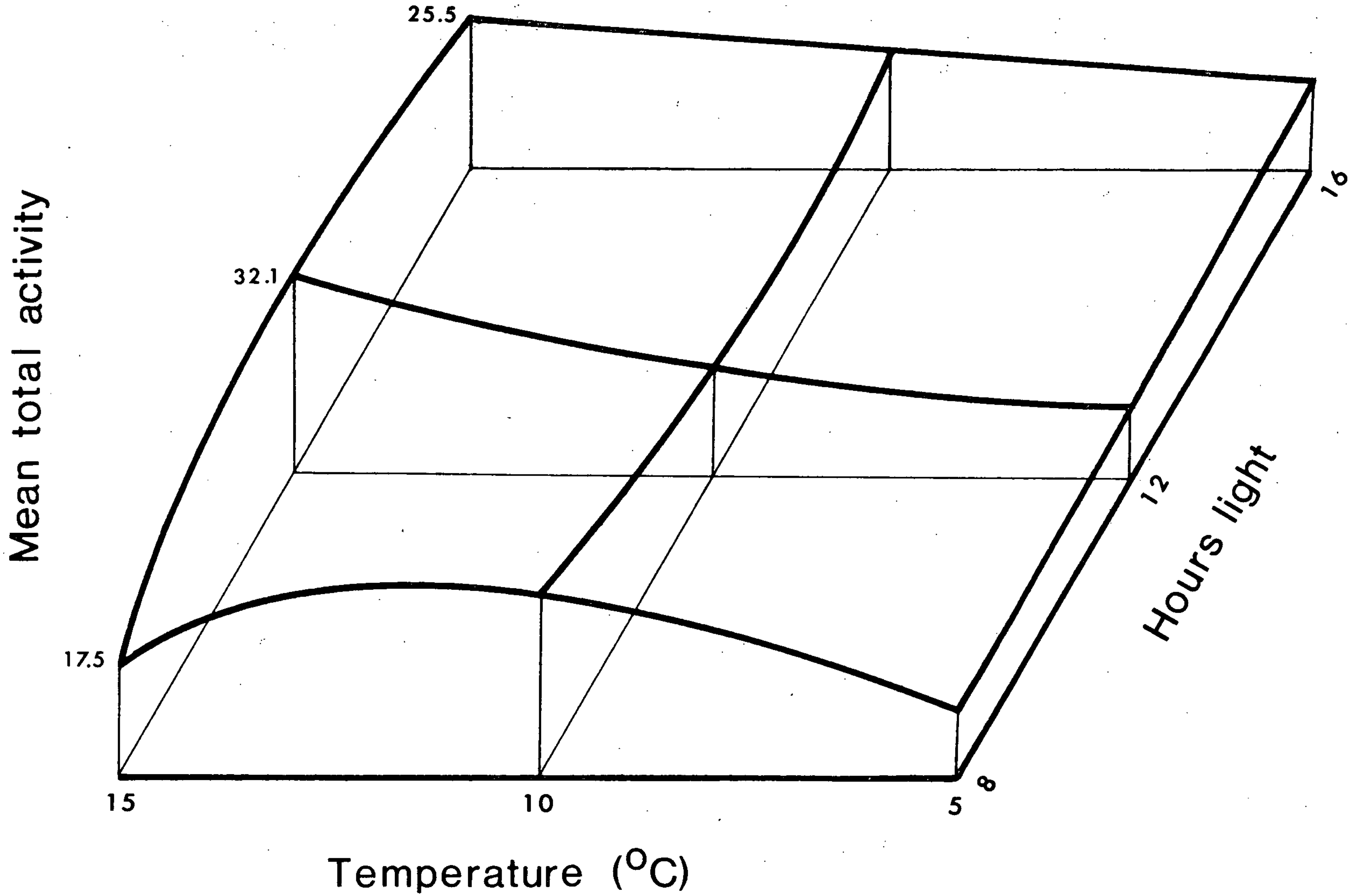
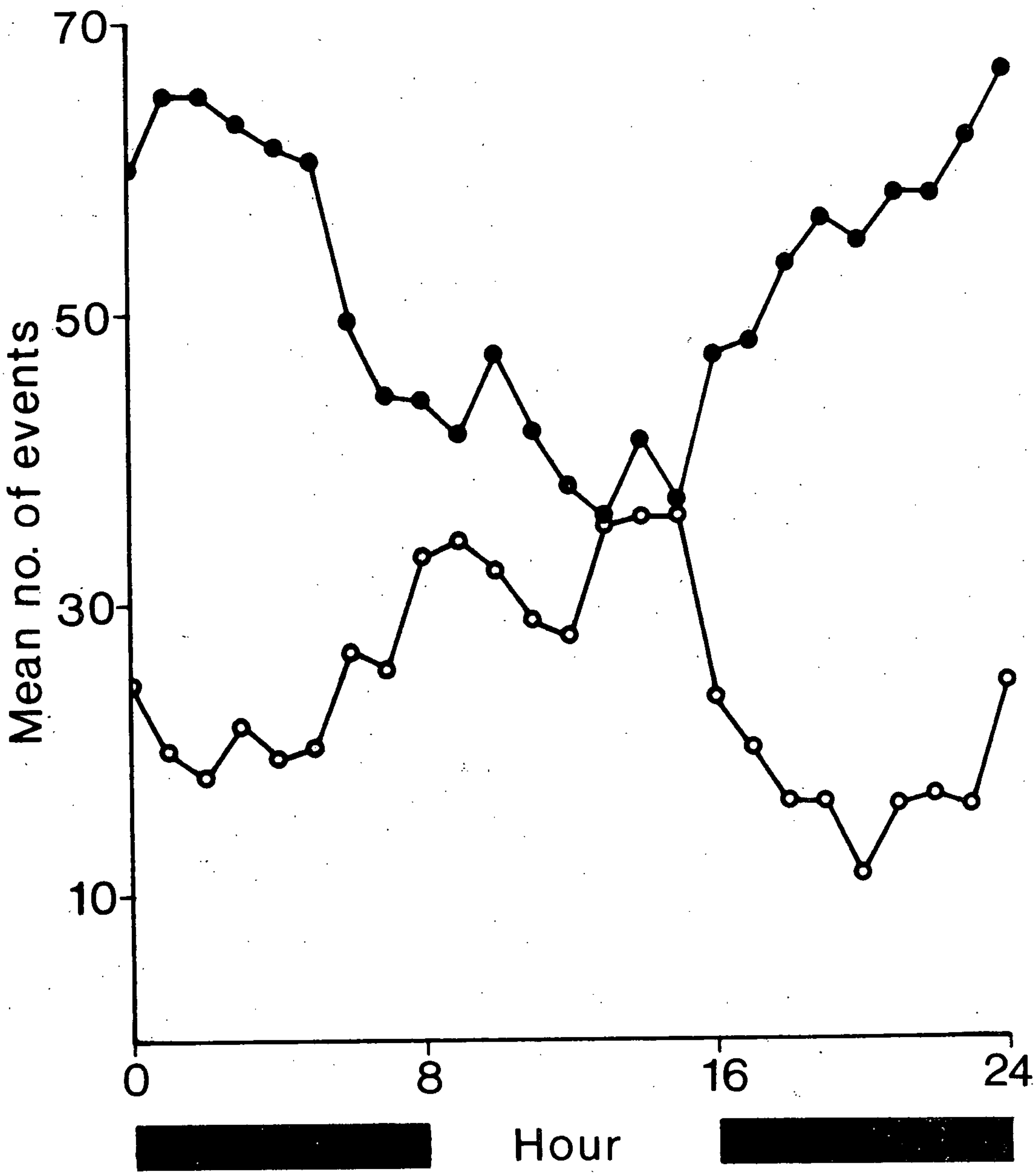

Mean total activity
25.5
32.1
17.5
15
10
5
Temperature (°C)
8
12
16
Hours light

Figure 15. Mean daily activity patterns for two individuals subjected to 8L 16D at 15°C.


70
50
30
10
Mean no. of events
0
8
16
24
Hour

reversal in the activity response at short daylengths and high temperatures.

A consideration of the percent frequency occurrence of nocturnal activity indicated a progressive activity increase which was emphasized where short photoperiods and high temperatures were combined (Fig. 16). It was also apparent that the increases were not parallel at all daylength and temperature combinations.

At 8L 16D the estimated 50% distribution between light and dark activity was at 8°C (Fig. 17, a, b, c). At 12L 12D this value was at 15°C, and at 16L 8D the extrapolated value was at 30°C. However, this latter value was unrealistic since it exceeded the tolerance limits of juvenile sockeye salmon. In each case, a four hour increase in daylength was sufficient to double the effective temperature where the 50% day/night activity distribution occurred.

It is hypothesized that for a certain daylength there is an upper temperature limit which if exceeded will reverse the activity response and juvenile sockeye salmon will express greater activity amplitudes during dark than during light. Lower temperature limits were not investigated, but are assumed to approximate 0°C in juvenile sockeye.

Figure 16. Plot of the percent occurrence of nocturnal activity at each daylength and temperature.

○ = 5°C

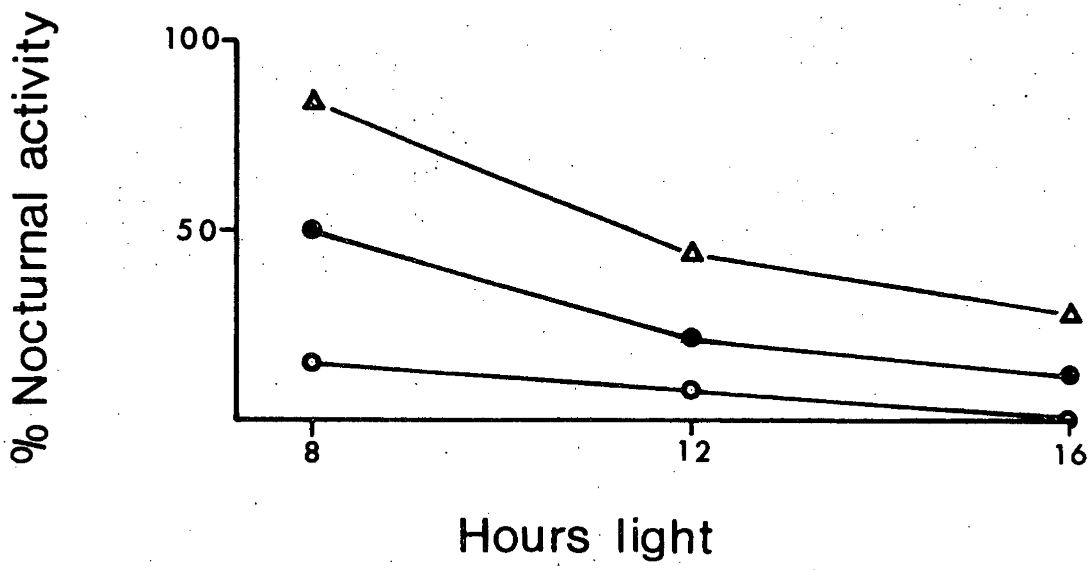
● = 10°C

△ = 15°C

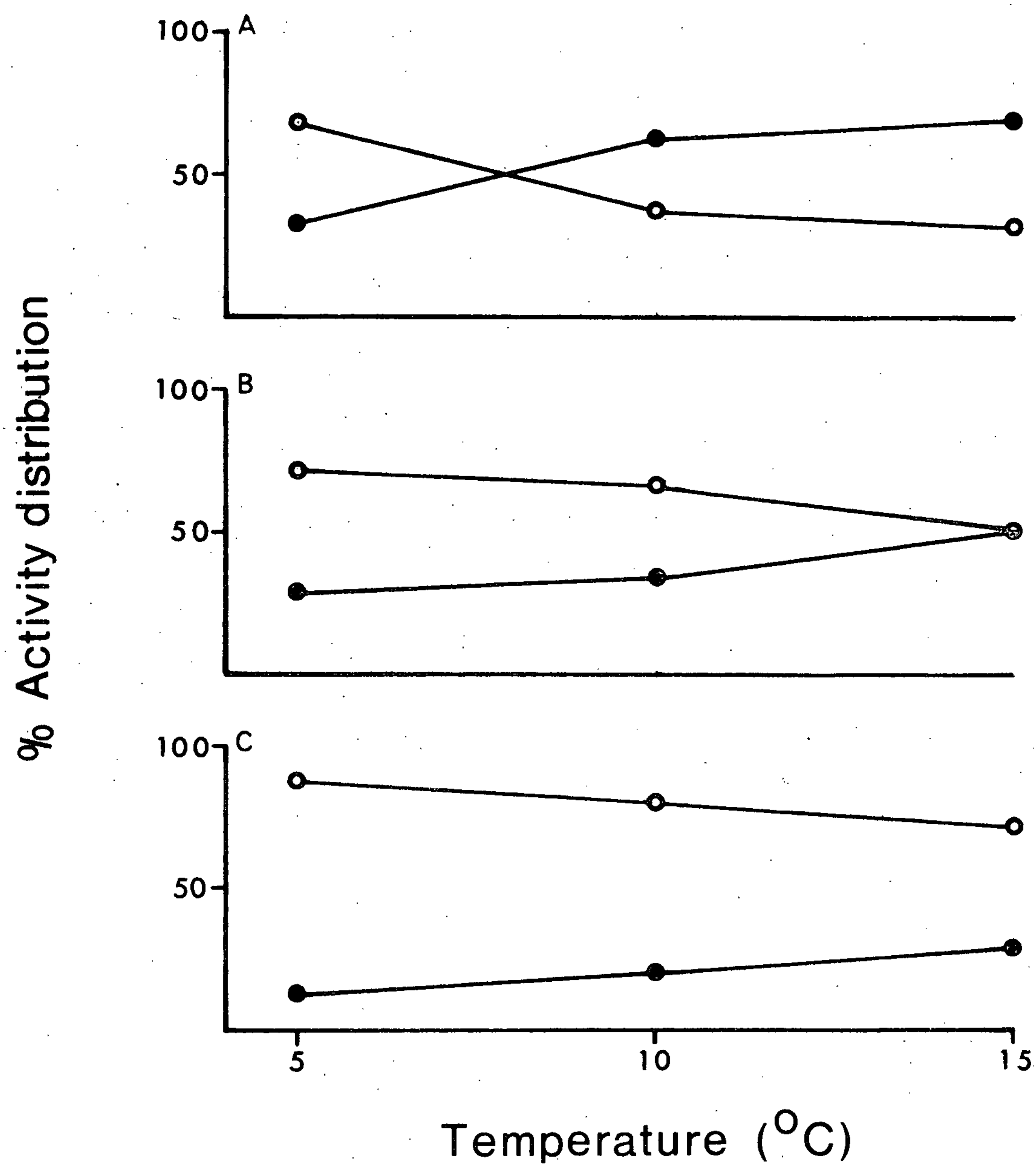
Figure 17 (a, b, c). Plots for each photoperiod illustrating the distribution of activity for light and dark. The intersecting lines represent the points where a 50% activity distribution occurs.

○ = Light

● = Dark



% Nocturnal activity
100
50
8
12
16
Hours light



% Activity distribution
A
B
C
100
50
5
10
15
Temperature (^{o}C)

3.3 Endogenous Activity Expressed in A Constant Environment.

An investigation considering the photoperiodic responses of an organism might assume the presence of an endogenous rhythmicity which is being synchronized by the light cycle. The description and analysis of the endogenous response in juvenile sockeye salmon might be valuable to past and future research which is focused either wholly or in part on the responses of these fish to an environmental light cycle.

Lakelse pre-smolts were obtained from the outdoor holding tanks and held in the laboratory aquaria for a minimum of two weeks. Individuals were then introduced into the activity chambers under a 12L 12D photoperiod. The transfer from the controlled environmental holding aquaria occurred during the light phase of the artificial photoperiod. At the end of the light period the timers were prevented from completing a revolution and the environmental conditions were maintained in a constant state (LL or DD). The temperature was held constant at 10°C and the environmental lighting was maintained at a constant level (34.4 lux, except in DD, or where specifically indicated).

These fish were allowed 48 hours for adjustment to the new environmental conditions. Locomotor activities were recorded for 5 or 6 days, but in isolated cases an individual was allowed to remain in the constant environmental conditions for a longer period.

