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by

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ABSTRACT

ENVIRONMENTAL CONTROL OF RHYTHMIC BEHAVIOR IN THE WEAK-ELECTRIC FISH, GNATHONEMUS PETERSII (MORMYRIDAE)

by

Susan Jane Cobert

Adviser: Professor Peter Moller

Rhythmic behavior of African weak-electric mormyrid fish is effected by light-dark cycles, temperature cycles and social contact. The present research was an analysis of these cues as potential zeitgebers for electric organ discharge rate and general locomotor activity rhythms in the mormyrid, Gnathonemus petersii. The fish's responses are discussed in the context of underlying oscillator systems.

A light-dark cycle (LD 12:12) entrained both locomotor activity and electric organ discharge rate. A phase shift (6-hour delay) of the light-dark cycle produced a 6-hour phase shift of both activities. Under constant dark conditions (0.7 or 0.09 lux) both electric organ discharge rate and locomotor activity became arhythmic. Locomotor activity and electric organ discharge rhythms exhibited inter- and intra-individual variability in response to the imposed light regimens. A

temperature cycle (warm:cool=12 hr:12 hr, $\Delta t=5^{\circ}$) was ineffective as an entraining cue for locomotor activity.

Social contact synchronizes rhythms in many animals including fish, birds, rodents, and primates. Social communication in G. petersii is primarily mediated through the electrosensory system. It is difficult to record electric organ discharges from individuals within a group. Therefore an experiment was designed that permitted only electrical contact between two fish. One fish was kept in constant darkness and its partner was entrained to a light-dark cycle. Only the arhythmic locomotor activity of the fish in constant darkness became rhythmic after electrical contact was established. The electric organ discharges of this fish did not entrain. Considering the interrelations between the activities of the two fish, minima and maxima in locomotor activity of the fish in constant darkness matched those of its entrained partner's locomotor activity and electric organ discharge activity. This suggested that locomotor activity entrained to cues from the electric organ discharge pattern of the entrained partner.

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Introduction

Almost all living organisms exhibit some form of endogenously controlled behavioral rhythmicity. The environmental cue that has the greatest influence on periodic behavior is light. In addition, there are other environmental factors of importance including temperature and social interaction. For many organisms, little is known of the underlying mechanisms that regulate rhythmic behavior and consequently, analysis is still in the descriptive stage. For this project I chose the African weak-electric fish, Gnathonemus petersii which shows such rhythms. G. petersii together with the neotropical gymnotids possess two daily rhythmic activities, electric organ discharge rate and locomotor activity. Field and laboratory data have shown daily water temperature variations of as much as 5°C and light changes from 0.03 lux to 300 lux in a 24 hour period (Moller, Serrier, Belbenoit, & Push, 1979). Light is an important cue for the control of electric organ discharge rate and locomotor activity (Harder, Schief, & Uhlemann, 1964; Moller, Serrier, & Belbenoit, 1976; Moller et al., 1979; Bässler, Helbig, & Rahmann, 1979). No work has been done analyzing the possible influence of a temperature cycle on electric organ discharge rate and locomotor activity. Social factors also synchronize rhythms in many species (Menaker & Eskin, 1966; Kavanau, 1967; Siegmund & Wolf,

1972, 1973; Rusak & Zucker, 1975; Bässler et al., 1979; Regal & Connolly, 1980). The fish species chosen, G. petersii, is also a social fish which uses its electric organ discharge for communication (Scheich & Bullock, 1974; Hopkins, 1980, 1981; Moller, 1980; Westby, 1981) and electrolocation (Heiligenberg, 1977).

This research project had three goals: 1) In an analysis of the environmental control of behavior, it is necessary to examine as many potential cues as possible that may influence the pattern and amplitude of a rhythm. For example, since the electric organ discharge rate and locomotor activity of G. petersii are influenced by light, social interaction, and temperature, a first goal was to define the relation among these three environmental cues and electric organ discharge rate and locomotor activity.

2) Often, only one behavior of an organism is studied at one time. When two or more activities are closely related, that is, in pattern, onset time, and amplitude, it is important to learn whether or not these activities respond similarly or differently to changes in the environment. Electric organ discharge rate and locomotor activity in G. petersii are two activities that have a high correspondence. Generally, high electric organ discharge rates occur with high levels of locomotor activity. The question arose here as to whether or not

electric organ discharge rate and locomotor activity would show the same kind of relation when cyclic environmental cues were experimentally altered.

3) Most behavioral periodicities are controlled by internal oscillators. Evidence for internal timing is the continuation of rhythmicity when all known environmental cues have been eliminated. By measuring electric organ discharge rate and locomotor activity under constant environmental conditions it was hoped to determine if one or both of these activities were under the control of an endogenous timing system.

Background

To understand the context within which the present investigation was conducted, some pertinent information about rhythms and their control is needed.

A. Rhythms - Introduction: The rhythmic nature of activities and physiological functions of many organisms, in the laboratory and in the field, is a well established fact (cf. Brown, Hastings, & Palmer, 1970; Cold Spring Harbor Symposium, 1960; Aschoff, 1965; 1981; Menaker, 1971; Rusak & Zucker, 1975; Thorpe, 1978; Aschoff, Daan, & Groos, 1982). Those rhythmic activities that persist in the laboratory under conditions of constant light, temperature, etc. have periods that closely resemble those of natural environmental rhythms and are referred to as "circa" rhythms (circa=about). For example, a behavior, such as general locomotor activity, whose period approximates 24 hours (a solar day) is referred to as a circadian rhythm (Cold Spring Harbor Symposium, 1960; Aschoff, 1965, 1981; Bünning, 1973; Rusak & Zucker, 1975). When a behavior follows a daily light-dark cycle or a temperature cycle, it is said to be entrained or synchronized to that environmental cue or zeitgeber (see Appendix 1 for further definitions). Entrainment involves both phase and period control. Period control occurs when the period of the behavior matches that of the cue. (In the case of

the light-dark (LD) cycle, the behavior will exhibit an exact 24 hour period.) Phase control is the result of daily adjustments of the behavioral cycle to the daily variations of the zeitgeber, such that the two maintain a steady phase angle relationship (Bruce, 1960). For example, the daily photoperiod is, at any particular time of the year, getting shorter or longer. A daily activity cycle must continually adjust its phase angle with the photoperiod to maintain its phase angle relationship. There are several criteria for rigorously establishing the influence of a zeitgeber (Aschoff, 1960, 1981; Enright, 1981a). First, the spontaneous frequency (or free running period) of a rhythm is measured under constant environmental conditions. This demonstrates whether or not an organism is capable of sustaining a free running rhythm and it eliminates unintended entraining cues. Second, the potential zeitgeber is introduced and the rhythm is then monitored. If the rhythm follows the cue, the cue may be a zeitgeber. Bluegill and largemouth bass, when presented with an LD 12:12 cycle show entrainment of the activity rhythm (Davis, 1965). Third, if entrainment occurs, a phase shift of the cue is presented. The behavior should phase shift immediately or within a few cycles and re-establish its phase angle with the LD cycle. In the field and in the laboratory it has been shown that fish phase shift their activity rhythms in response to seasonal changes in photoperiod (Kavaliers, 1981a,b). Fourth, the rhythm should be allowed a post-entrainment free run.

If the first cycle of the free run has a period and phase closely resembling those of the last entrainment, the cue is a zeitgeber, and the internal biological pacemaker has been altered. If phase and period are not similar to those measured during entrainment the internal pacemaker has not been altered, i.e., it has continued to free run and, only the overt rhythm has been changed. Finally, one should be able to entrain a rhythm to various frequencies or intensities of the zeitgeber (Aschoff, 1960). Brown bullheads were subjected to three different conditions of the LD cycle (100:.1 lux; 10:.01 lux; 1.0:.001 lux) and it was found that the bullheads entrained to each of the three intensities of the light-dark cycle. Furthermore, bullheads were nocturnal at the highest pair of intensities and diurnal at the lowest intensity. At the medium intensity the fish were nocturnal but their activity patterns were less stable (Eriksson, 1978).

A brief overview will highlight some of the data describing light and temperature as zeitgebers for general locomotor activity and electric organ discharge rate, and the effects of social stimuli on entrainment. The Cold Spring Harbor symposium (1960), Aschoff (1965; 1981), Schwassmann (1971a,b, 1979), Rusak & Zucker (1975), and Thorpe (1978) summarized much of the current information.

B. Major Zeitgebers

1. Light: The most effective zeitgeber for gross locomotor activity and other activities is light (Aschoff, 1960; 1981; Bruce, 1960; Schwassmann, 1971a; Bünning, 1973; Pittendrigh, 1974; Rusak & Zucker, 1975). The most important information conveyed by the LD cycle is the "light to dark transition" (indicating dusk) and the "dark to light transition" (indicating dawn). The period of plant rhythms depends upon light intensity and spectrum while light intensity is the major factor effecting vertebrate period length (Bruce, 1960; DeCoursey, 1960).

a. Entrainment to an LD cycle: The period and photofraction (ratio of the duration of light to the duration of dark) determine the entrainability of an activity cycle to an LD cycle (Bruce, 1960). The limits on period length vary from organism to organism. Non-mammalian organisms have a greater range of entrainability than mammals (Tribukait, 1956; Halberg, 1959; Bruce, 1960).

Lizards entrain to cycles of light and dark with periods of 12, 22, and 26 hours (Bruce, 1960). Fiddler crabs entrain to periods from 12 to 33 hours (Webb, 1950; Brown & Webb, 1949). The house sparrow will entrain to periods ranging from 17 to 28

hours (Menaker, 1968; Eskin, 1971) and finally, the eclosion rhythm of Drosophila pseudoobscura entrains to LD cycles ranging from 19 to 29 hours (Pittendrigh, 1965). The photofraction of an LD cycle also influences entrainability (Bruce, 1960). If the period of the LD cycle is 24 hours, then the photofraction can be changed considerably and the rhythm will remain entrained. Cockroaches maintained on an LD cycle totaling 24 hours can be entrained with 1, 12, 18, and 23 hours of light (Roberts, 1962). Mammals also entrain to LD cycles with 24 hour periods and varying photofractions (Bruce, 1960).

b. DD vs LL: Organisms typically free run in constant dim light or darkness (DD) while constant light (LL) inhibits free running either immediately or within a few cycles (Aschoff, 1960; Bruce, 1960; Enright, 1981a). For the period of time that a rhythm is present in LL, the value of the period will be different from the value in DD. For nocturnal organisms in LL, such as mice and rats, some fish and some birds, the period length generally increases (the frequency or the reciprocal of the period decreases) relative to the period length in DD (Aschoff, 1979). The animal becomes less active and there is less time spent active. (Activity time is figured as the amount of time spent active compared to the amount of time spent at rest.) Aschoff (1965) reported that the period of the activity rhythm in mice ranged from 23 to 23.5 hours in DD and increased

to 25 to 26 hours in LL. In general, for a diurnal or day active animal (lizards, some birds, mammals, fish) period length (in LL) decreases (frequency increases) and the amount of activity time and the amplitude of the activity rhythm increase (Aschoff, 1960, 1979). Bullfinches have an activity rhythm with a 24 hour period in DD and a 22 hour period in LL (Aschoff, 1960). This relationship of an increase in period length for nocturnal organisms in LL and a decrease for diurnal organisms is known as Aschoff's Rule. Exceptions to this rule have been found (see Aschoff, 1979). Menaker (1968, 1971) reported some interesting results from an LL study with sparrows, Passer domesticus. Sparrows, which free run in DD, become arhythmic in LL. If, however, light intensity is increased to 55 lux, the free running rhythm is masked. Menaker suggests that any rhythm will entrain to any extremely intense light source. Consequently, a free running rhythm will not be detected when excessive light intensities are used.

c. Single light pulses: A brief disturbance of light (meaning a pulse lasting from several minutes to several hours) applied to a free running rhythm in DD will cause a phase shift whose direction and size will depend upon phase of the cycle at which it is presented (Pittendrigh & Bruce, 1957; Bruce, 1960; Pittendrigh, 1958, 1960, 1961, 1966, 1974; Pittendrigh & Daan, 1976a,b; DeCoursey, 1960,1961). In general, a pulse of light

given at or near the end of the subjective night will produce a phase advance while a pulse early in the subjective night will produce a phase delay. If the pulse is presented during the subjective day or in the middle of the subjective night, little or no phase shifting occurs (Bruce, 1960; Pittendrigh, 1965). The differences in responsiveness of a free running rhythm to light pulses presented at different times in the dark period indicates a changing rhythm of sensitivity to the phase shifting effects of light. One 15-minute light pulse presented during the subjective night of the eclosion rhythm of Drosophila pseudoobscura (LD period ranged from 19 to 29 hours) produced phase shifts whose magnitudes were related to the length of the periods. A pulse presented in the dark phase of the 21 hour cycle caused a 3 hour phase advance when the pulse occurred late in the subjective night. A pulse early in the subjective night of a 25-hour cycle produced a 1 hour phase delay (Pittendrigh, 1965). Similar results have been reported for rodents (Rawson, 1959; Halberg, 1959; Pittendrigh & Daan, 1976a,b) and birds (Menaker, 1967; 1969; Aschoff, 1967; Eskin, 1971). Data from experiments involving single disturbances by a light pulse must also be analyzed with respect to the occurrence of transients. A transient is a temporary shortening or lengthening of the period following a pulse. This occurs before a steady-state entrainment is established. A transient may last several cycles before entrainment becomes evident. Hence, phase shifts that are

followed for only a few days may produce misleading results. Transients have been described by Pittendrigh, Bruce, and Kaus (1958) and Pittendrigh and Bruce (1959). D. pseudoobscura and the golden hamster (Pittendrigh, 1965) show these transients as do other species of rodents (Pittendrigh & Daan, 1976a,b).

2. Temperature: In nature, daily LD cycles are closely related to daily temperature changes. That is, high temperatures commonly occur in the early afternoon while low temperatures occur at dawn. Alone, temperature can act as an entraining agent in plants and in warm and cold blooded animals (Rhythmic events may be entrained, phase shifted, maintained in DD and LL, etc.) though the effectiveness of temperature variations is not as great as the effectiveness of LD cycles (Bruce, 1960; Sweeney & Hastings, 1960; Bünning, 1973; Rusak & Zucker, 1975). Period length is basically unaffected by temperature cycles because of a physical-chemical temperature compensation mechanism (Brown & Webb, 1948; Pittendrigh, 1954, 1960, 1961, 1974; Bruce, 1960; Sweeney & Hastings, 1960; Wilkins, 1965). Amplitude of a rhythm certainly can be influenced by temperature. As temperature increases animals tend to become more active (up to a certain point), so period length may increase slightly. If an organism tends to decrease activity as temperature increases, period length may decrease (Aschoff, 1960; Wilkins, 1965). Reviews of many studies (Bruce, 1960; Sweeney &

Hastings, 1960) have found that Q10's (the ratio of a period at temperature A to the period at temperature A+10) for many organisms, plants and animals, vary between 0.8 and 1.3. The rate of change of the period of a rhythm is small when compared to other events measured by the Q10 (Bruce, 1960; Rusak & Zucker, 1975). This is particularly true of mammals, less so for poikilotherms. In spite of temperature compensation, a free running circadian rhythm can be entrained to either a sinusoidal or square wave temperature cycle, with the limitation that the cycle is close to 24 hours (Bruce, 1960; Sweeney & Hastings, 1960; Bünning, 1973). The eclosion rhythm of Drosophila pseudoobscura is one of the most thoroughly studied circadian rhythms. A square wave temperature cycle, from 28^{O}C to 20^{O}C, will entrain the eclosion rhythm in DD. Further, if single brief pulses of temperature are presented phase shifts, similar to those caused by light, will occur. If the temperature is stepped down from 28^{O}C to 20^{O}C, a phase delay occurs while a step up from 20^{O}C to 28^{O}C produces a phase advance (Zimmerman, Pittendrigh & Pavlidis, 1968). Similar results have been found for the cockroach, Periplaneta sp., exposed to a sinusoidal temperature cycle of 19^{O}C to 29^{O}C in DD (Roberts, 1962),

The effectiveness of a temperature cycle is related to the amplitude of the cycle though homeotherms are little influenced, if at all, by temperature as compared to poikilotherms (Bruce,

1960; Sweeney & Hastings, 1960; Pittendrigh, 1974). Locomotor activity in the lizard, Lacerta sp., can be entrained by a temperature cycle with an amplitude variation as low as 1°C over a 24 hour period (Hoffmann, 1969a,b). Activity of the snail, Agrolimax reticulatus, is entrained by a temperature cycle with an amplitude of only 0.1°C (Dainton, 1954). Similar results have been obtained with the cockroach and other poikilotherms, including fish (Bruce, 1960; Sweeney & Hastings, 1960; Schwassmann, 1971a). For homeotherms, the amplitude of the temperature cycle must be much greater in order to obtain entrainment of a free running rhythm, if entrainment occurs at all. Activity of Glaucomys volans, the flying squirrel, was unaffected by temperature changes as large as 10° (15°- 25°C range) and 16°(14°- 30°C range) (Decoursey, 1960). Hoffmann (1969c) found no entrainment of circadian activity cycles in field mice to a temperature cycles with ranges of 15° and 26° though there was evidence that high temperatures can suppress activity without synchronizing it to the temperature cycle.

An interesting exception to the lack of entrainability to temperature of circadian rhythms in homeotherms was found in the house sparrow (Eskin, 1971). General locomotor activity was entrained to a temperature cycle with an amplitude of 32°(6°- 38° C range). Evidently, the very high temperature served as the light portion of the day while the very low temperature cued the night.

In many cases a temperature cycle can restore an entrained rhythm that has been lost under constant conditions. For example, the activity rhythm of the spider beetle disappears under LL but recurs in the presence of a temperature cycle (Bentley, Gunn, & Ewer, 1941). A temperature cycle can maintain the activity cycle of the cockroach when the LD cycle becomes DD, but if the rhythm is lost under DD, temperature will not restore the lost rhythm (Cloudsley-Thompson, 1953). Roberts (1962) found that light and temperature synchronize the activity rhythm of cockroaches. If placed in DD, running activity will persist if a sinusoidal temperature cycle is presented. The onset of activity coincides with the high temperature which represents dusk.

3. Light and temperature as zeitgebers for fish:

a. "Non-electric" fish: Field studies have demonstrated that some fish are diurnal and others are nocturnal, and that levels of activity (including not only circadian activity levels but also daily and annual migratory behavior and reproduction), as measured by gillnet catches, sonar, and underwater observation, are in many cases regulated by light and temperature cues (Schwassmann, 1971a; Thorpe, 1978).

Periodic gillnet catches in lakes in Minnesota and Iowa showed that yellow pikeperch, saugers, and tullibee are night

active while perch and northern pike are day active (Carlander & Cleary, 1949). The same catches revealed differences in daily migratory patterns among species. The authors suggested that these differences in daily activity patterns were likely to be related to changes in levels of illumination and temperature and to variations in internal cycles. Hasler & Villemonte (1953), using sound echoing techniques and divers, found that perch rested on the bottom of a lake at night and schooled during the day in deep waters. Variations in location and activity throughout the day were suggested to be related to the light cycle. Barlow (1958) found that activity of the mudpuppy was related to daily temperature variations rather than to light. Andreasson (1969), using freshwater sculpins, and Siegmund (1969) studying perch, red-eye, and tench, also related daily activity changes to temperature variations. Fry (1967) suggested that fish are adapted to be active in a particular range of optimum temperatures. Data for several species of temperate zone fish indicate an increase in swimming up to a particular temperature ($25^{\circ}C$ for goldfish) and then a decrease as temperature continues to increase (between $25^{\circ}C$ and $35^{\circ}C$ for goldfish) (See Schwassmann 1971a).

Laboratory studies have shed more light on the nature of locomotor activity in fish and the controlling factors. Spencer (1929, 1939) measured activity of several species and found

variations in levels of activity to be related to the light cycle. For example, goldfish maintained on an LD cycle are day active but when LD is changed to LL the fish become less active and diurnality fades. Damping of the daily rhythm under LL is to be expected but the decrease in activity does not follow Aschoff's predictions (1966) of the effects of LL on activity. Spencer's techniques were admittedly crude, hence the reliability of the results may be questioned. Spencer further found that feeding time influenced activity. For the species tested, fish were highly active for 1 to 3 hours following feeding. Davis and Bardach (1965) and Davis (1965) also looked at the effects of feeding time on activity. They found a pre-dawn, pre-feeding increase in activity. When bluegills are maintained on an LD 12:12 schedule and fed at the onset of the light period, a pre-dawn peak in locomotor activity appears and lasts for the final 1 to 3 hours of the dark period. This peak appears for the first time after 10 to 20 days (Davis, 1965). If the light period and feeding time are delayed by 6 hours, the pre-dawn peak also shows a phase delay over the next 4 to 6 days. The same results are obtained with killifish and various marine species (Davis & Bardach, 1965). Siegmund (1969) also reported the occurrance of a pre-feeding peak in activity for several species of freshwater fish. If the fish were fed every 2 days, the peak was also evident on the days when the fish were not fed. These results clearly indicate that in a circadian rhythms study

involving fish a randomized feeding schedule is necessary to get an accurate picture of when activity begins. Otherwise it would be necessary to consider the conditioning effect of a regular feeding schedule in the data analysis. That is, the pre-feeding peak found in the laboratory may be a conditioned effect that is cued by the LD cycle (as evidenced by the phase delays and advances demonstrated by Spencer, 1939). A pre-feeding peak of daily activity, in the field, could also indicate the conditioning effect of the LD cycle unless other cues also trigger the pre-feeding peak. This may be true of the pre-emergence peak of elecric organ discharge in Gnathonemus petersii, with respect temperature or social cues (Moller et al., 1979).

Temperature effects were studied by Alabaster & Robertson (1961). Roach, bream, and perch are day active, showing peaks in activity at dawn and dusk. When water temperature is decreased 2° C during the day, an increase in activity occurs. This would suggest that the dawn and dusk activity bursts are related to low or decreasing temperatures. However, if temperature is held constant throughout the day, the dawn activity burst still occurs. This indicates another cue, probably light, is related to the dawn and dusk activity peaks. Evidence thus far points to the interaction between light and temperature in producing the total activity picture. It is suspected that light and

temperature also interact in producing the "natural" activity and electric organ discharge rates of electric fish.

b. Electric fish: Similar results have been found relating light and temperature to rhythms in South American gymnotids and African mormyrids.

It is clearly established that all gymnotids and mormyrids studied in the laboratory are nocturnal. They become most active, both in their electric organ discharge rate and locomotor activity, when the level of illumination decreases. Light is apparently an effective cue for these rhythms in electric fish (Lissmann, 1958, 1961; Lissmann & Schwassmann, 1965; Kemmer, Baumann & Altmann, 1970; Steinbach, 1970; Schwassmann, 1971a,b; Dewsbury, 1966a,b; Moller et al., 1979; Bässler et al., 1979).

Among both mormyrids and gymnotids some fish show a pre-emergence increase in both locomotor activity and electric organ discharge rate prior to lights off and a decrease in activity after the lights have been turned on (Moller et al., 1979; Lissmann & Schwassmann, 1965). Lissmann & Schwassmann (1965) analyzed the behavior of several species of gymnotids. The data on the sandfish, Gymnorhampichthys hypostomus, are of particular interest here. During daylight hours, the sandfish is inactive, remaining buried in the sand. Its electric organ discharge rate

is correspondingly low (10-15Hz). About 2 hours before emergence from the sand, there is a gradual pre-emergence increase in electric organ discharge activity. This occurs before there is any change in light intensity. At sunset the sandfish is maximally active and electric organ discharge rate increases to 65-100Hz. At the end of the dark period, the sandfish re-enters the sand and its electric organ discharge rate decreases, no less than 1 hour before the light goes on. Hence, some factor other than light may be helping to cue the behavior. If the onset of the dark period is delayed, by keeping the lights on, the pre-emergence electric organ discharge rate increase continues and the frequency increases beyond the normal level. If the dark period is further delayed, the fish emerges from the sand and achieves a normal electric organ discharge frequency and activity pattern. Light introduced during the dark period results in a decrease in electric organ discharge frequency and the fish re-enters the sand. When the fish is in DD, the emergence time changes slightly from day to day demonstrating a free running period of approximately 24 hours. Subjects show different reactions to phase shifts, produced by 15 minute light pulses in DD, that may be related to individual differences in light sensitivity. The evidence clearly demonstrates that light is a strong cue for activity and electric organ discharge rate in a gymnotid fish. The electric organ discharge rhythm appears in its "best" form when environmental conditions are optimal, but

electric organ discharge is not completely modulated by light as activity and electric organ discharge continue in the presence of light if there is no sand for the fish to bury itself in.

Field and laboratory data also confirm the entraining effects of light for mormyrid fish. Harder, Schief, & Uhlemann (1964) reported that electric organ discharge rate was slightly lower during daylight than at night for Gnathonemus petersii. When placed in constant conditions, the fish showed a reversal of their electric organ discharge patterns during a 3 day period. Moller et al. (1979) studied a population of Swashi River mormyrids in the field (Lake Kainji, Nigeria). About 1 hour before sunset (Beginning at 1800 hours light dropped from about 300 lux to 0.03 lux in about 20 minutes at a depth of 50 cm) the electric organ discharge frequency increased from a daylight low of 15.4 + 8.2 Hz to the nighttime high of 26.6 + 12.7 Hz. Then at sunset, activity increased again. Hence, the electric organ discharge frequency increase preceded activity by 1 hour. A decrease in electric organ discharge rate occurred at sunrise (Light intensity increased from 0.03 lux to 300 lux in about 20 minutes at 50 cm depth) but the fish continued their motor activity for one half hour beyond sunrise. Associated with daily variations in light intensity were variations in temperature. At sunrise, when electric organ discharge rate and activity decreased, temperature was at the daily low of 27.2^{O}C. It

increased gradually throughout the day to a maximum of 30.0°C at sunset when electric organ discharge rate and activity increased. Temperature may also serve as a cue for the pre-emergence electric organ discharge rate increase (before the decrease in illumination) because temperature appeared to reach its maximum a short time before sunset. (Moller et al., 1979). It is possible that temperature signals an upcoming transition from either L to D or from D to L. The pre-emergence rise of electric organ discharge rate may be the result of a temperature related signal.

4. Social cues a zeitgebers: Of major importance in this research is an analysis of the use of social cues as zeitgebers for circadian cycles. Studies indicate that social interaction can play an important role in the entrainment of rhythms. Kavanau (1967) found that activity of mice in isolation differed from that of mice in social contact. Two females with distinctive activity patterns in isolation showed obvious shifts towards each other's cycles when placed in the same cage. When returned to isolation, each mouse resumed her own cycle. Meyer (1968) reported similar results for hamsters. Aschoff, Fatranska, Giedke, Doerr, Stamm and Wisser (1971) studied the effects of social contact on circadian cycles in human subjects. Humans, in 3 pairs, lived on artificial LD cycles for 4 days and then in DD for 4 days. From the results of physiological and psychological measures, Aschoff et al. concluded that social

interaction was a strong zeitgeber producing mutual entrainment of physiological and psychological functions.

Studies of non-mammalian species have also yielded interesting results. Webb, Brown, Bennett, Shriner, and Brown (1956) studied the circadian color change rhythm of fiddler crabs. After 3 days in individual isolation, 36 crabs showed a reduction of the color change rhythm by one half. By the tenth day, the rhythm was almost completely damped out. When 18 crabs were paired off (9 pairs) within 2 days the rhythm was restored to normal. The remaining isolated crabs continued to show almost no color change. When the last isolated crabs were paired off, the color change rhythm was restored within 1 day.

Menaker and Eskin (1966) have demonstrated the entrainment of singing and locomotor activity through bird song in the house sparrow. Ten birds were tested by playing recorded bird songs in the test area. Results showed that 3 out of 10 birds developed singing rhythms that followed the rhythm of the recorded song for both period and phase. When the song was discontinued, the birds became free running. Five other birds showed some of the characteristics of the song rhythm while 2 were unaffected. The entrainment of 1 bird's activity rhythm by another's was also demonstrated. Gwinner (1966) found social entrainment of activity in 3 female siskins and 1 female serin. Each was

enclosed in a sound proof stablimeter where the animals' movements were recorded. After 14 days they were exposed to a "12 hour on" - "12 hour off" cycle of the species specific song. Results showed that all 4 birds became synchronized to the song cycle. Cobert (1976) found that under DD conditions, female ring doves in visual and auditory contact were more active than isolated birds.

Among non-electric fish, Siegmund and Wolf (1972, 1973) suggested that grouped fish (Leucaspius delineatus and Carassius carassius) tend to synchronize their activty with each other using social and environmental cues.

Bässler et al. (1979) studied, in the laboratory, the effects of light change and social stimulation on electric organ discharge rate and general locomotor activity. For both individuals and grouped fish there was an increase in electric organ discharge rate and activity when lights went off and a decrease when lights went on, thus indicating the existence of two daily rhythms entrained to a LD cycle.

During electrical contact Halperin (1979) found that the electric organ dscharge activity of the electric eel, Electrophorus electricus, was suppressed when compared with the electric organ discharge activity of the same eel in isolation.

Rationale

This research project was designed to investigate the environmental control of daily rhythms in a mormyrid fish, Gnathonemus petersii. G. petersii are excellent subjects for this type of investigation because they possess two easily accessible and measurable rhythmic activities: electric organ discharge rate and locomotor activity.

In most studies of weak-electric fish locomotor activity is commonly measured as the number of times a fish is electrically "audible", passing through a pair of recording electrodes. That is, electric organ discharge rate was used as a measure of locomotor activity. It is possible, however, for a fish to become electrically silent but still be actively swimming or for a fish to be swimming too far from the electrodes for electric organ discharges to be recorded. In the present experiments, locomotor activity and electric organ discharge rate were recorded independently. Three environmental cues, light, temperature, and social interaction influence electric organ discharge rate and locomotor activity (see section 3B). A rigorous analysis of these cues as potential zeitgebers, however, was lacking. Using standard procedures for demonstrating the effectiveness of a cue as a zeitgeber (Enright, 1981a) an attempt was made to undertake such an analysis.

The specific aims of this research were:

1) to describe and analyze the roles of light and temperature as potential zeitgebers for locomotor activity in G. petersii and to determine if locomotor activity is circadian.

2) to record, independently, electric organ discharge rate and locomotor activity while manipulating a light-dark cycle to ascertain the effect of light on electric organ discharge rate and to determine if electric organ discharge rate is circadian.

3) to determine the degree of correspondence between locomotor activity and electric organ discharge rate.

4) to determine the effects of "limited" social contact on electric organ discharge rate and locomotor activity, and to assess the role of electric organ discharge rate as a social zeitgeber.

5) to explore the behavioral evidence for the existence of an oscillator system controlling electric organ discharge rate and locomotor activity in G. petersii.

General Methods of Procedure

Subjects. Twenty-seven weak-electric mormyrid fish, Gnathonemus petersii, were used as subjects. All were females ranging from about 1 to 2 years of age. They were obtained from a local fish importer and originally collected in Nigeria, West Africa. Gnathonemus petersii are found extensively throughout Central and West Africa, principally in the Niger and Congo River systems (Boulenger, 1901, 1909; Jackson, 1961). Four animals, two experimental and two replacement, were used in each experiment. Replacements were kept in the experimental chamber and exposed to the same manipulations as the experimental fish except for recording their electric organ discharges and locomotor activity. They were used in the event that an experimental fish died during a manipulation (Eriksson, 1978). If a replacement was used an additional 2 to 5 days of recording was done. Prior to the experimental treatment the fish were housed in three-209 liter (45.7 x 50.8 x 91.4 cm) communal tanks. The fish were sexed, weighed, measured and transferred into individual 20.9 liter (40 x 22 x 26 cm) experimental or replacement tanks. The fish were kept in a reversed light-dark cycle set at 12:12. This was to ensure that the fish were active when the experimenter was active. Temperature was held constant at $26^{\circ}C \pm 0.5^{\circ}C$ (Experiments 2 and 3) or $27^{\circ}C \pm 0.5^{\circ}C$ (Experiment 1). Water

conductivity in the experimental tanks was held within a range of 80 to 150 µS/cm. The pH ranged from 6.0 to 7.0. Routine measures were made at least every other day.

The fish were fed live tubifex worms or blackworms at least three times each week.

Apparatus. The experiments were conducted in a room measuring 2 m in length, 2.2 m in width and 1.5 m in height. The walls, floor, and ceiling were covered with aluminum foil and grounded. Layered on top of the foil and attached to a wooden frame was black plastic to ensure that the area was light proof. A light trap entrance shielded the area from outside light. Air was circulated by a low speed, low noise Rotron CFM Muffin fan. Light inside the chamber was provided by one Durolite plant light (Experiment 1) (incandescent, 150 watts) and two soft white light bulbs (Experiments 2 and 3) (General Electric incandescent, 75 watt).

The tanks were shielded with aluminum foil and mesh wire and grounded. The tanks were placed inside styrofoam boxes whose walls extended 20 cm above the walls of the tanks. The styrofoam attenuated light and noise. Each tank contained two pairs of recording electrodes and one ground electrode placed in 12 mm diameter plastic tubes. The activity paddles were

taped to the walls of each tank approximately in the center of each wall (4 paddles per tank). All tanks contained a thin layer of gravel and an unglazed clay shelter.

The aquarium water was filtered and aerated through a 209 liter reservoir located outside the experimental chamber. The experimental tanks were connected with the reservoir through a hose (2 cm in diameter). Water was pumped into and syphoned out of each experimental tank to maintain a constant flow. Half the reservoir water was exchanged when conductivity reached 150 µs/cm. A constant temperature was maintained by four 100 watt tank heaters. The use of the large water reservoir also served as a means of measuring pH and temperature to avoid entering the experimental chamber.

A conventional LD 12:12 schedule was controlled by a single timer. Constant darkness was produced by turning off all lights. Only the red indicator light of the amplifiers remained on. Light pulses of 15 minutes duration were controlled by timers (cf. Enright, 1965; Pittendrigh, 980, 1981). Maximum illumination (L) was measured at 2800 lux (Experiment 1) and 500 lux (Experiments 2 and 3) in the center of each tank at the waters surface. Minimum illumination (D) was 0.7 lux (Experiment 1) and 0.09 lux (Experiments 2 and 3).

Constant light (LL) was 25 lux. All measurements were made with a Gossen Luna Pro light meter.

The fish's locomotor activity was measured with a plastic activity paddle based on a design by Spoor (1941). Activity was measured as the number of paddle deflections per unit of time. Modifications of the original design included replacement of all metal parts, where possible, by plastic or insulated wire sealed with silicone rubber. Designed as a normally closed circuit, movement by the fish deflected the paddles and broke the circuit yielding a relative measure of locomotor activity. The paddles did not produce any electrical noise that could have interferred with the recording equipment (Figure 1). The paddles were calibrated as follows: the experimenter placed her hand, simulating a fish into the experimental tank parallel to the long end of the tank. One swing began at the center of the front wall of the tank, moved through the tank so the finger tips brushed the top of the shelter tube, to the center of the back wall, and then back again to the front wall. To simulate various speeds of movement, recording was done at 1, 2, 5, and 10 swings per 5 sec. Each speed was tested 10 times in each tank, after equilibrium was regained. The mean for both tanks as a function of speed is shown in Figure 2. An approximately asymptotic relationship can be seen. To equate this

Figure 1. Activity paddle. Figure is not drawn to scale. See text for details.

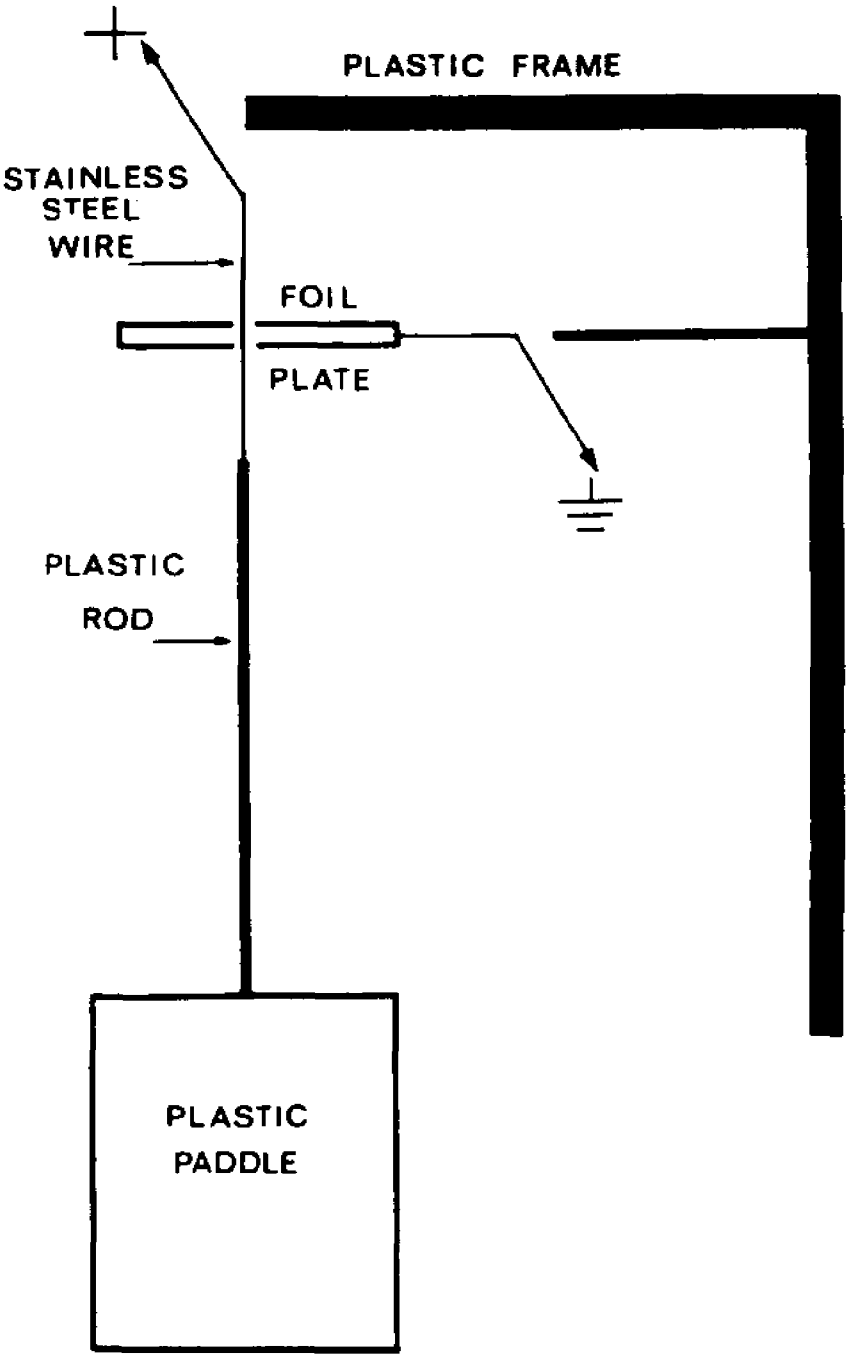
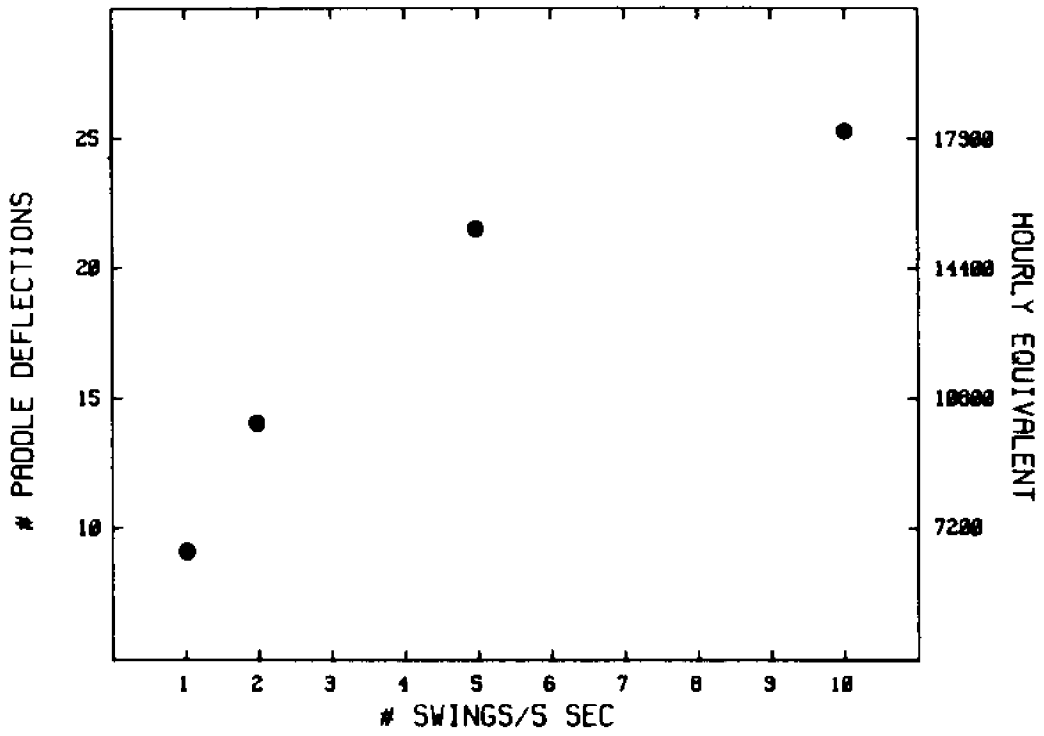

PLASTIC FRAME
STAINLESS STEEL WIRE
FOIL
PLATE
PLASTIC ROD
PLASTIC PADDLE

Figure 2. Activity paddle calibration. Mean number of paddle deflections plotted as function of number of swings per 5 sec. Hourly equivalent of mean number of paddle deflections appears on right ordinate. Note asymptotic relationship. Maximum number of paddle deflections recorded in one hour during manipulations fell in linear portion of curve.