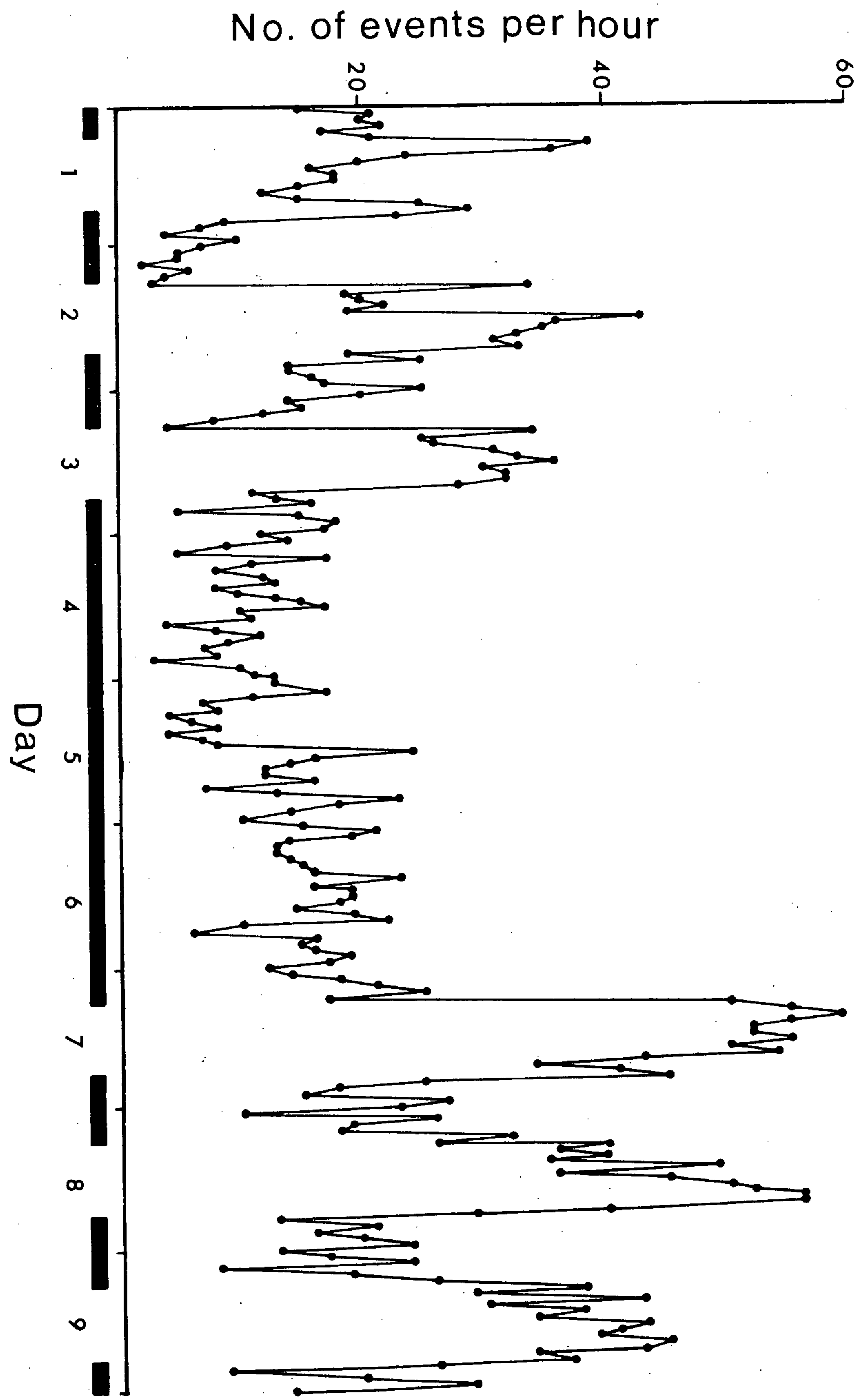
These data were analyzed at ten minute period estimates between the 20.00 and 28.00 hour range of the periodogram.

3.31 The endogenous response in constant darkness (DD) at a constant temperature of 10°C. A total of 33 fish representing 192 "days" of activity contributed to this series. The response to DD which represented most subjects indicated that entrainment to the photoperiod was apparent during the first and last three days of the test, but there was no indication of an obvious circadian component during days 4, 5, and 6 (Fig. 18). Further examination of these data indicated an endogenous component with an approximate 5 hour periodicity.

An exception was noted where a free-running rhythm was maintained with a low amplitude in one individual. The periodogram analysis of these data indicated a maximum increase in amplitude associated with a period of 22.90 hours (Fig. 19). This is a close approximation of the average value (22.8 hours) calculated for all fish showing a detectable rhythm (Table 5).

It was concluded that the endogenous circadian component was not readily expressed in constant darkness. In most cases where a detectable increase in amplitude was observed, it was expressed with a period value less than 24.00 hours.

Figure 18. A continuous nine day record for one individual in 12L 12D and constant dark at 10°C. The photoperiod is indicated by the dark bars at the base of the graph.



No. of events per hour
20
40
60
1
2
3
4
5
6
7
8
9
Day

Figure 19. Periodogram analysis representing the response of one individual in constant darkness.

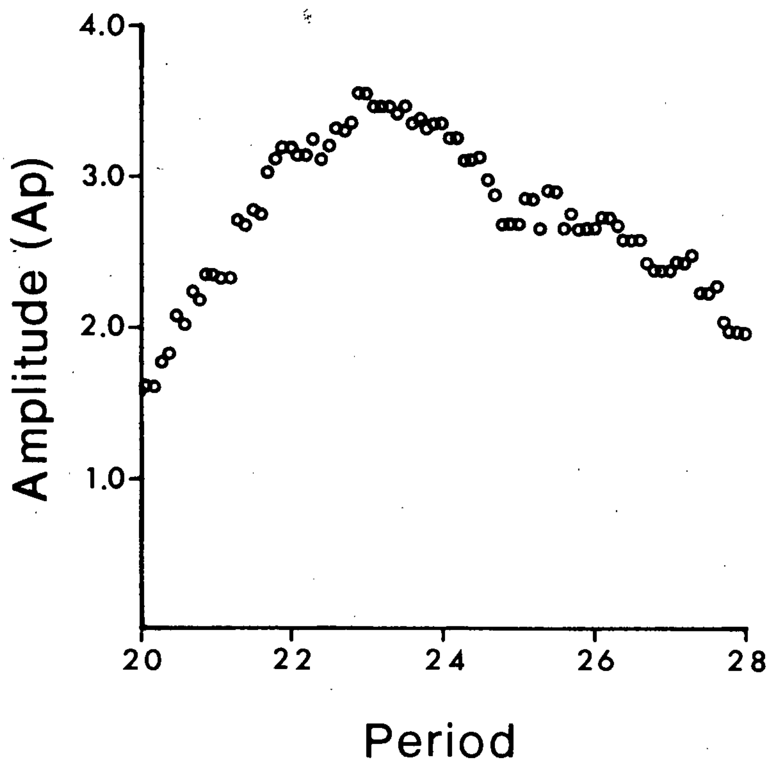

4.0
3.0
2.0
1.0
Amplitude (Ap)
20
22
24
26
28
Period

Table 5. Periodicity expressed by 16 fish in constant darkness at 10°C.

Fish No.	Period	Fish No.	Period
1	20.8	9	21.6
2	20.0	10	25.4
3	21.7	11	24.7
4	20.8	12	25.0
5	20.8	13	23.1
6	24.0	14	23.8
7	24.7	15	24.9
8	23.2	16	21.0

Avg. 22.8

This is not typical of diurnally active organisms which usually express a spontaneous frequency that is longer than 24.00 hours in constant darkness.

3.32 The endogenous response in constant light (LL) at a constant temperature of 10°C. Thirty-five subjects representing 183 "days" of activity were examined in this series. A particularly good example of the endogenous component is illustrated by the twelve day activity record for a single juvenile sockeye (Fig. 20). This record indicates that some individuals can maintain a rhythmic activity pattern in the absence of periodic environmental stimuli. However, the response representative of most fish tested indicated a dampening of the endogenous component after the first three or four days in constant light.

Periodogram analysis of the above data indicated a peak amplitude increase at 23.50 hours for this individual (Fig. 21). The data analyses for all fish showing a detectable rhythm between 20.00 and 28.00 hours revealed an average endogenous periodicity approaching 23.30 hours (Table 6). The endogenous component varied with each individual and period values ranged from 20.00 hours to 26.40 hours.

In constant light the mean periodicity expressed was less than 24.00 hours, which is characteristic of most diurnally active organisms (Aschoff, 1960). It appeared

Figure 20. A continuous 12 day record of one individual in constant light (34.4 lux at 10°C).

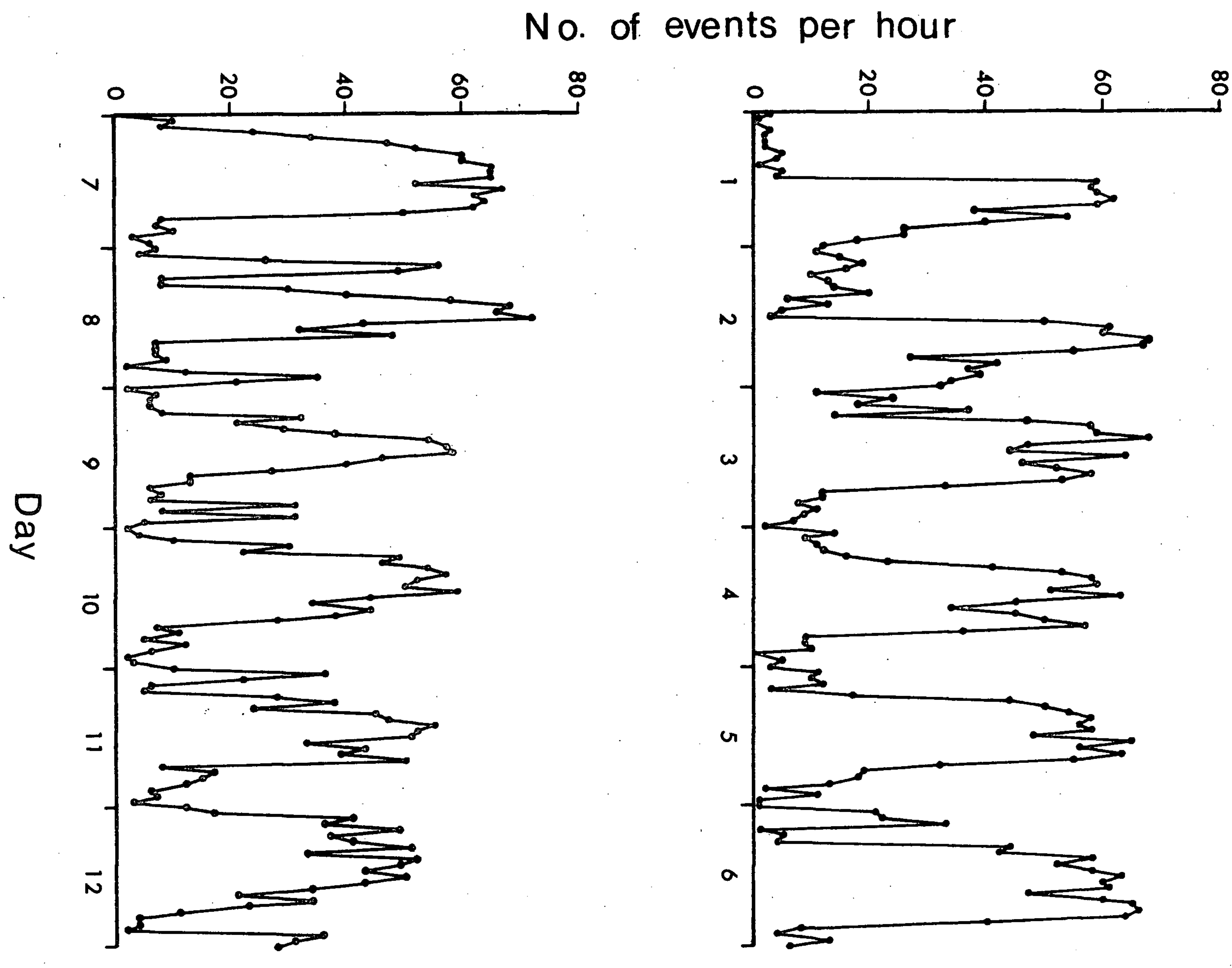
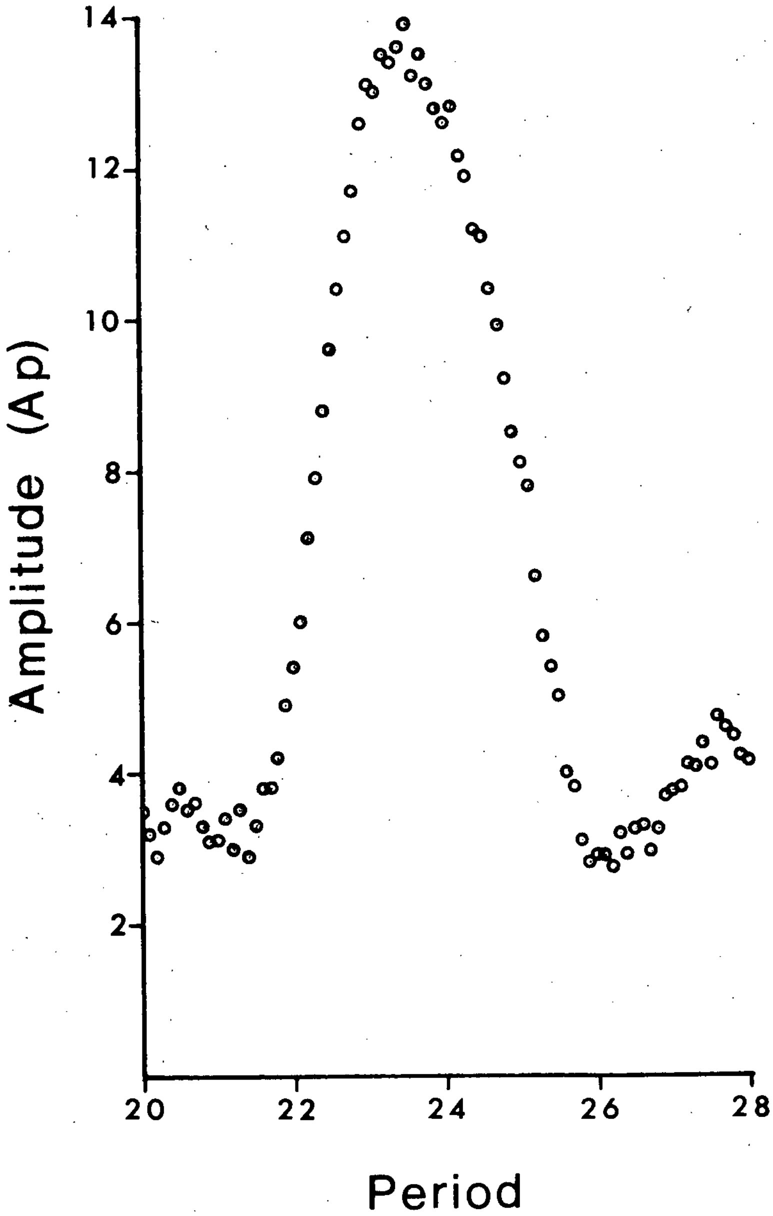

No. of events per hour
0
20
40
60
80
1
2
3
4
5
6
7
8
9
10
11
12
Day

Figure 21. Periodogram analysis of the data presented in Figure 20.



Amplitude (Ap)
14
12
10
8
6
4
2
20
22
24
26
28
Period

Table 6. Periodicity expressed by 30 fish in constant light at 10°C.

Fish No.	Period	Fish No.	Period
1	26.4	16	25.1
2	26.3	17	24.8
3	21.5	18	21.1
4	24.0	19	24.0
5	20.0	20	22.2
6	23.2	21	20.3
7	21.0	22	27.2
8	25.1	23	21.9
9	24.0	24	24.4
10	23.5	25	22.0
11	24.0	26	23.0
12	23.2	27	22.2
13	24.6	28	23.2
14	25.4	29	21.0
15	24.0	30	24.9

Avg. 23.3

that juvenile sockeye expressed an endogenous rhythm much more freely in constant light than in constant dark.

3.33 The response of the endogenous component to increasing light intensities. The endogenous periodicity was measured in six fish subjected to two light intensities (<1 lux and 34.4 lux). Environmental temperatures were maintained constant at 10°C and the lower light intensities were achieved by using a 1 watt neon lamp. Periodogram analysis was used to determine the characteristic period for each individual.

Table 7 illustrates the values obtained at each intensity. The general trend is for an increase in period value with increasing light intensities. This is not in accordance with previously observed responses in many other day active species (Aschoff, 1960), but exceptions have been reported in the literature (Hoffmann, 1965).

3.34 The entraining effects of periodic feeding in constant light at 10°C. Twenty four fish representing 166 "days" of activity contributed to this series. Food was introduced to the constant environment at noon each day. The objective was to determine whether or not periodic feeding could entrain the activity pattern.

The mean values for all tests indicated that introducing food to the constant environment produced a significant increase in activity and entrained the endogenous component.

Table 7. Periodicity (hours) expressed in six juvenile sockeye subjected to increasing light intensity at 10°C.

Fish	1 lux	34.4 lux
1	22.4	23.2
2	22.2	23.4
3	23.5	24.0
4	23.2	24.4
5	21.6	23.2
6	24.0	23.6
Avg.	22.8	23.6

The mean daily activity pattern for nine individuals indicated that the increased activity amplitude was maintained at a peak for approximately one hour and then gradually returned to the background level (Fig. 22). Apparently the synchronizing effects were stronger during the first three days of the test, and gradually weakened with time.

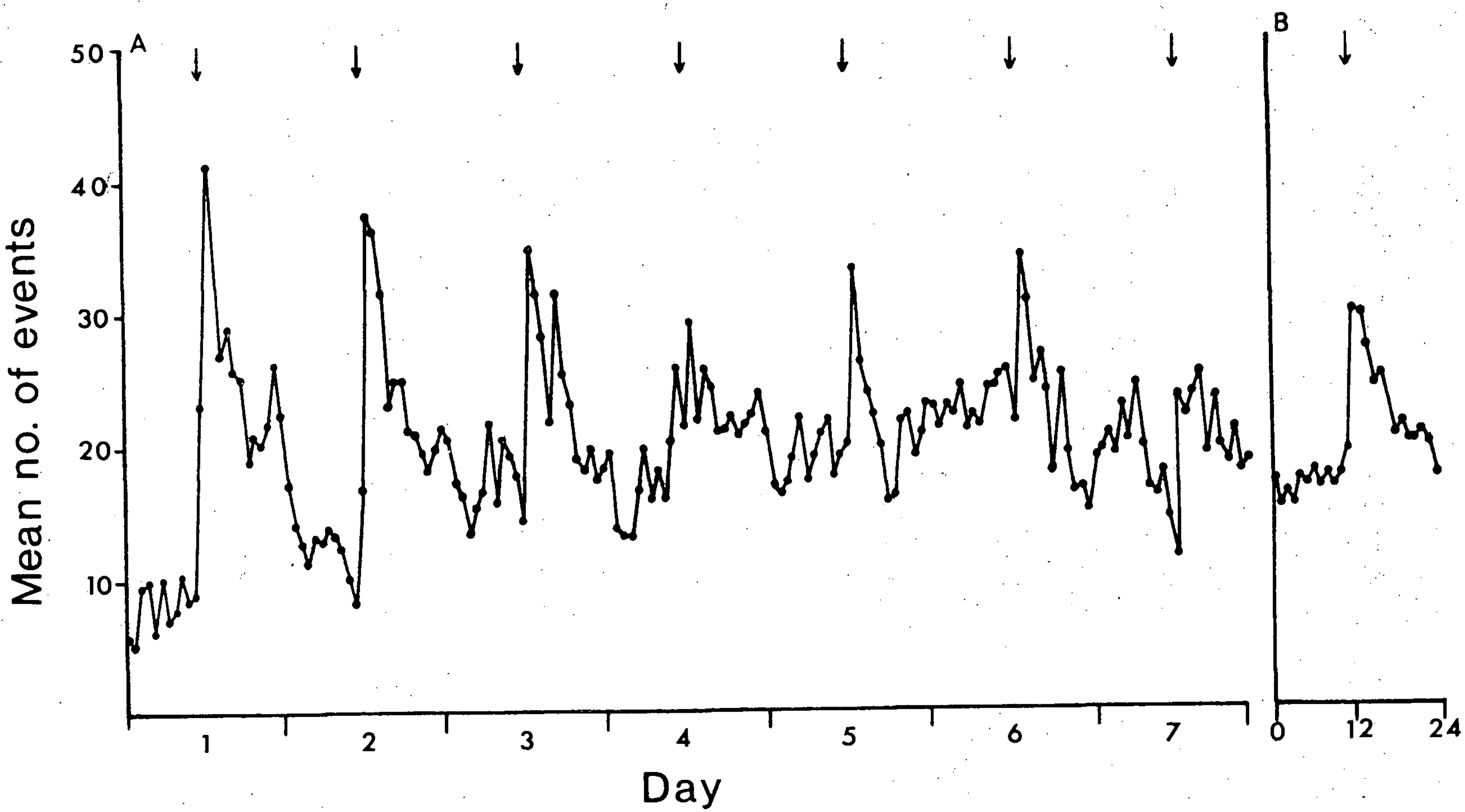
It was concluded that the stimulus of periodic feeding could entrain the endogenous rhythm and correspondingly alter any free-running rhythmicity that was present. The only alternative for future long-term experiments would be to maintain food available in the constant supply, or attempt to mask the effects of feeding by presenting food in a random manner during both light and dark. Since the majority of experiments reported here covered a duration of one week, it was considered more practical not to introduce food to the environment.

3.4 The Principal Sensory Receptors Involved in the Mediation of the Entrained Response.

The more obvious and accessible photoreceptive systems included the eyes and the pineal body. Less obvious systems might involve cutaneous photoreceptors or a general sensitivity of the nervous system to stimulation by light. Since the eyes and the pineal body are the more prominent and accessible systems, a series of experiments were designed to investigate the role of these organs in mediating the

Figure 22. (A) The mean activity response of 9 individuals for 7 days. The grand means are presented at the right (B).

↓ indicates time of feeding



Mean no. of events
50
40
30
20
10
A
B
1
2
3
4
5
6
7
0
12
24
Day

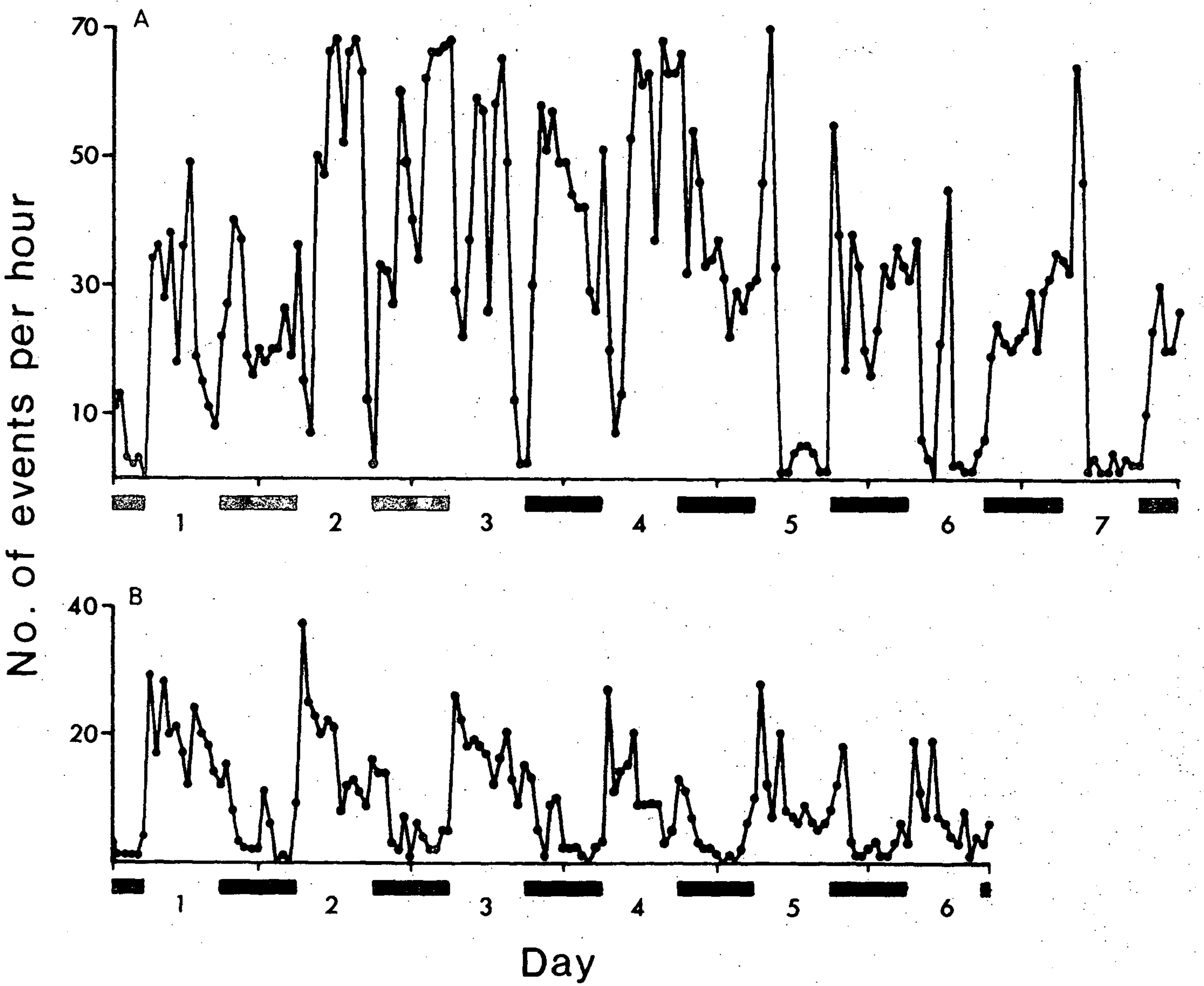
the entrained response.

These tests are focused upon the daily entrained response occurring in 12L 12D at 10°C. Longer term changes may occur but the data are not adequate for the analysis of seasonal or annual cycles. Mean daily records are presented and are adequate to indicate consistent increases or decreases in the locomotor activity cycle.

3.41 The entrained response in pinealectomized fish. High activity levels associated with the apparent loss of entrainment were common immediately after pinealectomy. Figure 23 a, b illustrates the response of one individual immediately after pinealectomy (a) and again, two weeks later (b). Hyperactivity and apparent loss of entrainment occurred on days one through four. The total daily activity was lessened in days 5, 6, and 7 with the appearance of a definite response to the environmental light cycle. Two weeks after pinealectomy (b) the response had stabilized and entrainment to the light cycle was demonstrated. All further experiments included a two week period of postoperative recovery in order to maintain an acceptable degree of comparison between each day of the experiment and also to prevent masking of the entrained response.

The sixteen fish tested for an interval of six days each indicated that a pinealectomized fish would respond

Figure 23. The activity response of one individual in 12L 12D at 10°C immediately after pinealectomy (A) and again two weeks later (B).


A
B
70
50
30
10
40
20
1
2
3
4
5
6
7
No. of events per hour
Day

to the light-dark cycle in a manner similar to that expressed by sham operants (Fig. 24). The average periodicity calculated for all pinealectomized individuals was 23.97 hours. Eight individuals were in exact synchrony with the light cycle and the other eight expressed periodicities ranging from 21.7 to 26.8 hours. It is suggested that these latter subjects demonstrated a scatter about the 24.00 hour mean, which might be attributed to post-operative effects. However, the exact nature of the disturbance was unknown.

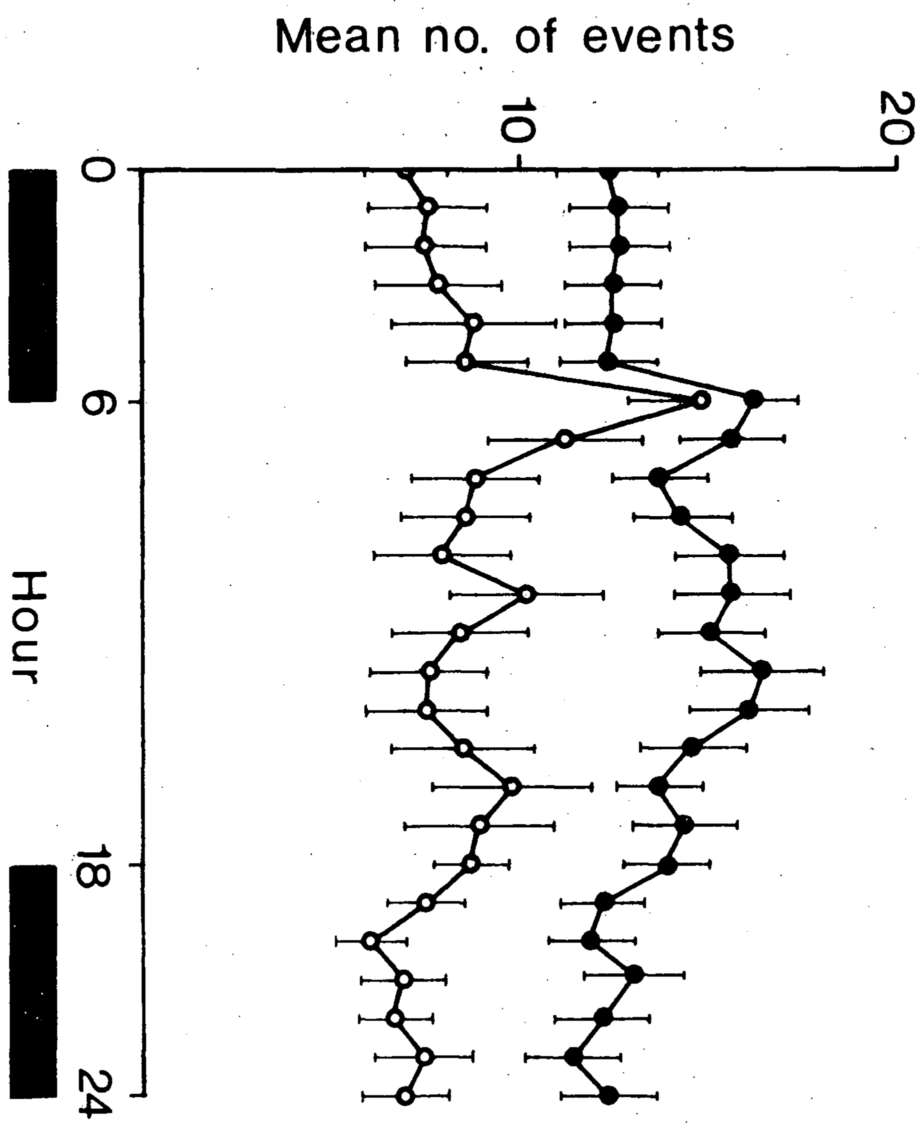
Eight sham operant fish were tested in the same manner as pinealectomized fish. All eight individuals expressed a 24.00 hour mean periodicity in a light-dark environment. Two individuals in this group had additional secondary peaks in the periodogram analysis, indicating that the rigours of the operation might result in some disturbance of the entrained response.

It was concluded that pinealectomy altered the daily response. A comparison of the total activity levels expressed by pinealectomized and sham operant fish indicated that pinealectomy resulted in significantly higher ($P<.01$) activity levels (see Fig. 24). However, even though the mean daily cycle was not well expressed in Fig. 24, periodogram analysis indicated that most individuals were entrained to the light cycle.

Figure 24. The mean daily activity patterns for pinealectomized and sham pinealectomized fish. Standard errors are indicated by the vertical brackets.

●—● Pinealectomized

○—○ Sham Pinealectomized



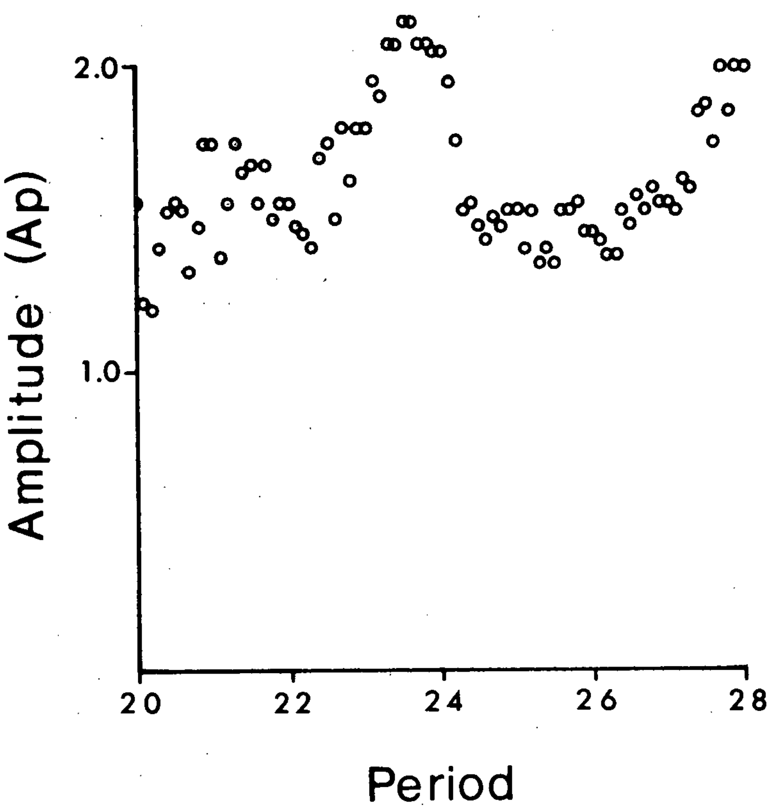
Mean no. of events
10
20
0
6
18
24
Hour

3.42 The endogenous response in pinealectomized fish. After a two week period of post-operative recovery, eight pinealectomized fish and four sham operants were subjected to constant environmental conditions of LL at 34.4 lux and 10°C. Periodogram analysis indicated that only two of the eight pinealectomized fish expressed a peak in amplitude that could be visually separated from the background. The low amplitudes between 20 and 28 hours for a single pinealectomized fish made the assumption of any periodic element questionable (Fig. 25). No sham operant fish expressed a periodicity between 20 and 28 hours.

Apparently the rigours of the operation would upset the endogenous component, since both pinealectomized and sham operants expressed similar disturbances. Continued investigation is necessary to determine the role of the pineal in the endogenous response, and it is suggested that longer periods of post-operative recovery might facilitate any such investigation.

3.43 The entrained response with opaque or transparent plastic shields over the site of the pineal. Since the previous experiments (sections 3.41, 3.42) indicated that penetration of the cranium could alter the entrained response, plastic shields were designed in an effort to simulate pinealectomy. Either clear or black plastic shields were inserted between the dermis and the ossified cranium, beneath the deepest layer of connective

Figure 25. Periodogram analysis of a single pinealectomized fish.


2.0
1.0
Amplitude (Ap)
20
22
24
26
28
Period

tissue. This placed the shields between the pineal body and the source of indirect overhead illumination. No lateral source of light was available to the subjects since the walls of the swimming channels were coated with non-reflective flat black paint. However, one cannot discount the possibility that the pineal could receive adequate lateral illumination resulting from the diffraction of light by either particulate material in the water or the tissues surrounding the skull.

All visible light was able to penetrate the clear plastic shields. The absorption curve of this material between 200 and 970 mμ indicated that only those wavelengths in the ultraviolet region were not transmitted. In the visible spectrum (380 to 700 mμ) transmittance was at a maximum.

(a) Black shield tests.

Six fish representing 36 "days" of activity contributed to this series. The mean daily response to a 12L 12D photoperiod at 10°C indicated that the presence of the black shields over the pineal region did not effectively alter the entrained response to the light-dark environment (Fig. 26). However, it was apparent that preventing illumination of the pineal resulted in higher levels of activity.