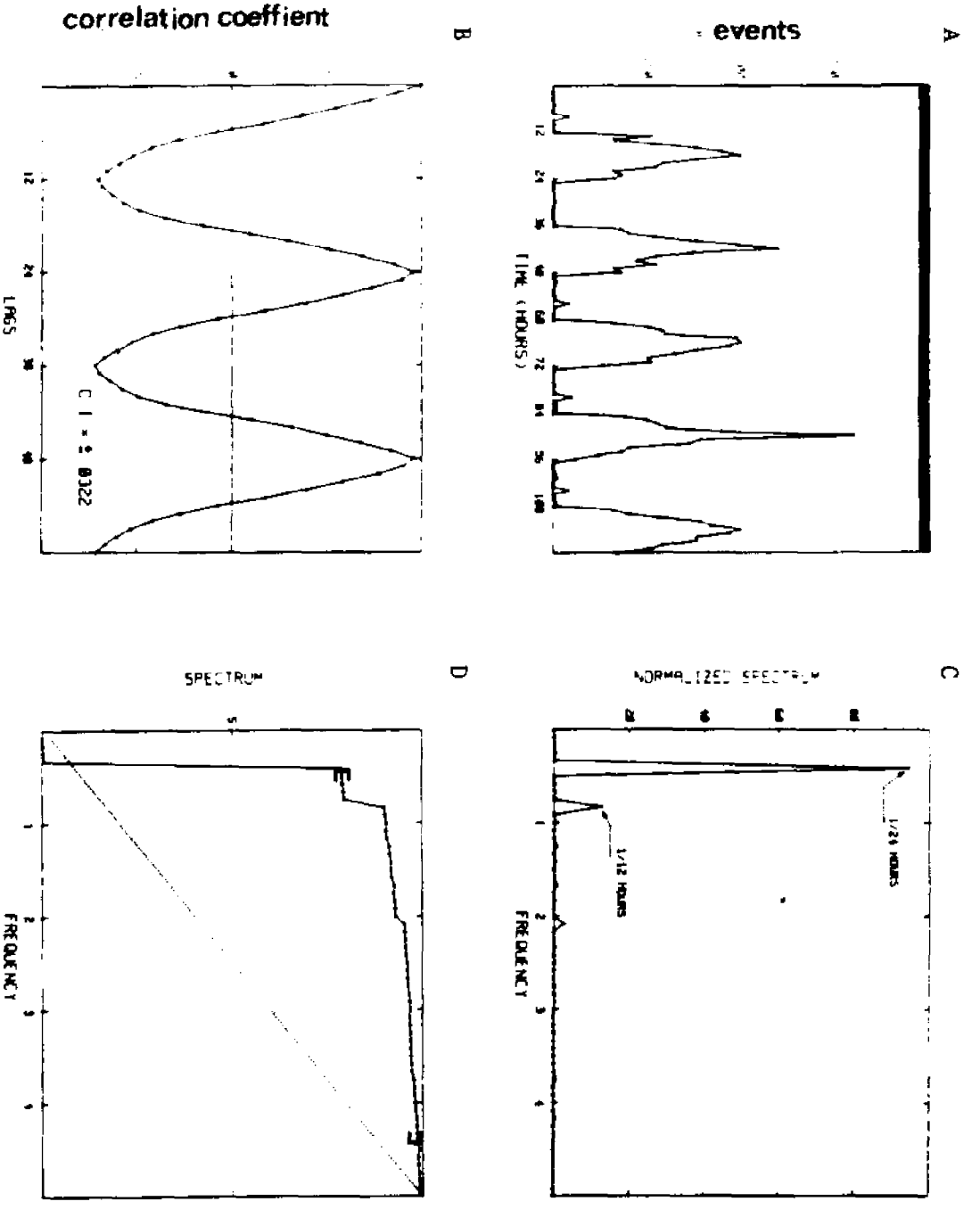

PADDLE DEFLECTIONS
25
20
15
10
17900
14400
10800
7200
HOURLY EQUIVALENT
1
2
3
4
5
6
7
8
9
10
SWINGS/5 SEC

calibration to recorded hourly rates of locomotor activity the mean per 5 sec for each speed was multiplied by 720, the number of 5 sec blocks in one hour. The maximum number of paddle deflections recorded for a fish was about 11,000. Since this number falls within the approximately linear portion of the curve, the actual recorded paddle deflections reflect a linear transduction of the fishes' locomotor activity.

Quantitative Analysis. The first step in the data analysis was to determine the presence of periodicity. The procedures are illustrated using a set of simulated data (Figure 3). Figure 3A shows the raw data indicating the presence of a 24 hour rhythm under constant darkness. If a rhythm could not be detected by visual inspection of the raw data or if confirmation of the presence of a rhythm was needed several alternative time series procedures were used (Broom, 1979; Enright, 1965, 1981b; Gottman, 1981).

Time series analysis is the study of variation of events over a discrete or continuous dimension, time. Time series analysis can be used to determine the presence of rhythmicity in a data set, and to identify the presence of one dominant frequency and/or several higher or lower frequencies. This type of analysis will also show if the process that is

Figure 3. Simulated data. A. Circadian rhythm. Number of events is plotted as function of time in hours. Black bar at top of graph is constant darkness. B. Autocorrelation function. Correlation coefficient is plotted as function of lags. Confidence intervals for 0 appear in lower right hand corner. Note 24 lags peak to peak and trough to trough indicating 24 hour periodicity. C. Spectral analysis. Normalized spectrum is plotted as function of frequency (1/period in hours). Large peak at 1/24 hours indicates presence of 24 hour periodicity. Peak at 1/12 hours is a submultiple of 1/24 hours. D. White noise test. Integrated spectrum is plotted as a function of frequency (1/period in hours). Dotted line is expected integrated spectrum for random data. Solid line is integrated spectrum of simulated data. Points between brackets are significantly different from values expected for random data.


correlation coeffient
B
LAGS
C I = ± 0122
events
A
TIME (HOURS)
SPECTRUM
D
FREQUENCY
NORMALIZED SPECTRUM
C
1/12 HOURS
1/24 HOURS
FREQUENCY

generating the rhythmicity is constant over time (stationary) or if it changes over time (non-stationary). Lack of variability in frequency is evidence of stationary data. Biological time series are almost never stationary. Frequencies vary over time. It is possible, however, to analyze non-stationary cycles by looking at non-overlapping sequential segments of the data set (Enright, 1965; Broom, 1979; Gottman, 1981).

There are two approaches to the study of time series processes, time domain and frequency domain analysis. Time domain analysis looks at data directly in terms of time and it enables the prediction of present or future cycling based on past events. One of the most common time domain statistics is the autocorrelation function. It is useful for detecting the presence of periodicity and for estimating period length (Box & Jenkins, 1970; Binkley, 1976; Enright, 1981b). A correlation coefficient is determined between the original data set and that same set lagged on itself over a fixed number of time units. If periodicity is present in a data set the autocorrelation function will show an oscillation with the period of the oscillation corresponding to the number of lags between successive peaks or troughs. Autocorrelations were calculated for all data using n/2 lags (Vandenbussche, 1969). Figure 3B shows the autocorrelation function for the simulated

data. There is an oscillation in the function and the number of lags between successive peaks or troughs is 24, corresponding to a period of 24 hours. This confirms the presence of a circadian rhythm in the simulated data.

Frequency domain analysis or spectral decomposition (periodogram) breaks a time series into its basic frequency components each with a different amount of variance for which it accounts (Enright, 1965; Box & Jenkins, 1976; Gottman, 1981). A rhythm may have a dominant 24 hour periodicity that might account for 40% of the variability in the time series and there may be a shorter 8 hour cycle that accounts for 10% of the variability. Figure 3C shows the spectral analysis for the simulated data. The normalized spectrum is an estimator of the intensity of the frequency measured. There is a large peak at a frequency of 0.0417 which corresponds to 1/24 hours. This frequency also accounts for 79% of the variance in the data. A smaller peak at a frequency of 0.0833 or 1/12 hours accounts for 11% of the variance.

Two other points regarding spectral peaks are important. First, if there is a trend in the data, either an increase or decrease in the number of events over time, this will be reflected by a peak at a frequency corresponding to the period of the entire distribution. That is, a peak at 0.0083 or

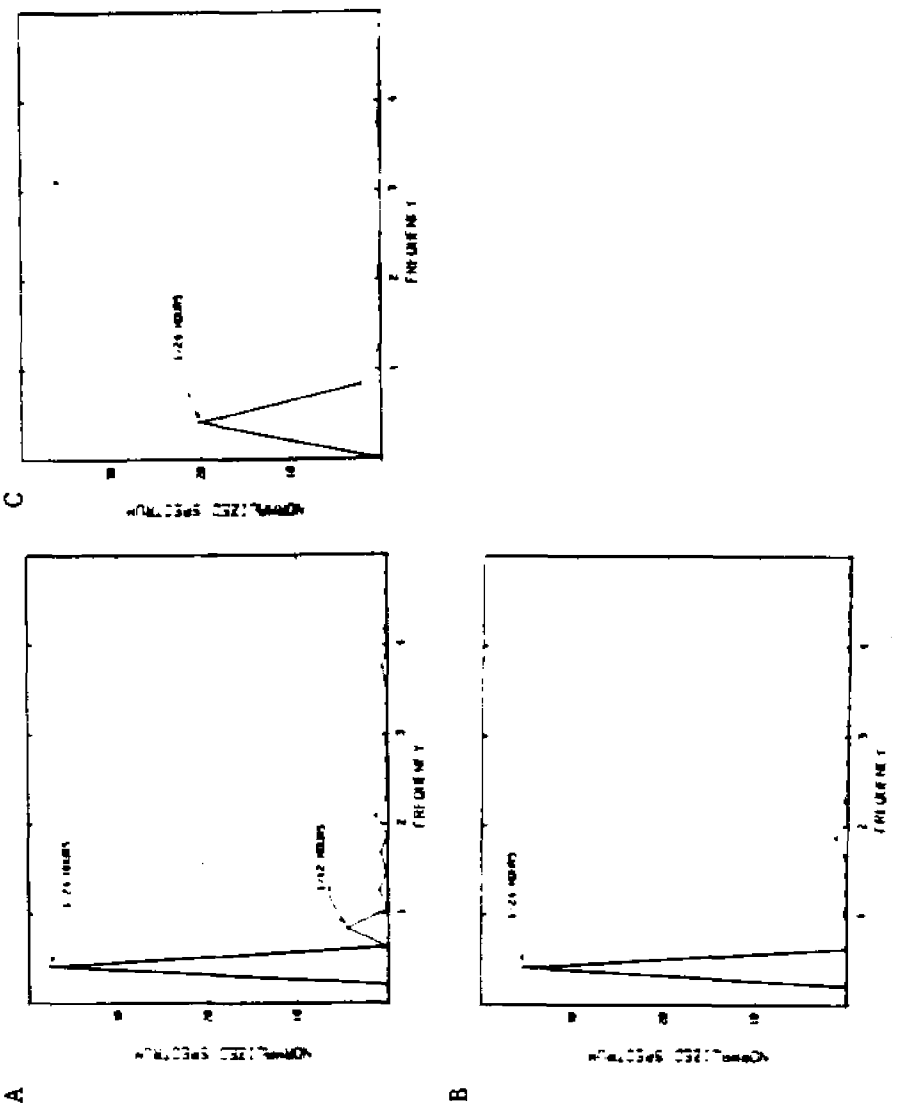
1/120 hours would indicate a trend. The simulated data do not show a trend.

Second, it is not uncommon for peaks to occur at multiples or submultiples of a particular frequency. If there is a dominant frequency of 1/24 hours, there may also be peaks at 1/12 hours, 1/8 hours or 1/48 hours. When these peaks occur, they generally reflect the natural harmonics of the rhythm (Enright, 1965).

If the reliability of a spectral peak needs to be determined, non-overlapping sequential segments of the data are reanalyzed. Persistent rhythmicity should produce a peak in the spectrum regardless of the amount of data analyzed (Enright, 1965). Figure 4 shows the spectral analysis for segments of the simulated data. The 120 data points were broken into two-48 hour segments and one-24 hour segment. The peak at 1/24 hours appears from segment to segment, confirming its reliability as a rhythmic component. The peak at 1/12 hours appears in two of the three segments but as stated above, this peak is most likely a reflection of the natural harmonics of the 24 hour rhythm.

For Experiment 1, locomotor activity recorded in all constant condition manipulations was analyzed by sequential

Figure 4. Simulated data. Spectral analysis of non-overlapping sequential segments of data. Normalized spectrum is plotted as function of frequency (1/period in hours). A. First 48-hour segment. Arrows indicate large peak at 1/24 hours confirming presence of 24 hour periodicity. Peak at 1/12 hours is submultiple of 1/24 hours. B. Second 48-hour segment. Arrow indicates peak at 1/24 hours. C. Twenty-four hour segment. Arrow indicates peak at 1/24 hours. Analysis confirms reliability of 24 hour periodicity


C
A
B

segments to determine if any peaks at 1/24 hours reflected reliable rhythmic components. For Experiments 2 and 3 a spectral peak was reanalyzed only if the variability for which it accounted (see above) was 15% or greater. Spectral peaks in the segments were considered reliable if they also accounted for 15% of the variabiltiy in a distribution. The value, 15%, was chosen for two reasons. First, in Experiment 1 the statistical analysis was performed for peaks that accounted for 0 to 10% of the variability in the data. None of the peaks was found to occur from segment to segment. Hence, it was felt that analysis of peaks accounting for 10% variability or less would not demonstrate reliable periodicity. Second, the variability accounted for by a spectral peak at 1/24 hours for entrainment data ranged from 20 to 65%. Fifteen per cent was chosen as a criterion above the maximum value tested that reflected unreliable periodicity and the minimum value found for entrainment.

In general, a thorough analysis of a time series uses statistics from both domains, time and frequency (Gottman, 1981).

Finally, when no rhythmicity or trend was present in the data a white noise test or test for randomness in the data, Kolmogorov-Smirnov statistic, was used (Jenkins & Watts,

1968). The white noise test compared the integrated spectrum of the data, another spectral estimator, with spectral values that would be generated by random data (expected integrated spectrum). If 5% of the integrated spectrum data are significantly different from values expected from random data, then the spectrum data are not random. For the simulated data, 80% of the points, Figure 3D, were significantly different from random data. This confirms the presence of rhythmicity in the simulated data.

When there is no apparent rhythmicity in the raw data and this lack of rhythmicity is confirmed by the autocorrelation function and spectral analysis, it is possible that the white noise test will show the data to be non-random. Lack of randomness, however, does not mean the presence of rhythmicity. It indicates the presence of a trend in the data.

These three tests were performed on the data from all experiments using a Fortran program compiled by Dr. D.P. Crockett and the CUNY Computer Center system.

Experiment 1

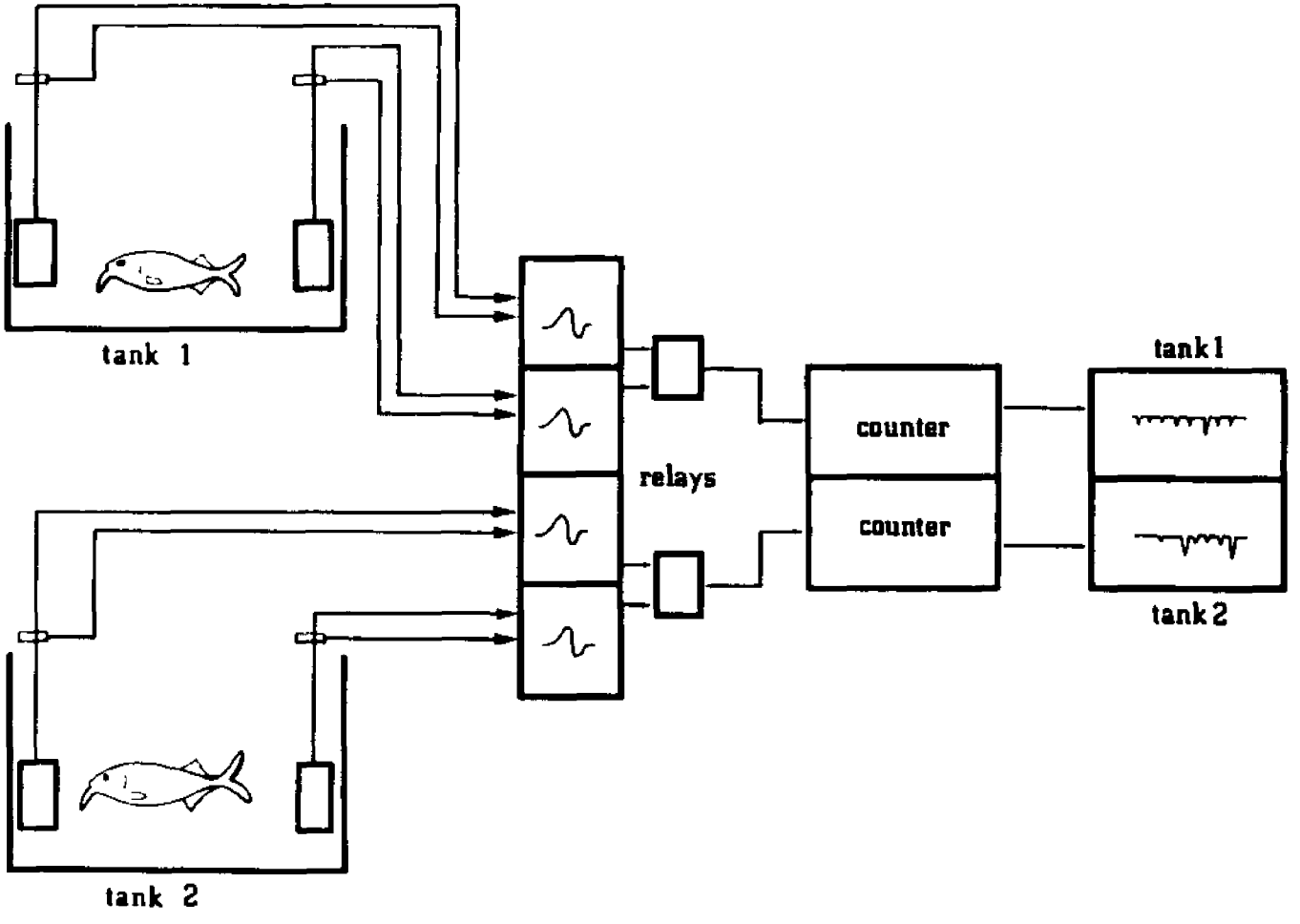
Experiment 1 examined light and temperature as possible zeitgebers for general locomotor activity in G. petersii. An attempt was made to establish whether or not the fish was able to maintain a free running circadian locomotor activity rhythm. The standard procedure for demonstrating the entraining abilities of an environmental cue (see Background) was followed: first with light as a cue (A) and subsequently temperature (B).

Methods

Subjects. Five G. petersii served as subjects for Experiment 1. The fish ranged from 30 to 82 g in weight and 16.5 to 20 cm in length.

Apparatus. General locomotor activity was monitored with a custom-made analog digital analog counter (see Appendix 2). Paddle deflections were converted into digital signals and counted (65 deflections/channel maximum capacity) and the sum of the output was received by the driver amplifiers of a 4-channel polygraph (Grass Instruments, Model 7). Sample times lasted 10 s and reset duration was 0.5 s (Figure 5).

Figure 5. Schematic of activity apparatus for Experiment 1. Figure is not drawn to scale.



ACTIVITY APPARATUS

The total number of paddle deflections per hour was obtained from the polygraph pen deflection and graphed as a function of time. Each daily record was divided into one-hour segments. The length of each pen deflection was determined and multiplied by a calibration constant. A one millimeter deflection on channel 1 equalled 6.5 paddle deflections and on activity channel 2, one millimeter was equal to 7.2 deflections.

To simulate natural conditions for Experiment 1/Part A, a bi-directional brushless motor was connected to a common household rotary light dimmer to generate dawn and dusk. Through programming with timers, the motor stepped the light down over a 30 min period to provide dusk and reversed 12 hours later to increase the light intensity to produce dawn (also over a 30 min period).

To produce a square wave temperature cycle (warm-cool 12:12) (cf. Bruce, 1960; Saunders, 1981) for Experiment 1/Part B, the water temperature was cooled from its original mean of 27^{O}C to 25^{O}C by adding ice cubes to the reservoir located outside the experimental room. Cooling took 15 minutes. This temperture was maintained for 12 hours using the tank heaters. Then the water was heated to 30^{O}C by a adding preheated water to the reservoir and maintained at this level for 12 hours

with the tank heaters. Warming also lasted 15 minutes. This temperature range, 25°C to 30°C, was based on field data from Moller et al. (1979).

Experiment 1/Part A. Procedure. This experiment consisted of ten manipulations. The same two fish, F1 and F2, (and one replacement, F3) participated in i-v; two new subjects, F5 and F6, were used in vi-x.

The manipulations were as follows:

i. Constant dim light: The fish were maintained in constant conditions with respect to light (15 watt G.E. red light bulb, 25 lux), temperature (27°C + .5°), pH (6-7), and conductivity (80-150 µs/cm).

ii. Alternated light intensities: Twelve hours of dim red light (25 lux) was alternated with 12 hours of bright white light (2800 lux) to determine if entrainment would occur.

iii. Entrainment: An LD 12:12 (2800 lux : 0.7 lux) cycle was imposed while the other factors remained constant to determine if the LD cycle exercised period control over locomotor activity.

iv. Phase shift of zeitgeber: The LD 12:12 cycle was shifted 6 hours (delayed) to demonstrate phase control.

v. Post-entrainment free run: DD with the other environmental cues held constant to determine if G. petersii were capable of rhythmic locomotor activity in the absence of time giving cues.

vi. Undisturbed free run: With two new fish as subjects, an undisturbed free run was carried out to establish whether or not they would free run in DD.

vii. Phase shift of rhythm: While all other cues were constant one 15 minute light pulse was presented late in the subjective night (at about 0200 hours) in an attempt to establish rhythmicity.

viii. Undisturbed free run: to return the fish to baseline .

ix. Entrainment: The fish were again entrained to an LD 12:12 cycle.

x. Entrainment with simulated sunrise and sunset: to determine the effects of gradual transitions from light to dark and dark to light on locomotor activity.

Experiment 1/Part B. This experiment examined temperature as a entraining cue for locomotor activity. Light was held constant (0.7 lux) throughout the experiment and temperature was manipulated.

i. Undisturbed free run: Constant temperature of 27^{O}+ $.5^{O}$to establish whether or not the fish were capable of a self-sustained locomotor activity rhythm.

ii. Entrainment: The temperature cycle, warm-cool 12:12 was presented to determine if a temperature cycle would exercise period control over locomotor activity.

Experiment 1/Part A

Results

Constant dim light. The first required manipulation in this series was intended as a free run in constant darkness. Two fish, F1, and F2, were maintained under dim red light (25 lux). It was initially assumed that red light at this intensity level would not affect the fish and thus constitute

darkness. Entrainment began by alternating 12 hours of the dim red light with 12 hours of bright (2800 lux) white light. Entrainment did not occur.

It was subsequently found that blackwater adapted fish, such as mormyrids, are sensitive to red light (Levine & MacNichol, 1982). Moller & Teyssedre (1982) tested the optomotor response of G. petersii and found following responses under light illumination levels of 6, 12, and 60 lux.

In the present experiment, when the red light was turned off, a rapid increase in activity occurred and entrainment followed (see Entrainment above). This suggested that the two light levels used were perceived by the fish as essentially the same possibly regardless of color. Therefore, the data from these two manipulations were analyzed as two constant light manipulations.

Figures 6A & 7A show the raw data from the two fish recorded from (F1 and F2) under dim red light. Visual inspection of the data revealed no rhythmic patterns. The autocorrelation functions did not oscillate (Figures 6B & 7B). The spectral analyses for both fish (Figures 6C & 7C) showed several peaks for each fish, but none was at 24 hours. Thus

Figure 6. Constant dim light, Fl. A. Raw data. Shaded bar at top of graph is constant dim light (25 lux). Number of paddle deflections is plotted as function of time in hours. No obvious 24 hour rhythmicity detected. Note decreasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. No oscillation present. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). No peak at 1/24 hours (see arrow) confirming absence of 24 hour periodicity. D. White noise test. Solid line is integrated spectrum of data. Dotted line is expected integrated spectrum for random data. Points between brackets are significantly different from random data. No 24 hour periodicity in constant dim light. Lack of randomness is due to decreasing trend.

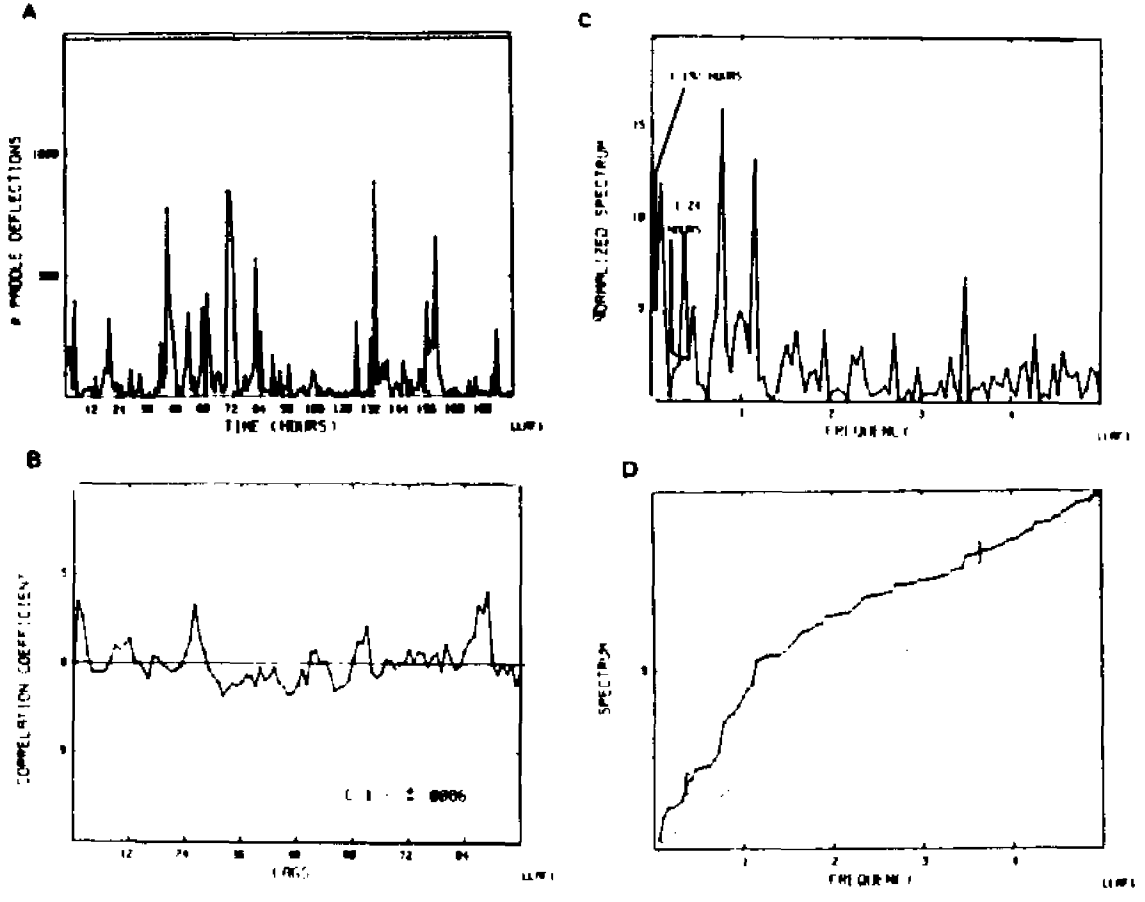
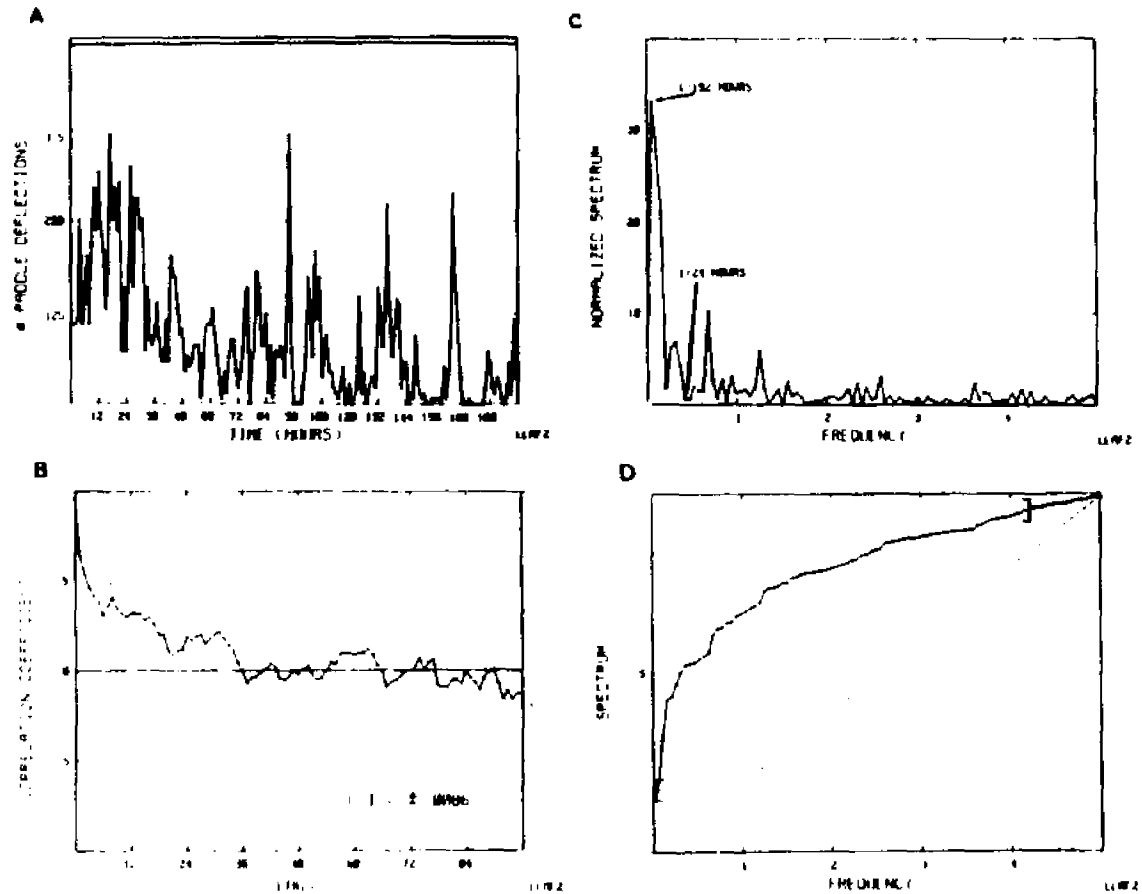

A
B
C
D

Figure 7. Constant dim light, F2. A. Raw data. Shaded bar at top of graph is constant dim light (25 lux). Number of paddle deflections is plotted as function of time in hours. No obvious 24 hour rhythmicity detected. Note decreasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. No oscillation present. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). No peak at 1/24 hours (see arrow) confirming absence of 24 hour periodicity. D. White noise test. Solid line is integrated spectrum of data. Dotted line is expected integrated spectrum for random data. Points between brackets are significantly different from random data. No 24 hour periodicity in constant dim light. Lack of randomness is due to decreasing trend.


A
C
B
D

there was no 24 hour circarhythm under constant dim red light conditions. The peak at 1/192 hours indicated the the presence of a decreasing trend. To determine if any of the peaks represented a reliable rhythmic component, 48 hour sequential segments were analyzed. Figures 8 & 9 show no peaks appearing from segment to segment and therefore no reliable rhythmic components in the data.

Finally, the white noise tests for both fish showed that more than 5% of the points were significantly different from values expected if the data were random (Figures 6D & 7D). Since none of the spectral peaks was reliable this lack of randomness is due to the decreasing trend in activity.

Alternated light intensities. When 12 hours of dim red light was alternated with 12 hours of bright white light no rhythmic pattern was seen in the raw data (Figures 10A & 11A) for either fish. The autocorrelation functions also show no rhythmicity (Figures 10B & 11B). The spectral analyses showed peaks at a frequency of 1/120 hours indicating again a decreasing trend in activity but neither fish showed a peak at 1/24 hours (Figures 10C & 11C). When the data were divided into non-overlapping 48 hour segments and analyzed none of the peaks appeared reliably from segment to segment (Figures 12 & 13). No rhythmicity was present when two light intensities

Figure 8. Constant dim light data, F1. Spectral analysis of non-overlapping, sequential segments of data. Normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. A. First 48-hour segment. B. Second 48-hour segment. C. Third 48-hour segment. D. Fourth 48-hour segment. Peak at 1/24 hours does not appear in all segments, indicating no reliable 24 hour periodicity.

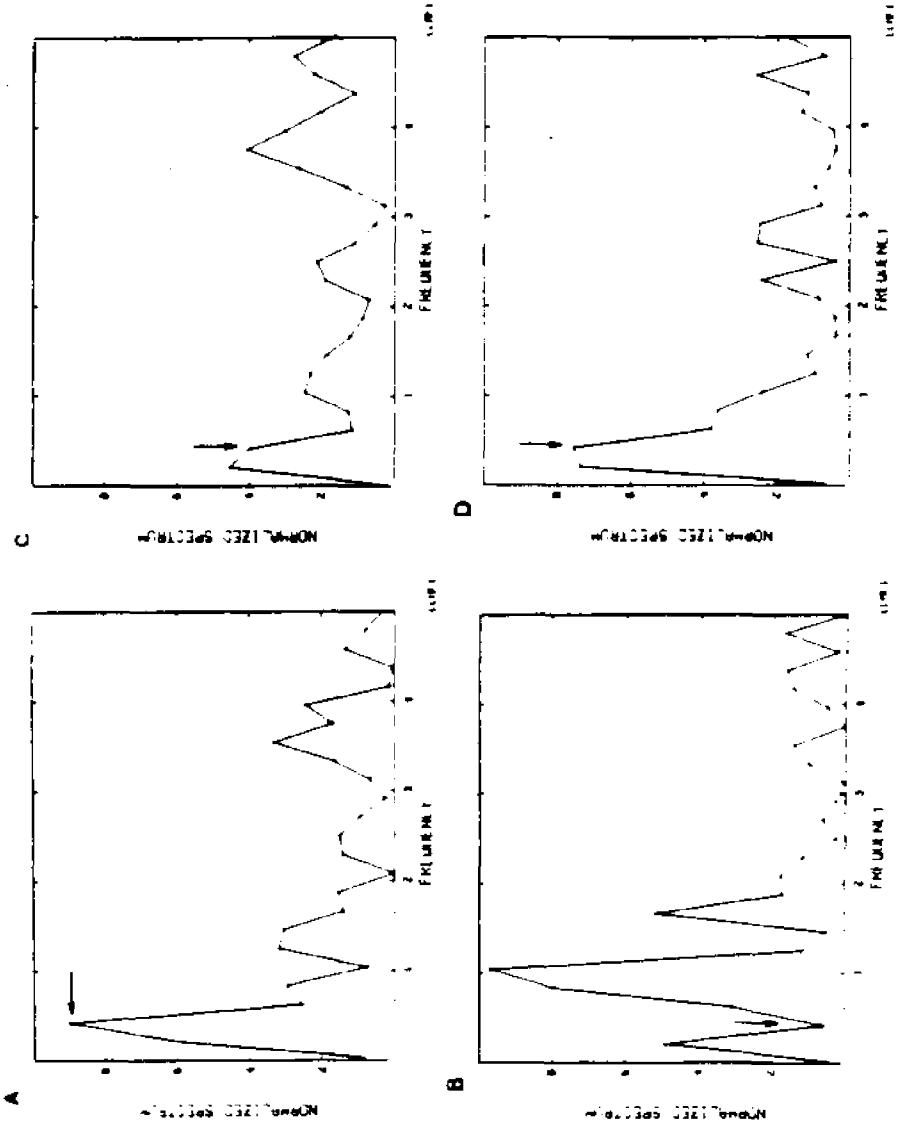

A
B
C
D

Figure 9. Constant dim light, F2. Spectral analysis of non-overlapping, sequential segments of data. Normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. A. First 48-hour segment. B. Second 48-hour segment. C. Third 48-hour segment. D. Fourth 48-hour segment. Peak at 1/24 hours does not appear in all segments, indicating no reliable 24 hour periodicity.

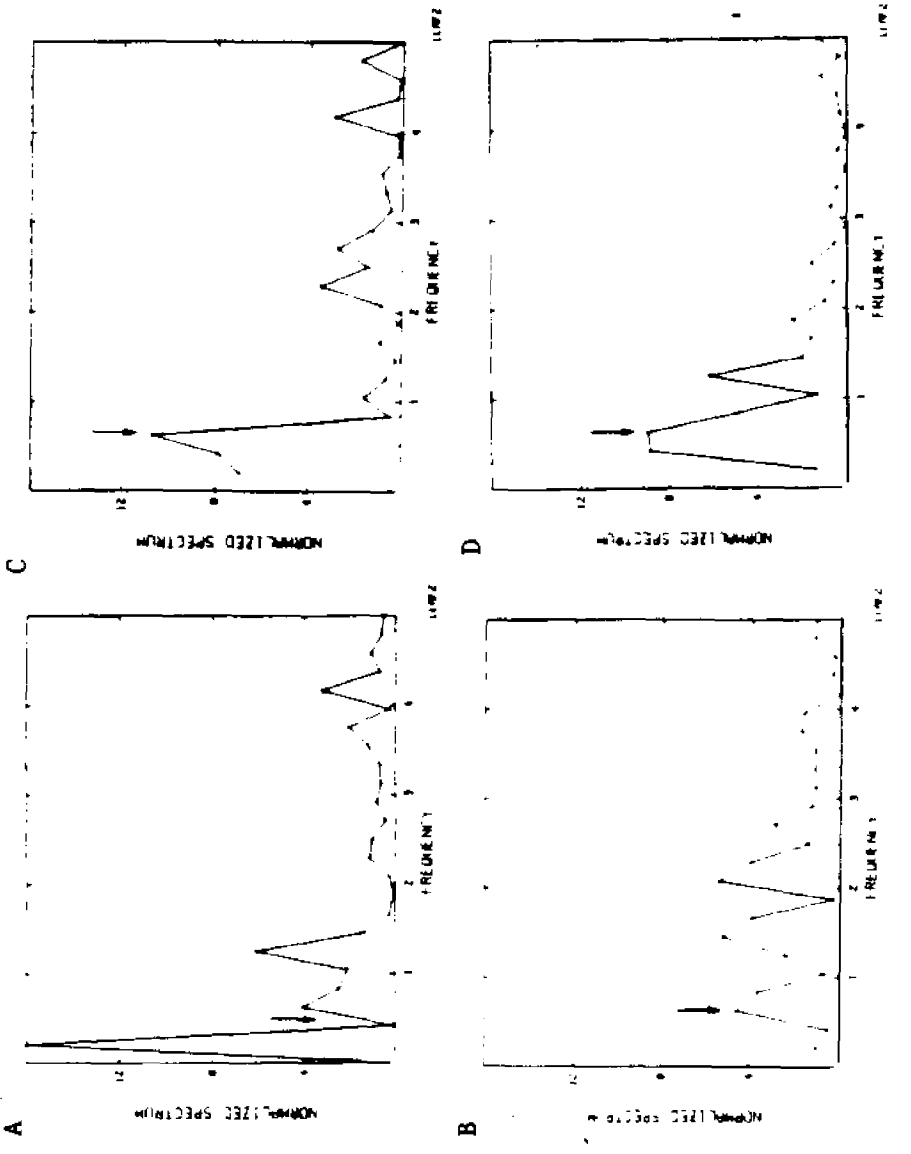

A
B
C
D

Figure 10. Alternated constant light intensities, F1. A. Raw data. Alternating shaded and open bar at top of graph is alternating dim and bright light (25 lux:2800 lux). Number of paddle deflections is plotted as function of time in hours. No obvious 24 hour rhythmicity was detected. Note increasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. No oscillation present. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). No peak at 1/24 hours (see arrow) confirming absence of 24 hour periodicity.

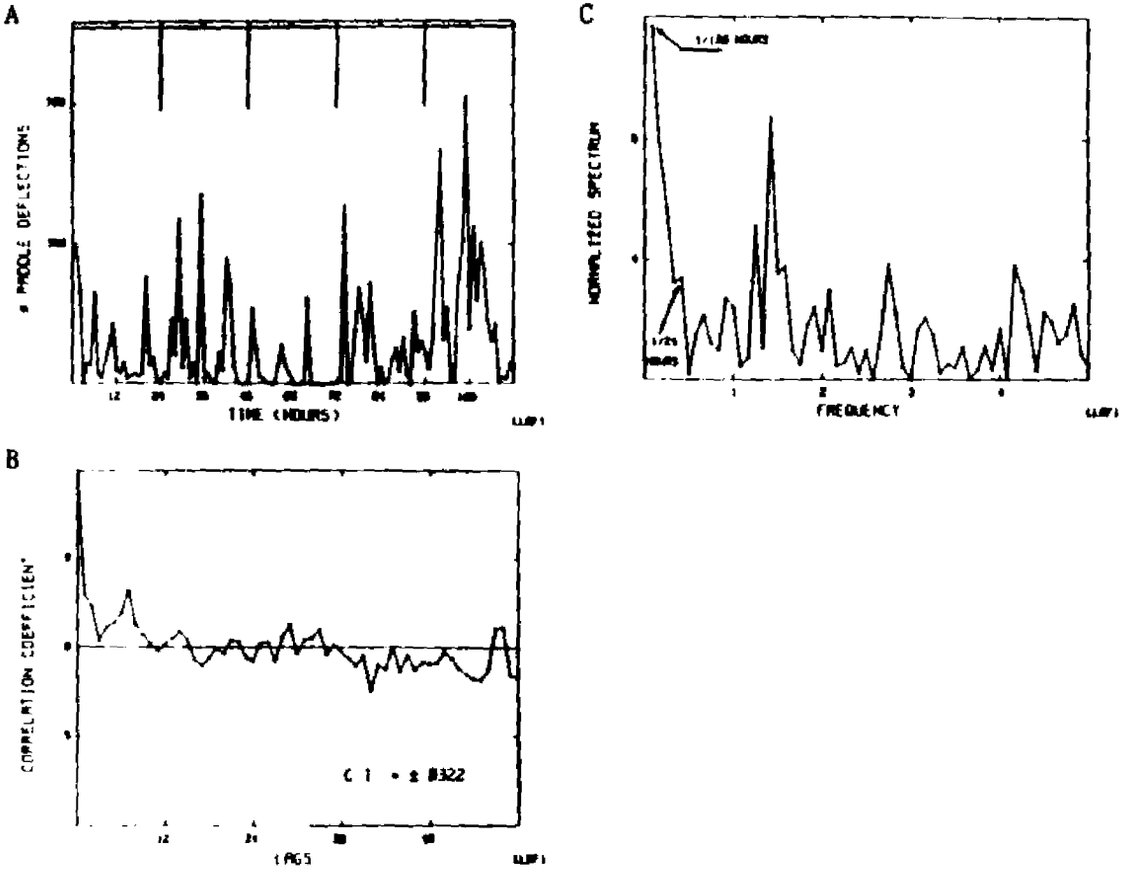

A
PADDLE DEFLECTIONS
TIME (HOURS)
B
CORRELATION COEFFICIENT
C 1 = ± 0322
LAGS
C
NORMALIZED SPECTRUM
FREQUENCY

Figure 11. Alternated constant light intensities, F2.

Raw data. Alternating shaded and open bar at top of graph is alternating dim and bright light (25 lux:2800 lux). Number of paddle deflections is plotted as function of time in hours. No obvious 24 hour rhythmicity detected. Note increasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. No oscillation present. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). Small peak at 1/24 hours (see arrow). (see Figure 13.)

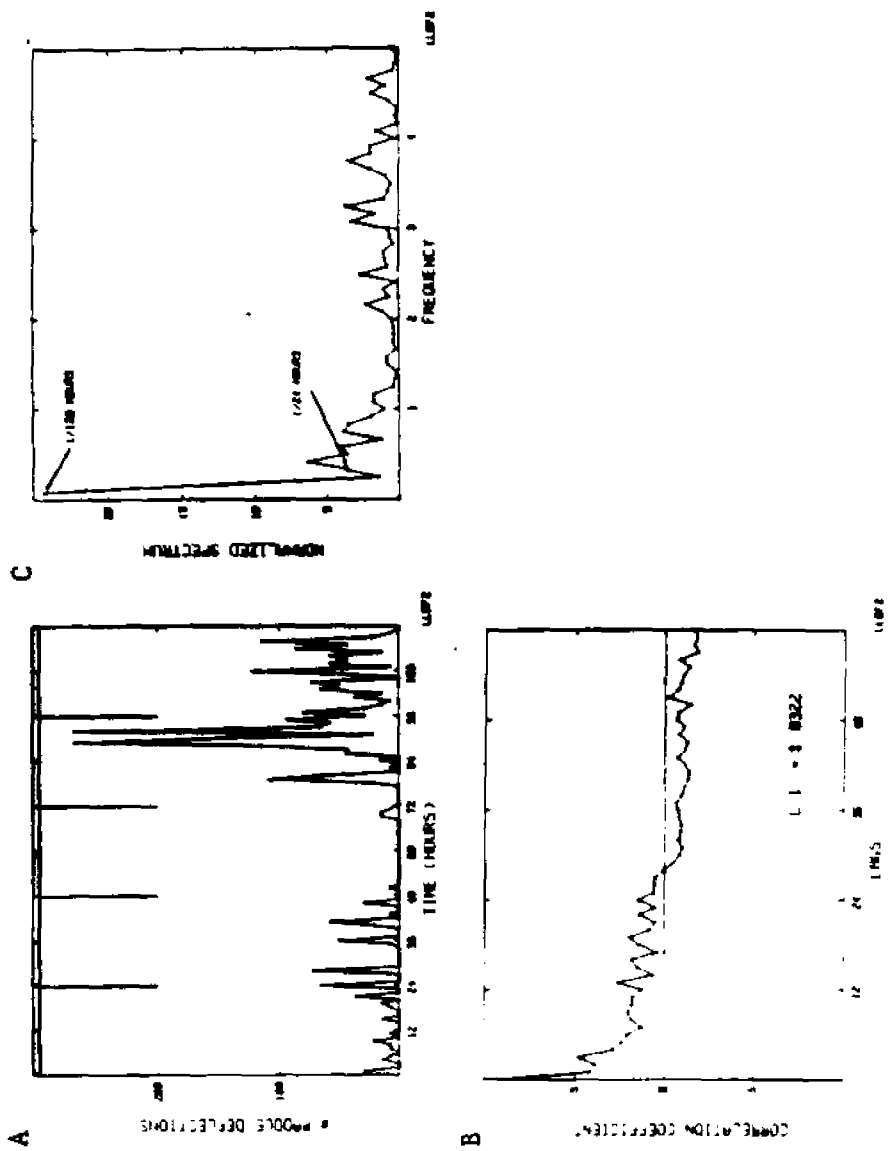

C
FREQUENCY
A
TIME (HOURS)
B

Figure 12. Alternated constant light intensities, F1. Spectral analysis of non-overlapping, sequential segments of data. Normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. A. First 48-hour segment. B. Second 48-hour segment. C. Twenty-four hour segment. Peak at 1/24 hours does not appear in all segments, indicating no reliable 24 hour periodicity.

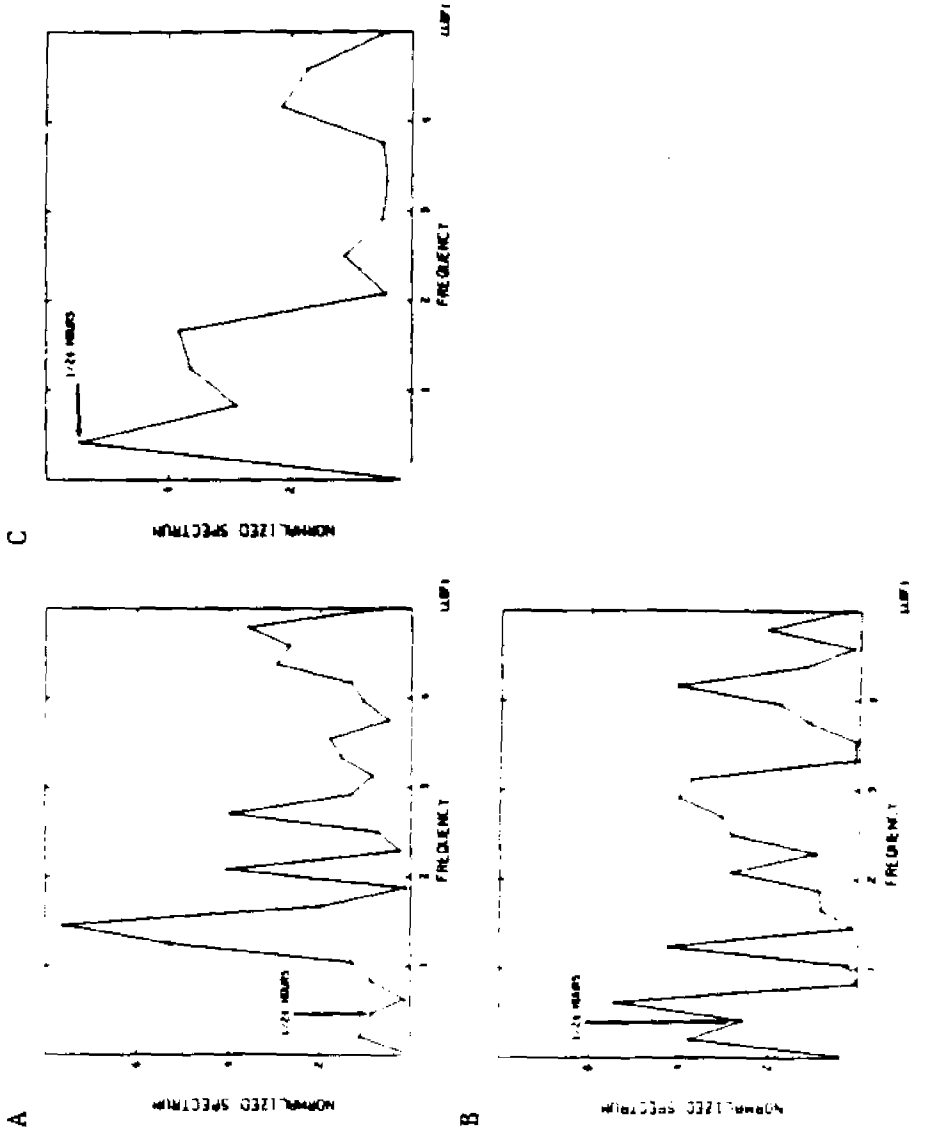
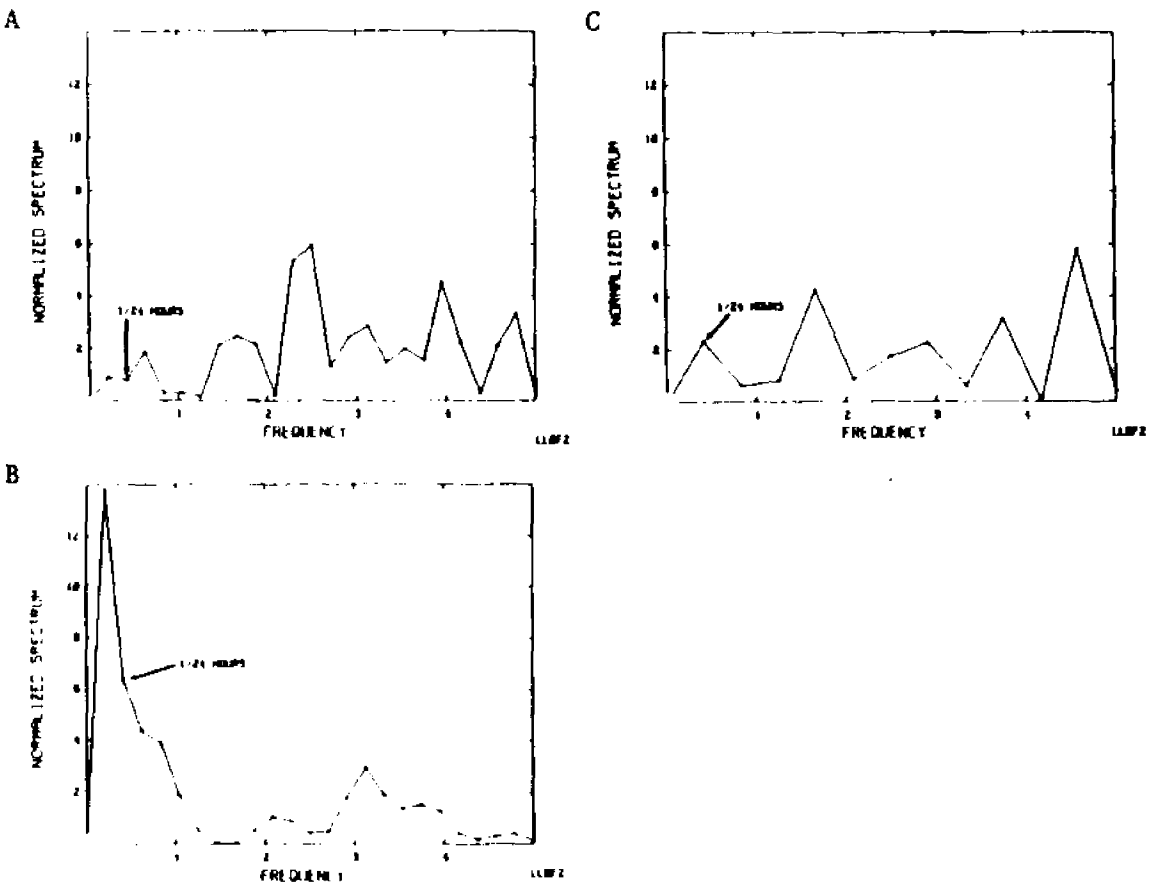

A
B
C
FREQUENCY
1/24 HOURS

Figure 13. Alternated constant light intensities, F2. Spectral analysis of non-overlapping, sequential segments of data. Normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. A. First 48-hour segment. B. Second 48-hour segment. C. Twenty-four hour segment. Peak at 1/24 hours does not appear in all segments, indicating no reliable 24 hour periodicity.


A
B
C
NORMALIZED SPECTRUM
FREQUENCY

were alternated. Wever (1980) found that birds became arhythmic under different intensities of constant light but would entrain to these same intensities if they were alternated with a 24 hour period. The two G. petersii exposed to alternated light intensities did not entrain.

Entrainment. Two fish, F1 and F2, and one replacement, F3, were studied. Data from subject F1 were compared with data from F2 for the entrainment manipulation. The replacement was used for the two subsequent manipulations after baseline had been established.

Figure 14 shows the data for F1 on an LD 12:12 schedule. There was more locomotor activity during the dark period than during the light period, thus confirming field and laboratory data (Moller et al., 1979; Bassler et al., 1979). Activity abruptly increased when the light was turned off and decreased less suddenly at light onset. While the light-dark rhythm was maintained, a decreasing trend in the amount of activity over time was evident. The second fish showed the same entrainment to the light-dark cycle (Figure 15) and also the same pattern over time as F1.

Figures 14B & 15B show the autocorrelation functions for both fish. The autocorrelation functions show clear

Figure 14. Entrainment, Fl. A. Raw data. Number of paddle deflections is plotted as function of time in hours. Alternating black and open bar at top of graph is LD 12:12. B. Autocorrelation function. Correlation coefficient is plotted as function of lags. Confidence intervals for 0 appear in lower right hand corner. Note 24 lags peak to peak and trough to trough indicating 24 hour periodicity. C. Spectral analysis. Normalized spectrum is plotted as function of frequency (1/period in hours). Large peak at 1/24 hours indicates presence of 24 hour periodicity. Peak at 1/192 hours reflects decreasing trend. Peak at 1/8 hours is submultiple of 1/24 hours.

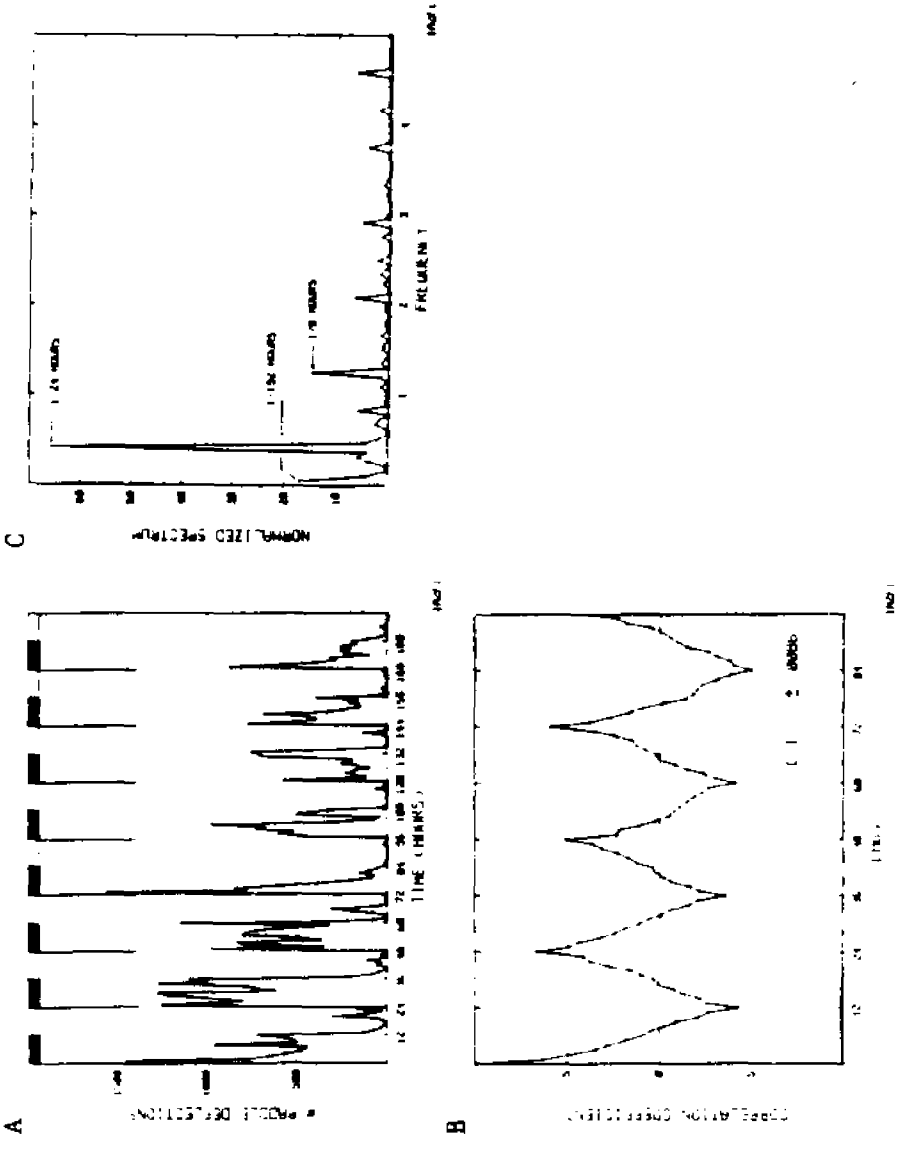
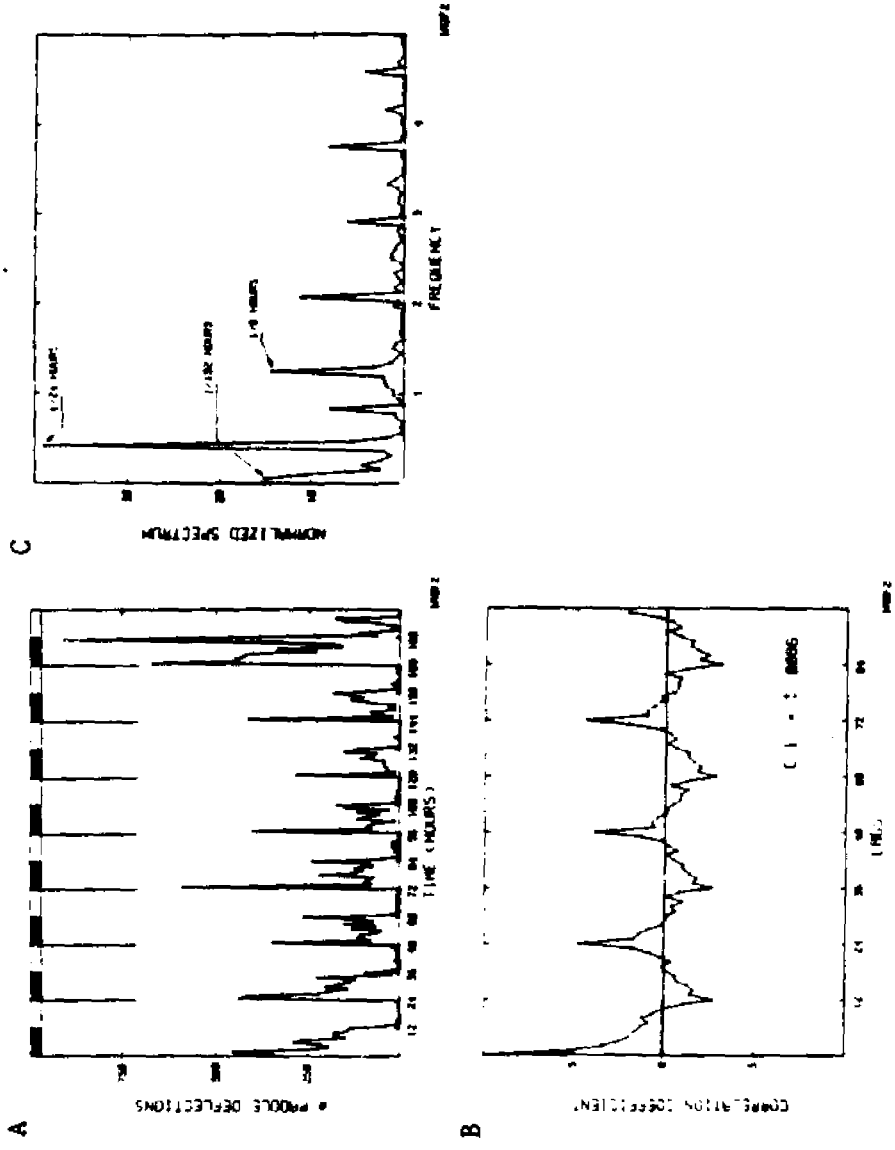

A
B
C

Figure 15. Entrainment, F2. A. Raw data. Number of paddle deflections is plotted as function of time in hours. Alternating black and open bar at top of graph is LD 12:12. B. Autocorrelation function. Correlation coefficient is plotted as function of lags. Confidence intervals for 0 appear in lower right hand corner. Note 24 lags peak to peak and trough to trough indicating 24 hour periodicity. C. Spectral analysis. Normalized spectrum is plotted as function of frequency (1/period in hours). Large peak at 1/24 hours indicates presence of 24 hour periodicity. Peak at 1/192 hours reflects decreasing trend. Peak at 1/8 hours is submultiple of 1/24 hours.


C
NORMALIZED SPECTRUM
FREQUENCY
A
MODULE DEFLECTIONS
TIME (HOURS)
B
CORRELATION COEFFICIENT

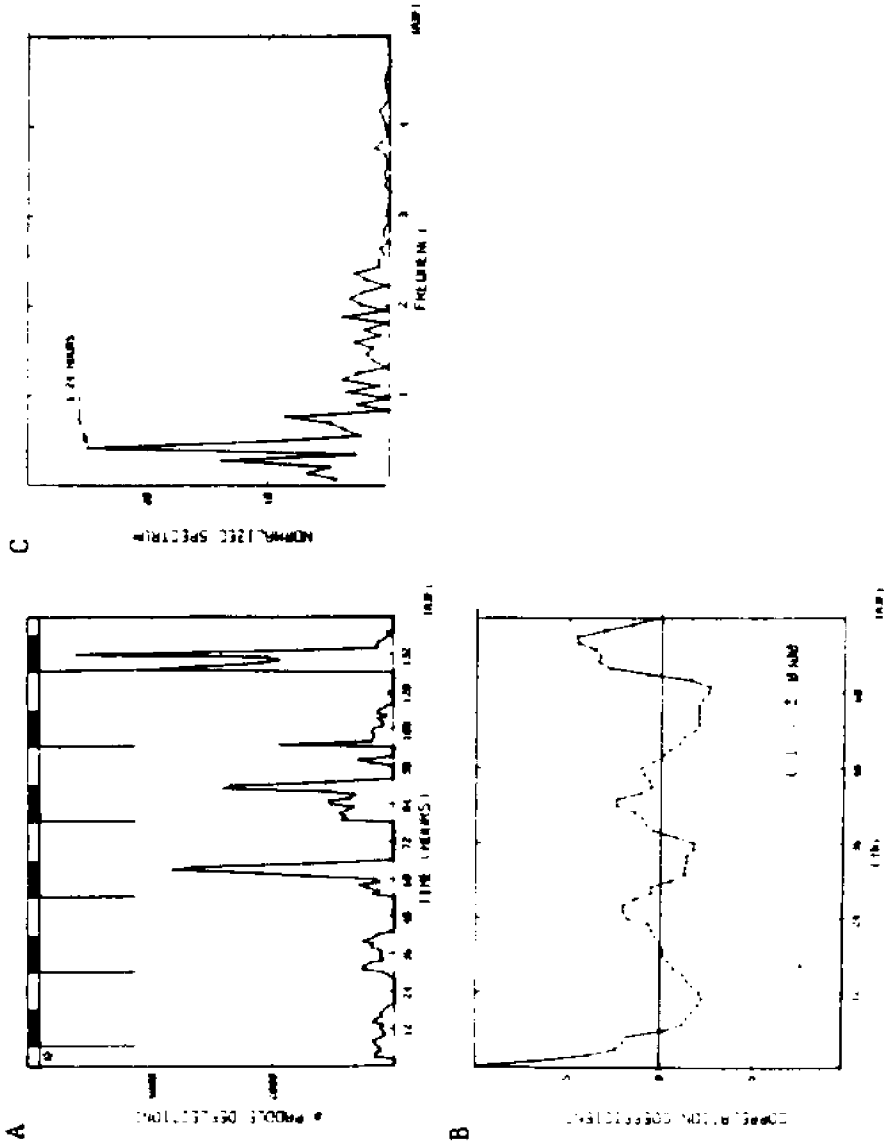
oscillations with the number of lags between successive peaks or troughs equalling 24, corresponding to a period of 24 hours. Thus the entrained locomotor activity rhythm of G. petersii equaled the period of the LD cycle of 24 hours. The imposed LD regimen controlled the fish's activity cycle.

Figures 14C & 15C show the power spectrum analyses. A large peak occurs at a frequency of 0.0417 (1/24 hours) which confirms the 24 hour periodicity of the entrainment data. Two other peaks stand out, one at a frequency of 0.0052 (1/192 hours) and one at 0.1250 (1/8 hours). The peak at 1/192 hours confirms the decrease in activity over time noted in the raw data. Since 8 hours is a submultiple of 24 hours, it probably reflects the natural harmonics of the 24 hour rhythm (Enright, 1965).

Under the LD 12:12 condition both fish entrained to the 24 hour cycle. Their activity cycles had period lengths of 24 hours, thus demonstrating period control. The time series statistics confirmed the presence of a persistant 24 hour rhythm.

Phase shift of zeitgeber. Following entrainment, the LD cycle was phase shifted by 6 hours following the last entrainment day. The raw data for F1 are shown in Figure 16A.

Figure 16. Phase shift of zeitgeber, Fl. A. Raw data. Number of paddle deflections is plotted as function of time in hours. Alternating black and open bar at top of graph is LD 12:12. 6-hour delay indicated by *. Note 2-hour/day delay over first 3 days as activity rhythm re-entrains to LD cycle
B. Autocorrelation function. Correlation coefficient is plotted as function of lags. Confidence intervals for 0 appear in lower right hand corner. Function oscillates but without 24 lags peak to peak. Reflects instability of 24-hour periodicity during shifting. C. Spectral analysis. Normalized spectrum is plotted as function of frequency (1/period in hours). Large peak at 1/24 hours indicates presence of 24 hour periodicity.


A
B
C

On day 1 of the phase shift, the fish became active 2 hours after the time when the light would have gone off under the previous entrainment schedule. The activity level was low compared to the last day of entrainment. During resynchronization the amplitude of a rhythm is commonly damped (Bunning, 1973). On day 2 the fish delayed activity onset for 4 hours and then showed an increase in activity for the last 2 hours before the light went off. On day 3, activity onset was delayed the full 6 hours of the phase shift. Days 4 and 6 show the same 6 hour delay. There was some activity during the first 3 hours before light offset on day 5, but the level dropped to zero for the second 3 hours before the light was turned off. F3 showed a similar shifting of activity though there was no clear 2 hour shift each day (Figure 17A). The observed phase shift demonstrated phase control of the activity rhythm by the LD cycle. The autocorrelation functions (Figures 16B & 17B) and spectral analyses (Figure 16C & 17C) confirm the 24 hour periodicity of locomotor activity for both fish.

Post-entrainment free run. The final manipulation was a free run under constant conditions. F1, during the first 2 days of constant darkness (DD) showed a peak of activity during what would have normally been the dark period of the LD cycle (Figure 18A). On day 1, the peak occurred in the middle

Figure 17. Phase shift of zeitgeber, F3. A. Raw data. Number of paddle deflections is plotted as function of time in hours. Alternating black and open bar at top of graph is LD 12:12. 6-hour delay indicated by *. Note immediate shift of activity rhythm. B. Autocorrelation function. Correlation coefficient is plotted as function of lags. Confidence intervals for 0 appear in lower right hand corner. Function oscillates but without 24 lags peak to peak. Reflects instability of 24-hour periodicity during shifting. C. Spectral analysis. Normalized spectrum is plotted as function of frequency (1/period in hours). Large peak at 1/24 hours indicates presence of 24 hour periodicity.

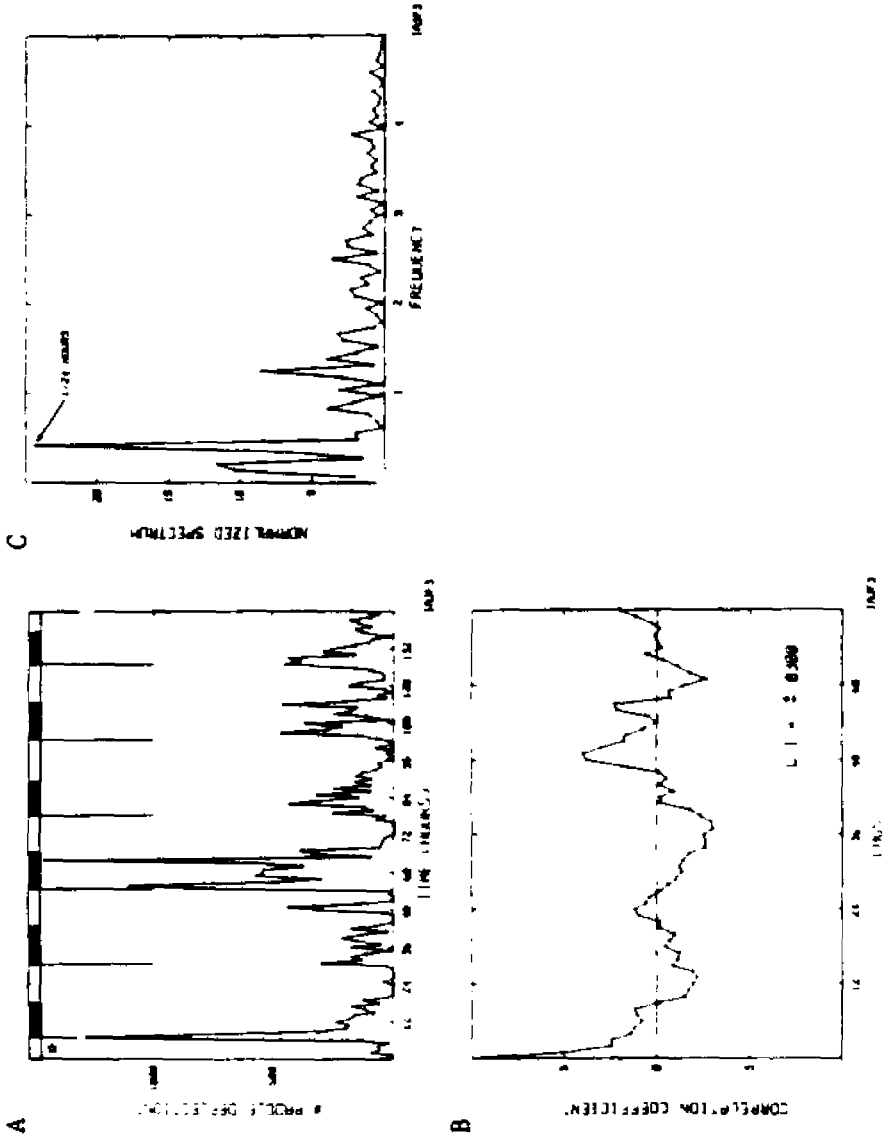
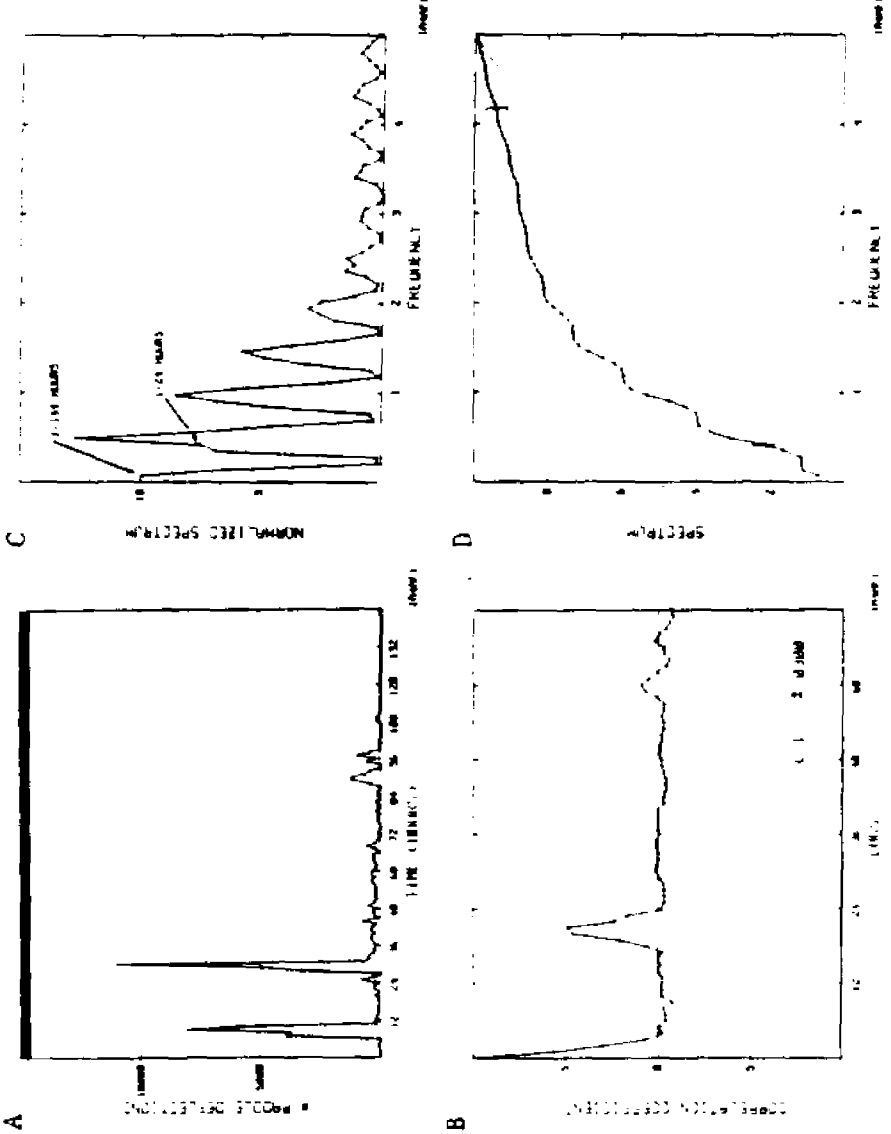

A
B
C

Figure 18. Post-entrainment free run, Fl. A. Raw data. Black bar at top of graph is constant darkness (0.7 lux). Number of paddle deflections is plotted as function of time in hours. No obvious 24 hour rhythmicity detected. Note decreasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. No oscillation present. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). No peak at 1/24 hours (see arrow) confirming absence of 24 hour periodicity. Peak at 1/144 reflects decreasing trend. D. White noise test. Solid line is integrated spectrum of data. Dotted line is expected integrated spectrum for random data. Points between brackets are significantly different from random data. No 24 hour periodicity in constant darkness. Lack of randomness is due to decreasing trend.


A
B
C
D

of what would have been the 12 hour dark period, and then the activity dropped for the next 14 hours. On day 2, there was a small burst during the hour when the light would have gone off on the previous entrainment cycle, Subsequently, a drop was followed by an increase of activity for 3 hours. There were several smaller bursts during what would have been the light period. On day 3, there was an even smaller burst at the time when the light would have gone off during entrainment. On day 4, there was no burst during the "dark" period while on day 6 there was. By day 6, the fish's locomotor activity had almost completely damped out.

Fish F3 showed a smaller peak at the time when the light would have gone off under entrainment (Figure 19A). Activity was generally higher then that of F1 and was not damped out by day 6, as it was for F1.

The autocorrelation functions for both fish (Figures 18B & 19B) revealed no rhythmicity. A spectral analysis for both sets of data showed several peaks (Figure 18C & 19C) but none of these peaks occurred at a frequency of 1/24 hours. Neither fish displayed a free running activity rhythm. The data were then broken into 48 hour non-overlapping segments to determine if any of the peaks were significant. No reliable rhythmic components could be discerned (Figures 20 & 21). Both spectra

Figure 19. Post-entrainment free run, F3. A. Raw data. Black bar at top of graph is constant darkness (0.7 lux). Number of paddle deflections is plotted as function of time in hours. No obvious 24 hour rhythmicity detected. Note decreasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. No oscillation present. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). No peak at 1/24 hours (see arrow) confirming absence of 24 hour periodicity. D. White noise test. Solid line is integrated spectrum of data. Dotted line is expected integrated spectrum for random data. Points between brackets are significantly different from random data. No 24 hour periodicity in constant darkness. Lack of randomness due to decreasing trend.

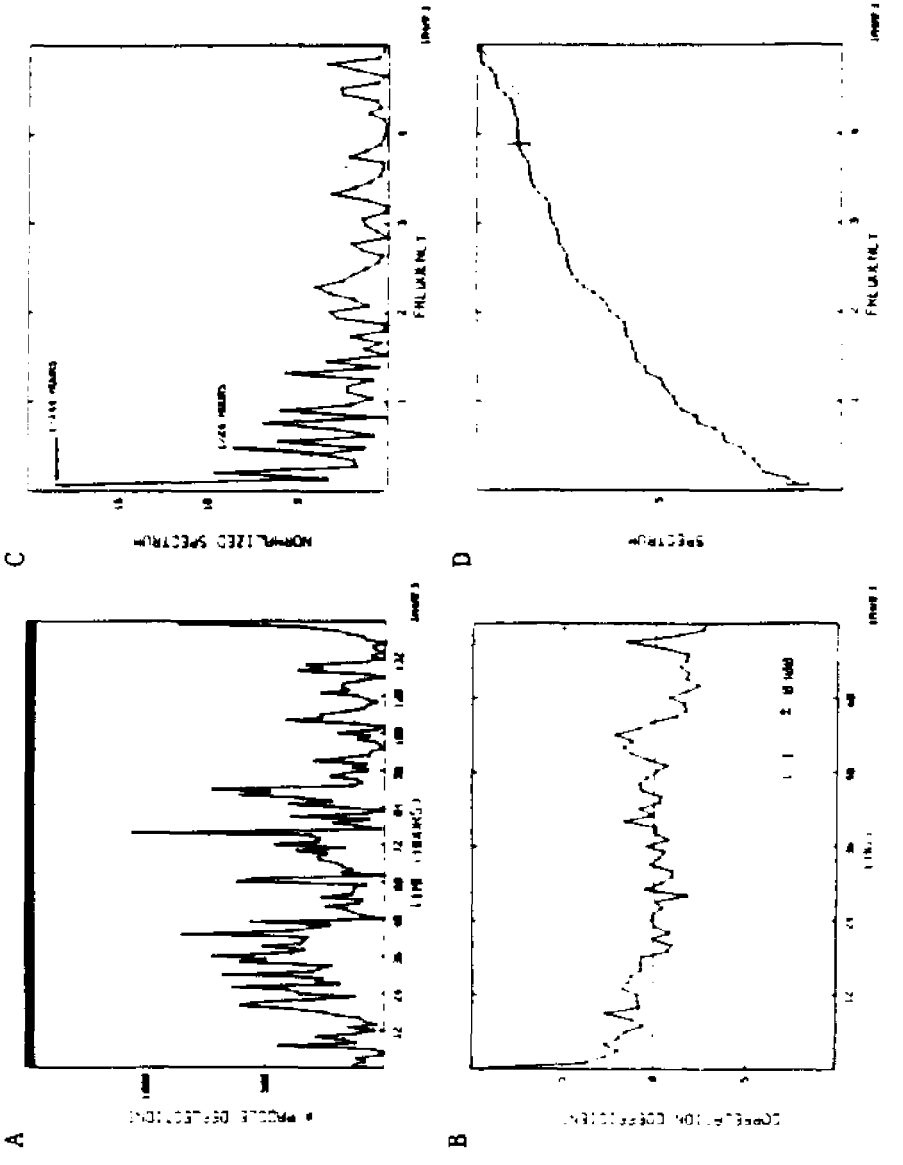

A
B
C
D

Figure 20. Post-entrainment free run, F1. Spectral analysis of non-overlapping, sequential segments of data. Normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. A. First 48-hour segment. B. Second 48-hour segment. C. Twenty-four hour segment. Peak at 1/24 hours does not appear in all segments, indicating no reliable 24 hour periodicity in constant darkness.