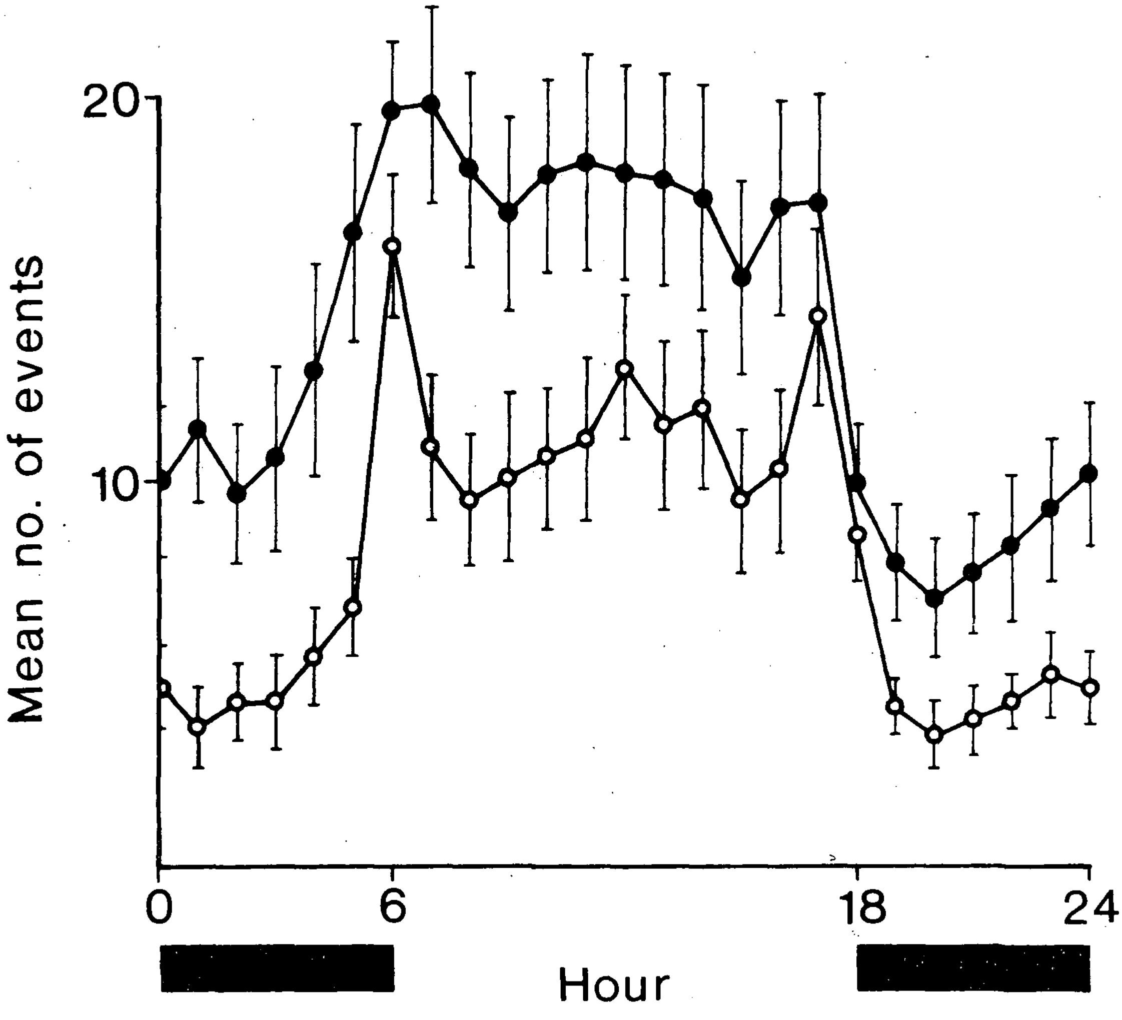
(b) Clear shield tests.

Four fish representing 24 "days" of activity contributed

Figure 26. Mean daily activity patterns of black shield and clear shield tests.

●—● Black shields

o—o Clear shields


Mean no. of events
20
10
0
6
18
24
Hour

to this series. The mean daily response indicated that clear shields did not interfere with the entrainment to the photoperiodic cycle (see Fig. 26). However, significantly lower levels of activity were obtained when these data were compared with the group tested with black shields.

These two experiments compliment the data obtained with pinealectomized fish by indicating the major difference exerted by the pineal is an alteration in the level of activity rather than determining the entrainment to photoperiod. These tests also provide further indication that penetration of the cranium alters the basic 24 hour pattern expressed by juvenile sockeye.

3.44 The entrained response in blinded fish. The experiments with pinealectomized fish suggested that mediation of the entrained response occurred via an extra-pineal information route. Attention was focused upon the eyes as the major (and perhaps most obvious) photoreceptor. Testing blinded individuals would clarify the role of the eyes, and a combined experiment with blinded and pinealectomized fish might indicate whether or not any extra-retinal, extra-pineal pathway was involved.

Four blinding techniques were used to determine the most suitable approach. These included the injection of phemerol chloride (1:1,000, Parke Davis) into the posterior chamber of each eye, sectioning of the optic nerve, bilateral enucleation of the eyes, and placing black plastic discs over the eyes. Those individuals subjected to surgical manipulation were allowed 2 weeks post-operative recovery before testing. Data were collected in a manner similar to previous tests.

(a) Chemical blinding.

Eight fish were prepared for this series of tests. The application of phemerol chloride followed the previously described technique (Hoar, 1955b; Gunning, 1959; Hasler, 1966).

Gunning (1959) questions the usefulness of this chemical, but bases his objections on altered behaviour patterns in the bluegill sunfish, and does not provide a measure of the organism's ability to detect light.

The majority of juvenile sockeye treated showed signs of recovery within one week. It was noted that the coloration was similar to normal individuals and not as dark as those fish subjected to bilateral enucleation. The fish responded to movements originating outside of the aquarium, and apparently detected these movements by vision.

The individual daily records indicated that most fish responded to the photoperiod and maintained a day active relationship to the light cycle.

One individual was apparently blinded by this technique. This observation was based upon coloration and response to movement outside of the aquarium. Subsequent testing in the activity chambers indicated a loss of entrainment to the environmental photoperiod.

This technique was discontinued due to the uncertain results. It was concluded that the chemical induced blindness in some, but not all individuals treated.

(b) The entrained response in fish after sectioning the optic nerve.

Eight fish were blinded by sectioning the optic nerve. The mean daily record indicated an activity increase after the onset of light (Fig. 27). A response to photoperiod was apparent and the predawn activity increase suggested the presence of an endogenous component. Periodogram analysis of the individual records indicated that six fish expressed an exact 24.00 hour periodicity. Slight deviations by the other two individuals resulted in an average 24.07 hour period for all fish tested.

These data suggest that the activity response and entrainment to photoperiod are not mediated via visual pathways such as the optic nerve.

(c) The entrained response in blinded and pinealectomized individuals.

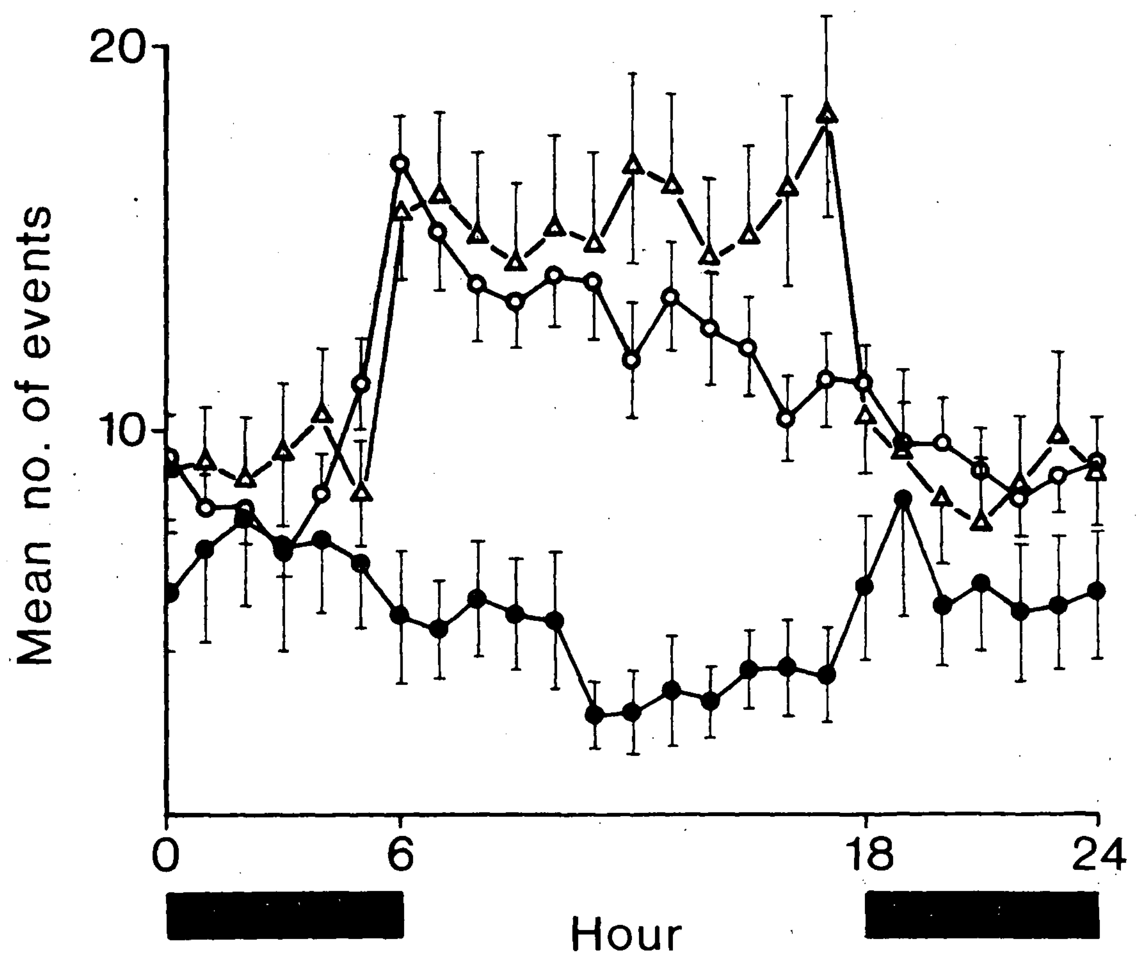
These experiments investigated the influence of photoreceptors other than the eyes or the pineal body. Blinding was induced by sectioning the optic nerve at least two weeks after pinealectomy. The tests were

Figure 27. Mean daily activity patterns for blinded and pinealectomized, blinded, and bilaterally enucleated groups. (Blinding = severing of optic nerve).

△—△ Blinded and Pinealectomized

○—○ Blinded

●—● Bilateral Enucleates


Mean no. of events
20
10
0
6
18
24
Hour

started after an additional two week period of post-operative recovery.

The mean daily activity pattern indicated that these fish were entrained to the environmental photoperiod (Fig. 27). A significant activity increase ($P<.01$) occurred at the onset of light and was terminated by a corresponding decrease at the onset of darkness. These data would tend to suggest the role of some extra-retinal, extra-pineal source of photoreception.

These tests were based upon the assumption that removal of a section of the optic nerve would effectively blind juvenile sockeye salmon. As far as is known, this process disrupts all visual connection between the retina and the brain, but non-visual afferent neural connections from the retina to the CNS might be present.

Gunning (1959) cautioned that although the optic nerve was severed, this would not completely omit the possibility of a retinal response to the changing light cycle. This led to an assumption that retinal mediation of the light cycle could still occur, but the transmission of information was via pathways other than the optic nerve.

(d) The entrained response with bilateral enucleation of the eyes.

Six fish were prepared for this series of tests. The surgical technique is listed in the appendix. Activity recordings were obtained two weeks after the operation but three fish failed to survive for the duration of the experiment. The remaining three individuals were tested for 11 days each, resulting in 33 "days" of activity representing the group.

These data indicated a very low amplitude of activity and the characteristic day active response to photoperiod was not present (Fig. 27). It was tentatively (due to the small sample size) concluded that the entrained response to the light cycle was mediated by the eyes.

If light falling on any region of extra-retinal photosensitivity was capable of eliciting the entrained response the results would be apparent in these tests. Both these and the following series of tests indicated that the extra-retinal entrainment was not involved.

(e) The entrained response with black eye covers.

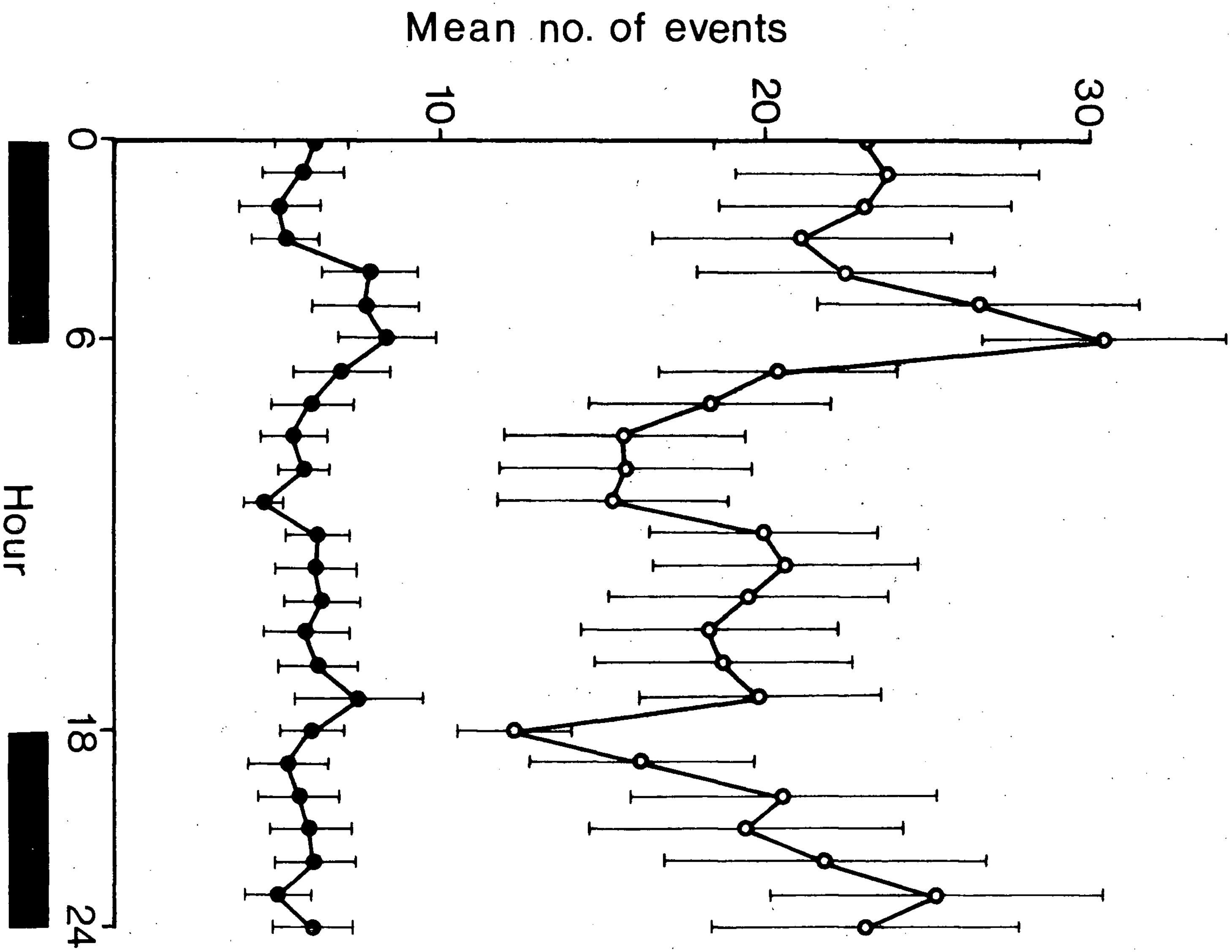
The fatalities associated with bilateral enucleation indicated the basic objection to that technique. Black eye caps were designed as an alternate technique which would prevent retinal stimulation by light, without removing the eyes. Clear plastic caps with the same absorption curve described for the pineal shields were used as a control. A total of 21 fish were used in these tests contributing a total of 146 "days" of activity.

The application of black caps abolished the entrained response to the light cycle (Fig. 28). The similarity of the response between normal fish and the fish after removal

Figure 28. Mean daily activity patterns for black and clear eye cap groups.

●—● Black Eye Caps

o—o Clear Eye Caps



Mean no. of events
10
20
30
Hour
0
6
18
24

of the black caps indicated that entrainment resulted from retinal photostimulation only (Fig. 29). If the eye caps were removed during the course of an experiment, the subject would respond immediately to the environmental photoperiod.

Before cap removal an endogenous four hour periodicity was noted. This was an ultradian response to the constant environment (less than a day, but ranging between 0 and 20.00 hours) and was similar to the short periodicity observed in the DD tests. No endogenous patterns with circadian characteristics were observed in fish with black caps in place, a factor supporting the assumption that DD might inhibit the expression of an endogenous rhythm by juvenile sockeye salmon.