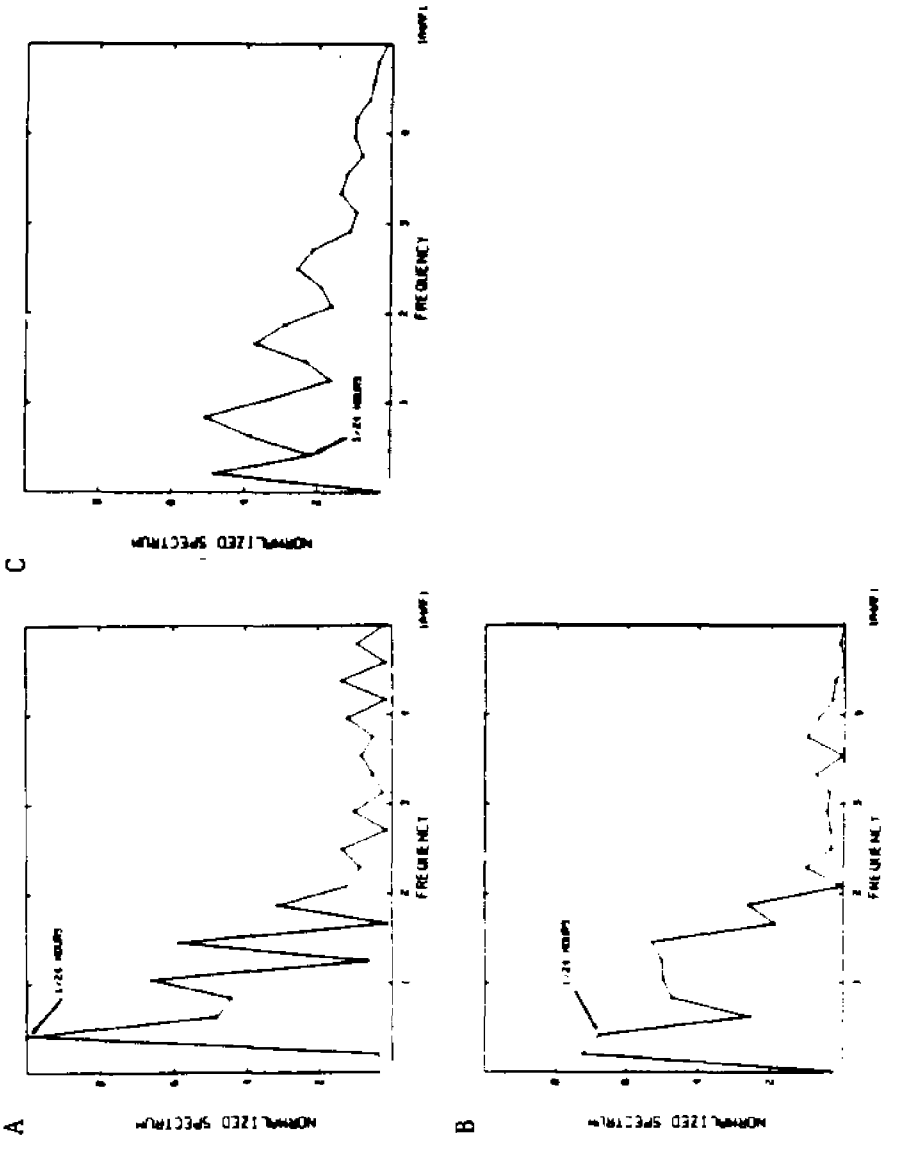
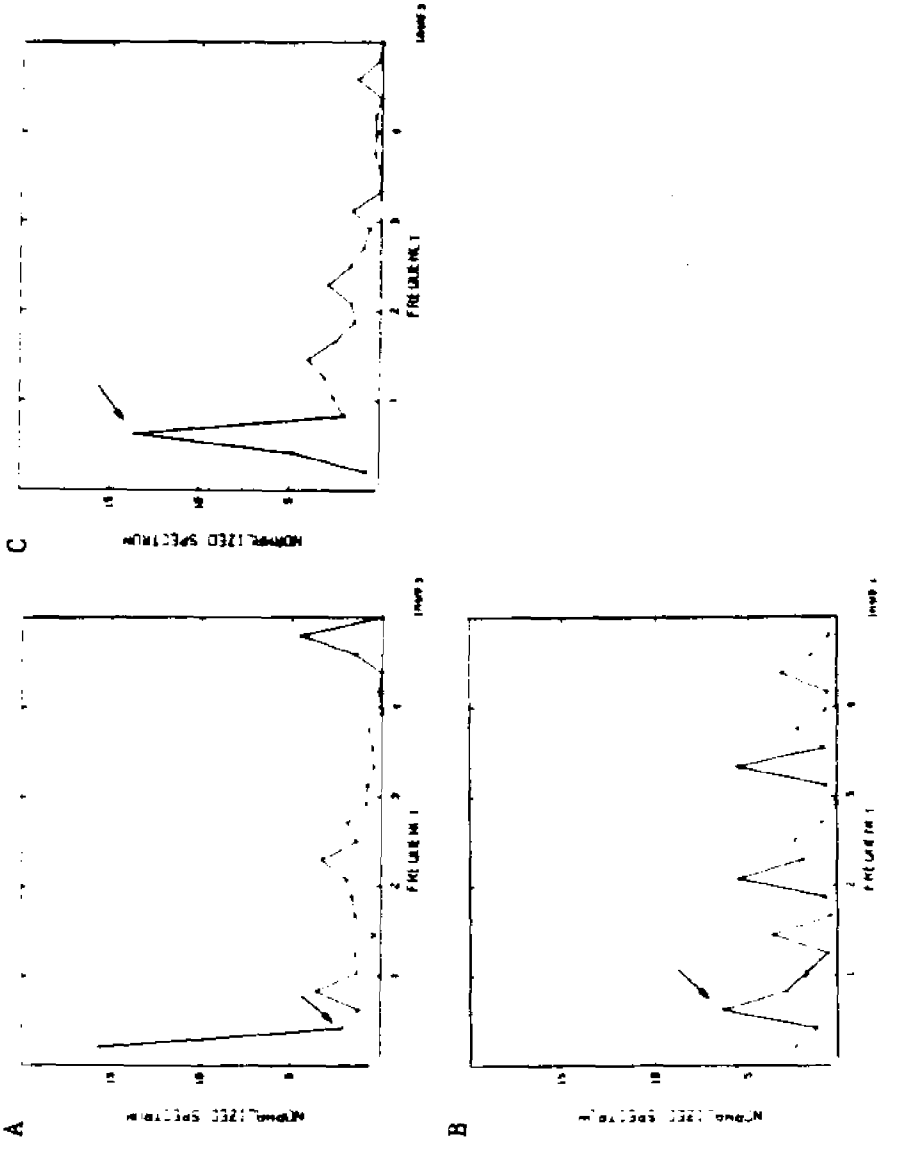

A
B
C
NORMALIZED SPECTRUM
FREQUENCY
1.24 HOURS

Figure 21. Post-entrainment free run, F3. Spectral analysis of non-overlapping, sequential segments of data. Normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. A. First 48-hour segment. B. Second 48-hour segment. C. Twenty-four hour segment. Peak at 1/24 hours does not appear in all segments, indicating no reliable 24 hour periodicity in constant darkness.


C
A
B

showed a decrease in the activity level over time. The white noise tests indicated locomotor activity in both fish was non-random (Figures 18D & 19D). Since rhythmicity could not be demonstrated by the spectral analysis, non-randomness was caused by the decreasing trend in activity, and not by a rhythmic component.

The results indicated the absence of a circadian activity rhythm in G. petersii.

The next set of five manipulations served two purposes. First, an attempt was made to establish a free running locomotor rhythm using two new subjects, F5 and F6. To do this, four manipulations, an undisturbed free run, a phase shift of the rhythm, a second undisturbed free run, and an entrainment, were done. Second, the last manipulation, entrainment to simulated sunset and sunrise, was introduced to compare the response of the fish to gradual, more natural, light-dark, dark-light transitions with their responses to abrupt changes in light intensity.

Undisturbed free run. The first manipulation in the series was a free run in constant darkness. There was no evidence of rhythmic locomotor activity in the raw data for either fish (Figures 22A & 23A). A decrease in the amount of

Figure 22. Post-entrainment free run, F5. A. Raw data. Black bar at top of graph is constant darkness (0.7 lux). Number of paddle deflections is plotted as function of time in hours. No obvious 24 hour rhythmicity detected. Note decreasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. No oscillation present. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). No peak at 1/24 hours (see arrow) confirming absence of 24 hour periodicity. Peak at 1/144 hours reflects decreasing trend. D. White noise test. Solid line is integrated spectrum of data. Dotted line is expected integrated spectrum for random data. Points between brackets are significantly different from random data. No 24 hour periodicity in constant darkness. Lack of randomness is due to decreasing trend.

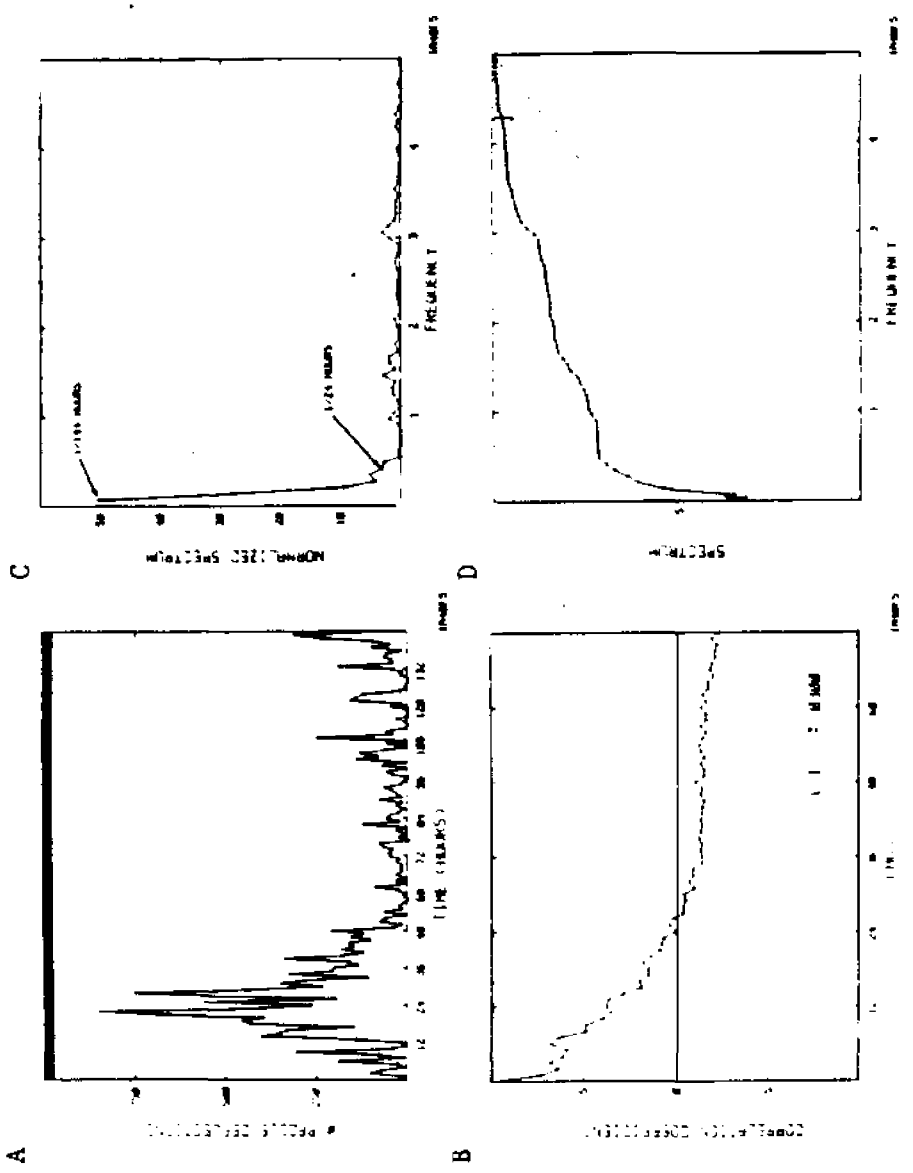
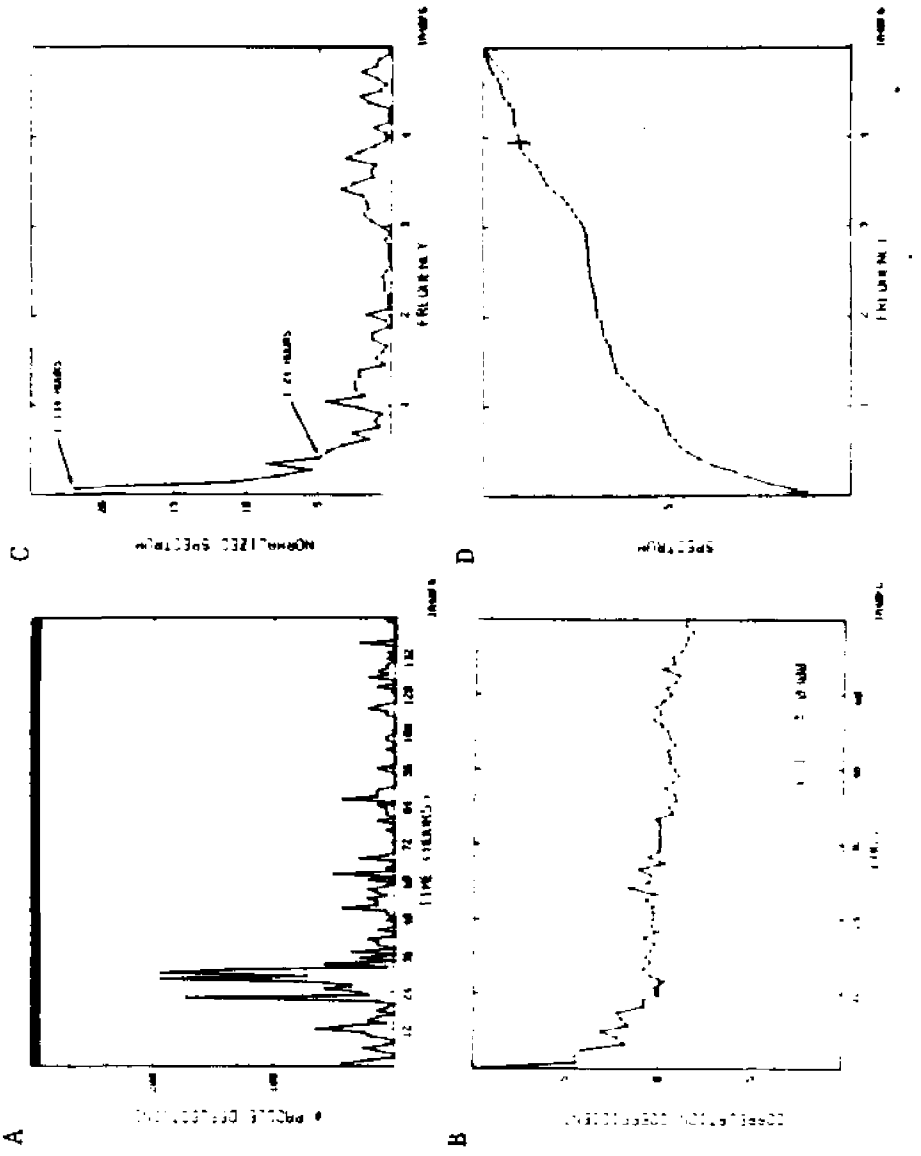

A
B
C
D

Figure 23. Post-entrainment free run, F6. A. Raw data. Black bar at top of graph is constant darkness (0.7 lux). Number of paddle deflections is plotted as function of time in hours. No obvious 24 hour rhythmicity detected. Note decreasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. No oscillation present. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). No peak at 1/24 hours (see arrow) confirming absence of 24 hour periodicity. Peak at 1/144 hours reflects decreasing trend. D. White noise test. Solid line is integrated spectrum of data. Dotted line is expected integrated spectrum for random data. Points between brackets are significantly different from random data. No 24 hour periodicity in constant darkness. Lack of randomness due to decreasing trend.


A
B
C
D

activity over time was seen. The autocorrelation functions (Figures 22B & 23B) and spectral analyses (Figures 22C & 23C) confirm the lack of periodicity. There was no peak at 1/24 hours for either fish. The decreasing trend was indicated by a peak at 1/144 hours. The white noise tests (Figures 22D & 23D) showed that more than 5% of the points were significantly different from values expected for random data. Based on the results of the spectral analyses, the lack of randomness in the white noise tests is due to the decreasing trend and not periodicity. The results again demonstrate the absence of a free running circadian activity rhythm in G. petersii.

Phase shift of rhythm. If a circadian rhythm is present, a brief pulse of light will produce either a phase delay or a phase advance depending upon when in the 24 hour period the pulse occurs. Although the above procedures did not show circadian rhythmicity, a 15 minute light pulse was, nevertheless, introduced late in the subjective night (6 hours into what would have been the dark period of an LD cycle). As with the previous manipulations the raw data did not show a rhythmic pattern for either fish (Figure 24A & 25A). Fish F5 responded to the light pulse with an increase in activity. Fish F6 did not exhibit this response. Both the autocorrelation functions (Figures 24B & 25B) and spectral tests (Figures 24C & 25C) confirm the lack of periodicity.

Figure 24. Phase shift of rhythm, F5. A. Raw data. Black bar at top of graph is constant darkness (0.7 lux). Arrow indicates hour when 15 min light pulse occurred. Number of paddle deflections is plotted as function of time in hours. No obvious 24 hour rhythmicity detected. Note decreasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. No oscillation present. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). No peak at 1/24 hours (see arrow) confirming absence of 24 hour periodicity. Peak at 1/120 hours reflects decreasing trend. D. White noise test. Solid line is integrated spectrum of data. Dotted line is expected integrated spectrum for random data. Points between brackets are significantly different from random data. No 24 hour periodicity in constant darkness. Lack of randomness is due to decreasing trend.

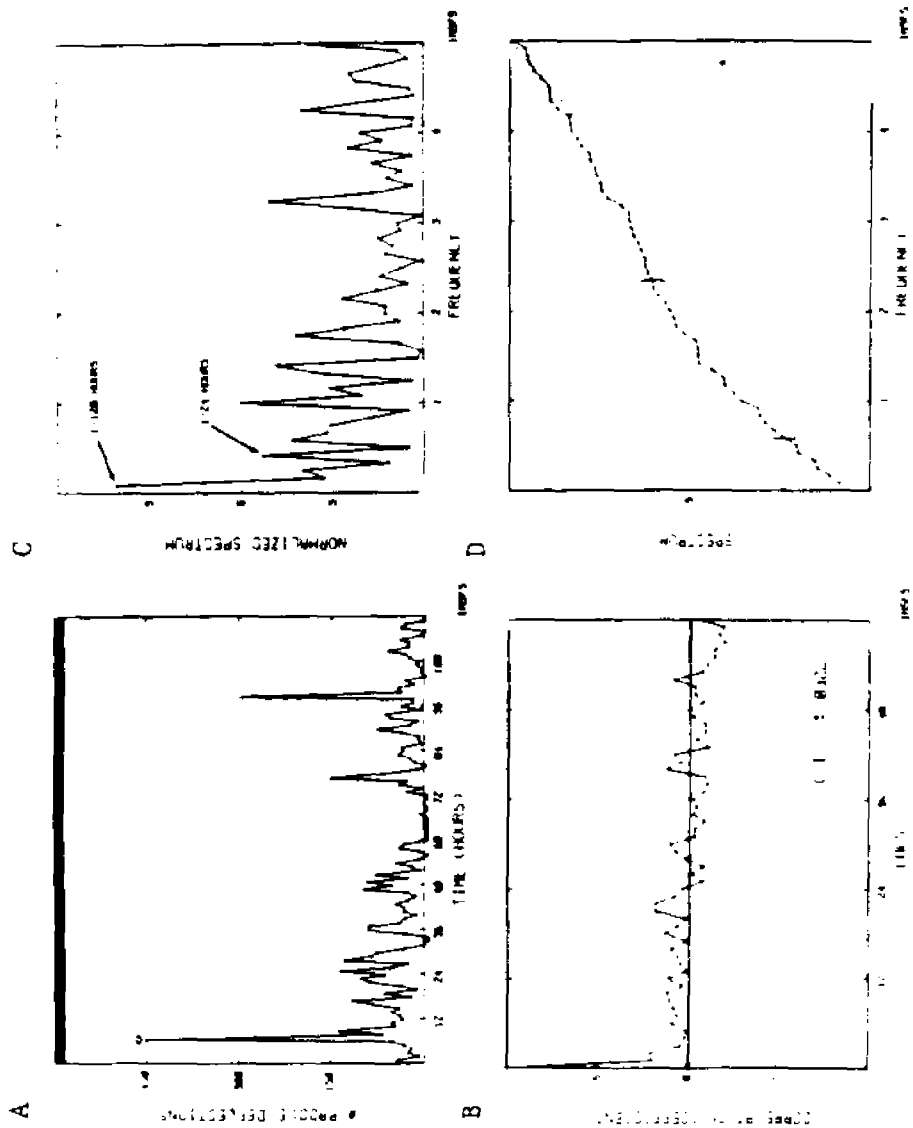
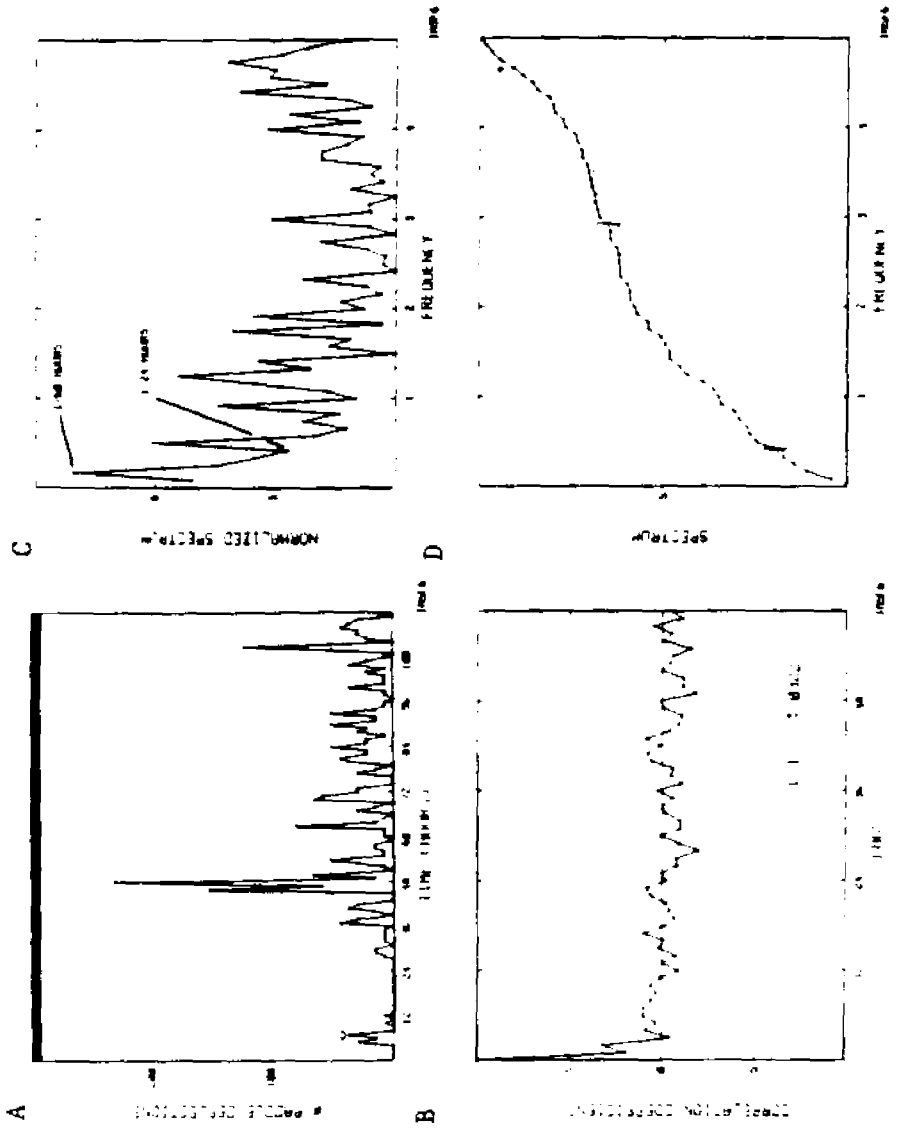

A
B
C
D
NORMALIZED SPECTRUM
FREQUENCY
TIME (HOURS)

Figure 25. Phase shift of rhythm, F6. A. Raw data. Black bar at top of graph is constant darkness (0.7 lux). Arrow indicates hour when 15 min light pulse occurred. Number of paddle deflections is plotted as function of time in hours. No obvious 24 hour rhythmicity detected. Note decreasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. No oscillation present. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). No peak at 1/24 hours (see arrow) confirming absence of 24 hour periodicity. Peak at 1/60 (submultiple of 1/120 hours) hours reflects decreasing trend. D. White noise test. Solid line is integrated spectrum of data. Dotted line is expected integrated spectrum for random data. Points between brackets are significantly different from random data. No 24 hour periodicity in constant darkness. No obvious effect of light pulse. Lack of randomness is due to decreasing trend.


A
B
C
D

There was a decreasing trend in the activity data and the white noise tests reflected the presence of this trend (Figures 24D & 25D).

Undisturbed free run. The next manipulation was another undisturbed free run in constant darkness. Again, the raw data showed no rhythmicity (Figures 26A & 27A), the autocorrelation functions no oscillations (Figures 26B & 27B) and there were no reliable peaks in the spectral analyses (Figures 26C & 27C). A small decrease in activity level was evident. For this free run condition, the white noise tests for both fish showed 5%, for F5, and 7%, for F6, of the points significantly different from expected values for random data (Figures 26D & 27D). When the spectral analysis for the non-overlapping segments was calculated, the white noise test was performed on each 48 hour segment. The points were not significantly different from expected values for random data. However, the lack of randomness for the complete data set indicated the small decreasing trend that did not appear in the smaller segments. Locomotor activity for both fish was random.

Entrainment. Following this free run condition, the fish were presented with an LD 12:12 entraining cycle. The alternating pattern of activity and rest is not as clear as

Figure 26. Undisturbed free run, F5. A. Raw data. Black bar at top of graph is constant darkness (0.7 lux). Number of paddle deflections is plotted as function of time in hours. No obvious 24 hour rhythmicity detected. Note decreasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. No oscillation present. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). No peak at 1/24 hours (see arrow) confirming absence of 24 hour periodicity. Peak at 1/120 hours reflects decreasing trend. D. White noise test. Solid line is integrated spectrum of data. Dotted line is expected integrated spectrum for random data. Points between brackets are significantly different from random data. No 24 hour periodicity in constant darkness. Lack of randomness is due to decreasing trend.

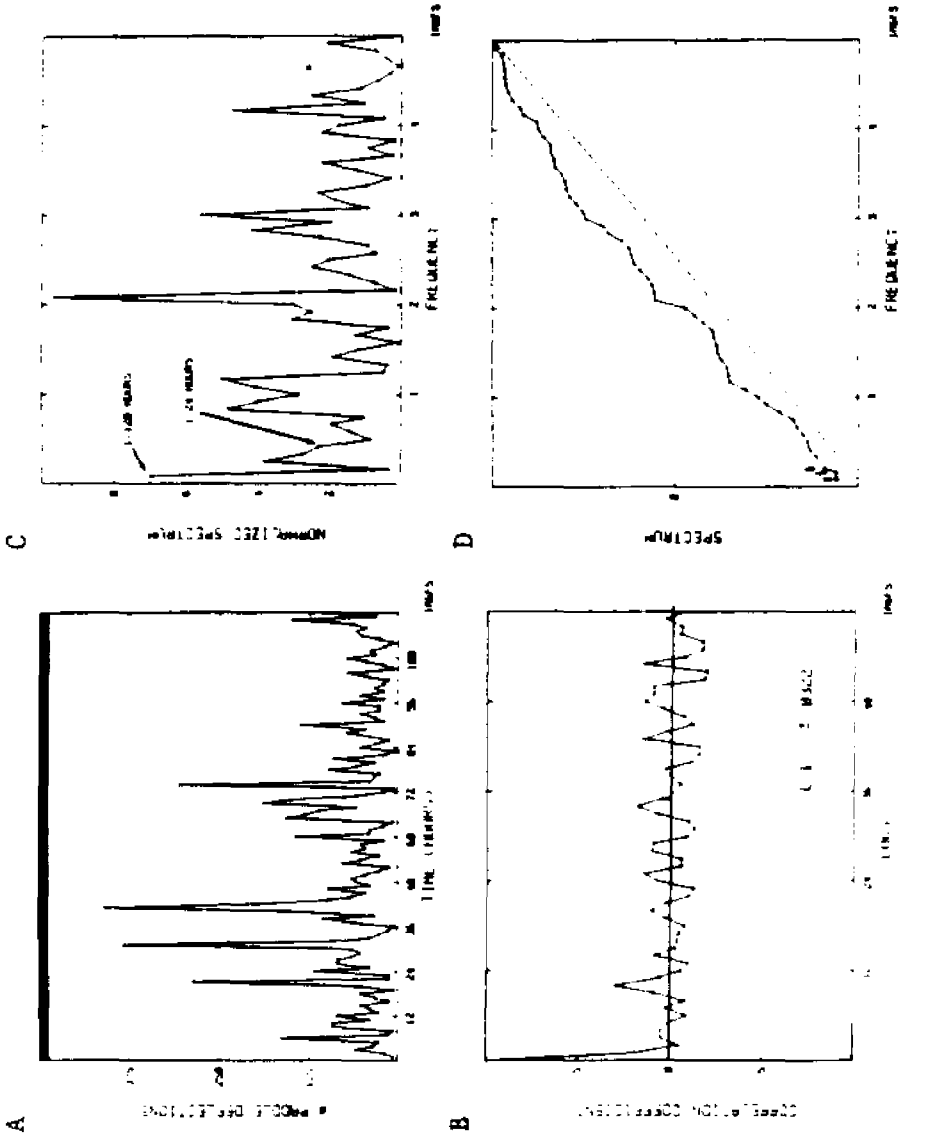
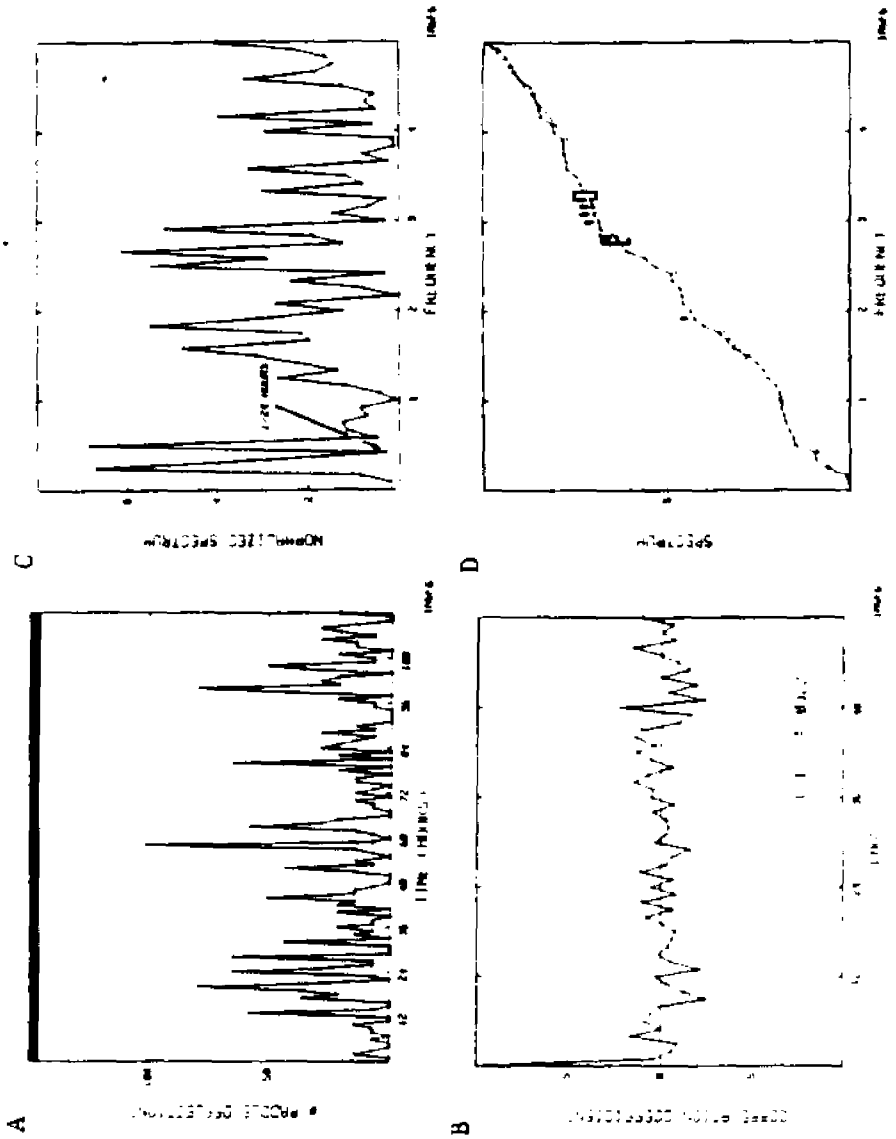

A
B
C
D
FREQUENCY

Figure 27. Undisturbed free run, F6. A. Raw data. Black bar at top of graph is constant darkness (0.7 lux). Number of paddle deflections is plotted as function of time in hours. No obvious 24 hour rhythmicity detected. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. No oscillation present. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). No peak at 1/24 hours (see arrow) confirming absence of 24 hour periodicity. D. White noise test. Solid line is integrated spectrum of data. Dotted line is expected integrated spectrum for random data. Points between brackets are significantly different from random data. No 24 hour periodicity in constant darkness. Lack of randomness is due to decreasing trend.


A
B
C
D

with the first entrainment. Fish F6 showed no obvious difference in the amount of activity between the dark and light periods on the first day (Figure 28A, B) but was more active during the dark period for the next 2 days. The pattern was reversed on day 4 but there was again more activity during the dark period for days 5,6 and 7. Then F6 became inactive for almost 2 full days. On day 9 there was more activity during the dark period and no activity for the next 22 hours. On the last 2 days of entrainment a clear dark-light, activity-rest pattern was seen. The same irregularity of patterning is shown by fish F5, though there were no periods longer than 1 hour when the fish was completely inactive (Figure 29A, B). The autocorrelation functions showed no oscillations (Figures 28C & 29C). This was not surprising since the fish's activity patterns were so irregular. The spectral analyses, (Figures 28D & 29D), however, did show peaks at 1/24 hours. This suggested that although the pattern was not well established there was 24 hour periodicity and entrainment had occurred. In contrast to this manipulation entrainment following exposure to constant light and alternating constant light intensities was clearly established on the first day for both fish. The irregular pattern entrainment just discribed or F5 and F6 followed 16 days of constant darkness with only one 15 minute light pulse on day 7 that had no apparent effect on paddle activity. This

Figure 28. Entrainment, F5. A,B. Raw data. Number of paddle deflections is plotted as function of time in hours. Alternating black and open bar at top of graph is LD 12:12. C. Autocorrelation function. Correlation coefficient is plotted as function of lags. Confidence intervals for 0 appear in lower right hand corner. Function oscillates but not with 24 lags peak to peak, reflecting instability of entrainment. D. Spectral analysis. Normalized spectrum is plotted as function of frequency (1/period in hours). Peak at 1/24 hours indicates presence of 24 hour periodicity. Peak at 1/144 hours reflects decreasing trend.

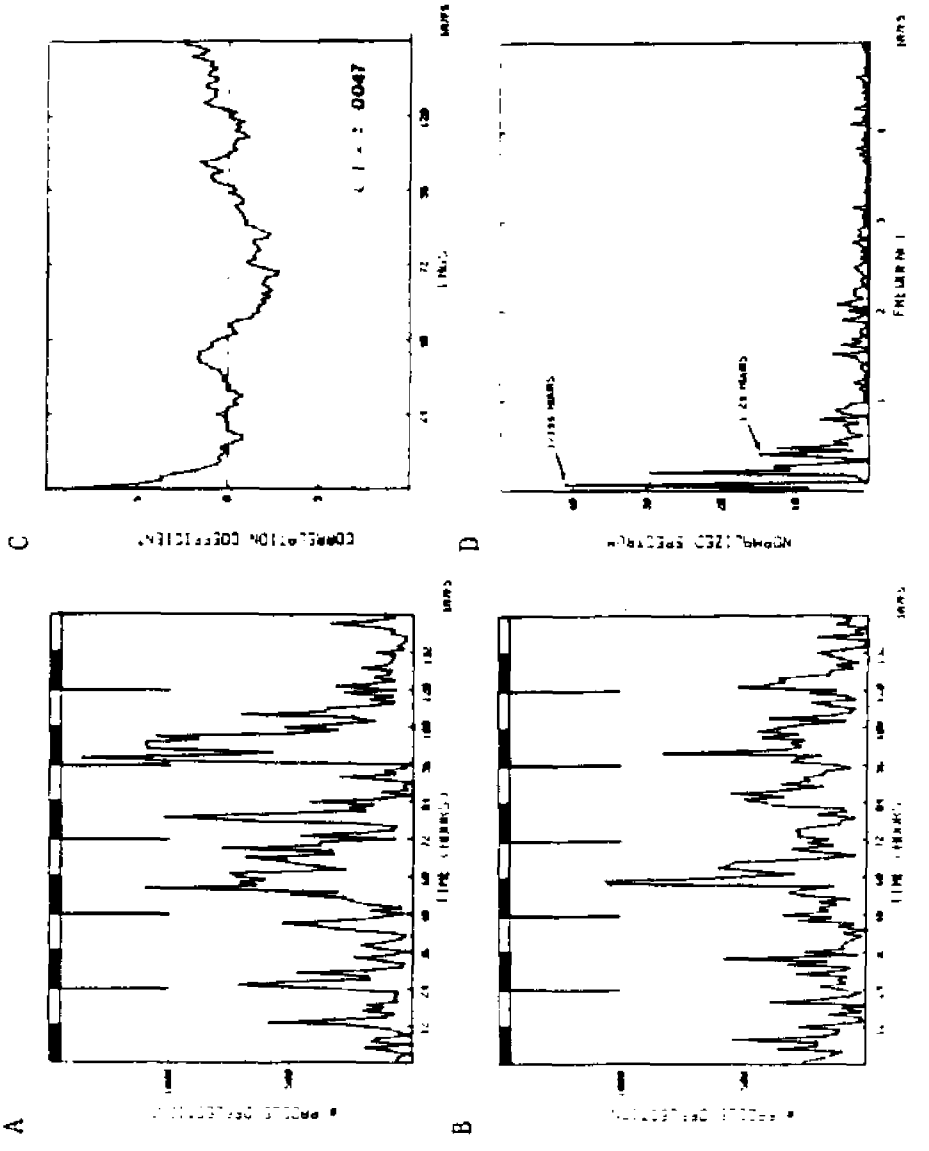
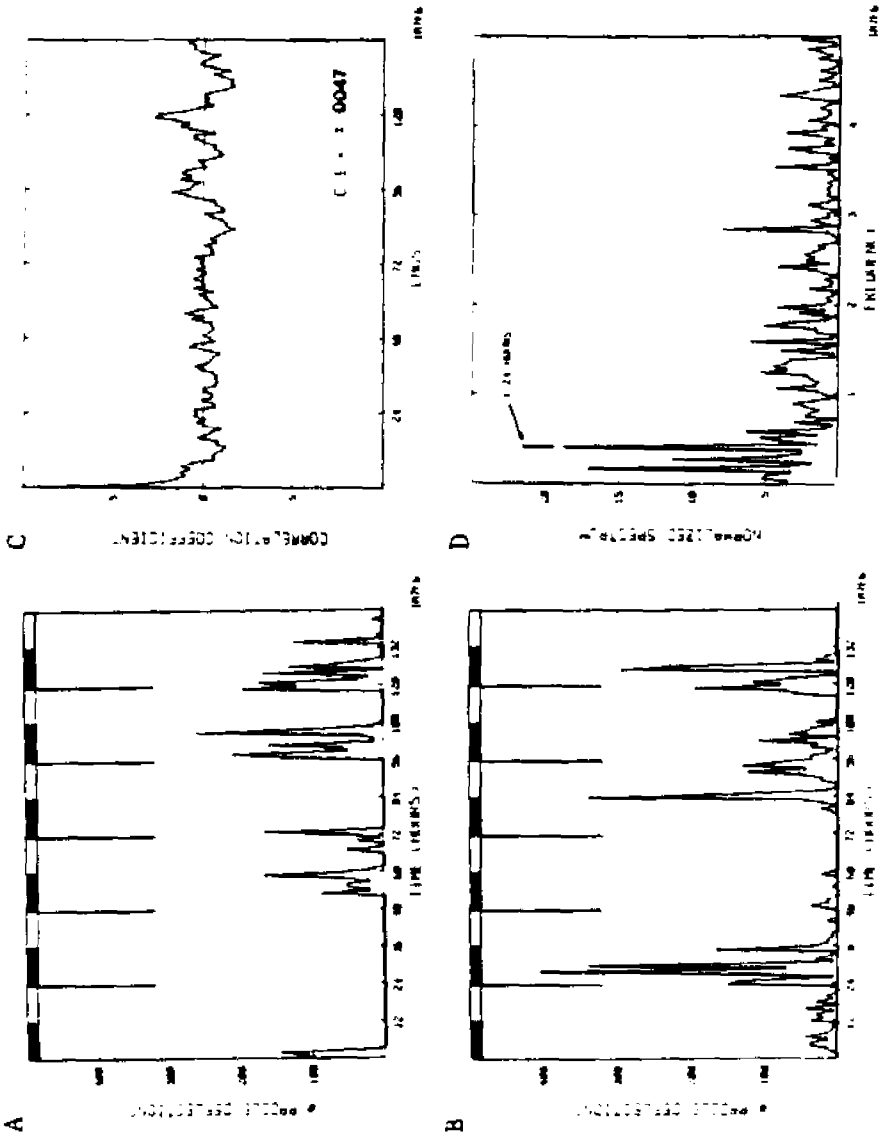

A
B
C
D

Figure 29. Entrainment, F6. A,B. Raw data. Number of paddle deflections is plotted as function of time in hours. Alternating black and open bar at top of graph is LD 12:12. C. Autocorrelation function. Correlation coefficient is plotted as function of lags. Confidence intervals for 0 appear in lower right hand corner. Function does not oscillate reflecting instability of entrainment. D. Spectral analysis. Normalized spectrum is plotted as function of frequency (1/period in hours). Large peak at 1/24 hours indicates presence of 24 hour periodicity.


A
B
C
D

difference in response may depend on the fish's prior history and could be indicative of internal rhythmic organization (see Discussion).

Entrainment with simulated sunrise and sunset. The last manipulation in this set was entrainment with a 30 minute sunset and 30 minute sunrise. Figures 30A & 31A show the raw data for both fish. There was clearly more activity during the dark period, but there was a dramatic decrease in activity over time. By the fifth day, F5 was almost completely inactive. The autocorrelation functions (Figures 30B & 31B) did not show a stable rhythmicity although the spectral analyses (Figures 30C & 31C) showed dominant peaks at 1/24 hours.

Figure 32 shows the transition data for F5 as compared to F1 during entrainment. To examine the effects of these transitions the number of paddle deflections was counted for 5 minute intervals for the 1/2 hour before the transition, for the 1/2 hour of the transition and for 1/2 hour after the transition. A mean was calculated for each 5 minute interval over the 5 days of recording. During the previous entrainment experiments the light was turned on and off. By providing a gradual increase and decrease in light intensity a more natural condition was simulated. When the light was turned

Figure 30. Entrainment with simulated sunrise and sunset, F5. A. Raw data. Number of paddle deflections is plotted as function of time in hours. Alternating black and open bar at top of graph is LD 12:12. B. Autocorrelation function. Correlation coefficient is plotted as function of lags. Confidence intervals for 0 appear in lower right hand corner. Function does not oscillate reflecting instability of entrainment. C. Spectral analysis. Normalized spectrum is plotted as function of frequency (1/period in hours). Peak at 1/24 hours indicates presence of 24 hour periodicity.

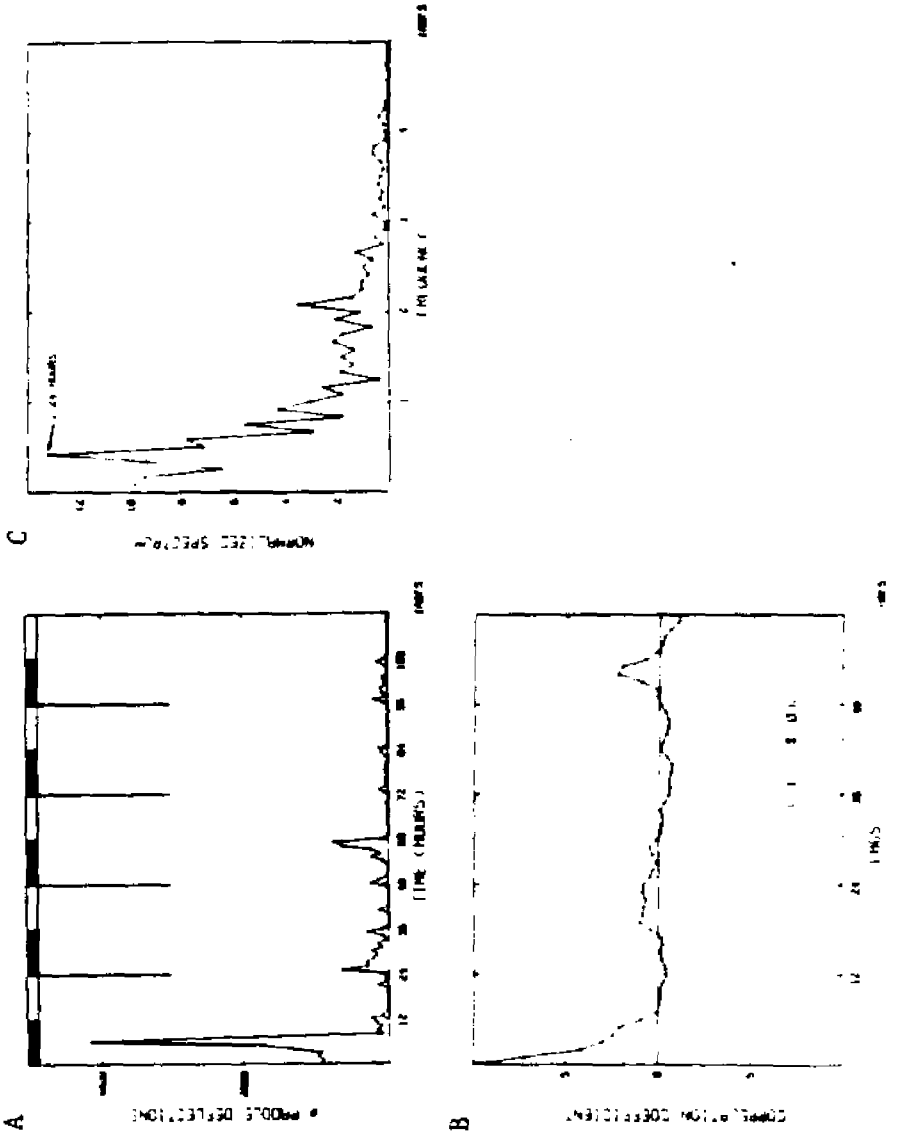

A
B
C

Figure 31. Entrainment with simulated sunrise and sunset, F6. A. Raw data. Number of paddle deflections is plotted as function of time in hours. Alternating black and open bar at top of graph is LD 12:12. B. Autocorrelation function. Correlation coefficient is plotted as function of lags. Confidence intervals for 0 appear in lower right hand corner. Function does not oscillate reflecting instability of entrainment. C. Spectral analysis. Normalized spectrum is plotted as function of frequency (1/period in hours). Peak at 1/24 hours indicates presence of 24 hour periodicity. Peak at 1/120 hours reflects decreasing trend.

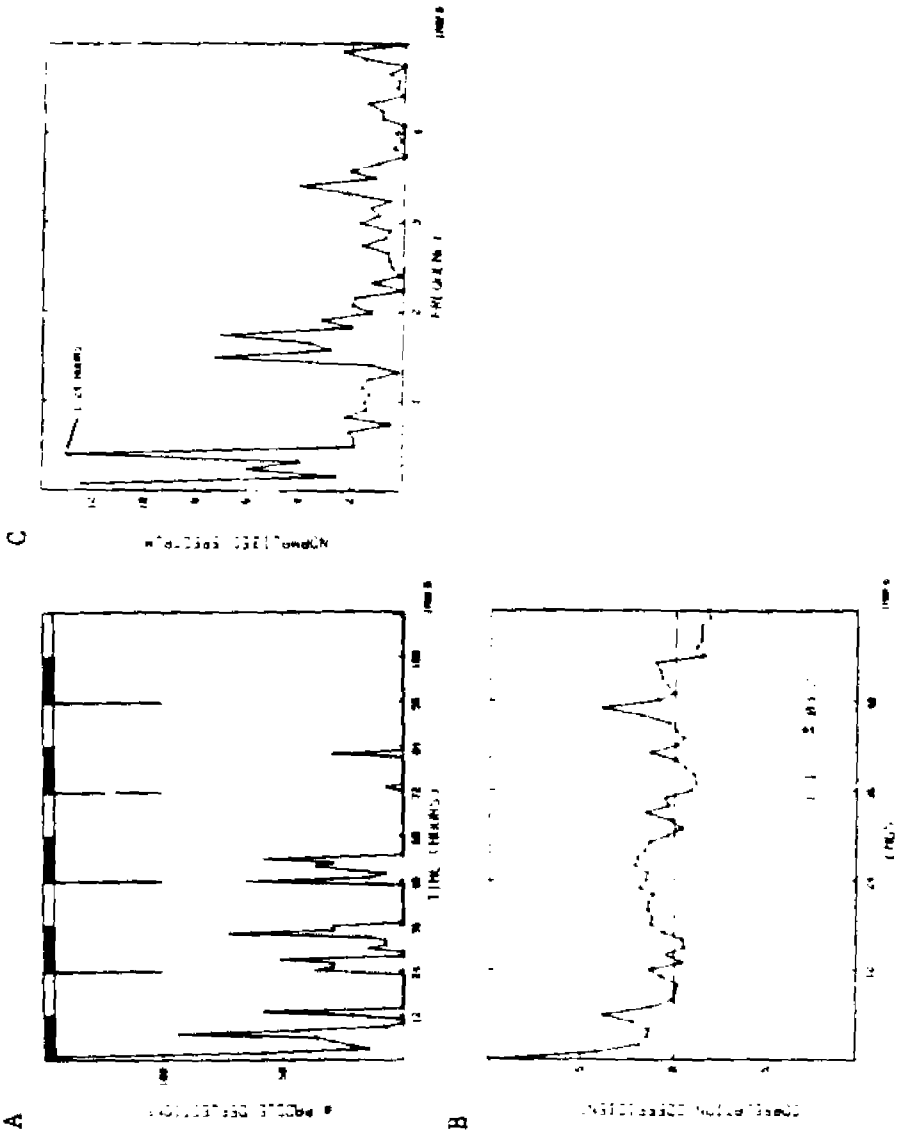
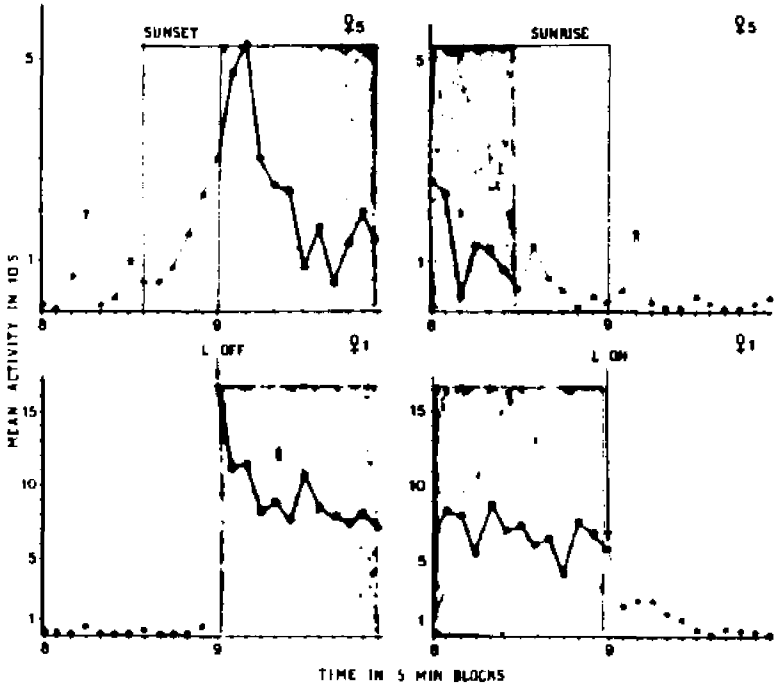

C
A
B

Figure 32. Sunrise-sunset transition (F5) compared with abrupt light-on, light-off of LD 12:12 cycle (F1). Top two graphs are transition data for F5. Dotted area is sunset on left graph, sunrise on right graph. Shaded area is dark period. Open area is light period. Bottom two graphs are abrupt light-off data (left graph) and light-on data (right graph). Shaded area is dark period. Open area is light period. Arrows indicate light-off and light-on. For all graphs mean number paddle deflections plotted as function of 5 min blocks. When light went off abruptly, (lower left graph) sudden increase in activity occurred for F1. When light was gradually dimmed (upper left graph) F5 showed gradual increase in activity over 30 min period. Note peak in activity occurred 10 min after sunset. With abrupt light-on (lower right graph), F1 showed sudden drop during first 5 min of light-on. Activity dropped to 0 during subsequent 25 min. For F5 exposed to sunrise (upper right graph) activity had begun to decrease before transition. Note increase in activity in second 5 min block followed by decrease. Gradual decrease in activity level was not found.


SUNSET
♀5
SUNRISE
♀5
L OFF
♀1
L ON
♀1
MEAN ACTIVITY IN 10 S
TIME IN 5 MIN BLOCKS

off abruptly, there was a sudden increase in the amount of activity. If this is compared with the response of F5 to sunset, there was a gradual increase in activity over the 30 minute period. A peak in activity was reached 10 minutes after sunset. F6 did not show the same gradual increase. Its activity increased when the light began to dim for the first 10 minutes, dropped to zero for the next 10 minutes and finally began to increase again for the remaining 10 minutes of sunset.

When the light was abruptly turned on, F1 responded with a drop in activity from 60 to 20 deflections for the first 5 minutes. Its activity continued to decline until it reached zero. For F5, activity began to decrease before sunrise. There was an increase in activity in the second 5 minute period followed by a decrease. There was a small burst of activity after sunrise and the pattern of activity was more variable than that of F1. F6 showed no obvious pattern during sunrise although there was a small burst of activity during the first 5 minutes of sunrise.

Only two fish were examined under transition conditions. Since their responses were not similar, a clear cut conclusion regarding the effects of dawn and dusk on the pattern of activity was not possible. The abrupt onset of activity at

light off was found in all fish for most days during entrainment to an LD cycle. F5 showed the more gradual response to the sunset transition. The reponses to light on and sunrise are less obvious. This was not surprising since the criteria for the onset of a behavior is generally more reliable than its cessation (Aschoff, 1960).

Experiment 1/Part B

Results

Experiment 1/Part B examined the effects of a square wave temperature cycle on the fish's locomotor activity. It consisted of an undisturbed free run under constant darkness and an entrainment.

Undisturbed free run. Figures 33A & 34A show the raw data for both fish. Only four days of the 19 day experiment are shown because locomotor activity for both fish ceased almost completely after a few days. Only the activity during the days graphed were subjected to statistical analysis. Fish F7 was more active than F8. No periodicity was detectable by visual inspection of the data. The autocorrelation functions for both fish are presented in Figures 33B & 34B. No oscillation was present in either function, indicating the

Figure 33. Undisturbed free run, F7. A. Raw data. Black bar at top of graph is constant darkness (0.7 lux). Number of paddle deflections is plotted as function of time in hours. No obvious 24 hour rhythmicity detected. Note decreasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. No oscillation present. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). No peak at 1/24 hours (see arrow) confirming absence of 24 hour periodicity. D. White noise test. Solid line is integrated spectrum of data. Dotted line is expected integrated spectrum for random data. Points between brackets are significantly different from random data. No 24 hour periodicity in constant conditions. Lack of randomness is due to decreasing trend.

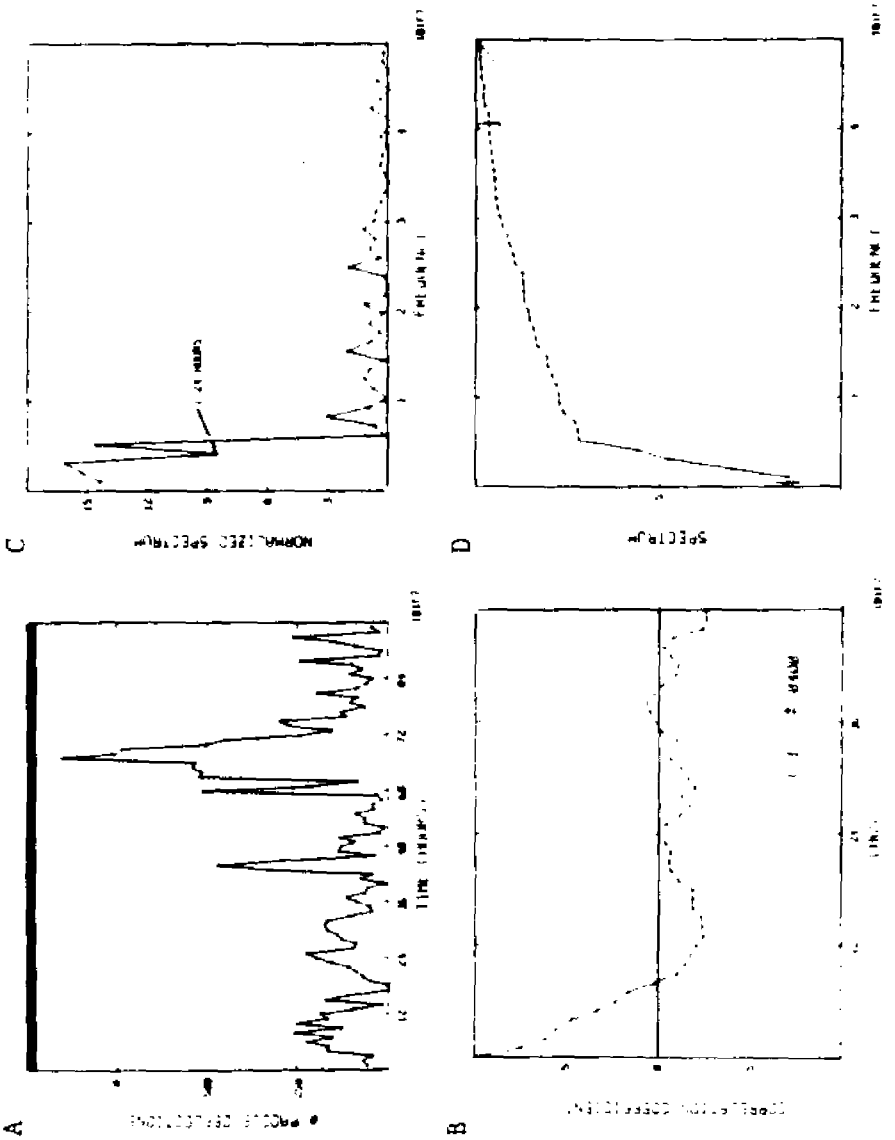
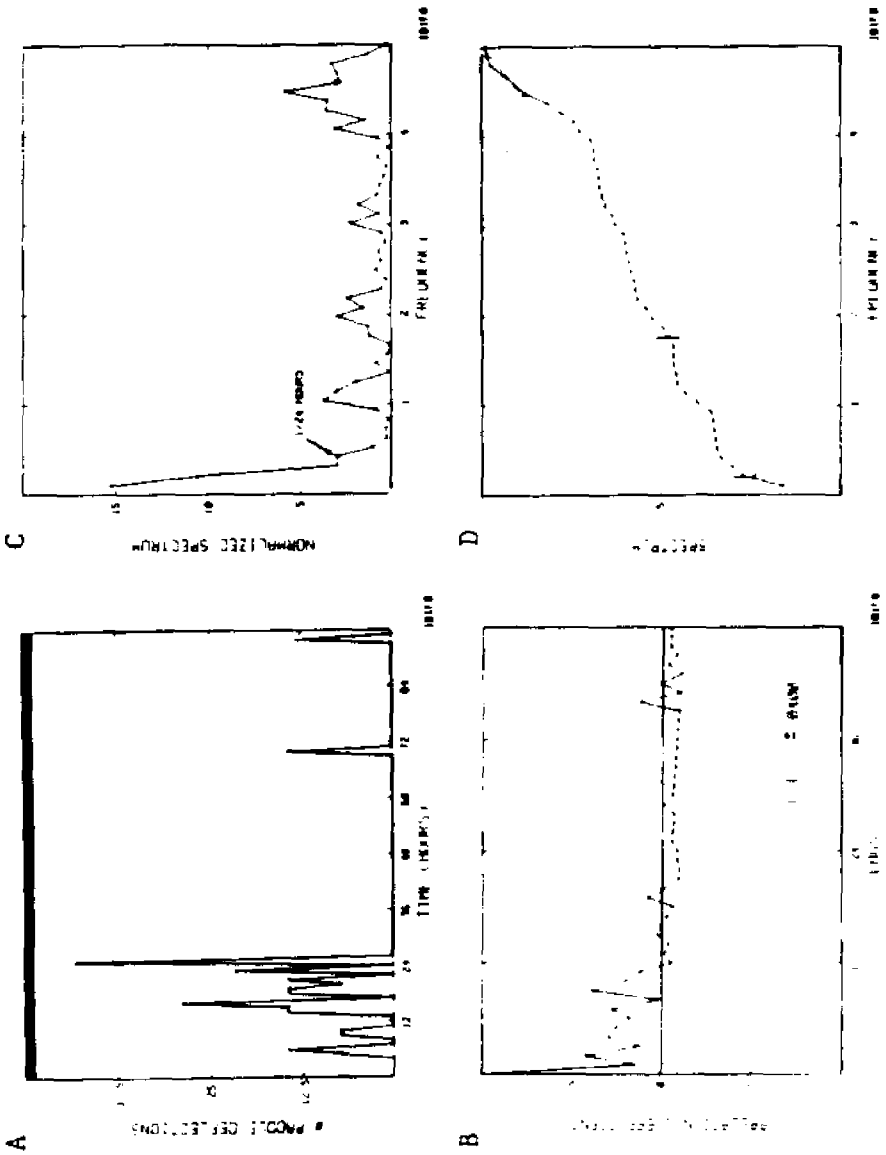

A
B
C
D

Figure 34. Undisturbed free run, F8. A. Raw data. Black bar at top of graph is constant darkness (0.7 lux). Number of paddle deflections is plotted as function of time in hours. No obvious 24 hour rhythmicity detected. Note decreasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. No oscillation present. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). No peak at 1/24 hours (see arrow) confirming absence of 24 hour periodicity. Peak at 1/96 reflects decreasing trend. D. White noise test. Solid line is integrated spectrum of data. Dotted line is expected integrated spectrum for random data. Points between brackets are significantly different from random data. No 24 hour periodicity in constant conditions. Lack of randomness is due to decreasing trend.

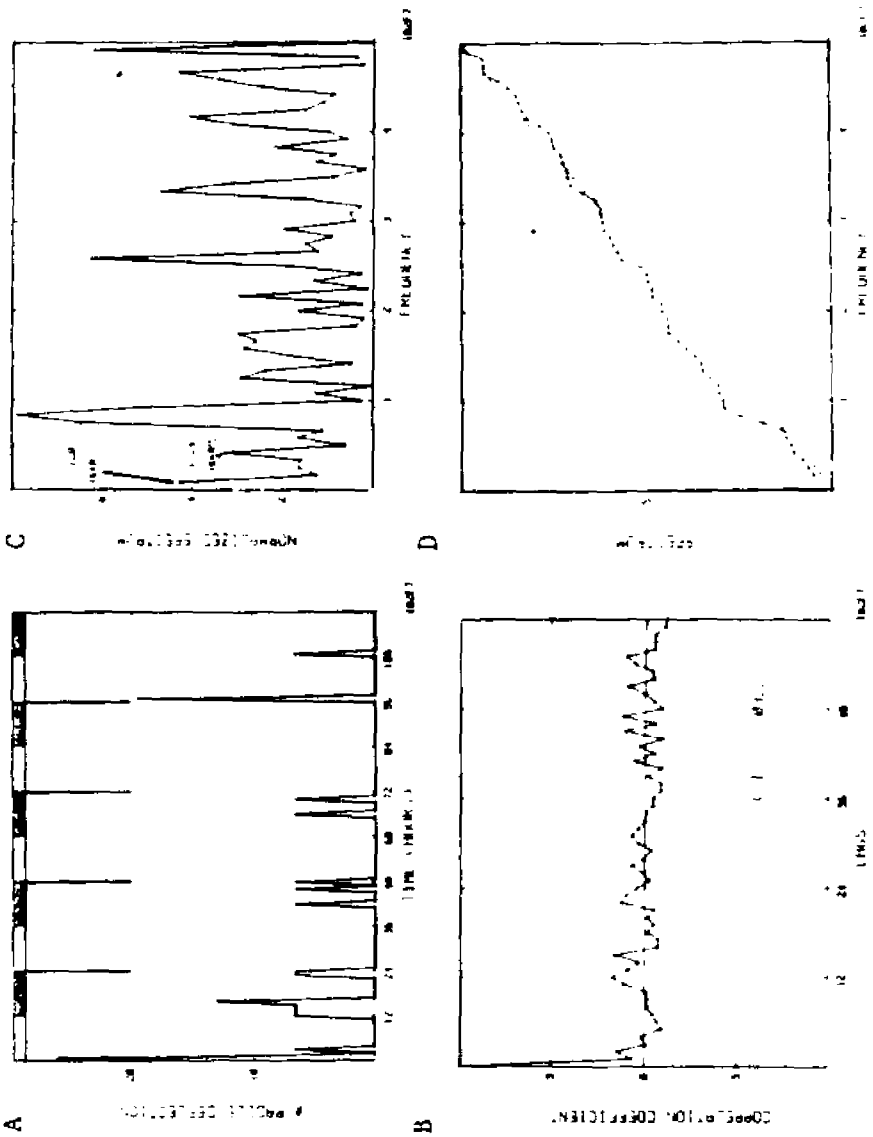

A
B
C
D

absence of periodicity. Spectral analysis confirmed the lack of rhythmic components in the data (Figure 33C & 34C). There was no peak at 1/24 hours for either fish and there were no other reliable rhythmic components. The white noise tests indicated that the data were not random (Figure 33D & 34D). Based on the results of the spectral analyses the lack of randomness was due to the decreasing trend in activity over time. Again in constant conditions, locomotor activity showed no periodicity.

Entrainment. At the end the 19 days in constant darkness a square wave temperature cycle was introduced. The fish were exposed to 12 hours of 25^{o}C alternated with 12 hours of 30^{o}C. Only one of the two fish, F7, was active enough to produce data for analysis. Fish F8 was inactive for several consecutive days. Figure 35A shows the raw data for F7. No consistent pattern or trends could be detected.

The autocorrelation function (Figure 35B) showed no oscillation, and spectral analysis confirmed the absence of a 24 hour periodicity (Figure 35C). There were no reliable rhythmic components appearing from segment to segment when the data were broken into 48 hour segments.

Figure 35. Entrainment to warm:cool 12:12 cycle, F7. A. Raw data. Number of paddle deflections is plotted as function of time in hours. Alternating open and shaded bar at top of graph is warm:cool 12:12. B. Autocorrelation function. Correlation coefficient is plotted as function of lags. Confidence intervals for 0 appear in lower right hand corner. No oscillation present. C. Spectral analysis. Normalized spectrum is plotted as function of frequency (1/period in hours). No peak at 1/24 hours indicating absense of 24 hour periodicity. D. White noise test. Integrated spectrum is plotted as a function of frequency (1/period in hours). Dotted line is expected integrated spectrum for random data. Solid line is integrated spectrum of data. No points were significantly different from random. Activity was arhythmic.


A
B
C
D

None of the points in the white noise test were significantly different from values expected for random data (Figure 35D). Locomotor activity for F7 remained arhythmic and random during exposure to a square wave temperature cycle. Fish F8 was inactive during exposure to the temperature cycle suggesting that this environmental cue was not powerful enough to restore its locomotor activity. The temperature cycle, using a range of 25°C to 30°C, as measured under field conditions (Moller et al., 1979) was not an effective entraining cue for locomotor activity in these two fish under the present experimental conditions.

Discussion

The results of this experiment demonstrated the effectiveness of changes in a light-dark cycle as entraining cues for locomotor activity in G. petersii. However, light cannot qualify as a zeitgeber for locomotor activity since a circadian activity rhythm could not be demonstrated. Of the six fish in constant darkness (8 manipulations), none displayed a free running circadian activity rhythm. The two fish tested in constant dim light also did not show a free running circadian activity rhythm.

The second environmental cue, temperature, was neither an entraining cue for locomotor activity nor was it powerful enough to restore the damped out activity of the fish.

Other points regarding the locomotor activity of G. petersii can be made. The activity patterns found were not stationary. The most dramatic change was a general decrease in the amount of activity over time in all conditions. A decline in activity in constant darkness and constant dim light was not surprising. Rhythms do decrease in amplitude in constant conditions, especially in constant light (Bunning, 1973). However, this decrease in amplitude occurred for both fish during the first entrainment (Figures 14A & 15A). This decline in amplitude could be related to the highly restricted and monotonous environment in which the fish were studied. They were housed in small aquaria (20.9 liter) with only a shelter tube, gravel, the activity paddles and electrodes present. Lissmann and Schwassmann (1965) studied rhythms in the gymnotid, Gymnorhamphichthys hypostomas, and found some waning of rhythmic behavior and more intermittent behavior patterns under laboratory conditions as compared with observations in the natural environment. These differences were attributed to the artificial environment and isolation from conspecifics.

It is also interesting to note that all of the fish used in this experiment died within 3 months following the end of their exposure to this environment and the experimental manipulations. The unnatural testing conditions may have been a significant stressor. Pittendrigh (1974) has pointed out that "organisms as innately oscillatory systems function most effectively when they are close to resonance" (p 456), that is, when they are driven by environmental cycles with periods very close to the natural periods of the system oscillators. It has been demonstrated that manipulation of the rhythmic organization of organisms can be harmful (Aschoff, 1981; Daan & Aschoff, 1982). Any manipulations, entrainment, phase shifting or constant conditions, disturb a circa-rhythm system. For example, weekly 6 hour phase shifts of the LD cycle reduced the life expectancy of blowflies 25% as compared with that of flies kept under normal LD cycles (Aschoff, Saint Paul & Wever, 1971).

Since the demonstration of free running rhythms in fish is difficult (see General Discussion below) (Eriksson & van Veen, 1980), some factors to optimize the conditions under which a circadian activity rhythm and electric organ discharge rhythm might be demonstrated, should be discussed.

One important factor to consider is the level of light used during constant darkness. An illumination of 0.7 lux was used for the six constant dark manipulations in this experiment. Lissmann & Schwassmann (1965) used a light intensity of less than 0.5 lux and found circadian activity and electric organ discharge rate rhythms in gymnotids. It is commonly found that constant light will inhibit a free running rhythm (Aschoff, 1960, 1965, 1981) and the level of light can be surprisingly low. An extreme example has been reported for the pigeon. Miselis & Walcott (1970) were able to demonstrate circadian activity at less than 0.5 lux, the same level used by Lissmann & Schwassmann (1965). The intensity of light at which rhythmicity disappears is also lower for nocturnal organisms as compared with diurnal organisms (Aschoff, 1981). In this experiment the constant darkness light intensity of 0.7 lux may have been too bright for G. petersii. Ecological data from the Swashi River, Lake Kainji, Nigeria report light levels less than 0.03 lux at 0.5 meters depth (Moller et al., 1979). Subsequent constant darkness manipulations (Experiments 2 and 3) used lower light intensities.

Some organisms will free run only in the presence of conspecifics (Enright, 1981a). For example, shoals of whitefish will free run with regard to locomotor activity when transferred from an LD 12:12 schedule to DD. However,

isolated, entrained whitefish will become arhythmic in DD (Muller, 1978). G. petersii are also social fish and they may become arhythmic when isolated in DD. This factor was considered in Experiment 3.

Finally, the measurement technique used can play a role in whether or not a circadian rhythm will be detected. For, example a running wheel for a rodent may be a more effective activity recording device than a stabilimeter cage (Enright, 1981a). Plastic activity paddles were chosen for a variety of reasons including the lack of electrical noise, simplicity of design and ease of production, but it is possible that an array of lights and photocells would be a more sensitive measuring technique. If reduction of the light level during constant darkness and the introduction of conspecifics are still not enough to demonstrate free running rhythms in G. petersii a different measurement technique including the use of photocells or a self-selection procedure making use of the fishes' photophobic response (Heppner & Farner, 1969; Colgan, 1975) would be a next step.

Experiment 2

Experiment 1 established a relation between locomotor activity and light, and locomotor activity and temperature. Again, using the standard procedures for demonstrating the entraining abilities of an environmental cue (see Experiment 1) the relationship between light and electric organ discharge rate was examined. An attempt was also made to assess the degree of correspondence between electric organ discharge rate and locomotor activity.

Method

Subjects. Two fish were used in this experiment. F18 weighed 40 g and measured 20 cm in length. F19 weighed 70 g and measured 19 cm.

Apparatus. Two pairs of recording electrodes plus one ground electrode were placed in each experimental tank. The electric organ discharges were differentially amplified. To record electric organ discharge rate and general locomotor activity simultaneously, an Apple II Plus computer with a timer interface card and pulse former card were used. Two Schmidt triggering chips (74LS221) were used as pulse formers for both electric organ discharges and paddle deflections.

Electric organ discharges were differentially amplified (355 chips) before entering the Schmidt trigger. The paddle signals were debounced (MC14490) before entering the second Schmidt trigger. (See Appendix 3). The four outputs of the pulse former card were counted by separate timers (8253 chips) on a second interface card. The capacity of the timers was 2 MHz thus capturing a maximum discharge rate of 140 Hz (See Appendix 4). To test the apparatus, known frequencies produced by a function generator were fed into the differential amplifiers and then into the Schmidt triggers and timers. The frequencies used were 1, 2, 5, 10, 20, 50, 100, and 200 Hz. Figure 36 shows a linear relation between the function generator input and the computer count. Two electric organ discharge totals and two activity totals were recorded every minute. Data were stored in the Apple and saved on 5 1/4 inch floppy disks (Figure 37).

Procedure. This experiment consisted of 4 manipulations.

i. Undisturbed free run: The fish were maintained in constant conditions with respect to light (DD at 0.09 lux), temperature ($26^{O}C$ + $.5^{O}$), pH (6-7) and conductivity (80-150 µs/cm) to determine if they were able to maintain a rhythm in the absence of time giving cues.

Figure 36. Electric organ discharge recording apparatus: Calibration. Signal generator output in hertz (Hz) appears on abcissa. Apple computer count in Hz appears on ordinate. Linear relationship between signal generator output and computer count was found.

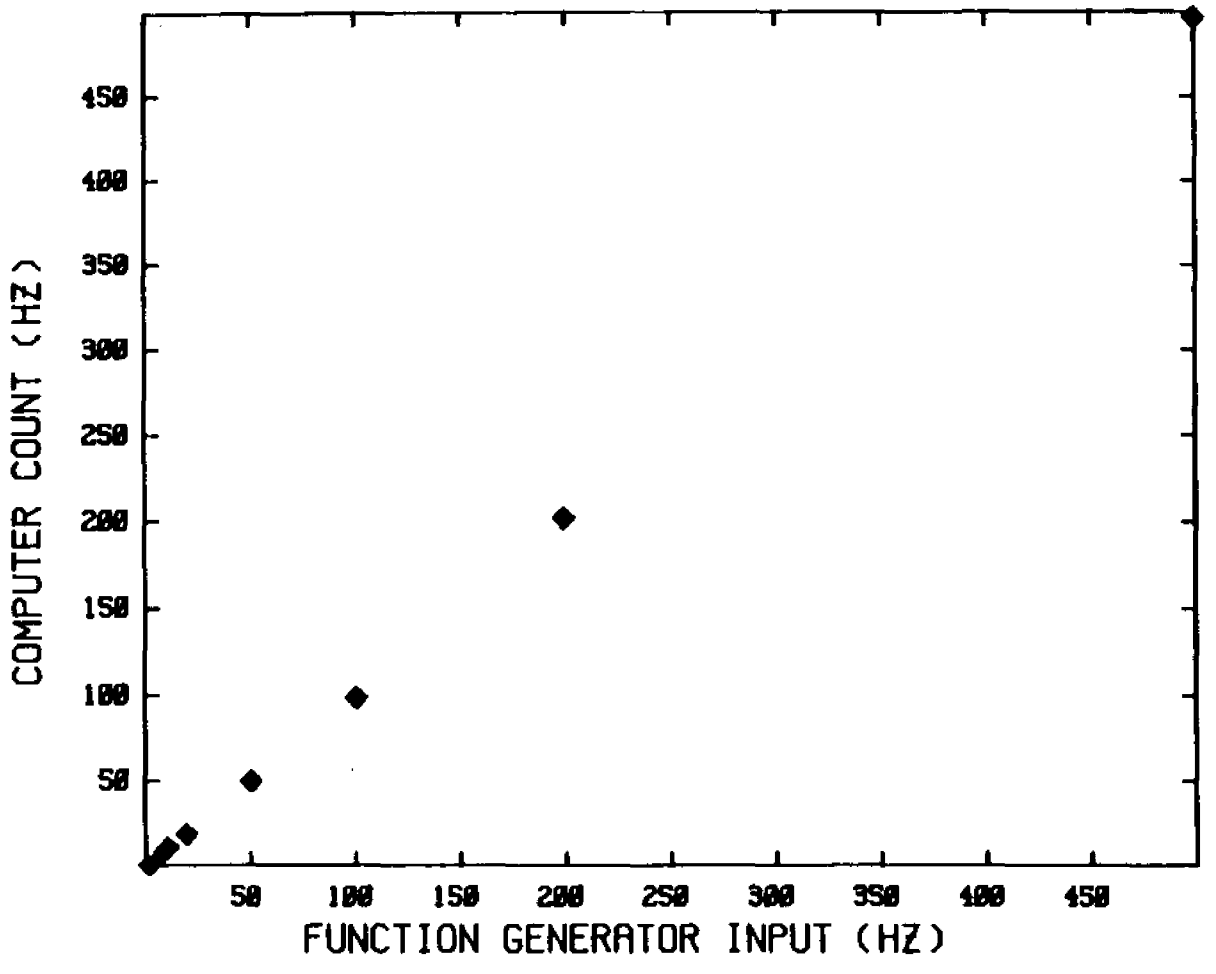
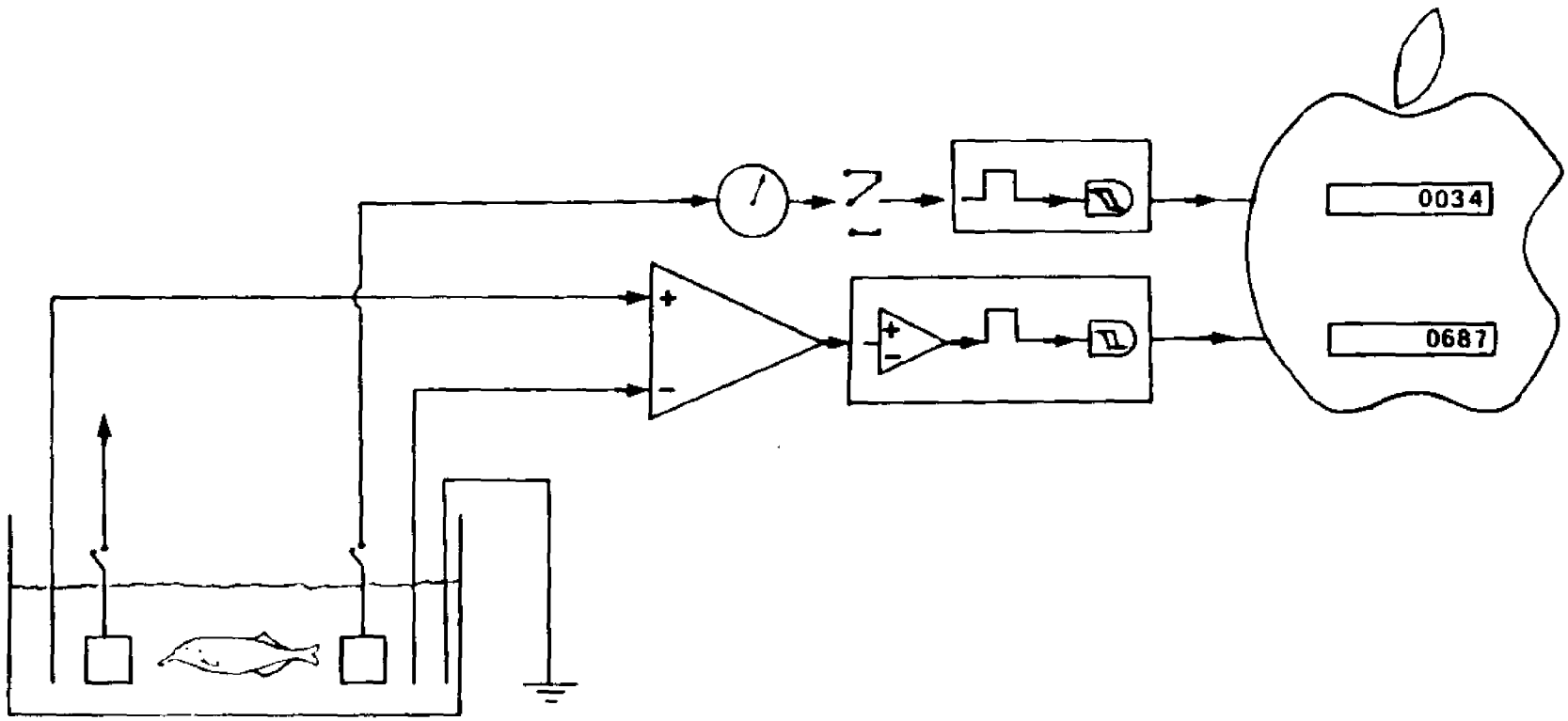

COMPUTER COUNT (HZ)
450
400
350
300
250
200
150
100
50
50
100
150
200
250
300
350
400
450
FUNCTION GENERATOR INPUT (HZ)

Figure 37. Schematic of Experiment 2 apparatus. Figure is not drawn to scale.