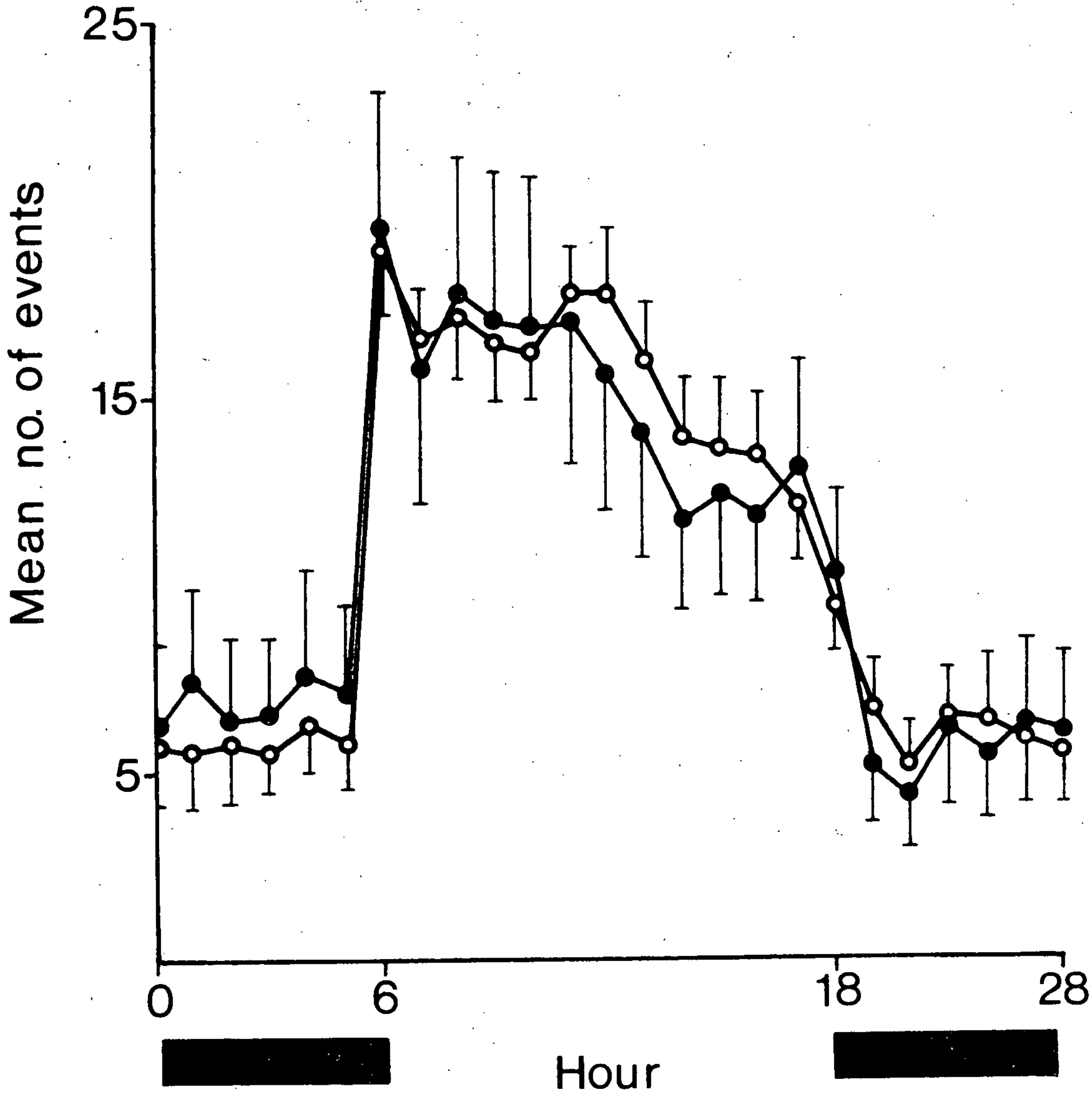
The response obtained with the clear plastic shields appeared to be characteristic of a day active organism in single individuals, but the grouped data indicated high levels of activity and an apparent loss of entrainment (see Fig. 28). The construction of the plastic discs for these tests undoubtedly created many visual aberrations which interfered with the optics of the intact eye, and might be responsible for these variations in activity.

These data indicated a definite source of reception for the entrained response to photoperiod in this species. Since the information about the light cycle is not transmitted via the optic nerve, it is suggested that the eye might

Figure 29. 12L 12D, 5°C response in normal fish and fish tested immediately after removal of the black eye caps.

o—o Normal fish

●—● Black eye caps removed


Mean no. of events
25
15
5
0
6
18
28
Hour

secrete a blood-born agent which is responsible for the cyclic activity response.

3.5 The Effects of Melatonin, Serotonin and Teleost Saline on the Entrained Response.

The data from the preceding sections on pinealectomized and blinded fish suggested that the production of certain chemical agents may be involved in entrainment. Those individuals subjected to pinealectomy or black shields over the pineal region exhibited significantly higher activity levels. The entrained response and the activity level were both altered by removal of the eyes and the application of black caps.

The objective of this series was to determine if the injection of melatonin and serotonin would induce significant changes in the activity levels of juvenile sockeye salmon. Since the pineal is generally considered as the major site of melatonin production, it is assumed that lower melatonin levels will be present following pinealectomy (unless compensatory increases occur in the retina). This decrease in the level of melatonin might be responsible for the higher activity levels observed. Alternatively, the increased activity level might be due to increased levels of serotonin, the precursor of melatonin. This information coupled with the findings of Wong and Whiteside (1968) led to the formulation of a working hypothesis predicting an increase in activity with serotonin, a decrease in activity with melatonin, and no change with injections of teleost saline.

A total of eighteen fish were tested in three groups of nine days each. The first three test days were focused upon the locomotor activities in a 12L 12D (10°C) environment before administering intraperitoneal injections. The second three days of the test were focused upon the expression following 0.25 cc daily injections (1μg per gram body weight) of melatonin, serotonin, or teleost saline. All injections were administered 1/2 hour before the dark phase of the photoperiodic cycle. The final three days examined the activity records of these individuals after the injections.

Significant activity increases occurred after injection of serotonin (Fig. 30). Melatonin injections resulted in decreased activity, whereas no gross changes occurred in the teleost saline treatment. There was an indication that slight activity decreases in all tests might be attributed to the method of removing the fish from the environment and injecting by hand (see control).

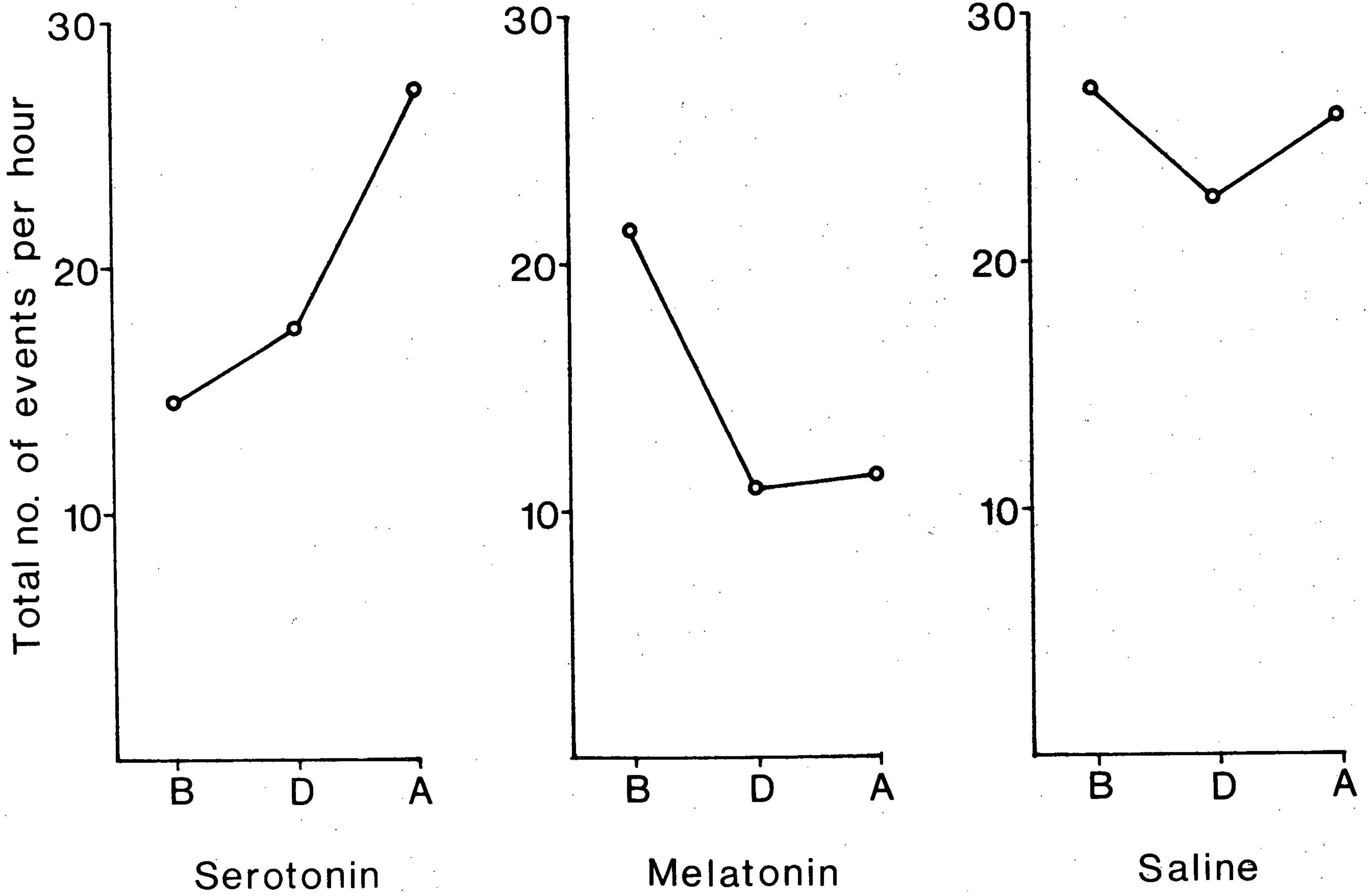
A comparison of the mean daily activity records for the control group and melatonin treatment indicated that the decrease in activity occurred mainly during the light phase of the photoperiodic cycle (Fig. 31). Conversely, the major area of activity increase for the serotonin treatment occurred during the dark phase of the photoperiod.

Figure 30. Mean total daily activity for Serotonin, Melatonin and teleost Saline injected groups.

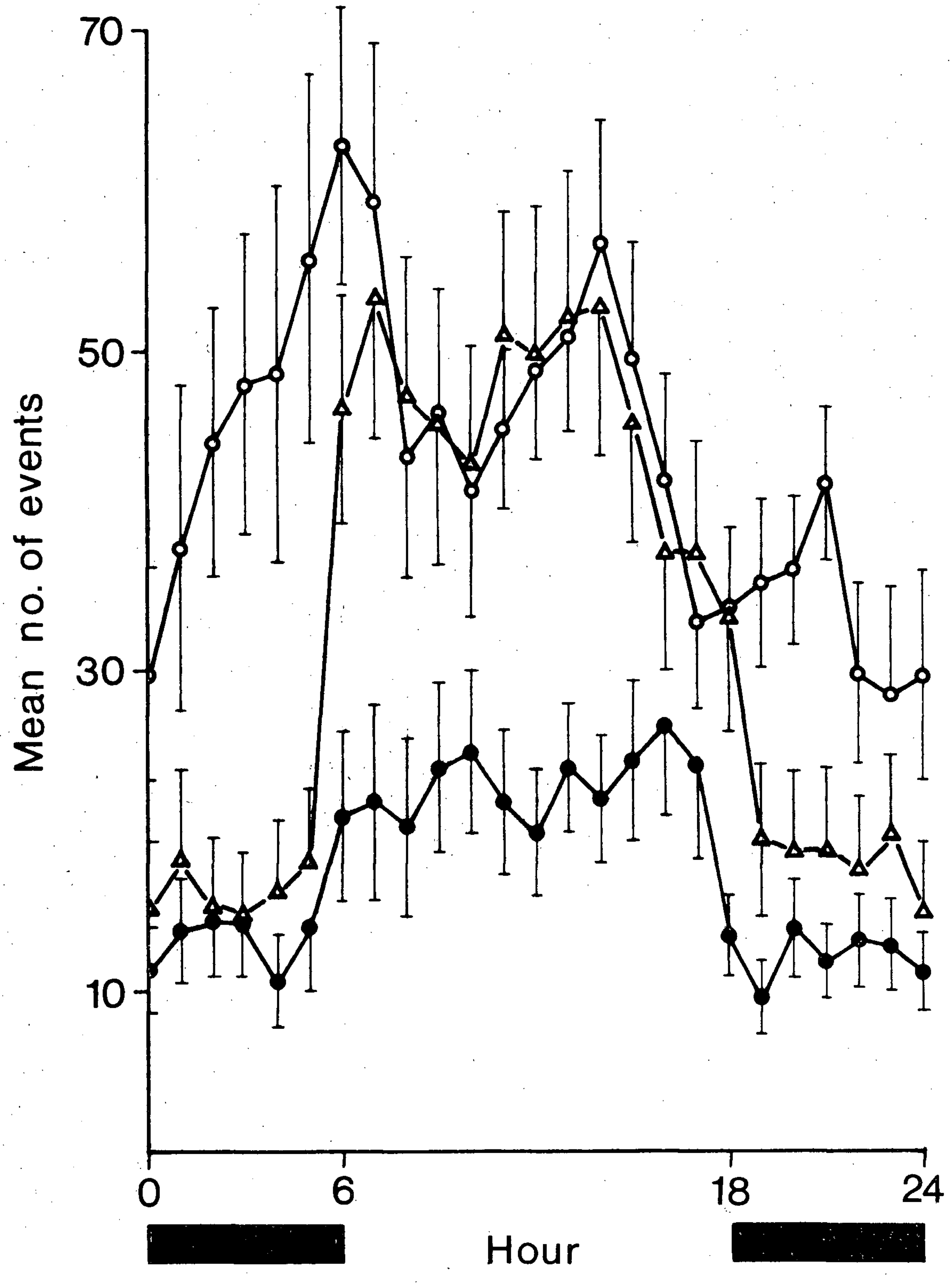
B = Before Injection

D = During Injection

A = After Injection



Total no. of events per hour
30
20
10
B
D
A
Serotonin
30
20
10
B
D
A
Melatonin
30
20
10
B
D
A
Saline


Mean no. of events
70
50
30
10
0
6
18
24
Hour

4. DISCUSSION

The general importance of biological rhythmicity is reflected by the amount of interest the subject has generated in the past fifteen years. Research has included examinations of daily, tidal, lunar and annual cycles, and how these relate to migration, hibernation, breeding, color changes, metabolic processes, feeding and locomotor activity patterns. The majority of interest has focused upon mammalian and avian forms, a factor reflecting their availability and usefulness in long term laboratory projects. As a result, most recording techniques have been developed for a few terrestrial organisms.

The apparent lack of interest in teleosts has resulted from the meagre supply of adequate instrumentation for recording locomotor activity. However, recent advances in the design of electronic components and their application (Cummings, 1963; Muir, et al., 1965; Meffert, 1968) have provided the opportunity to proceed with investigations in a variety of aquatic organisms.

Environmental control was of paramount importance to this investigation, and represented a factor not attainable in the field. However, the ultimate goal was to apply the laboratory based hypotheses to the field situation, thus indicating the general ecological significance of biological rhythmicities in juvenile sockeye salmon.

The control systems used in the laboratory were adequate and fulfilled the requirements outlined by Bunning (1960):

> "Provisions for constant temperature and exact control of light and darkness are particularly important since we know that in some cases temperature fluctuations of less than 1°C can significantly interfere with experiments, or that an observation light, even if applied for only a few seconds can also upset an experiment".

The use of electronic transducers facilitated the construction of an artificial environment and activity monitoring system which enabled the investigation to proceed without adding any instruments to the controlled environment. The general applicability of the transducer was tested by recording activity before, during and after instrument operation. These data indicated that use of high frequency sound would facilitate a long term study of locomotor activity in these fish.

Spontaneous locomotor activity is an easily measured behavioural response to endogenous and exogenous factors which has been investigated in a variety of organisms (reviews: Aschoff, 1960, 1965a). This particular measure of behaviour was used to indicate the entrained response to photoperiod and also served as an easily obtained assay of light perception.

4.1 The Basic Activity Pattern

A definite entrained response was obtained in an artificial (LD) environment. The activity rhythm was expressed as a light active relationship to the environmental photoperiod which also served as the primary entraining agent for either single individuals or groups of fish. The response at different developmental stages indicated that the activity pattern was firmly established at an early age and maintained for at least one year. Sufficient information was not available to determine the general activity pattern after one year of age. Manzer (1964) showed evidence of diel vertical movements in the Gulf of Alaska, and Groot (1965) suggested that there was a basic activity rhythm of sockeye salmon, young or old, migrating or not.

Fish obtained from a different locality (Kamchatka Peninsula) expressed a similar activity pattern at 5°C. However, both stocks (Lakelse and Kamchatka) were reared under identical laboratory conditions, a factor which may have contributed to the expression of similar responses. It was not known if similar data would be obtained if identical stocks were reared at their native geographical locations.

4.11 The influence of temperature and photoperiod. Both temperature and photoperiod altered the entrained response. Increasing temperatures usually resulted in higher activity amplitudes during both light and dark, but when short daylengths were combined with high temperatures, decreased activity occurred. A unimodal activity pattern was expressed when short daylengths and low temperatures were used. When either daylength or temperature increased, a bimodal pattern appeared.

It is concluded that certain combinations of temperature and photoperiod can produce changes in the contour of the activity response and can even initiate a complete reversal of the photobehavioural response. It is hypothesized that for each daylength there is a certain temperature, above which juvenile sockeye salmon will reverse the characteristic light active pattern and respond with dark activity. It is suggested that increasing temperatures will function as the major "reversing" agent, whereas the photoperiod will function as a "controlling" agent. In this way, increasing daylengths will stabilize the light active response.

The data presented refer only to the laboratory investigation, but are supported by the work of Gibson and Keenleyside (1966) who suggested that temperature might be responsible for a reversed photobehavioural response in young Atlantic salmon.

Poikilotherms generally respond to increasing temperatures (within the limits of tolerance) with greater metabolic activity. Increased locomotor activity might then be interpreted as a "reflection" of the metabolic state. The data presented in section 3.24 clearly indicate this generalized response in young sockeye salmon.

The apparent temperature-daylength interaction complicates the interpretation of the data. Hoar (1965) suggests that the temperature-photoperiod link may be associated with some pituitary factor, but the actual mechanisms remain to be determined. It appears that for each daylength there is an optimum temperature for certain metabolic processes such as fat deposition or resistance to heat. Seasonally changing photoperiods might adjust the metabolism of fish to anticipate sharp temperature changes in the spring and autumn. However, conflicting environmental stimuli (short days and high temperatures) apparently result in a reversal of the photobehavioural response.