0034
0687

ii. Entrainment: An LD 12:12 cycle was imposed while all other environmental factors remained constant to establish entrainment of electric organ discharge rate and locomotor activity and test for period control.

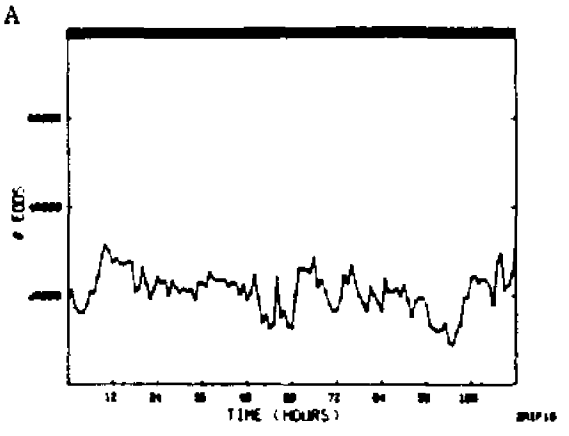
iii. Phase shift of zeitgeber: The LD 12:12 cycle was shifted 6 hours (delayed) to test for phase control of the LD cycle over electric organ discharge rate and locomotor activity.

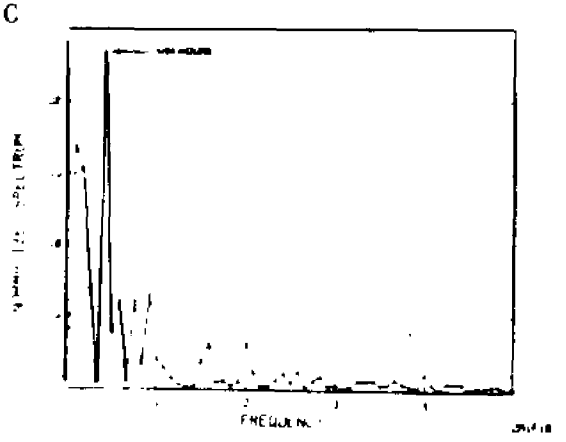
iv. Post-entrainment free run: Light was again held constant (DD at 0.09 lux) as were all other environmental cues.

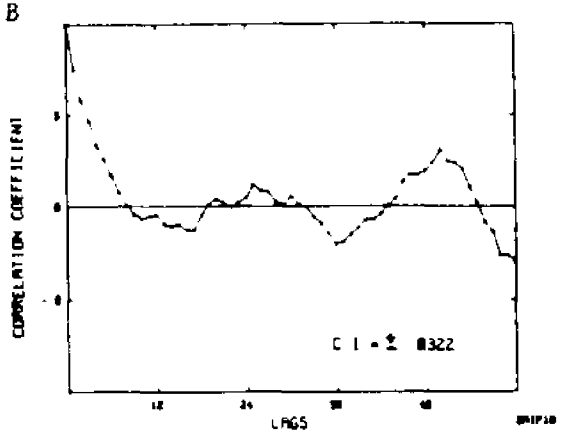
Results

Undisturbed free run. Figures 38 & 40 show the electric organ discharge data and locomotor activity data for F18. Visual inspection of the raw data (Figure 38A) suggested the presence of periodicity as well as a decreasing trend in electric organ discharge rate over time. The autocorrelation function, Figure 38B, showed an oscillation though the number of lags from peak to peak and trough to trough was not consistantly 24. The spectral analysis, Figure 38C, showed the largest peak occurring at 1/24 hours, thus suggesting the

Figure 38. Undisturbed free run, F18. Electric organ discharge rate. A. Raw data. Black bar at top of graph is constant darkness. Number of electric organ discharges plotted as function of time in hours. No obvious 24 hour periodicity was detected. Note decreasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. Function oscillated but not with 24 lags peak to peak. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). Peak at 1/24 hours suggested presence of 24 hour periodicity. Peak at 1/120 hours confirmed decreasing trend.


A
TIME (HOURS)


C


B
LAGS

presence of 24 hour periodicity in electric organ discharge rate for F18. The spectral analysis also showed the decrease in electric organ discharge rate over time with two smaller peaks at 1/120 hours and 1/60 hours. To test the reliability of the peak at 1/24 hours, the data were broken into three segments and reanalyzed, Figure 39. The peak at 1/24 hours appeared in two of the three segments suggesting that the 24 hour rhythm was not a reliable component of the data.

When locomotor activity of the same fish was analyzed, no rhythmicity could be detected in the raw data though there seemed to be an increase in the amount of activity over time (Figure 40A). The autocorrelation function did not oscillate (Figure 40B) and there was no peak at 1/24 hours in the spectral analysis (Figure 40C). The peak at 1/120 hours confirmed the increasing trend in locomotor activity.

For F18, under constant conditions, electric organ discharge rate showed an unreliable 24 hour periodicity. Locomotor activity did not show 24 hour periodicity.

The second fish under constant conditions, F19, became arhythmic with respect to both electric organ discharge rate and locomotor activity. Visual inspection of the raw data revealed no pattern in the electric organ discharge rate

Figure 39. Undisturbed free run, F18. Electric organ discharge rate. Spectral analysis of non-overlapping segments of data. Normalized spectrum plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. A. First 48 hour segment. B. Second 48 hour segment. C. Twenty-four hour segment. Peak at 1/24 hours appeared in two of three segments indicating unreliability of 24 hour periodicity of electric organ discharge rate in constant conditions.

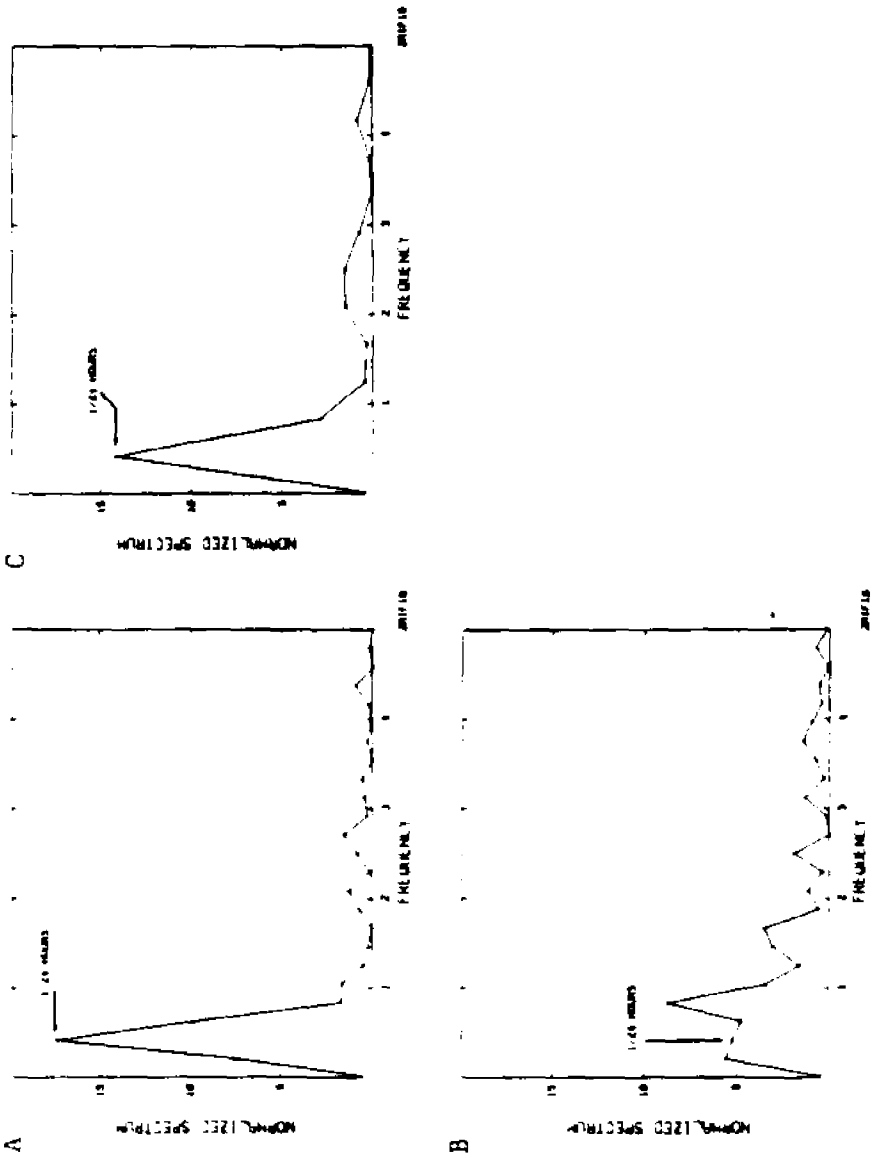
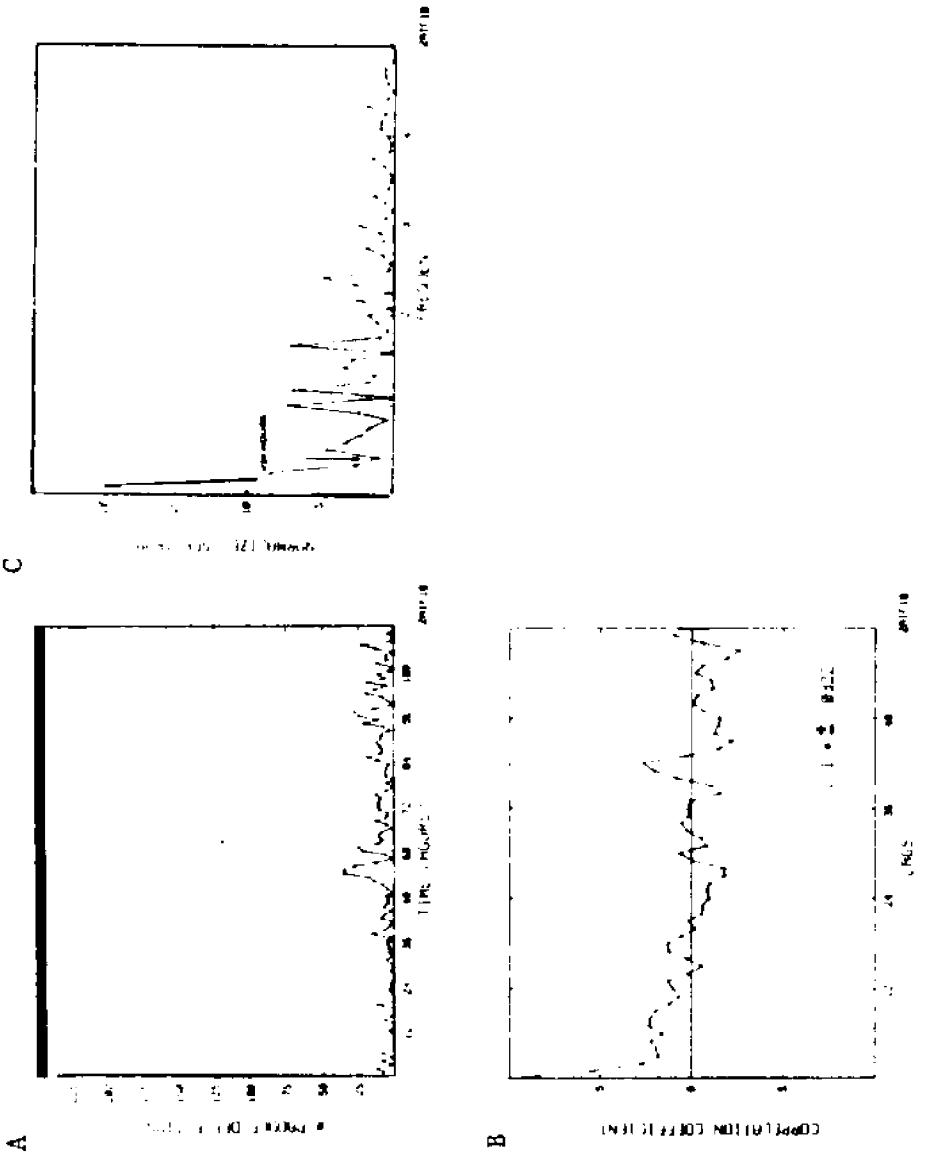

C
A
B

Figure 40. Undisturbed free run, F18. Locomotor activity. A. Raw data. Black bar at top of graph is constant darkness. Number of paddle deflections plotted as function of time in hours. No obvious 24 hour periodicity. Note increasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. Function did not oscillate. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). No peak at 1/24 hours. Peak at 1/120 hours confirmed increase in locomotor activity No periodicity of locomotor activity in constant conditions.


A
B
C

though there was a decrease in rate over time (Figure 41A). The autocorrelation function (Figure 41B) and the spectral analysis (Figure 41C) confirmed the absence of rhythmicity.

Locomotor activity showed similar results. There was no periodicity in the raw data (Figure 42A). A decreasing trend in the amount of locomotor activity was present. The autocorrelation function (Figure 42B) and spectral analysis (Figure 42C) confirmed the lack of periodicity.

Under constant conditions, locomotor activity became arhythmic for both fish, confirming the results of Experiment 1. Electric organ discharge rate was arhythmic for F19. For F18, the 24 hour periodicity of electric organ discharge rate was unreliable.

Entrainment. When 12 hours of darkness was alternated with 12 hours of light, both electric organ discharge rate and locomotor activity of F18 entrained. There were more electric organ discharges during darkness that during light (Figure 43A) confirming field and laboratory data (Moller et al., 1979; Bässler et al., 1979). When the number of electric organ discharges during the light period was examined, the raw data suggested there were more discharges during the second half of the light period as compared with the first half. A

Figure 41. Undisturbed free run, F19. Electric organ discharge rate. A. Raw data. Black bar at top of graph is constant darkness. Number of electric organ discharges plotted as function of time in hours. No obvious 24 hour periodicity was detected. Note decreasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. Function did not oscillate. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). Small peak at 1/24 hours was below criterion for analysis (see General Methods). No reliable 24 hour periodicity of electric organ discharge rate.

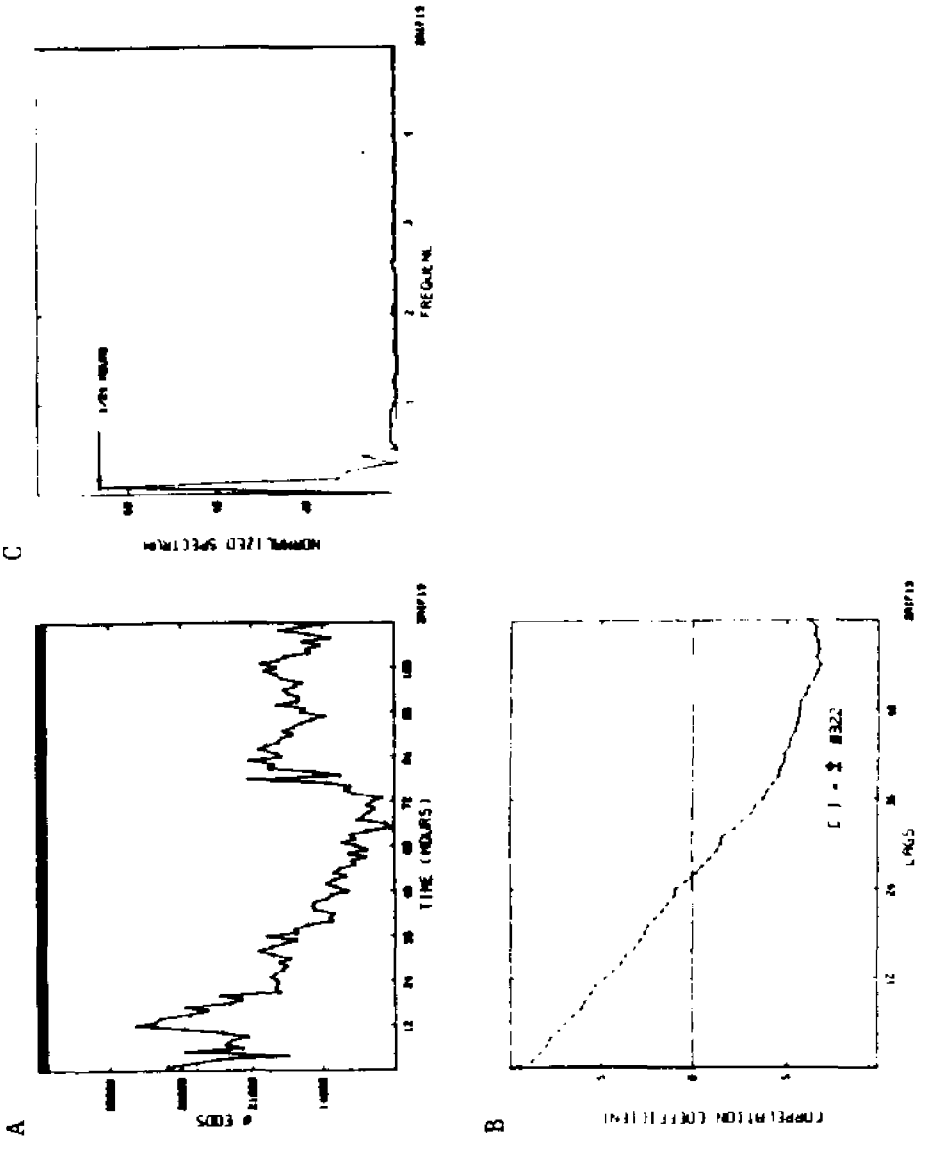

A
B
C
TIME (HOURS)
FREQUENCY

Figure 42. Undisturbed free run, F19. Locomotor activity. A. Raw data. Black bar at top of graph is constant darkness. Number of paddle deflections plotted as function of time in hours. No obvious 24 hour periodicity. Note decreasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. Function did not oscillate. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). No peak at 1/24 hours. Peak at 1/120 hours confirmed decrease in locomotor activity. No periodicity of locomotor activity in constant conditions.

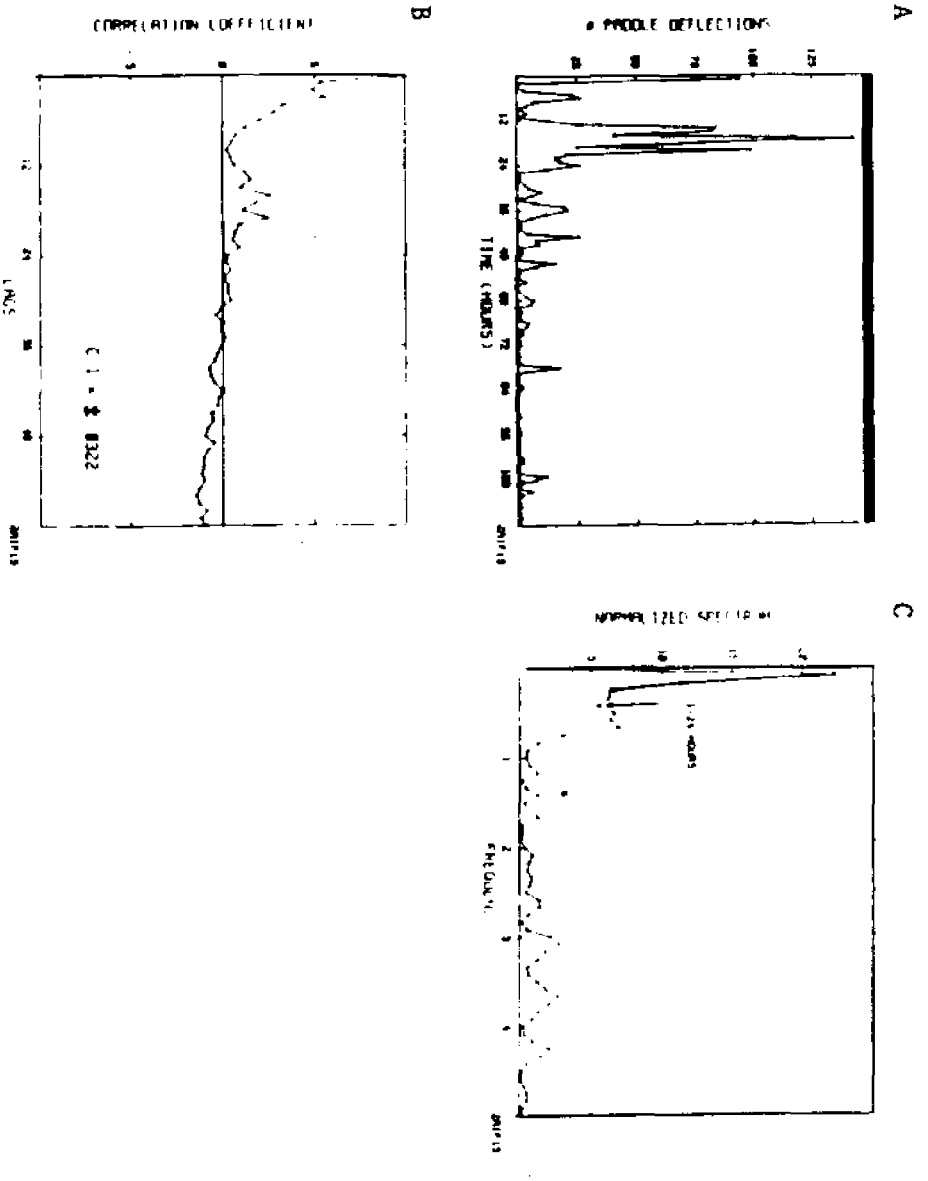
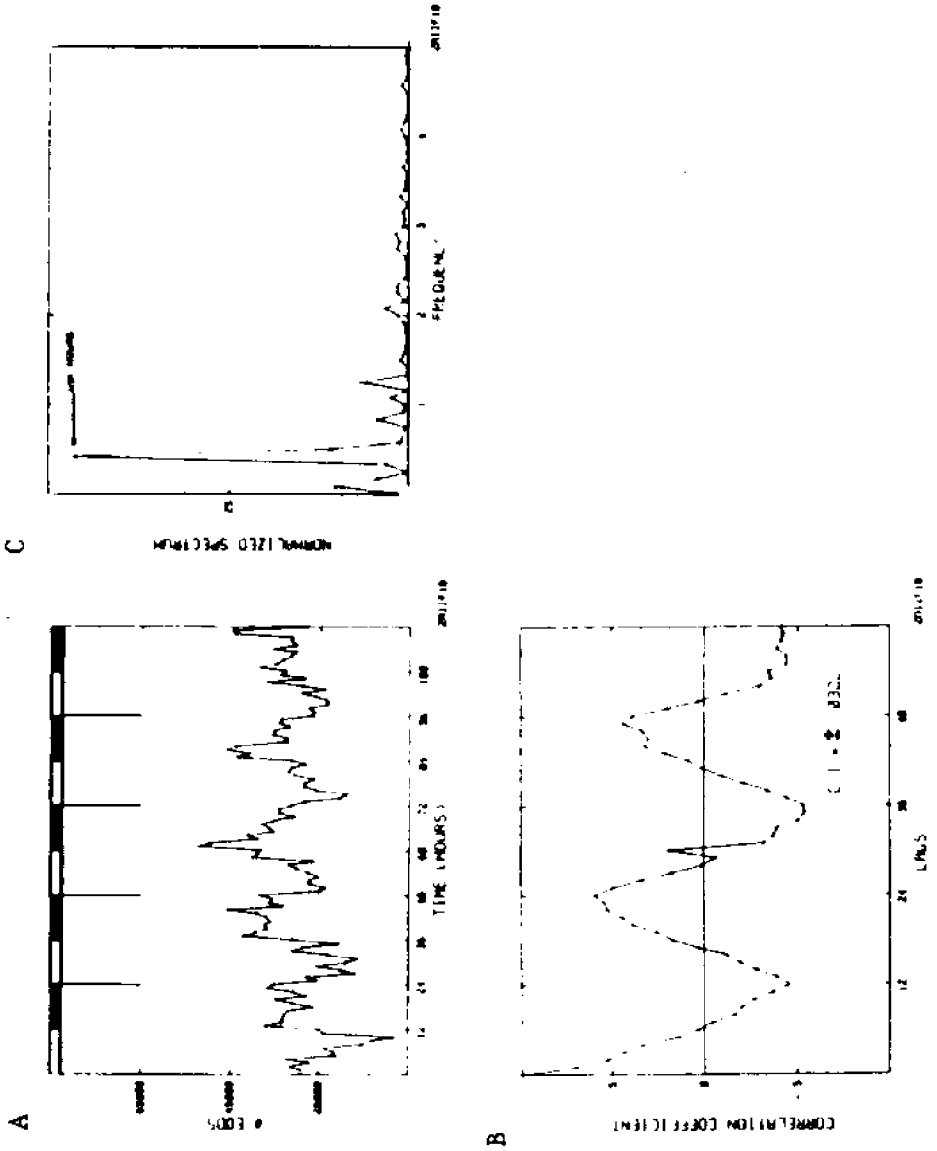

A
PADDLE DEFLECTIONS
TIME (HOURS)
B
CORRELATION COEFFICIENT
C

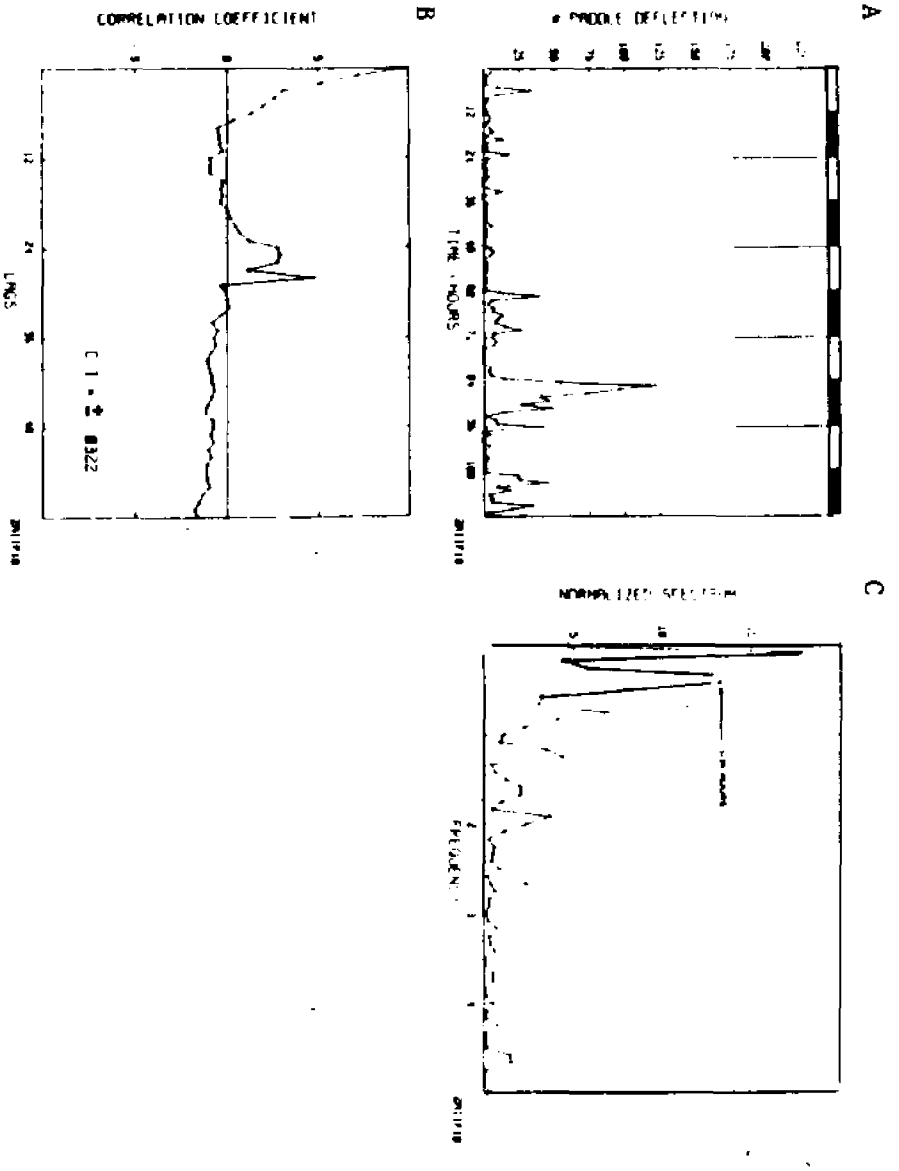
Figure 43. Entrainment, F18. Electric organ discharge rate. A. Raw data. Alternating black and open bar is LD 12:12. Number of electric organ discharges plotted as function of time in hours. More electric organ discharges occurred in dark period as compared with light period. Twenty-four hour periodicity of electric organ discharge rate was evident. Also note more electric organ discharges occurred in second half of light period as compared with first half of light period indicating presence of pre-emergence electric organ discharge rate increase. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. Note oscillation with 24 lags peak to peak. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). Largest peak occurred at 1/24 hours confirming 24 hour periodicity.


A
B
C
TIME (HOURS)
LAGS

Student's t test confirmed this observation ($t = 3.23$, $p < .05$, $df = 5$). At light on electric organ discharge rate decreased, it remained low for about 6 hours and then gradually increased. This result supports field reports of a pre-emergence electric organ discharge rate increase (Moller et al., 1979), independent of a temperature cue (see Background), among mormyrids. The electric organ discharge rate during the last hour of light was always greater than that during the first hour, after the first day of entrainment. There was also a burst in electric organ discharge rate when the light went off. The autocorrelation function (Figure 43B) and the spectral analysis (Figure 43C) confirmed the presence of a 24 hour periodicity.

Locomotor activity for F18 did not show the same pattern of entrainment. During the first 2 days of entrainment, there was more activity during the light period than during the dark period (Figure 44A) and there was no obvious response to the light going off. For days 3, 4, and 5 there was more locomotor activity during the dark period and a burst in activity occurred when the light went off. An increase in the amount of locomotor activity over time was evident. The autocorrelation function (Figure 44B) did not show an oscillation but the spectral analysis (Figure 44C) showed a peak at 1/24 hours, confirming the entrainment of locomotor activity to the LD cycle.

Figure 44. Entrainment, F18. Locomotor activity. A. Raw data. Alternating black and open bar is LD 12:12. Number of paddle deflections plotted as function of time in hours. First 2 days showed more activity during light period compared with dark period. More activity during dark period for remaining 3 days. Note increase in activity over time. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. Function did not oscillate. C. Spectral analysis. Normalized spectrum plotted as function frequency (1/period in hours). Largest peak occurred at 1/24 hours. Peak at 1/120 hours reflected increasing trend in locomotor activity. Autocorrelation function and spectral analysis reflect instability of enrainment of locomotor activity.


A
B
C
CORRELATION COEFFICIENT
LAGS
TIME, HOURS
FREQUENCY
8322

Electric organ discharge rate for F19 also entrained to the LD cycle (Figure 45A). When the light went off, there was an increase in electric organ discharge rate followed by a decline in rate for the rest of the dark period. This fish did not show the increase in electric organ discharge rate for the second of the light period that F18 did (t= 1.75, NS). The only day that clearly showed a higher electric organ discharge rate during the dark period was day 5. For days 2, 3, and 4, electric organ discharge rate dropped below that during the light period. The autocorrelation function (Figure 45B) confirmed the 24 hour cycle and the spectral analysis (Figure 45C) showed the largest peak occurring at 1/24 hours.

Locomotor activity of F19 did not entrain to the LD cycle. There appeared to be some pattern in the raw data, (Figure 46A), more activity at night for the first 3 days, though the autocorrelation function did not oscillate (Figure 46B) and there was no peak at 1/24 hours in the spectral analysis (Figure 46C).

In contrast to F18 whose electric organ discharge rate and locomotor activity entrained to the 24 hour LD cycle, only electric organ discharge rate for F19 entrained. Locomotor activity remained arhythmic.

Figure 45. Entrainment, F19. Electric organ discharge rate.

A. Raw data. Alternating black and open bar is LD 12:12. Number of electric organ discharges plotted as function of time in hours. More electric organ discharges occurred in dark period only on day 5. Note burst of electric organ discharges when light went off and then subsequent decline.

B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. Function oscillated with 24 lags peak to peak.

C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). Large peak at 1/24 hours confirmed entrainment of electric organ discharge rate to LD cycle.

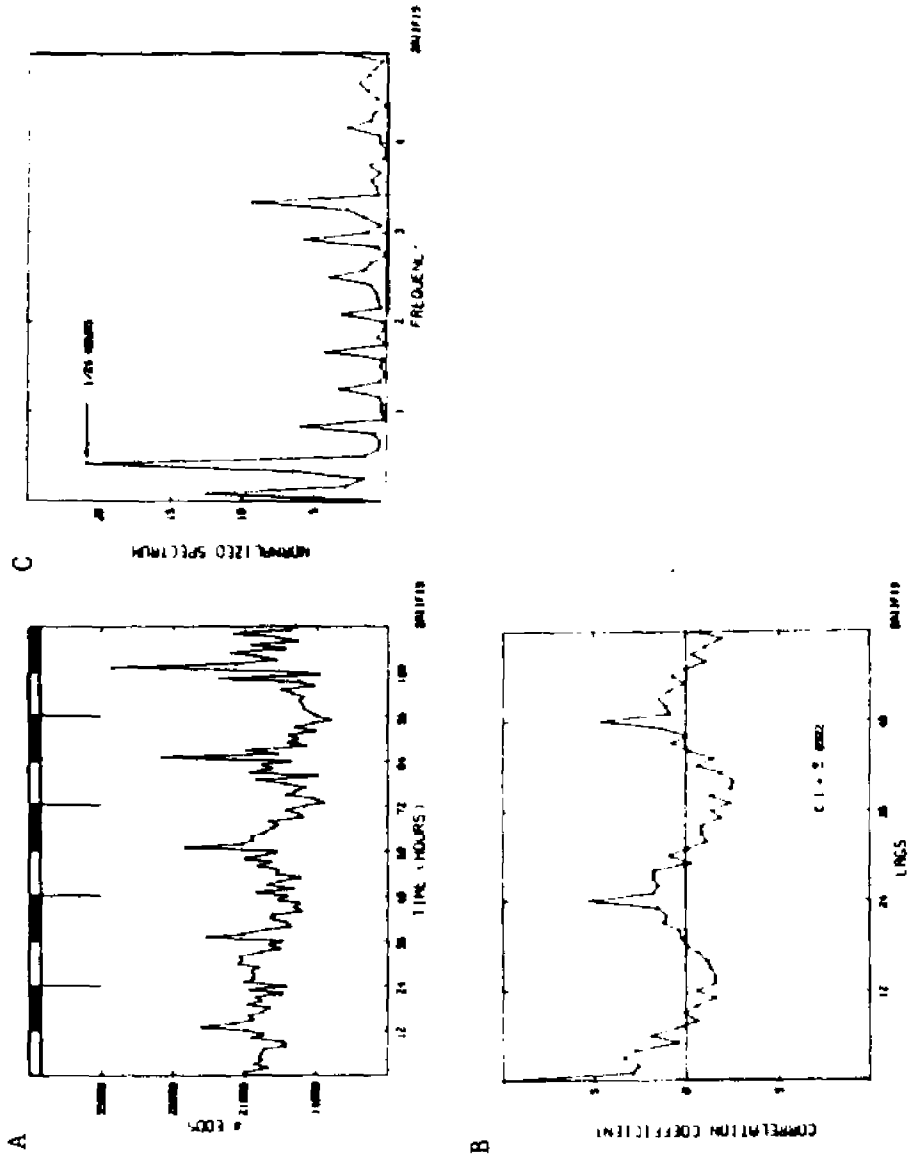
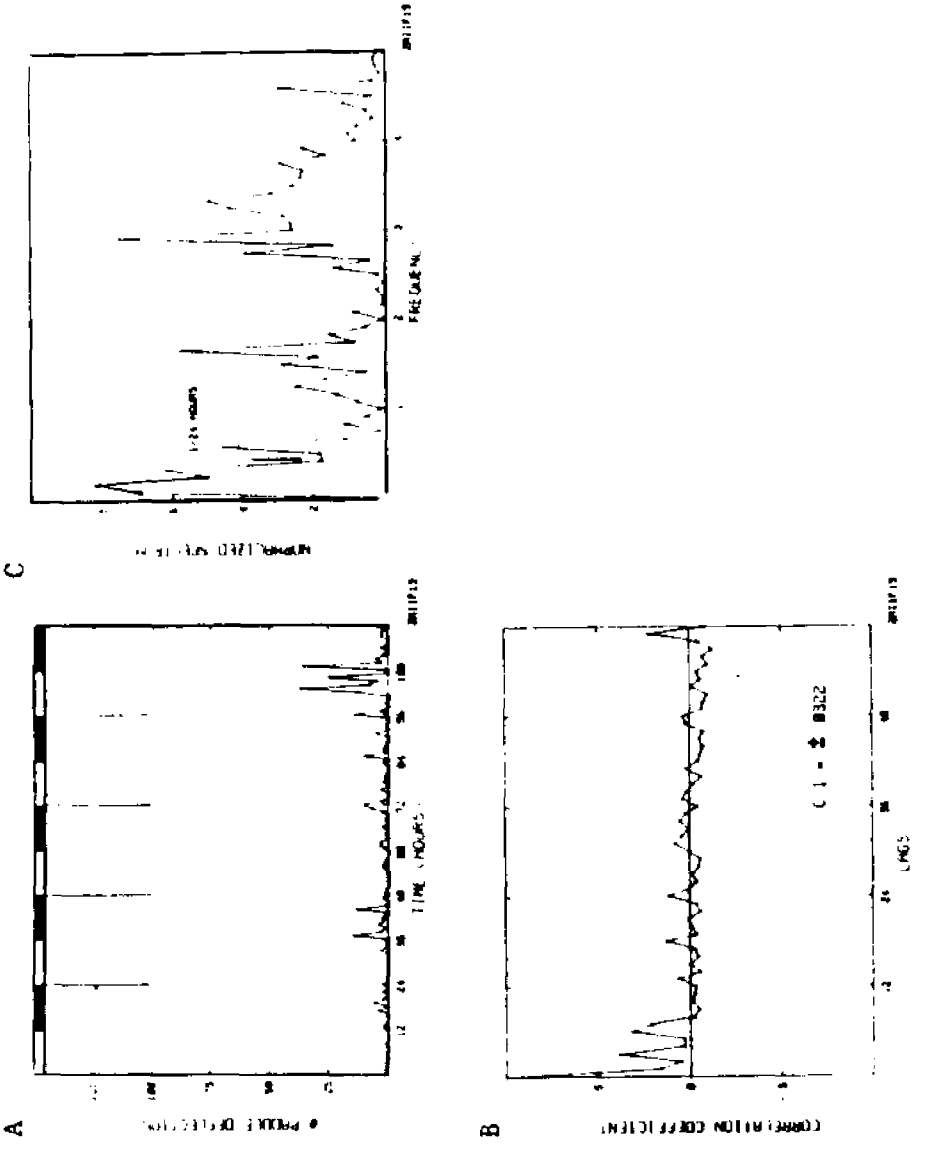

A
B
C
TIME (HOURS)
LAGS
FREQUENCY

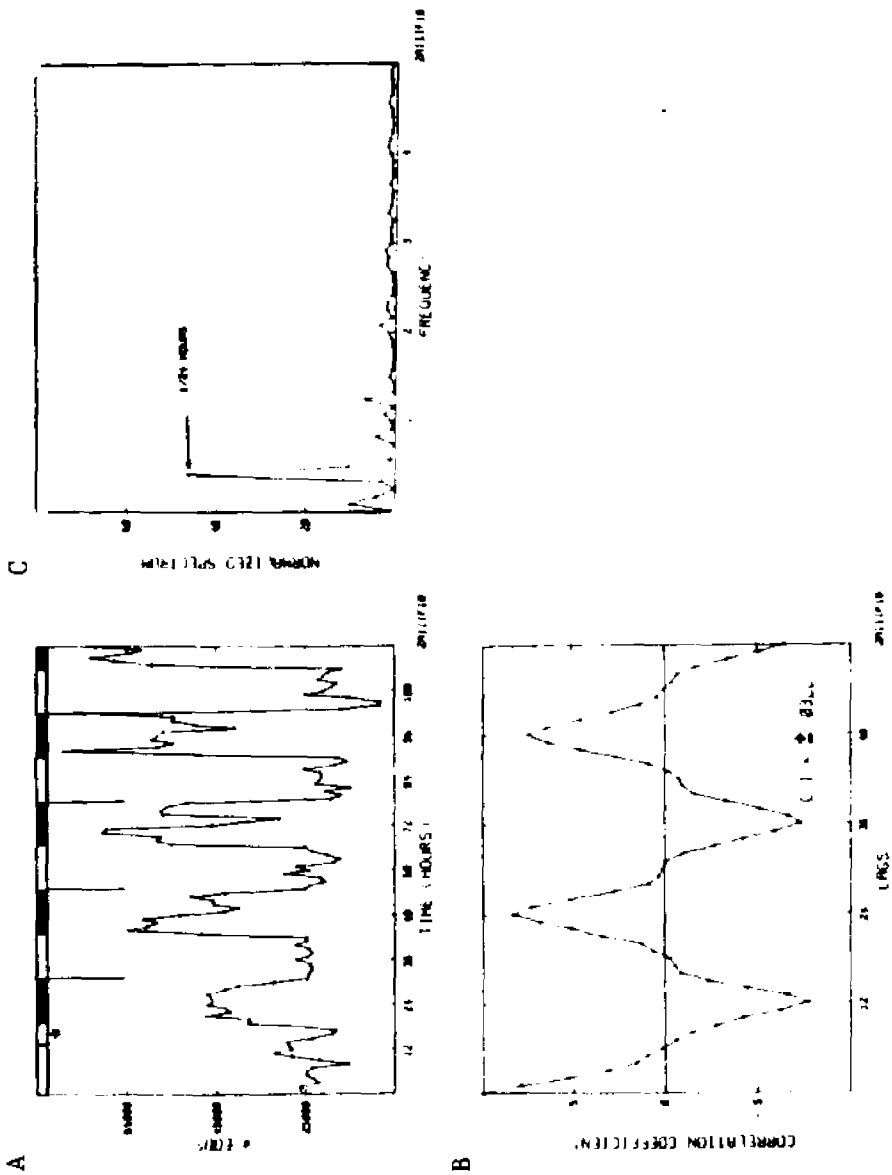
Figure 46. Entrainment, F19. Locomotor activity. A. Raw data. Alternating black and open bar is LD 12:12. Number of paddle deflections plotted as function of time in hours. Appeared to be more activity during darkness for first 3 days but pattern disappeared. Note increasing trend in activity. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. Function did not oscillate. C. Spectral analysis. Normalized spectrum plotted as function frequency (1/period in hours). No peak at 1/24 hours. Peak at 1/60 hours confirmed increasing trend. Locomotor activity of F19 did not entrain.