Aschoff (1960) suggested that at different light intensities there was an optimum level, above which an organism would seek the opposite response in the activity/rest ratio. A similar mechanism might occur here, where a manipulation of the temperature-daylength relationship might regulate the expression of light or dark activity.

These different activity responses to temperature and photoperiod might also account for some of the conflicting reports on salmonid activity which have occurred at separate geographical location (Groot, 1965; Thompson and Burgner, 1967).

4.2 The Endogenous Activity Response

The anticipatory increase in activity prior to the onset of light and a corresponding decrease in activity before dark suggested an endogenous cycle with a characteristic period that was less than 24.00 hours. Those experiments conducted in constant light indicated that the response was maintained in absence of periodic environmental stimuli. A free-running rhythm with a period approximating 23.30 hours was identified. These data supported the hypothesis of Groot (1965) and Hoar (1965) which suggested the presence of an endogenous clock in this species.

The response to increasing light intensities was not in accordance with Aschoff's general rule (1960) which states:

> "With increasing light intensities the period of a light active organism will decrease".

However, this is a gross generalization and several exceptions have been noted in the literature (review: Hoffmann, 1965).

The increasing period in these juvenile sockeye might be associated with their characteristic ability to express both diurnal and nocturnal activity patterns at different stages in the life cycle. It would be interesting to determine whether or not any changes occur in the endogenous component during the course of development, especially during the periods when migrations coincide with nocturnal activity.

Periodic feeding could also affect the endogenous cycle in an otherwise constant environment. It is concluded that food will have to be either continuously present or continuously absent for short term experiments.

4.3 Mechanism of Entrainment

A consideration of the eyes and pineal body indicated that entrainment occurred through retinal mediation. Since sectioning of the optic nerve did not abolish the entrained response, but presumably induced "blindness", the visual response in image formation and detection of light intensity might be based upon entirely different processes. It is hypothesized that photoperiodic information might be transmitted by some system other than the optic nerve (possibly vascular). However, it is equally feasible to consider the possibility of some unknown afferent nervous pathways from the retina.

The hypothesis was based upon the following information. First, removal of the eye, or placing black discs over it suppressed the entrained response (i.e. apparently the fish could not detect the photoperiodic environment). Secondly, sectioning the optic nerve did not result in loss of entrainment (i.e. the fish could detect the light cycle). This information indicated that some change occurred within the eye, and the resulting activity response was not mediated by pathways within the optic nerve. The possibility of entrainment by other photoreceptors such as the pineal body was rejected since entrainment did not occur in bilaterally enucleated fish with this organ intact. However, Menaker (1968) and Gaster and Menaker (1968) demonstrated that illumination of the pineal could serve to entrain blinded sparrows. Conversely, Quay (1968) failed to demonstrate any pineal mediation in the activity response of the laboratory rat, but did reveal that entrainment was a response to illumination of the retina.

My investigation indicated that the pineal body in juvenile sockeye salmon was not involved in the entrained response. However, removal of the pineal or shading it resulted with increased activity levels during both light and dark. Since entrainment is possible in some forms via pineal illumination, and in others by retinal mediation, one might first look for similar mechanisms within the two organs.

Quay (1965) demonstrated the presence of pineal and retinal HIOMT (hydroxyindole-o-methyl transferase) in lower vertebrates, including fish. This is the methylating enzyme responsible for the formation of melatonin (N-acetyl-5-methoxy-tryptamine) from its precursor N-acetyl serotonin (N-acetyl-5-hydroxy-tryptamine). Fenwick (in press) has demonstrated the presence of both retinal and pineal melatonin and serotonin in two closely related species of Pacific salmon. Bagnara (1960), Wurtman, et al., (1963) and Quay (1964) have shown that an apparent correlation exists between the environmental light cycle and variations in the level of pineal melatonin and serotonin in several higher vertebrates.

Quay's experiments with the laboratory rat indicated low serotonin levels during dark, with a 900% increase during light. Reiss, et al., (1963) and Wong and Whiteside (1968) have demonstrated that injections of pineal extracts or melatonin will significantly reduce the total wheel-running activity of laboratory rats.

It was hypothesized that intraperitoneal injections of melatonin might reduce locomotor activity in juvenile sockeye salmon if similar correlations existed between the production of this chemical and the environmental light cycle. If an opposing response could be attributed to serotonin, an increased amplitude of activity might be expected. The laboratory tests indicated that serotonin increased the activity level during the dark phase of the photoperiod and melatonin decreased the activity level during the light phase.

Since the pineal is generally considered to be the major site of melatonin production, the triggered release by either retinal information or direct illumination may involved. However, the mechanisms by which this release occurs remain to be determined.

The onset of darkness might facilitate melatonin synthesis from serotonin. The increased melatonin levels may be correlated with the decreased activity levels during darkness, however, the actual function within the organism remains to be determined. It is not known whether the melatonin formed is secreted as a hormone, or where the specific sites of action are located.

Serotonin (5-HT) originates as a product of tryptophan metabolism. The absolute amounts in the brain are small, but appear to have great importance (Quay, 1965). Its distribution is not confined to the central nervous system. In mammals, 5-HT has been identified in many tissues and large amounts have been found in the blood platelets.

Circumstantial evidence indicates that 5-HT may act as a transmitter substance (Roche, 1964). However, current research on the overall function of 5-HT is complicated by its nearly ubiquitous distribution.

In juvenile sockeye salmon, intraperitoneal injections of 5-HT increased dark activity. Here again, it is not known whether the sites of action were in the brain or at peripheral areas. Administration of 5-hydroxytryptophan (5-HTP), the precursor of serotonin, will increase the amounts of 5-HT in most tissues. Roche (1964) indicates that dogs and cats respond to increased 5-HT levels with somatic, autonomic and behavioural changes resembling those produced by lysergic acid diethylamide (i.e. excitement with loss of reflexes, apparent fear, sham rage, blindness and disorientation).

My data suggest a profitable avenue of future research. A collaborative approach providing a synthesis of information on the overt entrained response with the chemical and cellular changes occurring in the central nervous system would be most valuable.

4.4 The Adaptive Significance of a Daily Activity Cycle.

The expression of an activity rhythm is not unique, but the functional significance of such a response is evasive. One obvious advantage might be to synchronize the various physiological processes with daily, seasonal and yearly cycles, thus enabling the organism to fully exploit its environment.

If certain levels of sensitivity are synchronized with the environmental photoperiod (Aschoff, 1965b), an organism could make full use of its sensory information in performing such diverse activities as feeding, schooling, migrating or evading predators. However, the interaction between endogenous and exogenous components complicates the situation and obscures any simple answer to the general significance of biological rhythmicity.

The interaction observed between temperature and photoperiod supported the hypothesis that thermal resistance could be modified by the light cycle (Hoar, 1965b) (i.e. long photoperiods could increase resistance to heat while short photoperiods could increase resistance to chilling).

My study indicated that long photoperiods increased the temperature value at which there was a 50% distribution of light/dark activity. It was further demonstrated that if the environmental temperature exceeded a certain limit, there was a reversal of the photobehavioural response and juvenile sockeye salmon became dark active. The characteristic nocturnal smolt outmigration might represent a similar response to the rapidly increasing water temperatures in spring.

4.41 Emergence and migration. Under natural conditions sockeye fry are negatively phototactic and remain hidden in the gravel during the day. Those fry ready to migrate emerge at dusk, exhibit an apparent negative rheotaxis and move downstream to nursery lakes. The migrants express a bimodal activity pattern and do not school. After reaching the lake the fish form schools, exhibit a positive rheotaxis and remain swimming and feeding during the day (Hoar, 1954, 1958; McDonald, 1960; Hartman, et al., 1962; Heard, 1965).

My laboratory tests offered parallel evidence for a bimodal activity pattern immediately after hatching. A small morning activity peak occurred at the onset of light, and a major evening activity peak occurred after the onset of darkness. These peaks were separated by a midday depression of activity which persisted for approximately ten days. Newly emerged fry in a swiftly flowing stream, unable to perform visual landmark orientation, might be subjected to a passive downstream displacement. The high amplitudes of nocturnal activity in the newly emerged fry, coupled with reduced visual acuity (Ali, 1959) suggest a mechanism for downstream displacement.

Upstream migrants (a situation where the spawning grounds are in tributaries flowing out of the nursery lake) express a pattern of day active migration (McCart, 1967; Calaprice, pers. comm.). Andrew and Geen (1960) report that in the Chilco lake system, the newly emerged fry move downstream until reaching areas of low velocity along the river edge. The fry evidently hold in these areas and move upstream seven to ten days after emergence.

My investigation indicated that after ten days, downstream migrant fry became light active. The increased levels of day activity coupled with a strong rheotaxis would be of paramount importance to upstream migration during the day. This study would favour the hypothesis that upstream migrations are facilitated by changes in behaviour coupled with an increase in size (7 to 10 days additional development) and swimming ability (Hoar, 1958). However, Calaprice (pers. comm.) also considers equally feasible a genetically regulated light and current response for upstream and downstream migrants (see also Raleigh, 1967; Brannon, 1967).

4.42 Pelagic stage. After entering the nursery lakes, fry disperse along shore and later become entirely pelagic. This study suggested that light activity was maintained in those age groups normally resident in the nursery lakes. The experiments examining the effect of age on the entrained response, and the two year analysis of related data indicated the general applicability of a light active pattern to this age group.

Either single individuals or groups could be entrained to the same environmental cycle. This provided a mechanism enabling individuals to become correspondingly active and inactive at approximately the same times of day. This mechanism might be valuable in maintaining the cohesiveness of a school and may also provide greater accuracy in timing the onset of daily activity.

The investigation at Babine Lake, B.C. with "sea scanar" echo soundings indicates a relative inactivity and absence of dense schools at night (Narver, unpublished). Marshall (1965) and Denton and Nicol (1966) emphasized the role of vision in schooling species, but indicated that other sensory systems were undoubtedly involved. Schooling is apparently a visual response in this species (Hoar, 1958; Ali, 1959), a factor suggesting the general importance of light activity.

4.43 Feeding. The importance of vision in the location and capture of food is a characteristic of Pacific salmon (Hoar, 1958; Ali, 1959; Brett and Groot, 1963). Field observations indicated that feeding occurred during the day in upstream migrant fry (McCart, 1967) and Babine lake pre-smolts (Narver, pers. comm.). The Babine lake investigation indicated that feeding activity occurred when the fish were 150 to 200 feet deep, however, some feeding also occurred at dawn and dusk before and after the diel vertical migration (Foerster, 1968). Groot (1965) demonstrates dawn and dusk peaks in surface feeding activity for migrating smolts (at 14°C avg. temperature).

The high levels of activity during the day, coupled with increased visual acuity might facilitate the location and capture of prey. The feeding response is apparently established at an early age after closure of the yolk sac, when the fish have developed a light active pattern. Certain combinations of temperature and photoperiod may determine a bimodal pattern of feeding activity in the summer, and a unimodal pattern of feeding activity in the winter.

4.44 Diel vertical migrations. The vertical movements of this species have been well documented (Johnson, 1956, 1961; Burgner, 1962), however the patterns of movement and distribution are not identical in all sockeye lakes. In Babine Lake, B.C. the migration pattern can be divided into four phases (Narver, pers. comm.).

1. Dawn, vertical descent to depths approximating 150 to 200 feet.

2. Midday, holding at depth.

3. Dusk, vertical ascent with a short period of activity at the surface.

4. Night, descent to 10 to 25 feet and relative inactivity.

The responses obtained in the laboratory parallel the various activities observed in the field. It appeared that light activity was associated with feeding, schooling and diel vertical migration, while dark inactivity was associated with the holding response in the lake, the cessation of feeding, and the nocturnal dispersal of individuals (i.e. the breakdown of schools). The availability of juvenile sockeye salmon to surface sampling techniques at night (Burgner, 1962) provided additional support for their presence in this layer. However, my investigation suggested that individuals were relatively inactive at night while present at or near the surface.

4.5 Seasonal Regulation of the Entrained Response

This investigation indicated that short photoperiods and low temperatures (8L 16D, 5°C) resulted in a unimodal activity pattern. Long photoperiods and high temperatures (16L 8D, 15°C) resulted in a bimodal pattern with increased amplitudes of activity during both light and dark. Combining short photoperiods with high temperatures (8L 16D, 15°C) resulted in an apparent reversal of the photobehavioural response.

At Lakelse Lake, B.C. the temperatures from December 15th to April 15th approximate 3.5°C between 20 feet and 80 feet depths. May and June are characterized by a rapid temperature increase (10°C to 15°C) in the upper forty feet. The water temperatures drop to the winter levels from September to November (Brett, 1950). The laboratory temperatures approximated the range measured at Lakelse Lake, a factor suggesting the general applicability of this study to field conditions. Assuming that the laboratory study approximated the general pattern in the field, we can speculate upon the following seasonal activity response.

Winter: Short photoperiods and low temperatures in most sockeye lakes may result in a unimodal activity pattern. Light intensity can be further decreased by ice and snow cover. A unimodal activity pattern would enable resident sockeye to utilize all available daylight for the capture of food. Growth and energy expenditure are at their lowest annual level, responding to the low temperatures and decreased food supply.

Spring: Increasing photoperiod and temperature might result in higher levels and increased durations of activity. Ice breakup precedes the smolt migrations to a marine environment. Foerster (1937) provided a significant correlation between temperature conditions and seaward migrations from Cultus Lake, B.C. The increased nocturnal activity may be a response to the rapid temperature increases in spring and could impart a characteristic dark active pattern of movement to many smolt migrations. Further increases in photoperiod could act to stabilize the day active relationship of the activity pattern, thus bringing a cessation to the outmigration.

Summer: The response to long photoperiods and high temperatures indicates that the activity pattern expressed by new residents and residuals might be day active, and bimodal, with activity peaks occurring at dawn and at dusk.

Fall: Decreasing photoperiod and temperature could regulated a reversal of the process, resulting in the expression of a unimodal activity pattern during the winter.

To state that the organism is a complete subject of its environment is an oversimplification of the problem. Entrainment is apparently the response to an endogenous pattern of physiological excitability that is reflected by increases and decreases in the locomotor activity. The entrained response is a synchronization of this endogenous pattern with certain environmental cues acting as stimuli for the onset and termination of the activity cycle (Aschoff, et al., 1965).

5. SUMMARY AND CONCLUSIONS

1. The objective of this study was to provide a description and analysis of the endogenous and exogenous factors contributing to a daily pattern of locomotor activity in juvenile sockeye salmon.

2. The basic measure employed throughout the investigation was spontaneous locomotor activity. High frequency sound was used to continuously monitor activity in 36" (91.4 cm) diameter swimming channels. The apparatus provided a monitoring system without any apparent disturbance to either the controlled environment or the experimental subjects.

3. The influence of photoperiod and temperature on the entrained activity pattern was investigated. The artificial photoperiod functioned as the primary entraining agent for single individuals or groups of fish. The activity records indicated that sockeye fry were dark active immediately after emergence. A light active pattern was developed two weeks after emergence and was maintained for at least one year.

4. Both photoperiod and temperature influenced the 24 hour activity pattern in yearling fish. Low temperatures (5°C) facilitated light activity at all photoperiods, whereas high temperatures (15°C) combined with short photoperiods (8L 16D) increased dark activity.

5. The daily entrained activity pattern of sockeye fry was bimodal immediately after emergence. Yearlings responded to low temperatures and short photoperiods with a unimodal activity pattern, but increasing temperature and/or photoperiod resulted in a bimodal activity pattern.

6. Total activity was increased at the higher temperatures used. The temperature-photoperiod interaction indicated that longer photoperiods (16L 8D) facilitated a light active pattern at 15°C.

7. The endogenous rhythm was examined in a constant environment. The period value was 23.30 hours in constant light, but could be lengthened by increasing light intensity. Periodic feeding could function as an entraining agent.

8. The eyes were identified as the primary sensory receptors mediating information about the environmental photoperiod. Transfer of information was not via the optic nerve. This suggested the possibility of other afferent nervous pathways from the retina, or mediation by a blood-born agent.

9. The pineal body had no entraining role in this species. However, both pinealectomy and inserting shields over the pineal region increased the total daily activity.

10. Intraperitoneal melatonin injections decreased activity during light, whereas similar injections of serotonin increased activity during dark. This experiment supported the hypothesis based upon an apparent correlation in other species that melatonin and serotonin may be involved in the regulation of activity levels.

11. The laboratory findings were discussed in relation to published field observations on juvenile salmonids.

12. The functional significance of the activity cycle was discussed in relation to migration, orientation, feeding, and schooling behaviour.