A
B
C
FREQUENCY

Phase shift of the zeitgeber. When the LD cycle was shifted 6 hours (light offset was delayed 6 hours relative to the last day of entrainment) the electric organ discharge rate of F18 shifted immediately (Figure 47A). During the last 12 hours of light, before the 6 hour delay began, electric organ discharge rate decreased for the first 8 hours and then showed an increase for the next 3 hours. There was a slight drop during the last hour of light. When the light did not go off electric organ discharge rate decreased. At light off, electric organ discharge rate increased and remained high for the 12 hours of darkness. When the light went on, electric organ discharge rate decreased and remained low for the 12 hours of light. The same pattern was seen for the other days. When the amount of activity during the second 6 hours was compared with the amount of activity during the first 6 hours of light, no significant difference was found ($t = .211$, NS). An increasing trend in electric organ discharge rate was seen. The autocorrelation function oscillated (Figure 47B) with 24 lags from peak to peak and trough to trough, corresponding to a period of 24 hours. The largest peak in the spectral analysis (Figure 47C) occurred at 1/24 hours.

Locomotor activity for F18 also phase shifted. When the 6 hour phase delay began there was a small increase in the hour when the light would have gone off under the previous

Figure 47. Phase shift of zeitgeber, F18. Electric organ discharge rate. A. Raw data. Alternating black and open bar is LD 12:12. * shows 6 hour phase delay. Number of electric organ discharges plotted as function of time in hours. Electric organ discharge rate shifted immediately. Note absence of electric organ discharge rate increase in second half of light period that had appeared during entrainment. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. Function oscillated with 24 lags peak to peak and trough to trough. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). Large peak appeared at 1/24 hours. Autocorrelation and spectral analysis confirmed immediate phase shift.


A
B
C

entrainment schedule (Figure 48A). Then activity dropped to zero. Each time the light went off, there was an increase in the amount of activity. The autocorrelation function oscillated (Figure 48B) with 24 lags from peak to peak and trough to trough. The largest peak in the spectral analysis occurred at 1/24 hours (Figure 48C).

For F18, both locomotor activity and electric organ discharge rate shifted immediately when the LD cycle was delayed 6 hours.

The second fish, F19, shifted electric organ discharge rate immediately also. During the third hour of the 6 hour delay electric organ discharge rate increased and then decreased for the rest of the delay period (Figure 49A). When the light went off, electric organ discharge rate immediately increased. On each successive day a large burst occurred when the light went off. For 3 of the 4 days, however, electric organ discharge rate during the dark period dropped to rates lower than those during the light period. The autocorrelation function (Figure 49B) and spectral analysis (Figure 49C) confirmed the entrainment of electric organ discharge rate to the phase shifted LD cycle.

Figure 48. Phase shift of zeitgeber, F18. Locomotor activity.
A. Raw data. Alternating black and open bar is LD 12:12.
* indicates 6 hour phase delay. Number of paddle deflections plotted as function of time in hours. Note small increase during hour when light would have gone off in previous entrainment. Locomotor activity shifted immediately.
B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. Function oscillated with 24 lags peak to peak and trough to trough. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). Largest peak occurred at 1/24 hours. Locomotor activity of F18 phase shifted and re-entrained to LD cycle.

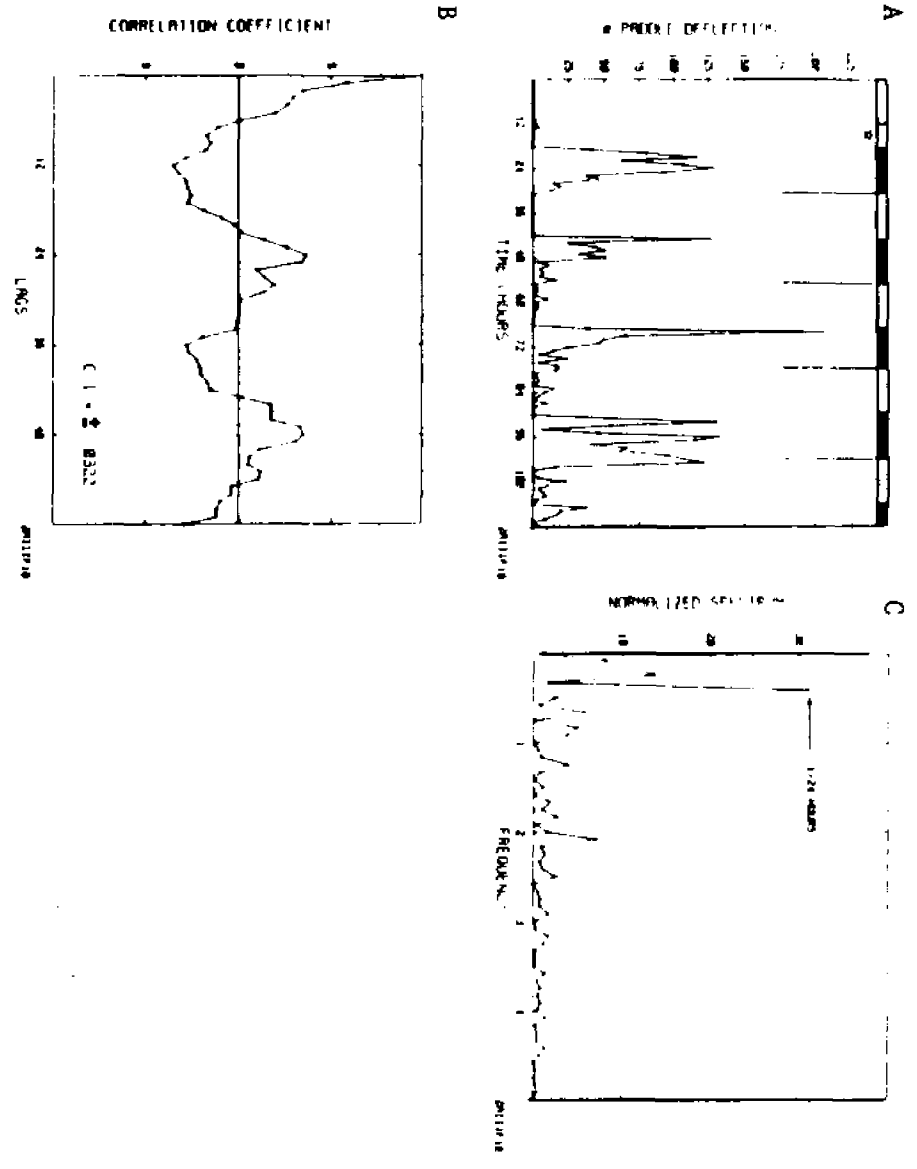
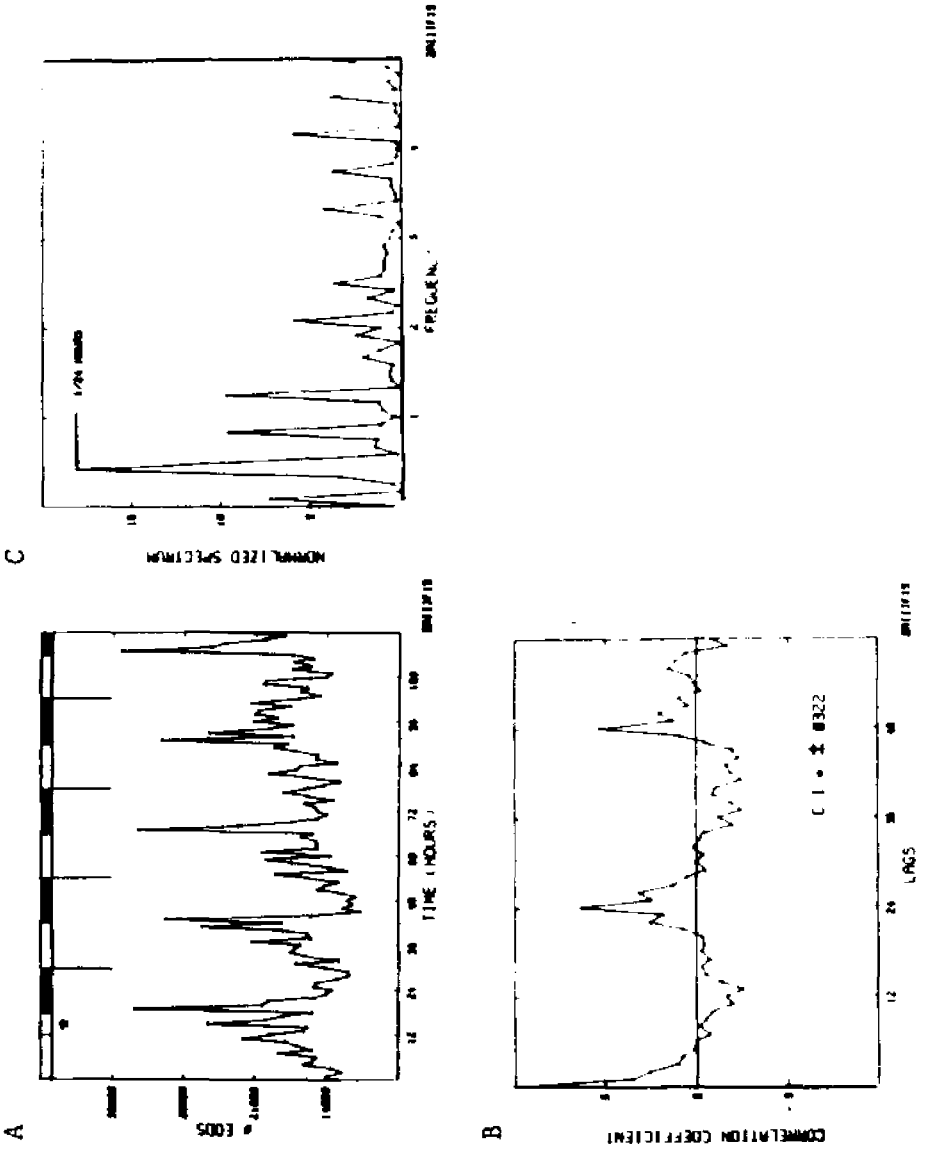

A
B
C
CORRELATION COEFFICIENT
LAGS
TIME, HOURS
FREQUENCY
1.24 HOURS

Figure 49. Phase shift of zeitgeber, F19. Electric organ discharge rate. Alternating black and open bar is LD 12:12. * indicates 6 hour phase delay. Number of electric organ discharges plotted as function of time in hours. Electric organ discharge rate shifted immediately. Note large burst of discharges occurred during hour of light off but fewer discharges occurred during rest of dark period for each day compared with light period. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. Function oscillated with 24 lags peak to peak. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). Large peak appeared at 1/24 hours. Phase shift occurred immediately and electric organ discharge rate re-entrained to LD cycle.


A
EDDS
TIME (HOURS)
B
CORRELATION COEFFICIENT
LAGS
C
NORMALIZED SPECTRUM
FREQUENCY

In the previous entrainment, locomotor activity of F19 did not entrain. When the LD cycle was shifted, locomotor activity remained arhythmic (Figure 50A). For the first 2 days of the phase shift, there was a burst of activity during the first hour after the light went off but this burst disappeared in subsequent days. The autocorrelation function (Figure 50B) did not oscillate and the small peak at 1/24 hours in the spectral analysis (Figure 50C) was not reliable. None of the points in the white noise test was significantly different from values expected for random data (Figure 50D). Locomotor activity became arhythmic after the phase shift.

For F19, under the 6 hour phase delay condition, only electric organ discharge rate phase shifted and remained entrained to the LD cycle. Locomotor activity did not entrain.

Post-entrainment free run. The final manipulation was a free run under constant conditions. During the previous free run, the electric organ discharge rate of F18 displayed an unreliable 24 hour periodicity. Figure 51A shows the raw data for F18. Visual inspection of the raw data suggested the presence of periodicity. There was also a decrease in the electric organ discharge rate over time. The autocorrelation function (Figure 51B) did not oscillate and there was a very

Figure 50. Phase shift of zeitgeber, F19. Locomotor activity. A. Raw data. Alternating black and open bar is LD 12:12. * indicates 6 hour phase delay. Number of paddle deflections plotted as function of time in hours. Note large peak at first light off after 6 hour delay. Bursts occurred at light off on days 2 and 5, also. No bursts on days 3 and 4. No obvious entrainment. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. No oscillation around 0 but 12 lags between peaks. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). Small peak at 1/24 hours.

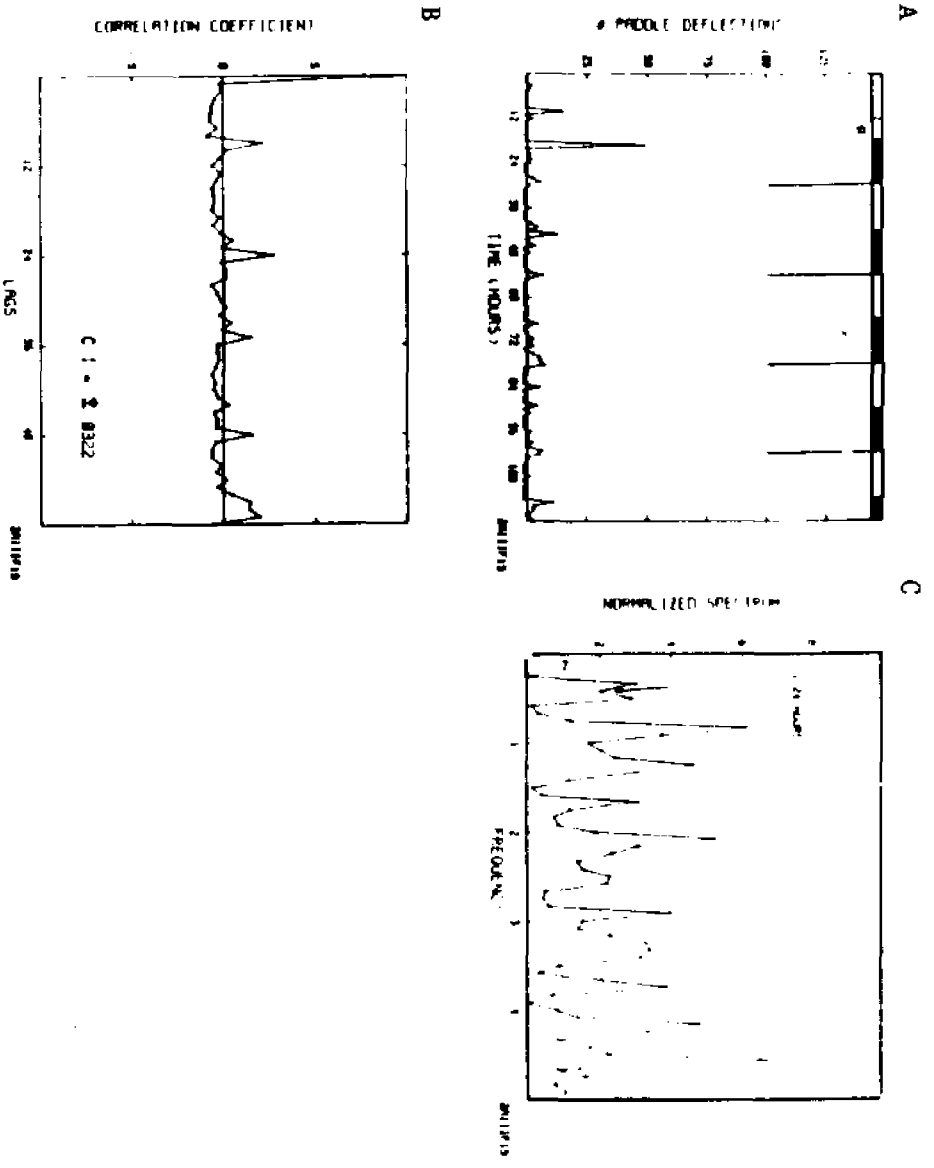
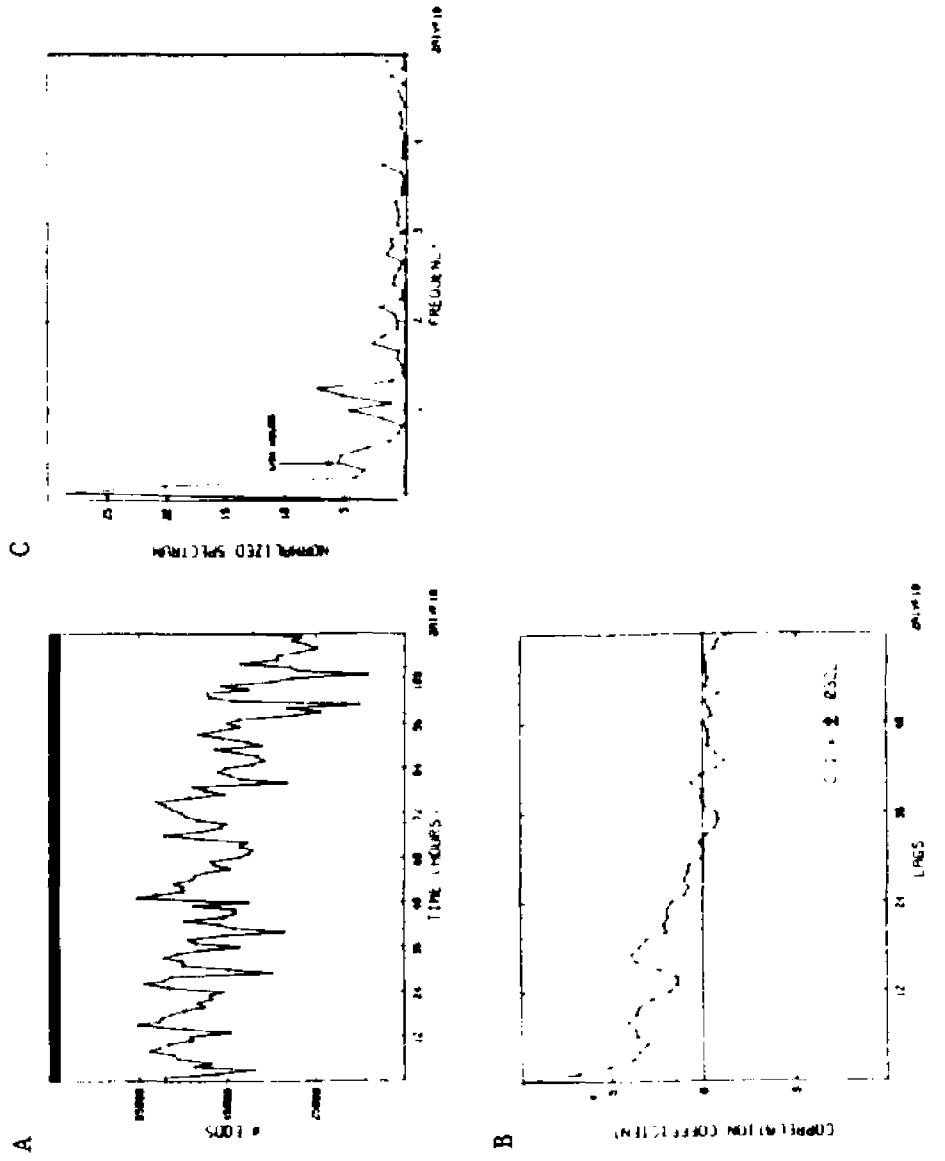

A
B
C
CORRELATION COEFFICIENT
TIME (HOURS)
FREQUENCY

Figure 51. Post-entrainment free run, F18. Electric organ discharge rate. A. Raw data. Black bar at top of graph is constant darkness. Number of electric organ discharges plotted as function of time in hours. Appeared to be periodicity in raw data indicated by alternating periods of high and low discharge rate. Note decreasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. Function did not oscillate. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). Note small peak at 1/24. Peak at 1/120 hours confirms decreasing trend. No obvious periodicity of electric organ discharge rate in constant conditions.


A
B
C

small peak at 1/24 hours in the spectral analysis (Figure 51C). The peak at 1/120 hours confirmed the presence of the decreasing trend. When the data were reanalyzed in segments, the 24 hour component appeared in only one of the three segments (Figure 52). Electric organ discharge rate of F18 did free run under constant conditions.

When the raw data for locomotor activity were examined, no rhythmicity could be detected (Figure 53A). The autocorrelation function did not oscillate (Figure 53B) and the spectral analysis confirmed the lack of 24 hour periodicity (Figure 53C). An increasing trend in locomotor activity was confirmed by the spectral analysis.

Electric organ discharge rate and locomotor activity data for F19 are shown in Figures 54 and 56. Visual inspection of the electric organ discharge data suggested the presence of rhythmicity (Figure 54A). The autocorrelation function did oscillate (Figure 54B). There were 24 lags from peak to peak but not from trough to trough. The two largest peaks in the spectral analysis occurred at 1/60 and 1/24 hours (Figure 54C). The peak at 1/60 hours reflected the decreasing trend in the data. When the data were reanalyzed as sequential segments, the peak at 1/24 hours occurred in two of the three segments (Figure 55) indicating the unreliability of the 24 hour periodicity.

Figure 52. Post-entrainment free run, F18. Electric organ discharge rate. Spectral analysis of non-overlapping segments of data. Normalized spectrum plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. A. First 48 hour segment. B. Second 48 hour segment. C. Twenty-four hour segment. Peak at 1/24 hours appeared in one of three segments indicating unreliability of 24 hour periodicity of electric organ discharge rate in constant conditions.

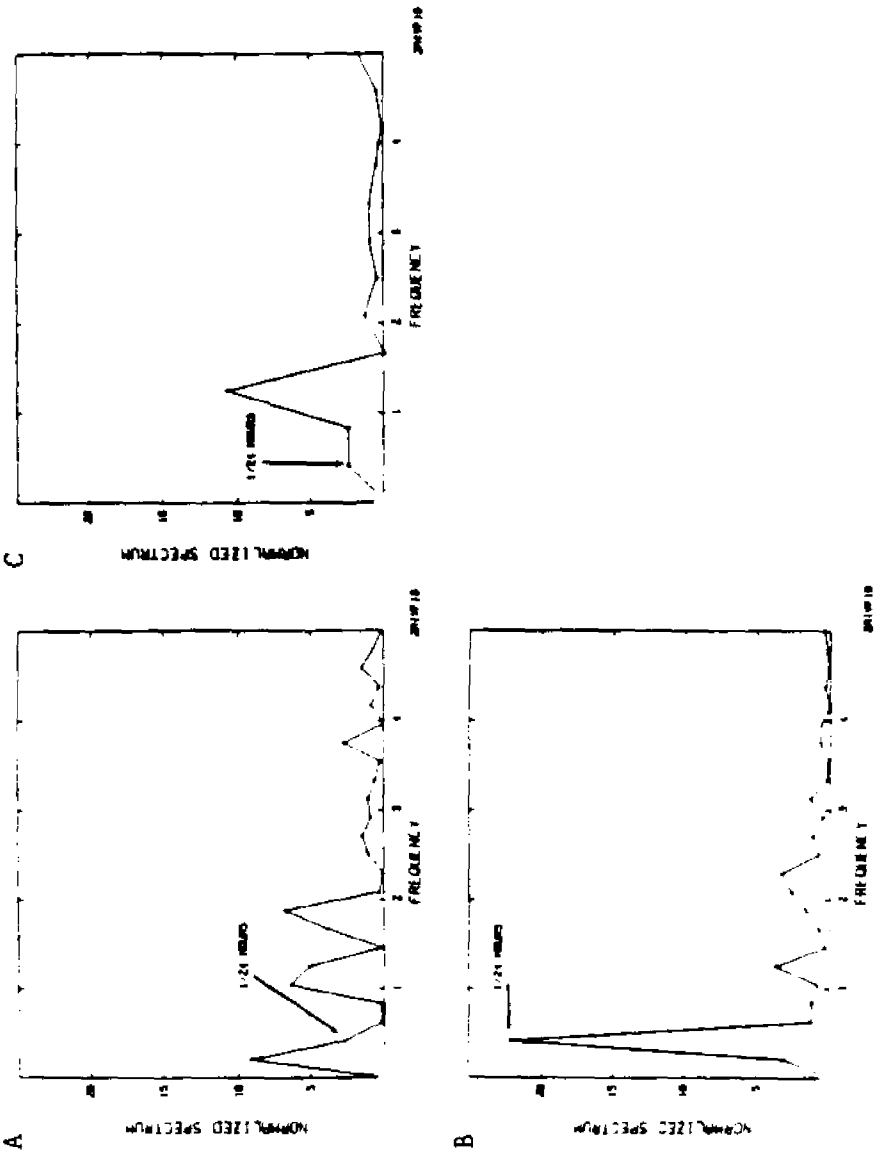

C
A
B

Figure 53. Post-entrainment free run, F18. Locomotor activity. A. Raw data. Black bar is constant darkness. Number paddle deflections plotted as function of time in hours. No obvious periodicity. Note increasing, then decreasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. Function did not oscillate. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). No peak at 1/24 hours. Peak at 1/120 hours confirmed trend. Locomotor activity did not free run in constant darkness.

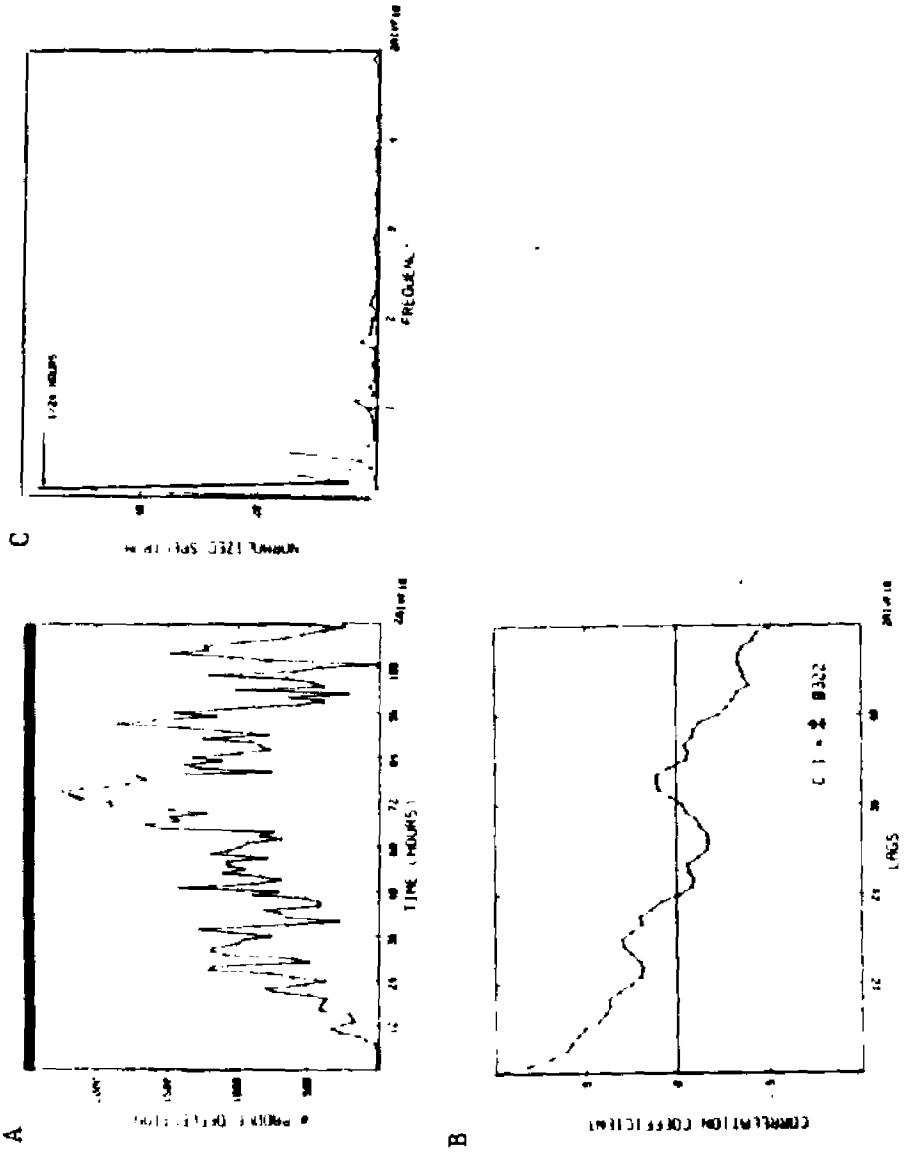

C
FREQUENCY
1.26 HOURS
A
TIME (HOURS)
B
LAGS

Figure 54. Post-entrainment free run, F19. Electric organ discharge rate. A. Raw data. Black bar at top of graph is constant darkness. Number of electric organ discharges plotted as function of time in hours. Note alternating periods of high and low discharge rates. Note decreasing trend. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. Function oscillated but not with 24 lags peak to peak. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). Peak at 1/24 indicated 24 hour periodicity. Peak at 1/60 hours confimed presence of trend.

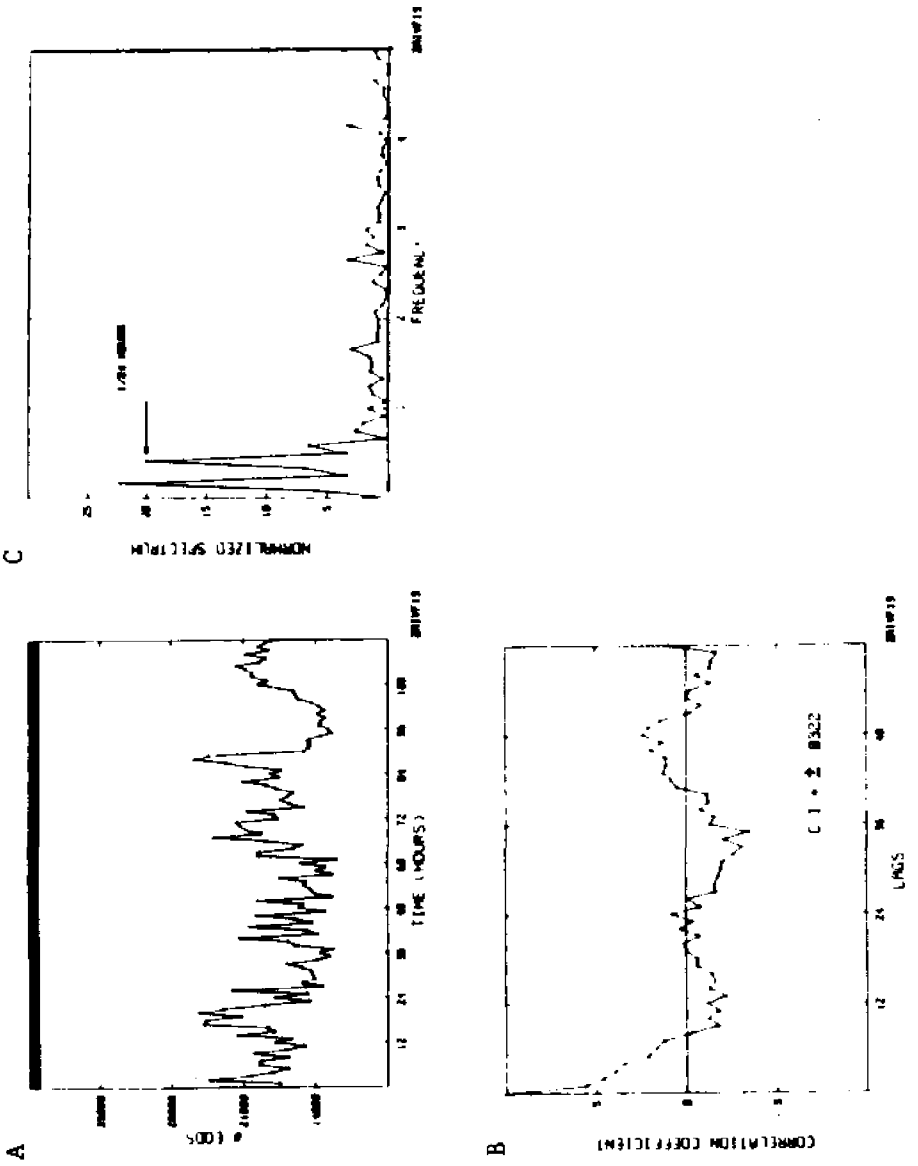
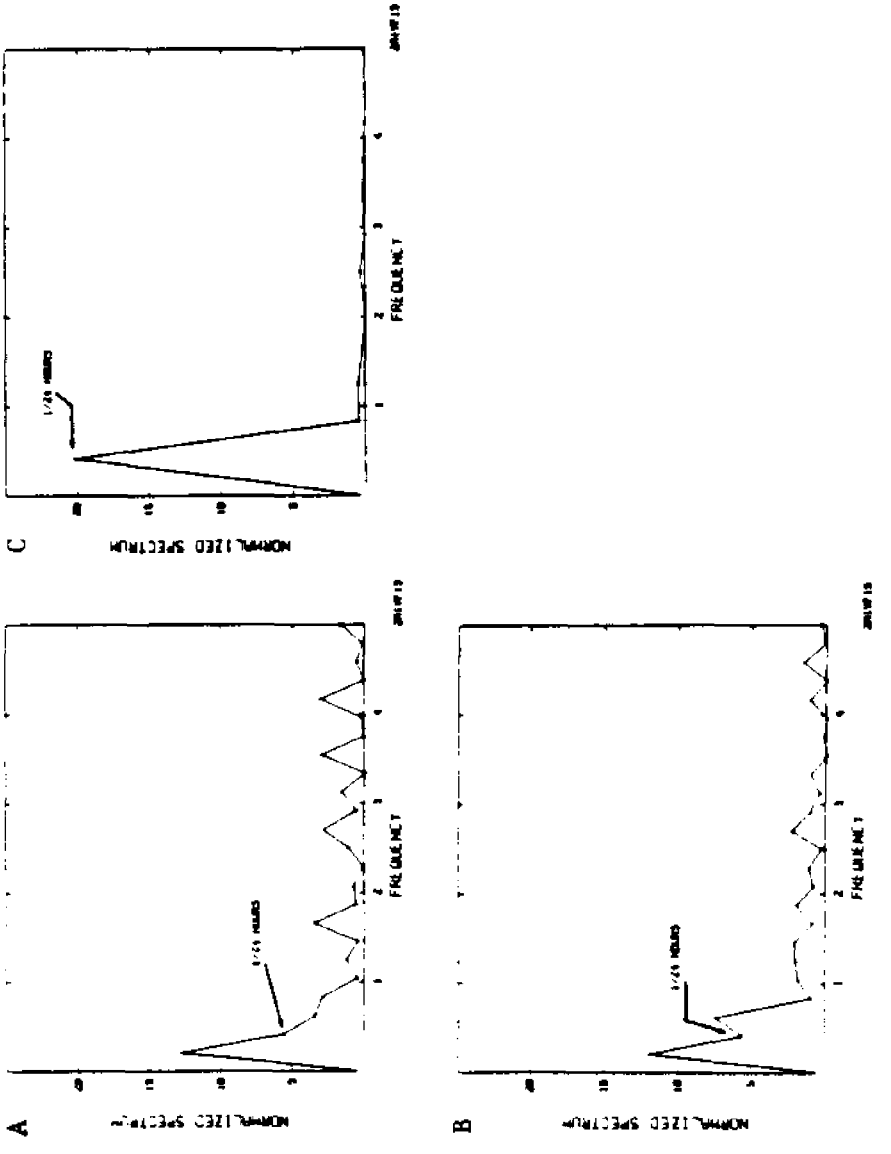

A
TIME (HOURS)
B
LAGS
C
FREQUENCY

Figure 55. Undisturbed free run, F19. Electric organ discharge rate. Spectral analysis of non-overlapping segments of data. Normalized spectrum plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. A. First 48 hour segment. B. Second 48 hour segment. C. Twenty-four hour segment. Peak at 1/24 hours appeared in two of three segments indicating unreliability of 24 hour periodicity of electric organ discharge rate in constant conditions.


C
A
B

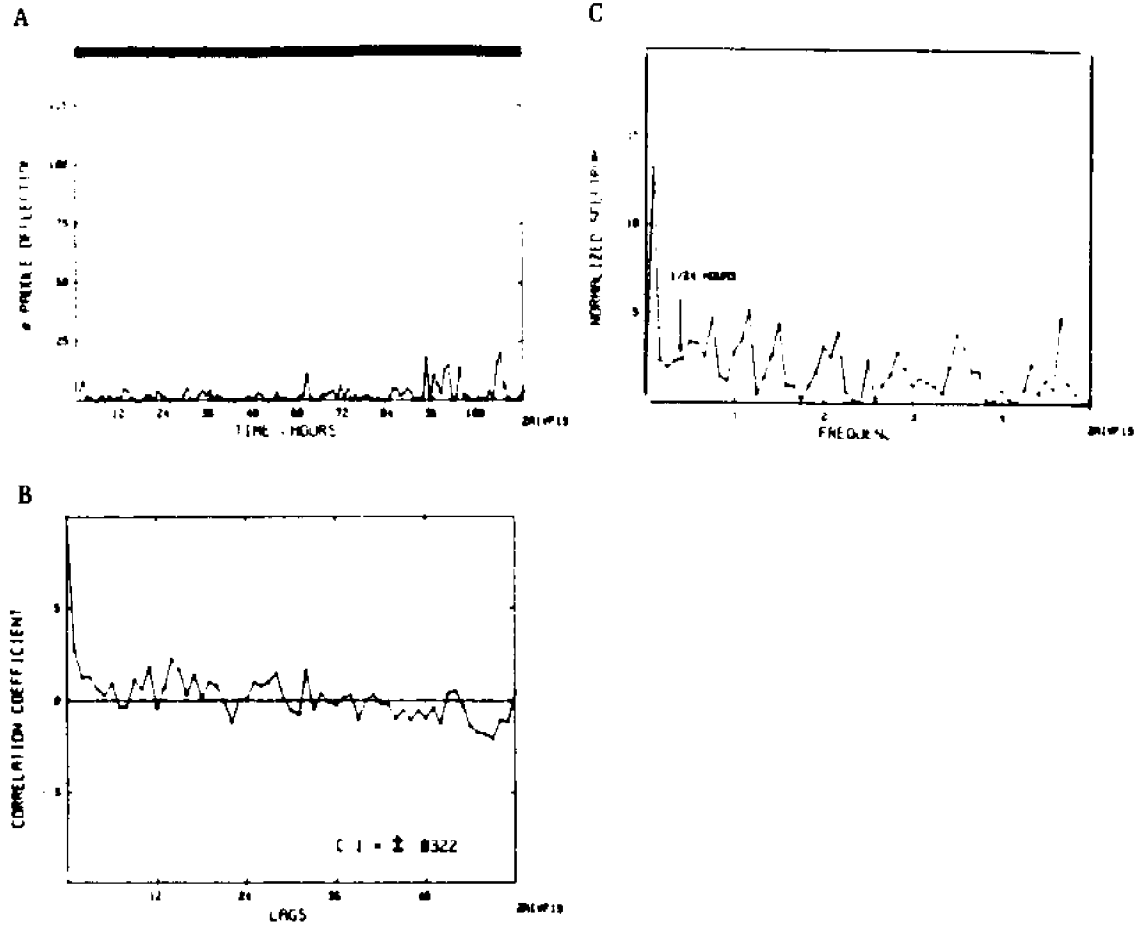
Locomotor activity remained arhythmic for F19 under constant conditions (Figure 56A). The autocorrelation function did not oscillate (Figure 56B) and there was no peak at 1/24 hours in the spectral analysis (Figure 56C). A peak at 1/120 hours confirmed the increasing trend observed in the raw data.

For both fish the electric organ discharge rate exhibited an unreliable periodicity. The locomotor activity was arhythmic.

Discussion

The results of this experiment demonstrated the effectiveness of light as an entraining cue for electric organ discharge rate in the two G. petersii studied. However, no reliable 24 hour periodicity was found under constant conditions and consequently, a conclusion about the presence or absence of a circadian electric organ discharge rate rhythm is difficult to make. Part of the difficulty may lie with the definition of rhythmicity used. Rhythmicity can be loosely defined as an event that occurs twice. The period length then equals the amount of time between the two events. If this definition were to be used, then every peak in a spectral analysis would represent a significant periodicity. A more

Figure 56. Post-entrainment free run, F19. Locomotor activity. A. Raw data. Black bar is constant darkness. Number paddle deflections plotted as function of time in hours. No obvious periodicity. B. Autocorrelation function. Correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. Function did not oscillate. C. Spectral analysis. Normalized spectrum plotted as function of frequency (1/period in hours). No peak at 1/24 hours. Locomotor activity of F19 was arhythmic in constant conditions.