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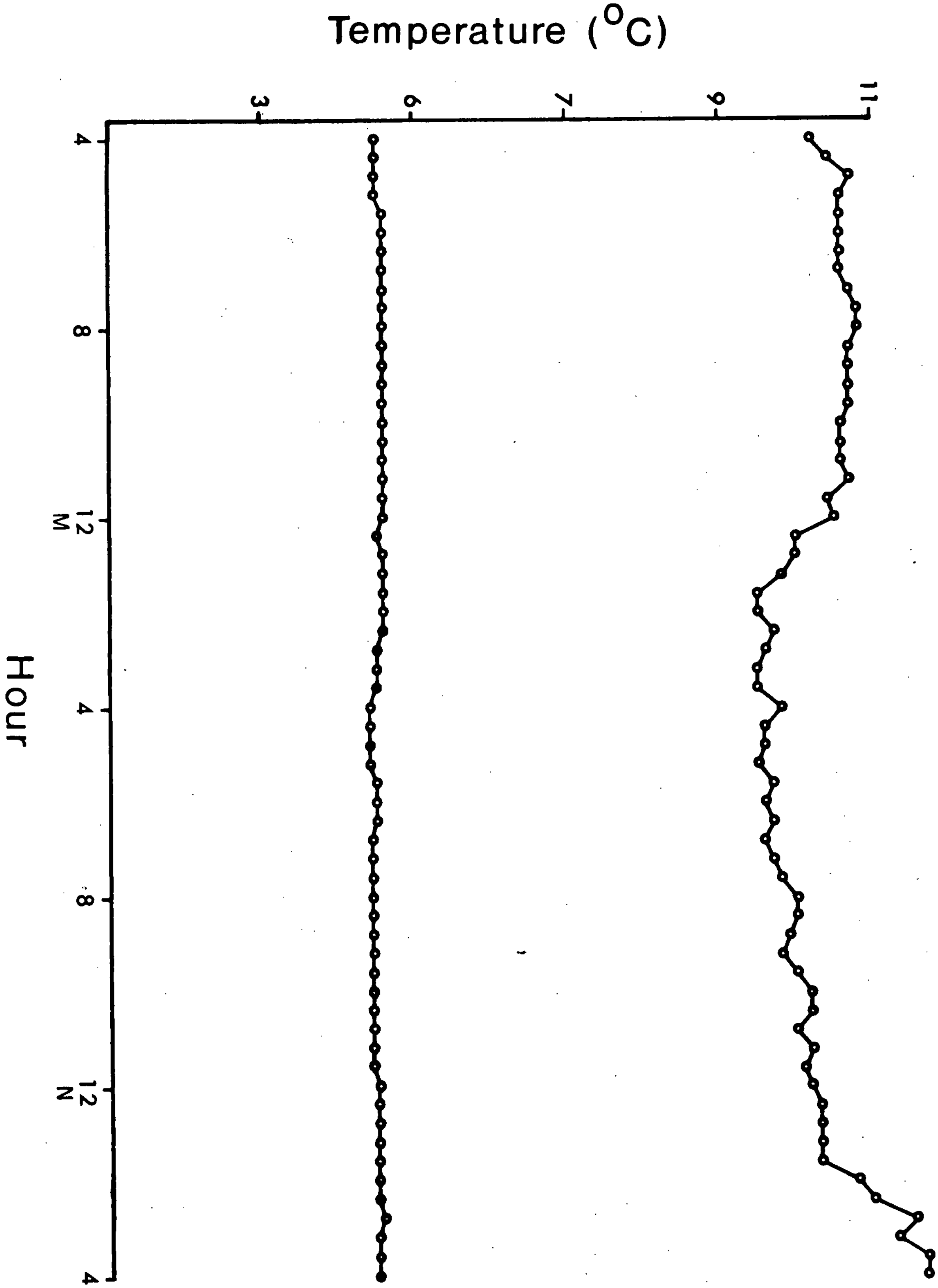
APPENDIX

Figure 1. A continuous 24 hour record of room temperature and water temperature in the swimming channels during March, 1967.



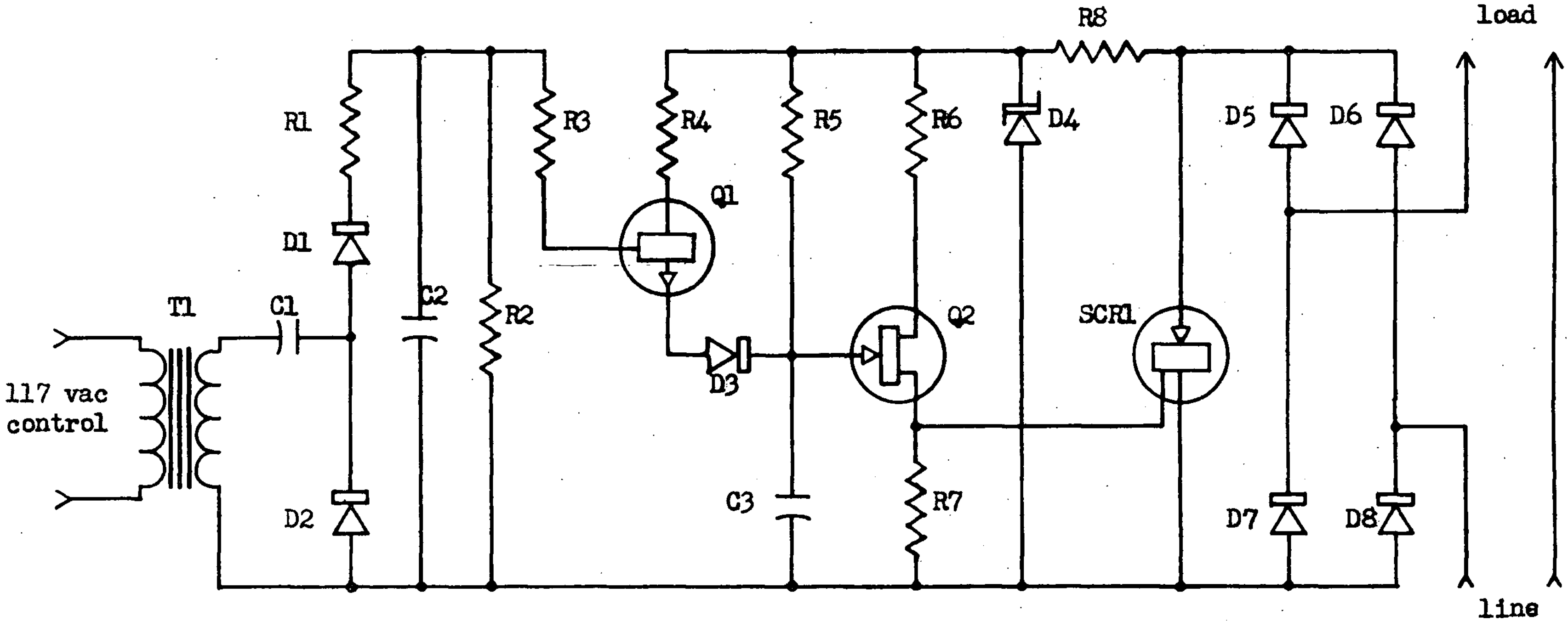
Temperature (°C)
11
9
7
6
3
4
8
12
M
4
8
12
N
4
Hour

Figure 2. Circuit diagram of 600 watt lamp controller.

Key:

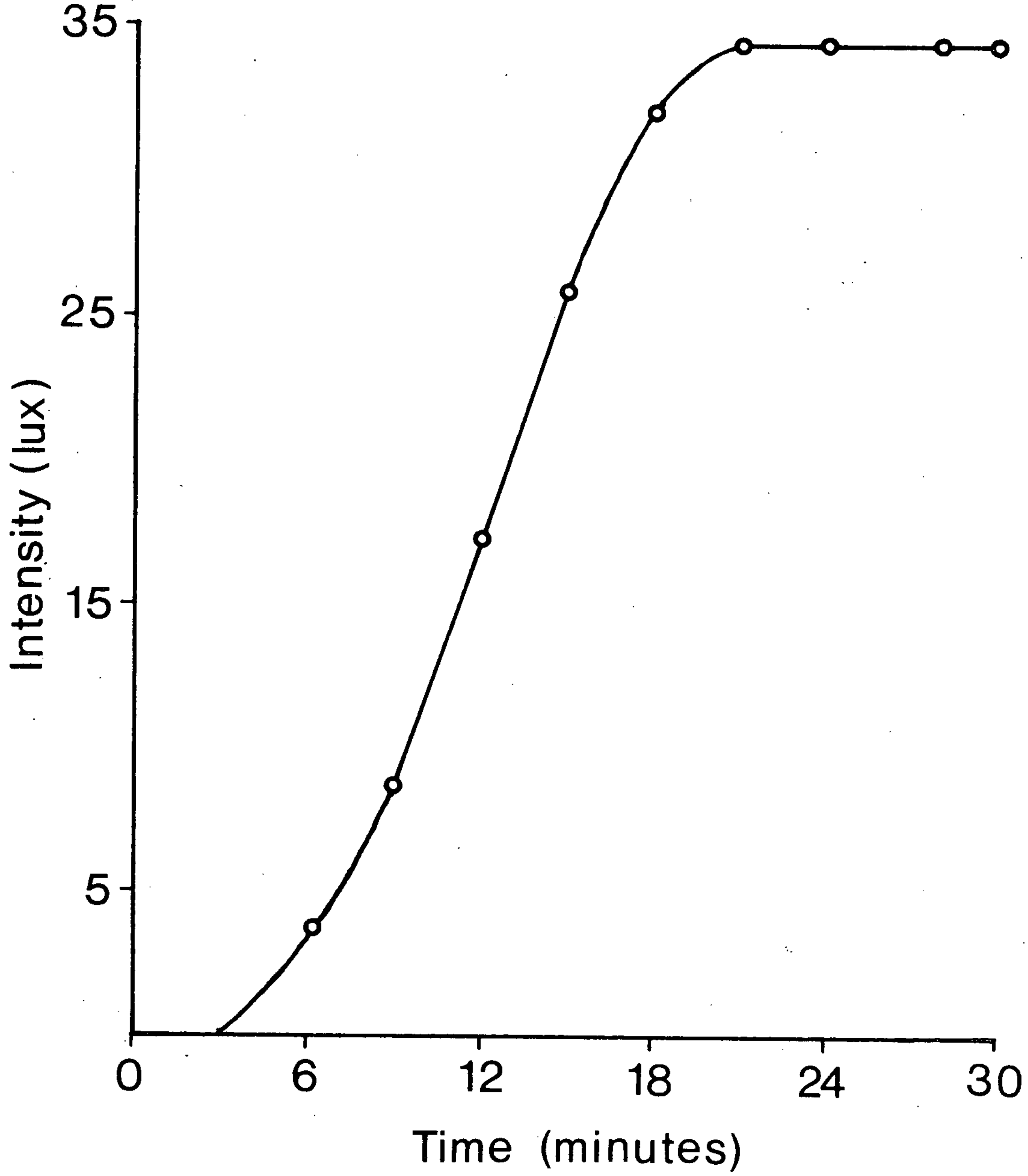
R1 - 470K 1/2w	C2 - 1000/25v
R2 - 1.5M "	C3 - 0.1/100v
R3 - 1.0M "	D1,D2,D3 - IN681
R4 - 2.2K "	D4 - Z4XL20
R5 - 68K "	D5,D6,D7,D8 - IN3571
R6 - 220 "	Q1 - 2N2925
R7 - 47 "	Q2 - 2N2646
R8 - 4700 2w	SCR1 - C20C
C1 - 1/25v	T1 - 12.6v Hammond 166F12

Unless otherwise noted, all cap. in mfd., resistors in ohms 1/2w and 2w, 10%

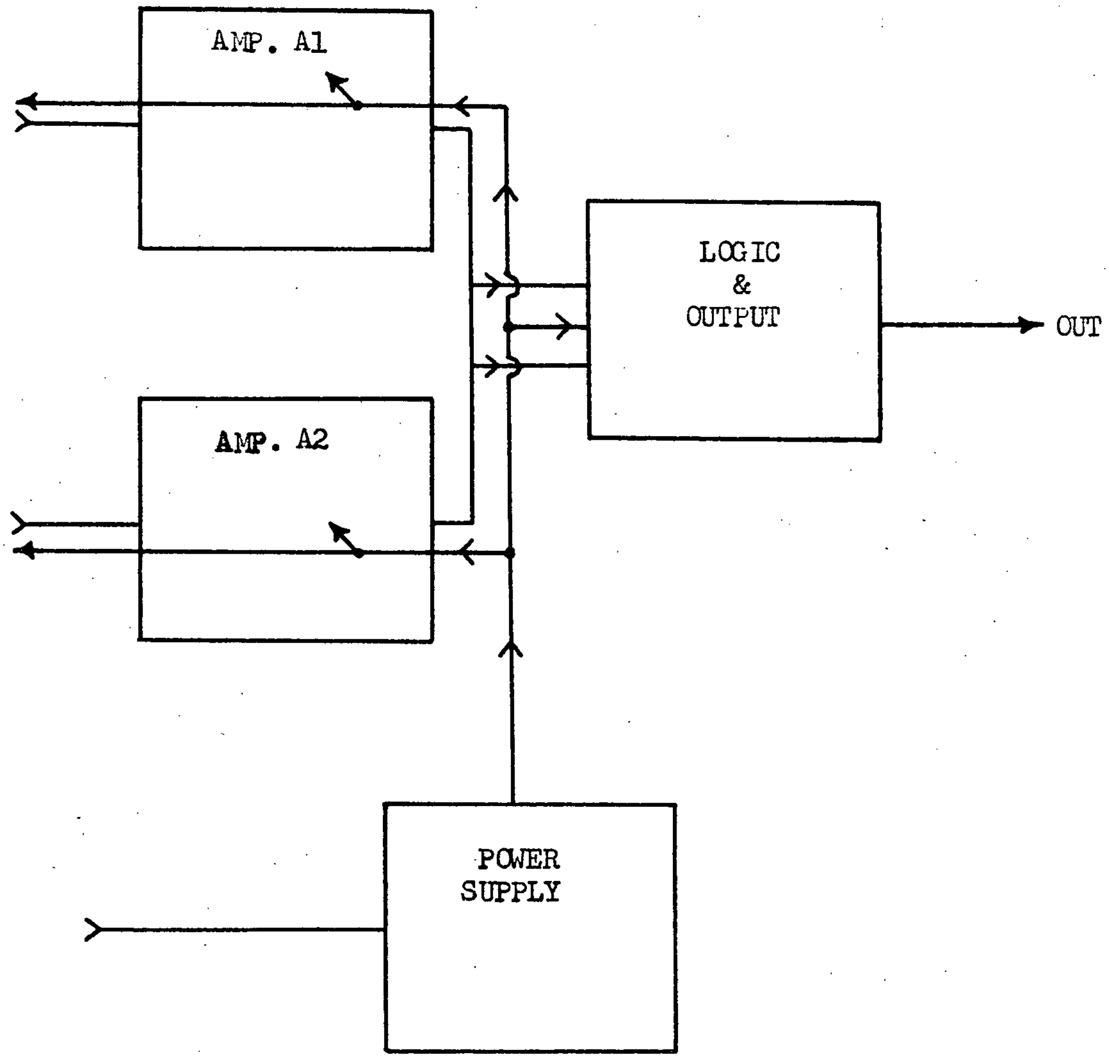


LAMP CONTROLLER

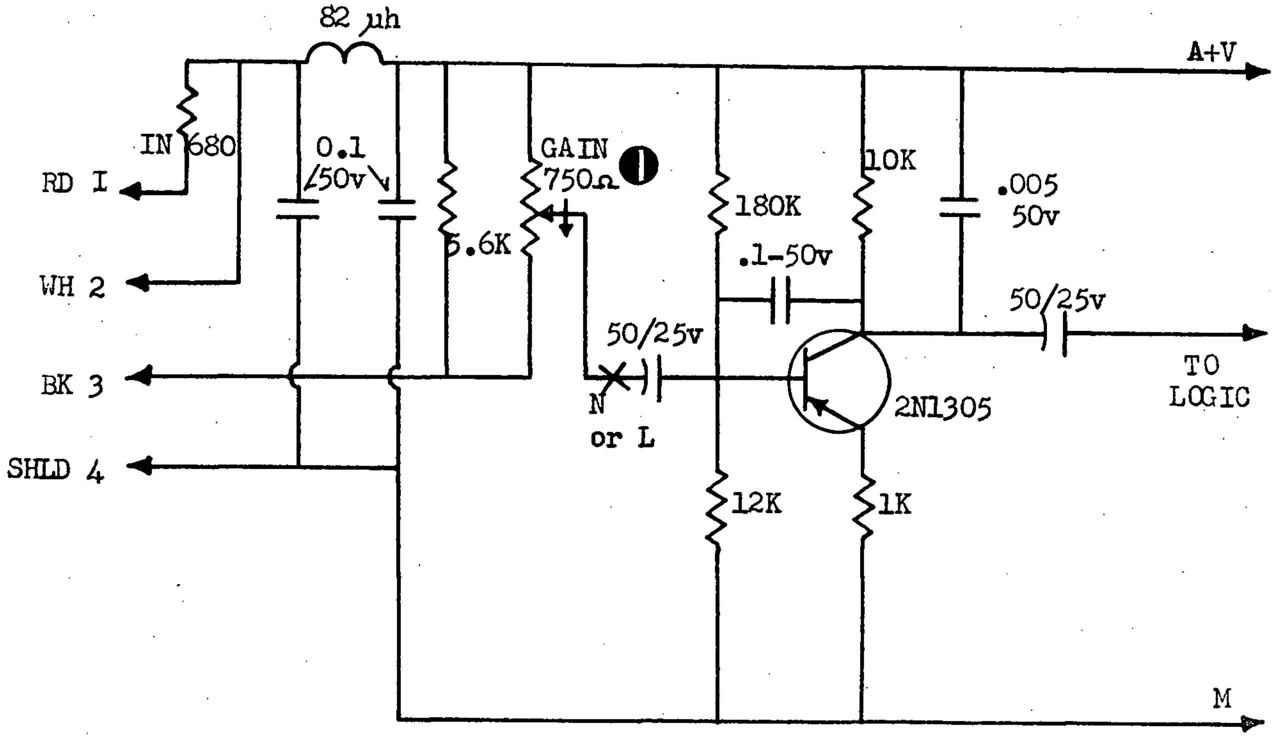
Figure 3. Plot of light intensity against time, indicating the duration of the twilight period regulated by the lamp controller.


35
25
15
5
Intensity (lux)
0
6
12
18
24
30
Time (minutes)

Figures 4-8. Circuit diagrams of the fish movement counter.

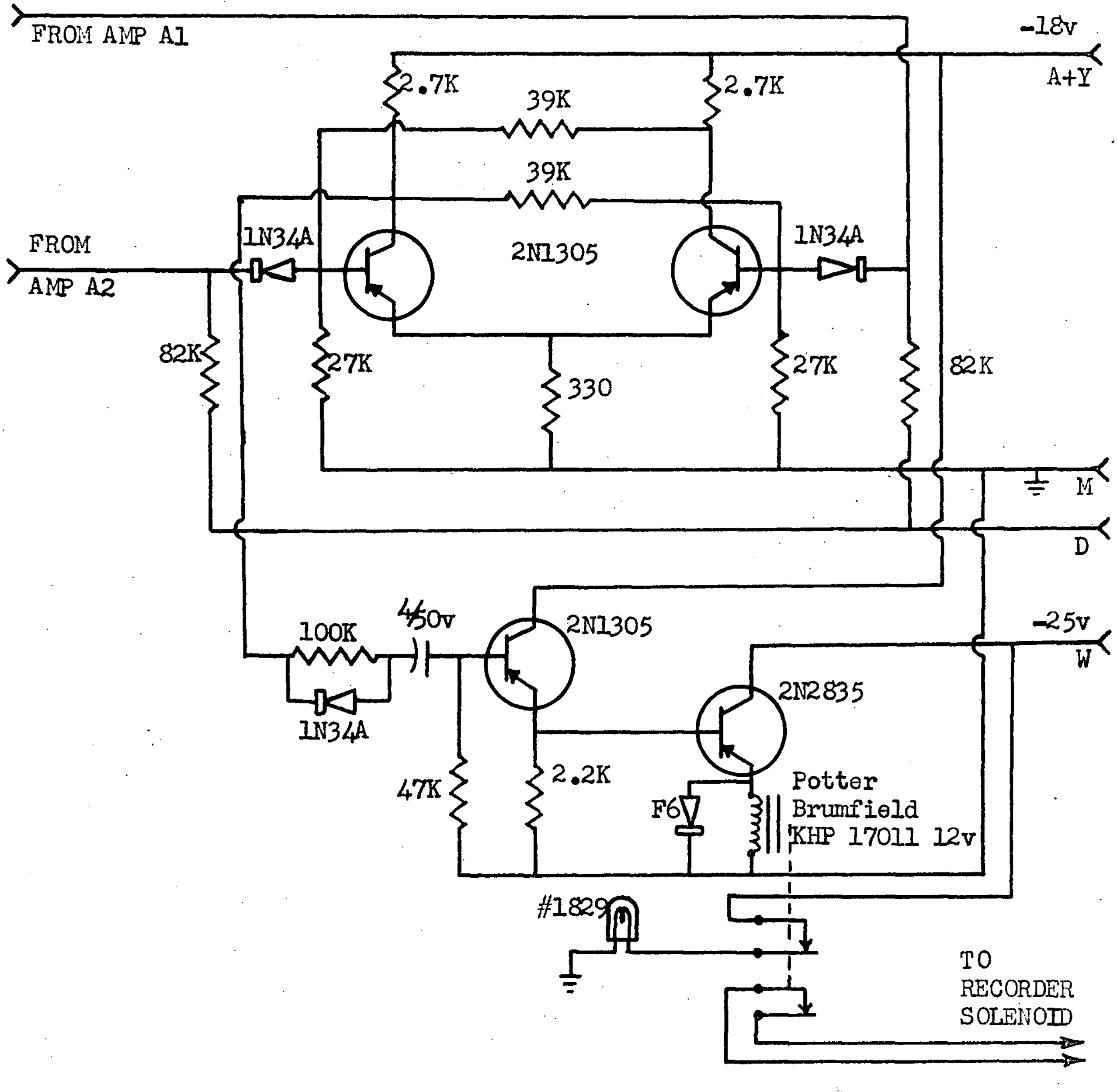


SYSTEM BLOCK DIAGRAM



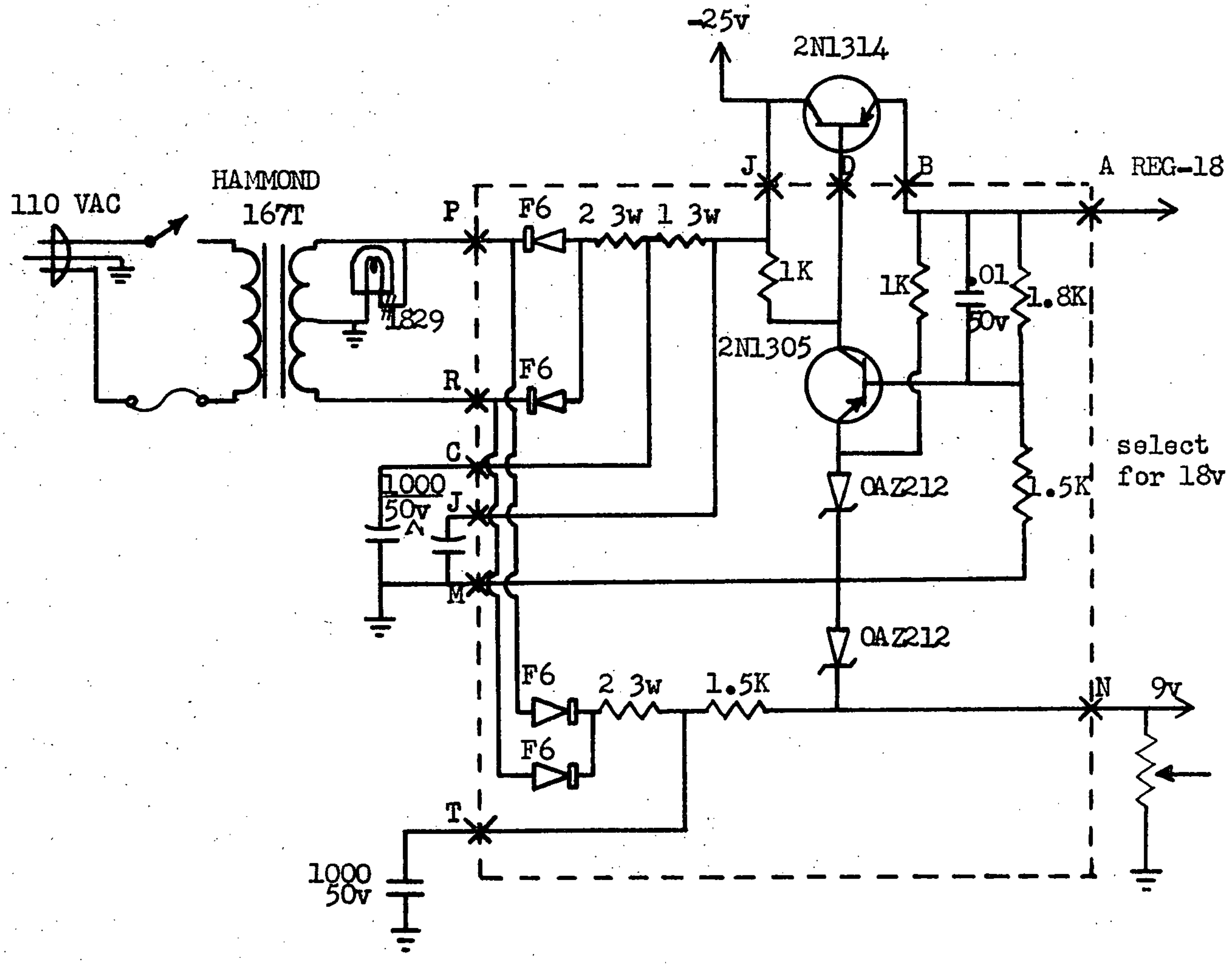
AMPLIFIER

Unless otherwise noted, all cap. in mfd.,
resistors in ohms ½w 10%.
Front panel adj. pot ● cw rotation.



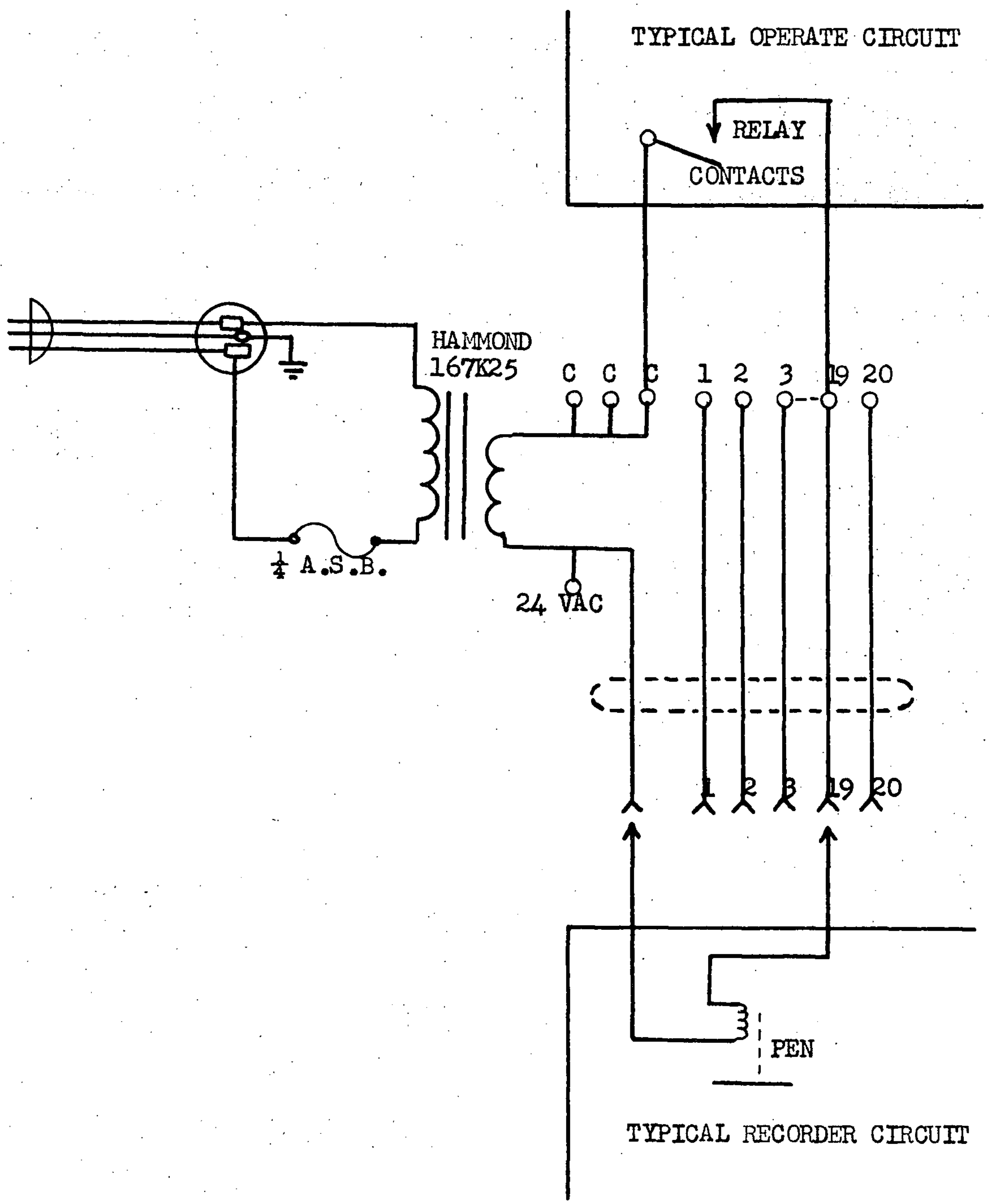
LOGIC & OUTPUT DRIVER

Unless otherwise noted
all caps. in mfd.
All resistors $\frac{1}{2}$w 10%
Letters indicate circuit board terminal



POWER SUPPLY

Unless otherwise noted,
cap. in mfd.
Resistors ½w 10%



JUNCTION BOX

Surgical techniques.

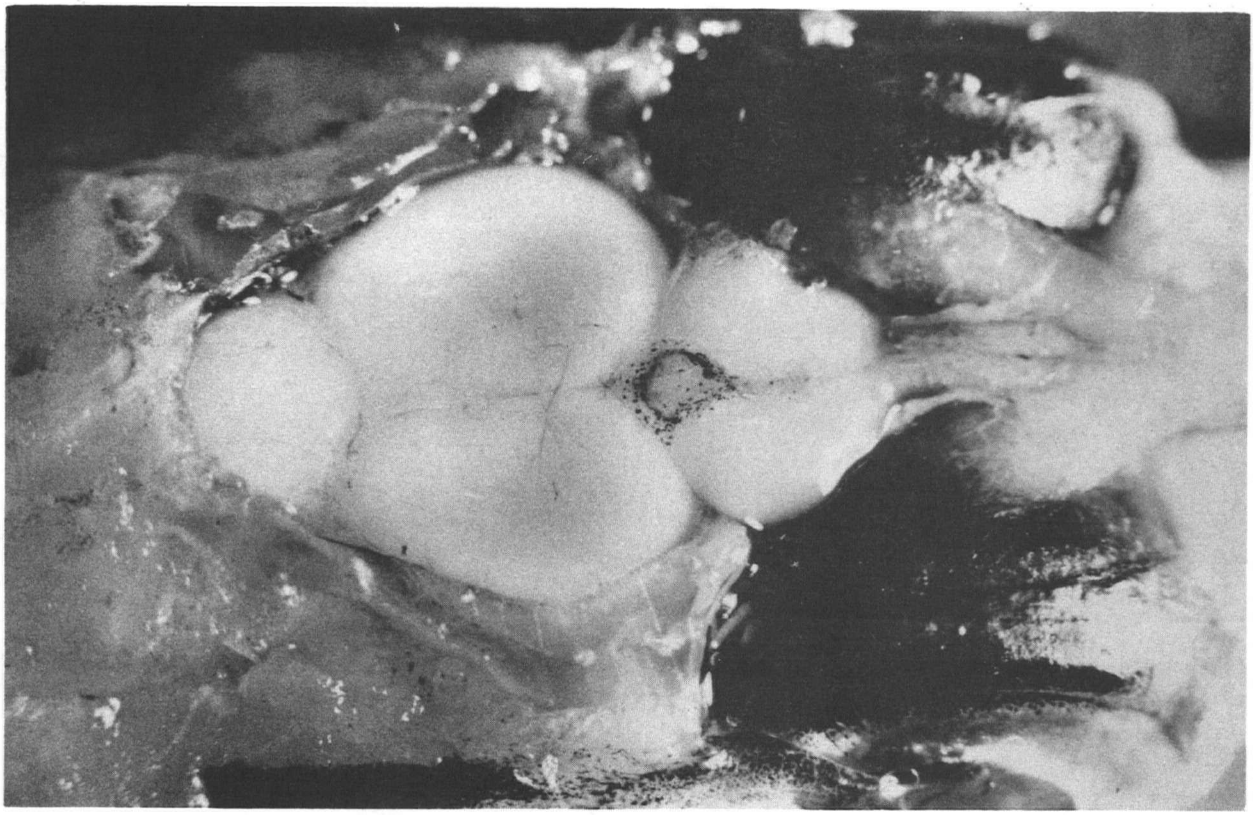
All fish subjected to operations were anaesthetized in a solution of M.S. 222 (Tricaine Methane Sulfonate; Kent Chemical Co., Vancouver, B.C.). The amount used for anaesthesia was 0.2 gram per gallon, which represented a concentration of 1:19,230. This was within the range generally tolerated by this species (Bell, 1967).

General anaesthesia was assumed when the subject ceased movement and floated ventral side up. If no reflex action was observed when the tail was grasped with a pair of forceps, the fish was removed from the solution and placed on a portable operating platform. A glass tube was connected to the dechlorinated water supply and inserted into the mouth or opercular chamber to irrigate the gills.

A. Pinealectomy

The pineal of these fish is a small structure located on the roof of the brain between the telencephalon and the optic lobes (Fig. 9). The location was easily visible through the top of the skull (Hoar, 1955), and a flap of tissue was laid back in this region to expose the cranium. The skull was penetrated with a sharp glass tube and a cylinder of ossified and cartilaginous material was removed and discarded. A fine, blunt, hollow glass tube that was connected to an aspirator was touched to the top of the exposed pineal. The organ was drawn into the tube and

Figure 9. Dorsal view of the exposed brain, illustrating the location and general appearance of the pineal body. Magnification 6.5X.



separation invariably occurred at the base of the stalk.

The site was sealed with Squibb orabase (a dental medication that contains no antibiotics) and the epidermal flap was replaced in position. The incision was then sealed with another layer of Squibb orabase.

Sham operations consisted of following similar procedures for removing the pineal body, however the organ was not removed. Penetration of the cranium was immediately posterior to the pineal. This modification was necessary to prevent damage to the organ. The incision was sealed in a manner identical to normal pinealectomy.

The subjects were revived and placed in postoperative holding aquaria (10 gallons) which were maintained under controlled photoperiodic conditions. Healing was completed within two weeks and the subjects were then introduced to the activity chambers for experimentation.

Success of removal was verified by observations with a dissecting microscope during the operation and by histological sections which were prepared to demonstrate the extent of pinealectomy. All material for these slides were preserved in Weber's solution and stained with hematoxylin and eosin.

B. Plastic screens

A similar preparatory procedure was followed for the placement of transparent and opaque plastic screens over the region of the pineal. In this case, only the skin tissue was laid back and the plastic screen inserted immediately superior to the skull. The tissues were then sutured back into position and the fish were revived and allowed to recover in the holding aquaria.

C. Blinding

Surgical techniques for blinding were as follows. The fish were anaesthetized and placed on the operating platform with the ventral side up. The lower jaw was held open by a blunt hook and a small incision was made in the oral epithelium. This exposed the optic nerve which was lifted slightly with a fine glass hook. It was severed medially with a pair of scissors. The nerve was severed again laterally and a section estimated at 1/16 to 1/8 inch was removed. Recovery followed the same procedure described above.

A limited number of individuals were subjected to bilateral enucleation of the eyes. All fluid was removed from the posterior chamber, the musculature and the optic nerve severed, and the complete eye was lifted from the socket