A
TIME - HOURS
B
CORRELATION COEFFICIENT
LAGS
C
FREQUENCY

conservative definition would require the persistance of an oscillation for some 'reasonable' number of cycles without major changes in the average period length and without attenuation (Enright, 1965, 1981). Using this definition, the data from this experiment may now be placed in context. The spectral analysis of the electric organ discharge data produced by F18 during the first free run showed a large peak at 1/24 hours that did not appear reliable when the data were broken into sequential segments. The same was true of the electric organ discharge rate data for F19 during the post-entrainment free run. Several interpretations are possible. If no peak had occurred at 1/24 hours for the electric organ discharge rate of all the fish in DD, it would probably be safe to conclude that under these conditions, no large-amplitude, 24- hour periodicity was detectable, or the 24-hour component was there but with an amplitude too small to be detected above the noise in the data. When a peak at 1/24 hours does rise above the background in a spectral analysis, as it did for these two fish, the peak may be due to a persistant stable component. However, reanalysis of segments of the data indicated the unreliability of this rhythmicity. In this case, the large peak at 1/24 hours in the spectral analysis and visual inspection of the raw data (Figures 38 & 54) may indicate the presence of a rhythmic component that appeared, disappeared or changed during the course of the

experiment, and more data may be necessary to demonstrate the reality of the 24 hour component. Aschoff (1979) has stated that it may take several months for a stable period to be established, after an organism is released from entrainment into DD or LL. Since the longest recording time for electric organ discharge rate in DD was 5 days a next step in determining if electric organ discharge rate is circadian would require longer recording periods and more subjects.

Though circadian rhythmicity of electric organ discharge rate was not established, the data could still be examined to determine their degree of correspondence with locomotor activity. Electric organ discharge rate and locomotor activity were plotted on the same graph and visually inspected for similarity of pattern and rate (Figures 57 and 58). Field and laboratory data (Moller et al., 1979; Bassler et al., 1979) report high rates of electric organ discharge and locomotor activity at night and low rates during the day for G. petersii and other mormyrids. In this experiment, in general, for F18, when electric organ discharge rate was high, locomotor activity was high. This relationship was discerned under all manipulations but was most evident in fish, F18, beginning at about the 60th hour (day 3) of entrainment (Figure 57B) and continuing through the phase shift and post-entrainment free run (Figure 57C and D). Peaks in

Figure 57. Electric organ discharge rate and locomotor activity for F18. Number electric organ discharges on left ordinate. Number paddle deflections on right ordinate. Both plotted as function of time in hours. Solid line is electric organ discharge rate. Dashed line is locomotor activity. A. Undisturbed free run. Black bar is constant darkness. No obvious relationship between electric organ discharge rate and locomotor activity. B. Entrainment. Alternating open and black bar is LD 12:12. Note similarity of pattern beginning at 60th hour (day 3). High rates of electric organ discharges correspond to high rates of locomotor activity. C. Phase shift of zeitgeber. Alternating open and black bar is LD 12:12. * indicates 6 hour delay. Note immediate shift of both locomotor activity and electric organ discharge rate. High correspondence between high and low rates of activity and electric organ discharges. D. Post-entrainment free run. Black bar at top of graph is constant darkness. Note high discharge rates and low locomotor activity level at start of constant darkness. Locomotor activity exhibited a decreasing trend for first 60 hours. Then from 60th hour on, both electric organ discharge rate and locomotor activity showed a decrease with high correspondence between peaks and between troughs.

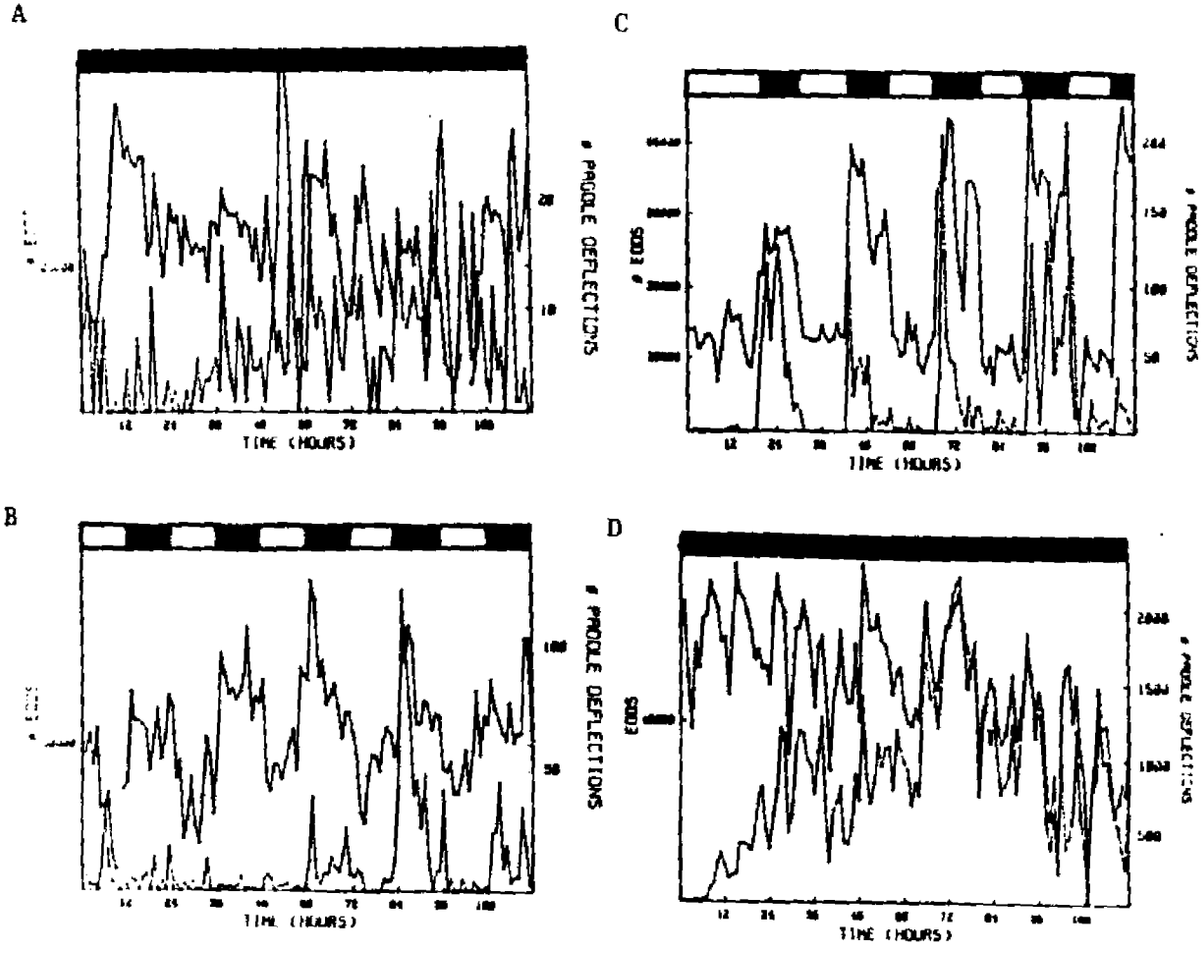
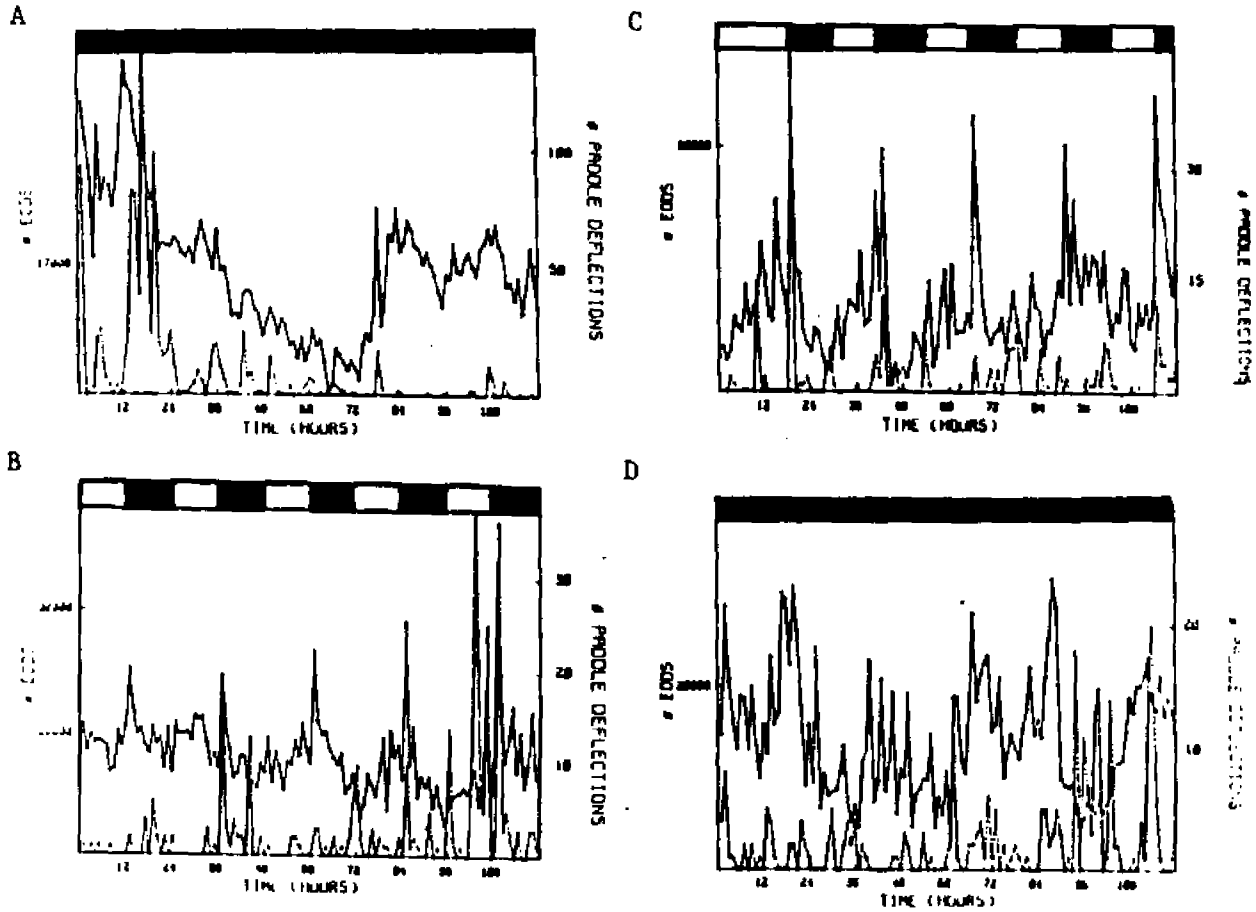

A
B
C
D
TIME (HOURS)
PADDLE DEFLECTIONS

Figure 58. Electric organ discharge rate and locomotor activity for F19. Number electric organ discharges on left ordinate. Number paddle deflections on right ordinate. Both plotted as function of time in hours. Solid line is electric organ discharge rate. Dashed line is locomotor activity. A. Undisturbed free run. Black bar is constant darkness. Some correspondence between peaks of activity and peaks of electric organ discharges. Both showed decreasing trend in first 72 hours. No relationship seen for last 48 hours.B. Entrainment. Alternating open and black bar is LD 12:12. Locomotor activity and electric organ discharge rate show correspondence for first 72 hours, low activity levels corresponded to low discharge rates. No discernable relationship for remainder of entrainment. C. Phase shift of zeitgeber. Alternating open and black bar is LD 12:12. * indicates 6 hour delay. No relationship between locomotor activity and electric organ discharge rate after phase shift of LD cycle. Locomotor activity did not re-entrain. D. Post-entrainment free run. Black bar at top of graph is constant darkness. No correspondence of electric organ discharge rates and locomotor activity during post-entrainment free run.


A
B
C
D
TIME (HOURS)
TIME (HOURS)
TIME (HOURS)
TIME (HOURS)
PADDLE DEFLECTIONS
PADDLE DEFLECTIONS
PADDLE DEFLECTIONS

electric organ discharge rate corresponded to peaks in locomotor activity. During the post-entrainment free run (Figure 57D) electric organ discharge rate was high and gradually decreased relative to locomotor activity. Locomotor activity was low but increased over the first 60 hours. From that point on, the patterns of locomotor activity and electric organ discharge rate were almost identical. Both exhibited a decreasing trend from about the 72nd hour (day 3) until the 120th hour (day 5).

The correspondence between electric organ discharge rate and locomotor activity was not as great for F19. During the undisturbed free run (Figure 58A) peaks in electric organ discharge rate corresponded to peaks in locomotor activity for the first 72 hours (3 days). This relationship disappeared in the subsequent 48 hours. Locomotor activity of F19 did not entrain to the LD 12:12 cycle (Figure 46). However, for the first 3 days there did appear to be less locomotor activity during the light periods which did correspond to low electric organ discharge rates (Figure 58B). No relationship was seen for the remainder of entrainment. Following the phase shift of the LD cycle, locomotor activity became arhythmic. Consequently, no relationship was found between electric organ discharge rate and locomotor activity during the phase shift (Figure 58C) and the post-entrainment free run (Figure 58D).

Experiment 3

This experiment examined the effects of social contact on the patterning of electric organ discharge rate and general locomotor activity. Social communication in G. petersii is primarily mediated through the fish's electrosensory system (Moller, 1970a; Moller & Bauer, 1973; Hopkins, 1977). Since it is extremely difficult to record electric organ discharges and locomotor activity, separately, from individuals within a group, an experiment was designed that prevented the fish from interacting, physically. The two fish were housed in separate tanks connected by a water-filled plastic pipe. This allowed the recording of electric organ discharges and locomotor activity for each fish. Hence, social contact was limited to the electrosensory modality.

Methods

Subjects. Twelve subjects ranging in length from 17cm to 21 cm and weighing from 30 g to 70 g were used.

Apparatus. The experimental tanks were connected with a 40 cm U-shaped, water-filled pipe. To test the apparatus, a fish was placed in one tank and the pipe was placed between the two tanks. The fish's signal was then recorded from both

tanks. The shape of the signal was unchanged. However, a sixty fold decrease of the peak to peak amplitude of the signal was found when recording was done in the corner furthest from the pipe opening (24 cm). Consequently, two fish would perceive each others' discharges as if they were interacting, electrically, at a distance of 6 to 10 cm (Moller & Bauer, 1973). To break contact the pipe was removed. The tanks were separated by a light proof barrier. Each tank had its own light system as described in the General Methods section.

The recording apparatus was the same as in Experiment 2.

Procedure

The social contact study consisted of 3 manipulations:

3A. Social contact- 2 replications

In Experiments 1 and 2 observations were made on fish housed in isolation. None of them free ran with respect to electric organ discharge rate or locomotor activity. Since some organisms will exhibit circadian rhythmicity only in the presence of conspecifics, the following manipulation was designed to investigate the effects of limited social contact

on the electric organ discharge rate and locomotor activity of fish kept in constant darkness (and with temperature, pH, and conductivity also held constant).

i. No contact. Both fish were exposed to constant darkness, temperature, pH, and conductivity conditions (see Experiment 1).

ii. Contact. The plastic pipe was placed in the tanks. Constant darkness, temperature, pH, and conductivity were maintained.

3B. Social contact-Conflict- 3 replications

In this manipulation one fish was placed in constant darkness (with temperature, pH, and conductivity held constant) and the other was kept on an LD 12:12 schedule (also with temperature, pH, and conductivity held constant). The fish were allowed to interact only through their electrosensory modality. The aim was to determine if locomotor activity and electric organ discharge rate of the fish in constant darkness would entrain to the LD cycle of its partner, via cues provided by the electric organ dicharge rate of the LD fish.

i. No contact. One fish was exposed to constant conditions of darkness, temperature, pH, and conductivity. The second animal was entrained to an LD 12:12 cycle with temperature, pH, and conductivity held constant.

ii. Contact. The two fish were connected via the plastic pipe. The individual light schedules were continued with one fish in constant darkness and the other on the LD schedule. Water temperature, pH, and conductivity were held constant.

3C. Social contact-Conflict- 1 replication

In this manipulation two fish were exposed to LD 12:12 schedules that were 90 out of phase. Social contact was again limited to the electrosensory modality. The aim was to ascertain whether or not social contact would effect the locomotor activity and electric organ discharge rate entrainment patterns established prior to contact.

i. No contact. Both fish were entrained to LD 12:12 cycles but the schedules were 90 out of phase. Water temperature, pH, and conductivity were held constant.

ii. Contact. The two fish were connected with the plastic pipe. The out-of-phase light schedules were continued. Constant water temperature, pH, and conductivity were maintained.

Results

For this experiment, each manipulation was examined for three possible effects of contact through the electric modality:

1) The raw data were visually inspected for the occurrence of periodicity of electric organ discharge rate and locomotor activity. If no periodicity was detected or verification of periodicity was needed, autocorrelation and spectral analyses were done.

2) The data were examined for changes in the amount of locomotor activity as reflected by a general increase or decrease over time. These changes are referred to as trends.

3) A determination was made as to whether an increase or decrease in variability of electric organ discharge rate and locomotor activity occurred. Variability was defined as an increase or decrease in the number of peaks and troughs

relative to the pre-contact condition. The coefficient of variation was used, descriptively, as an indicator of change.

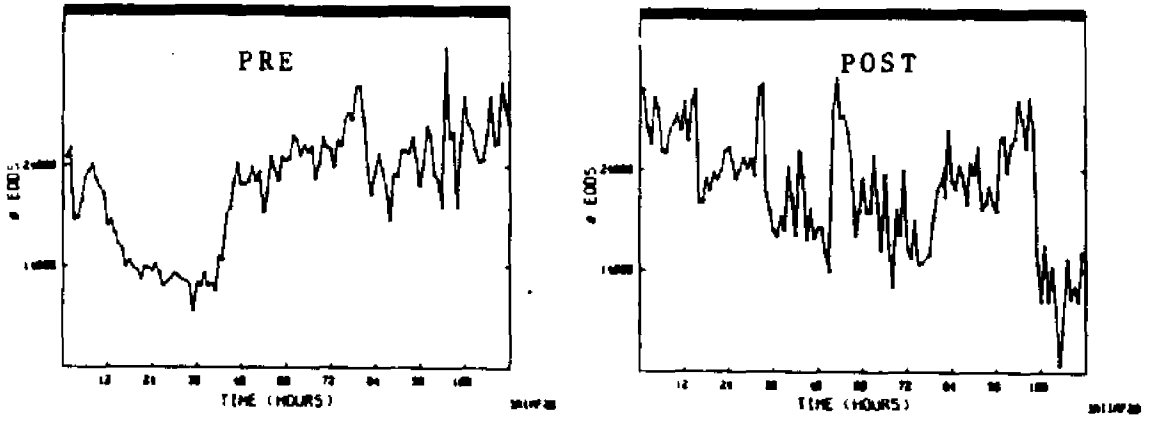
A. Experiment A (DD vs DD)

1. Periodicity. Of the four fish tested, one showed unreliable periodicity of electric organ discharge rate following contact. Figure 59A shows the raw electric organ discharge rate data for F20 prior to contact. No rhythmicity was detected. The autocorrelation function and spectral analysis confirmed this (see Appendix Figure 1A & C). After contact, a pattern was seen in the raw data (Figure 59A). The autocorrelation function oscillated but there were not 24 lags from peak to peak. However, the spectral analysis showed a large peak at 1/24 hours (see Appendix Figure 1C & D). The peak appeared in two of the three spectral analyses when the data were reanalyzed by sequential segments suggesting unreliable periodicity.

A second, F22, fish showed rhythmicity of electric organ discharge rate prior to contact but none during contact. Periodicity was detected by visual inspection of the raw data (Figure 60A). The autocorrelation function did not oscillate but a peak at 1/24 hours did appear in the spectral analysis (see Appendix Figure 3A & C). When the data were reanalyzed

Figure 59. Electric organ discharge rate and locomotor activity, F20, before and during contact. Black bar at tops of graphs is constant darkness. Pre is before contact. Post is during contact. A. Electric organ discharge rate. Discharges plotted as function of time in hours. Pre contact data show no obvious rhythmicity. Note increasing trend. Post contact show alternating periods of high and low discharge rates. Analysis indicated periodicity during contact was unreliable. Note decreasing trend during contact. B. Locomotor activity. Activity plotted as function of time in hours. No periodicity of activity before or during contact. Before contact, activity was random. During contact activity showed increasing trend. Activity increased in variability after contact was established.

A


PRE
POST
EODS
TIME (HOURS)

B

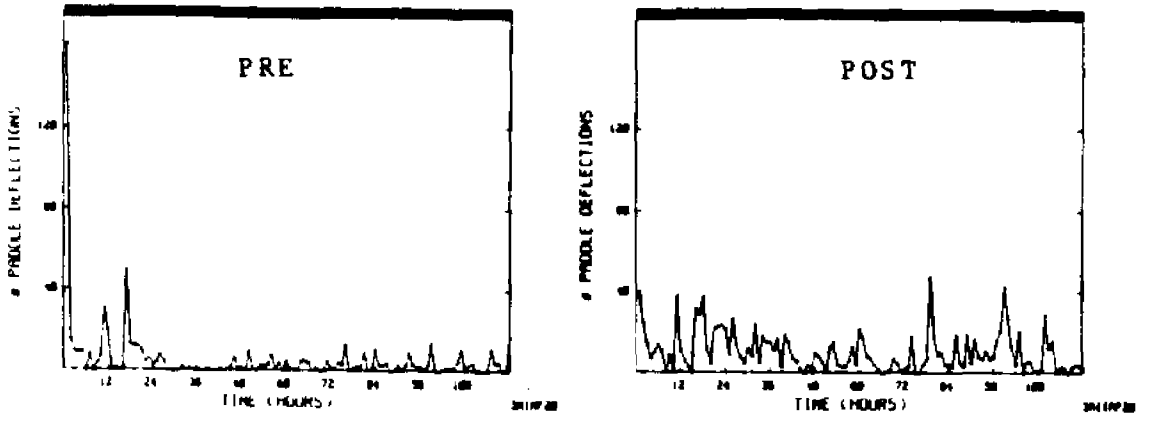
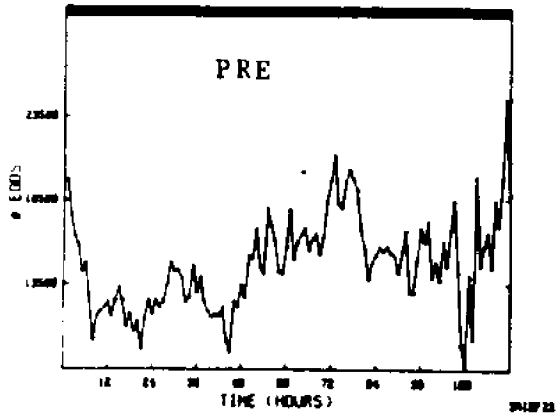
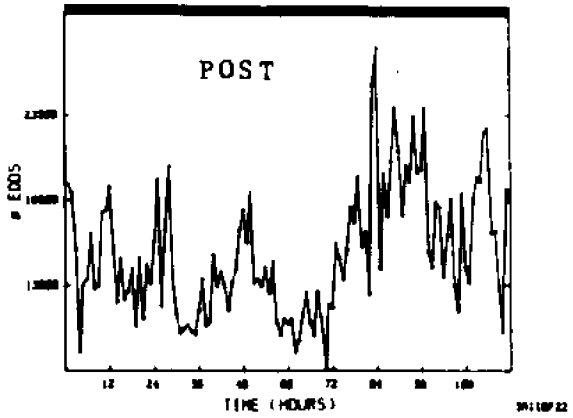

PRE
POST
PROBE DEFLECTIONS
TIME (HOURS)

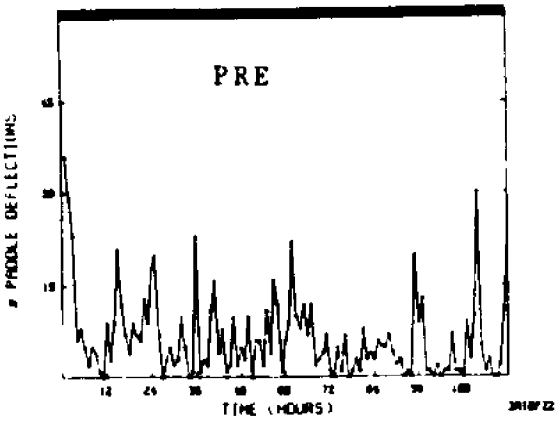
Figure 60. Electric organ discharge rate and locomotor activity, F22, before and during contact. Black bar at tops of graphs is constant darkness. Pre is before contact. Post is during contact. A. Electric organ discharge rate. Discharges plotted as function of time in hours. Pre contact data showed unreliable 24 hour rhythmicity. Note decreasing trend. No periodicity during contact. Variability increased during contact. Note increasing trend after contact was established. B. Locomotor activity. Activity plotted as function of time in hours. No periodicity of activity before or during contact. Before contact, activity was decreasing. During contact activity increased. Activity decreased in variability after contact was established.

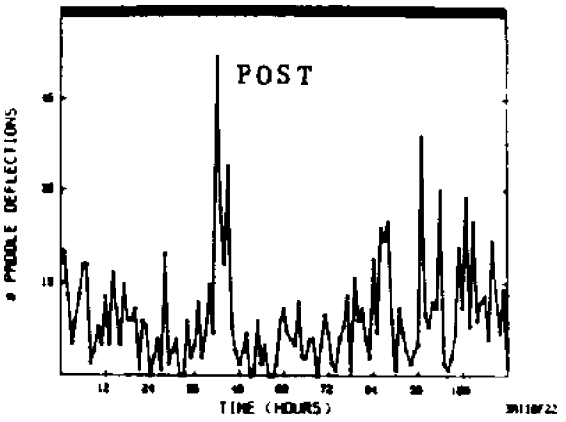
A


PRE
EDDS
TIME (HOURS)


POST
EDDS
TIME (HOURS)

B


PRE
PADDLE DEFLECTIONS
TIME (HOURS)


POST
PADDLE DEFLECTIONS
TIME (HOURS)

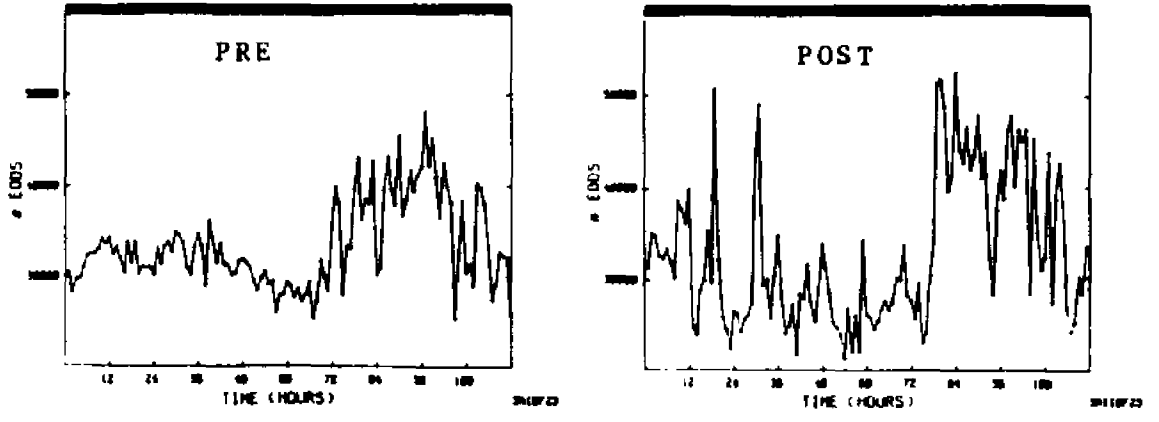
by segments, a large peak appeared in the first segment and 2 small peaks appeared in the remaining 2 segments. After contact, there was no discernible pattern in the raw data (Figure 60A) and this lack of periodicity was confirmed by the autocorrelation function and the spectral analysis (see Appendix Figure 3B & D).

There was no periodicity of locomotor activity before or during contact for any of the fish (Figures 59-62 and Appendix Figures 2, 4, & 6).

2. Trend. For electric organ discharge rate, one fish, F20, showed an overall increase in the number of discharges per hour before contact (Figure 59A). After contact the number of electric organ dischargs per hour decreased (Figure 59A). The three remaining fish showed no changes. Locomotor activity of F20 was random before contact (Figure 59B). During contact an overall increase in the number of paddle deflections was detected (Figure 59B). F22 decreased activity over time before contact and increased during contact (Figure 60B). F23 was inactive during contact but showed a small increasing trend in locomotor activity before contact (Figure 61B). F21 did not produce enough activity data for analysis (Figure 62B).

Figure 61. Electric organ discharge rate and locomotor activity, F23, before and during contact. Black bar at tops of graphs is constant darkness. Pre is before contact. Post is during contact. A. Electric organ discharge rate. Discharges plotted as function of time in hours. No periodicity detected before or during contaft. Variability of electric organ discharge rate increased during contact. B. Locomotor activity. Activity plotted as function of time in hours. No periodicity of activity before or during contact. Note small increase in activity level before contact and lack of activity after contact. Also note decrease in variability after contact was established.

A


PRE
POST
TIME (HOURS)
TIME (HOURS)

B

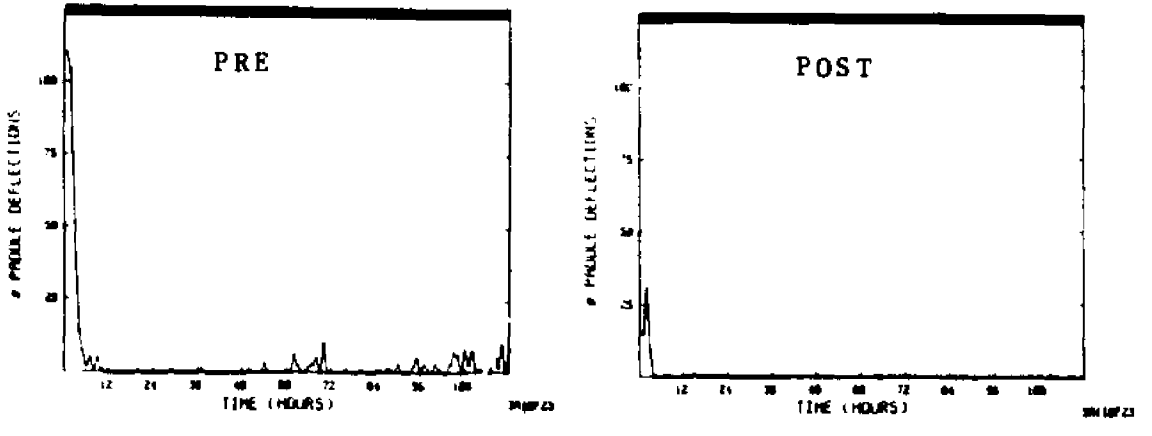
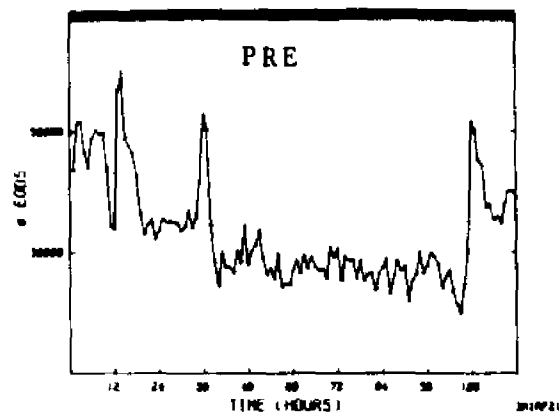
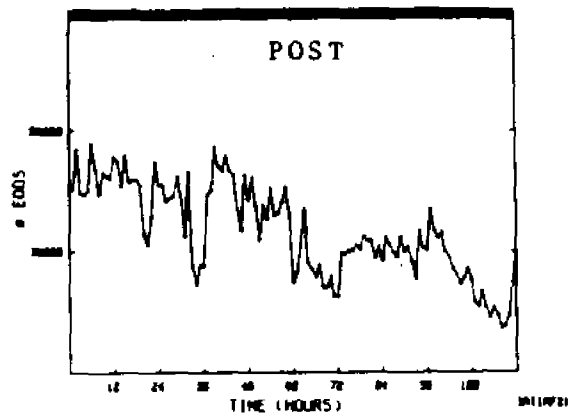

PRE
POST
TIME (HOURS)
TIME (HOURS)

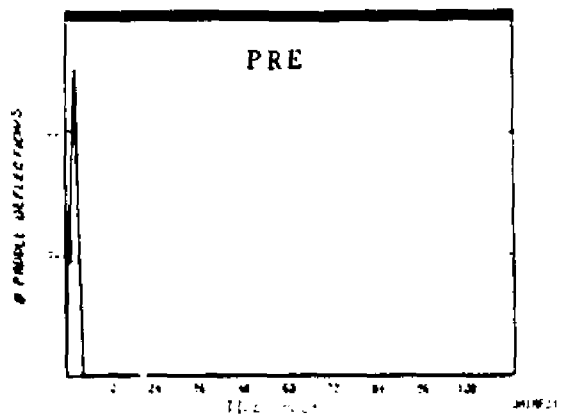
Figure 62. Electric organ discharge rate and locomotor activity, F21, before and during contact. Black bar at tops of graphs is constant darkness. Pre is before contact. Post is during contact. A. Electric organ discharge rate. Discharges plotted as function of time in hours. No periodicity of electric organ discharge rate before or during contact. Note variability of electric organ discharge rate decreased after contact was established. B. Locomotor activity. Activity plotted as function of time in hours. No periodicity of activity before or during contact. F21 was not active enough to produce data for analysis.

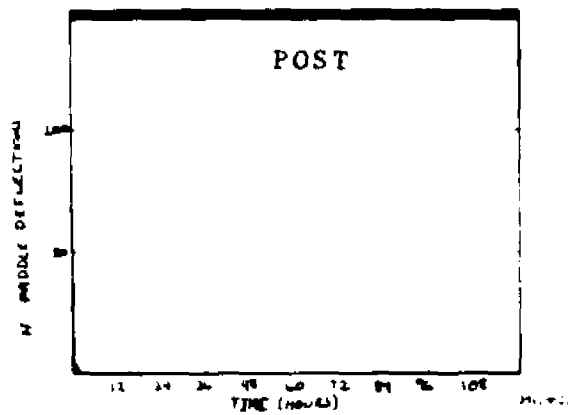
A





B





3. Variability. Of the two pairs of fish, one pair, F22 and F23, showed an increase in variability of electric organ discharge rate during contact (Figures 60 & 61A). This was verified by an increase in the coefficients of variation (Table 1). Locomotor activity did not show the same change. Variability of activity for F23 increased during contact (Figure 61B) while it decreased for F22 (Figure 60B). For the second pair, F20 and F21, the variability of the electric organ discharge rate decreased for F21 (Figure 62A) and did not change for F20 (Figure 59A). Locomotor activity of F20 decreased in variability during contact (Figure 59B). F21 was not active enough to produce data for analysis (Figure 62B).

B. Experiment B (DD vs LD)

1. Periodicity. All three fish under DD showed unreliable 24 hour periodicity of electric organ discharge rate during contact. Figures 63A, 65A & 66A show the electric organ discharge rate data for these fish. F9 and F24 both had small peaks at 1/24 hours in the spectral analyses (see Appendix Figures 8D & 9D) following contact. F26 showed small peaks at 1/24 hours before and during contact (see Appendix Figure 11).

TABLE 1

Comparison of Coefficients of Variation for Electric Organ Discharge Rate (EOD) and Locomotor Activity (Activity) Before (Pre) and During (Post) Contact in Experiment 3.

				EOD			ACTIVITY		
EXP #	PAIR#	S#	CONDI-TION	PRE	POST	DIR	PRE	POST	DIR
3A	1	20	DD	26.4	26.6	-	294.6	94.7	
		21	DD	26.1	22.9		-	-	
	2	22	DD	18.9	24.4		105.5	89.5	
		23	DD	14.4	24.5		408.6	680.0	
3B	1	9	DD	19.1	23.2		185	37.9	
		10	LD	17.8	25.0		122.2	191.7	
	2	24	DD	15.9	25.9		125.0	176.0	
		25	LD	12.9	9.4		537.8	278.0	
	3	26	DD	9.3	20.9		127.6	99.0	
		27	LD	25.1	24.3		483.6	185.8	
3C	1	11	LD	24.4	27.9		168.5	147.6	
		12	LD	24.6	25.8		227.9	119.0	

Note. EXP# is Experiment number
S# is Subject number
Condition is: DD- fish exposed to constant darkness
LD- fish exposed to light-dark cycle 12:12
DIR is Direction of change: increase
decrease
- no change

Figure 63. Electric organ discharge rate and locomotor activity, F9, before and during contact. Black bar at tops of graphs constant darkness. Pre is before contact. Post is during contact. A. Electric organ discharge rate. Discharges plotted as function of time in hours. Pre contact data show no obvious rhythmicity. Unreliable periodicity detected during contact. Note alternating periods of high and low discharge rates. Electric organ discharge rate increased before contact and reversed trend during contact. Note increase in variability during contact. B. Locomotor activity. Activity plotted as function of time in hours. No periodicity of activity before contact. Note increasing trend. During contact, periods of high and low activity corresponded to periods of high and low activity of LD partner, F10 (Figure 67). Note decreasing trend and decrease in variability during contact.

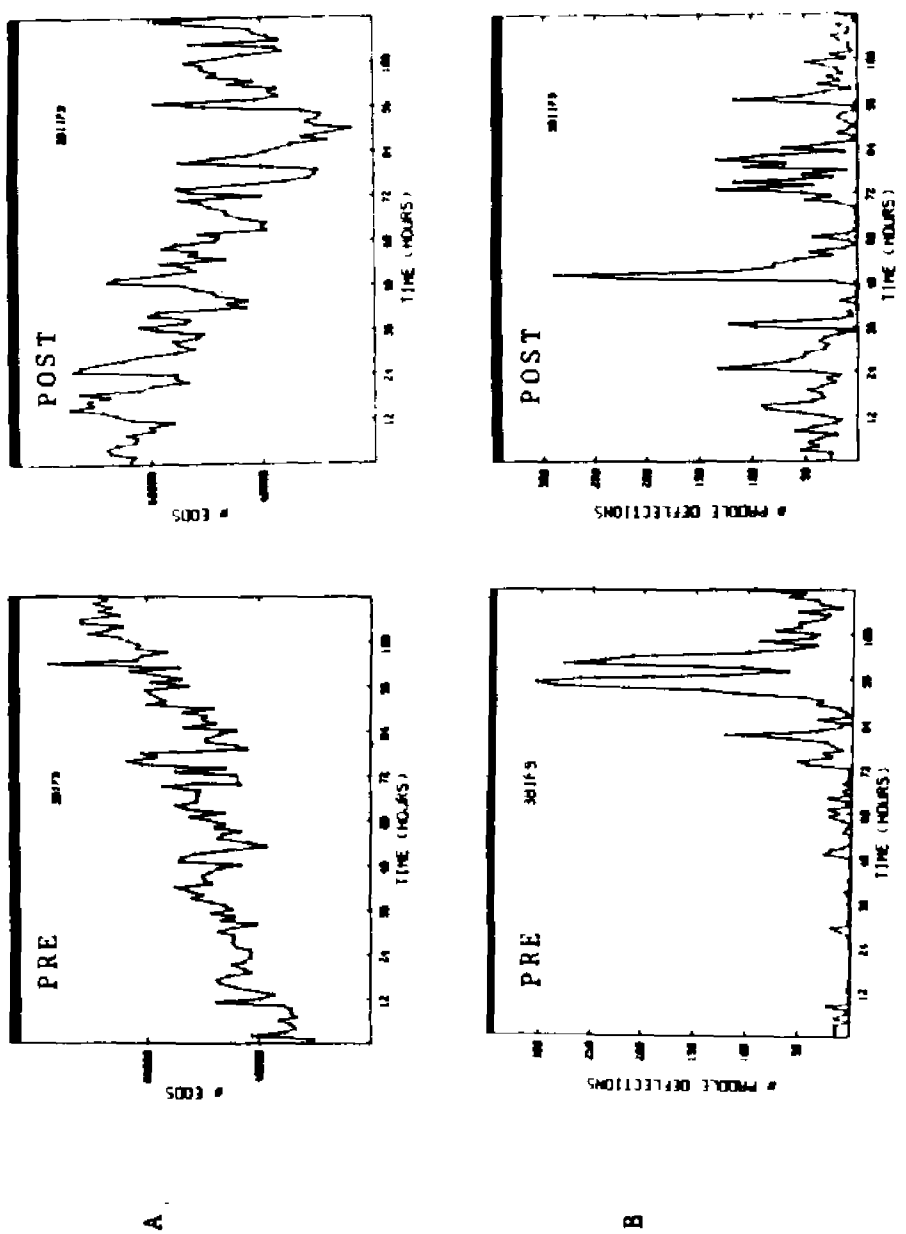

POST
PRE
TIME (HOURS)
PVCs
PVC DEFLECTIONS
A
B

Figure 64. Locomotor activity, F9. Autocorrelation and spectral analysis of locomotor activity before (Pre) and during (Post) contact. For autocorrelation function, correlation coefficient plotted as function of lags. C.I. for 0 appear in lower right hand corner. For spectral analysis, normalized spectrum plotted as function of frequency (1/period in hours). A. Note lack of oscillation of autocorrelation function before contact. During contact (B), function oscillated though not consistantly with 24 lags peak to peak. Before contact, small peak at 1/24 hours appeared in spectral analysis (A). After contact was established (B), largest peak appeared at 1/24 hours confirming entrainment of locomotor activity of F9 to cycle of its LD partner, F10.

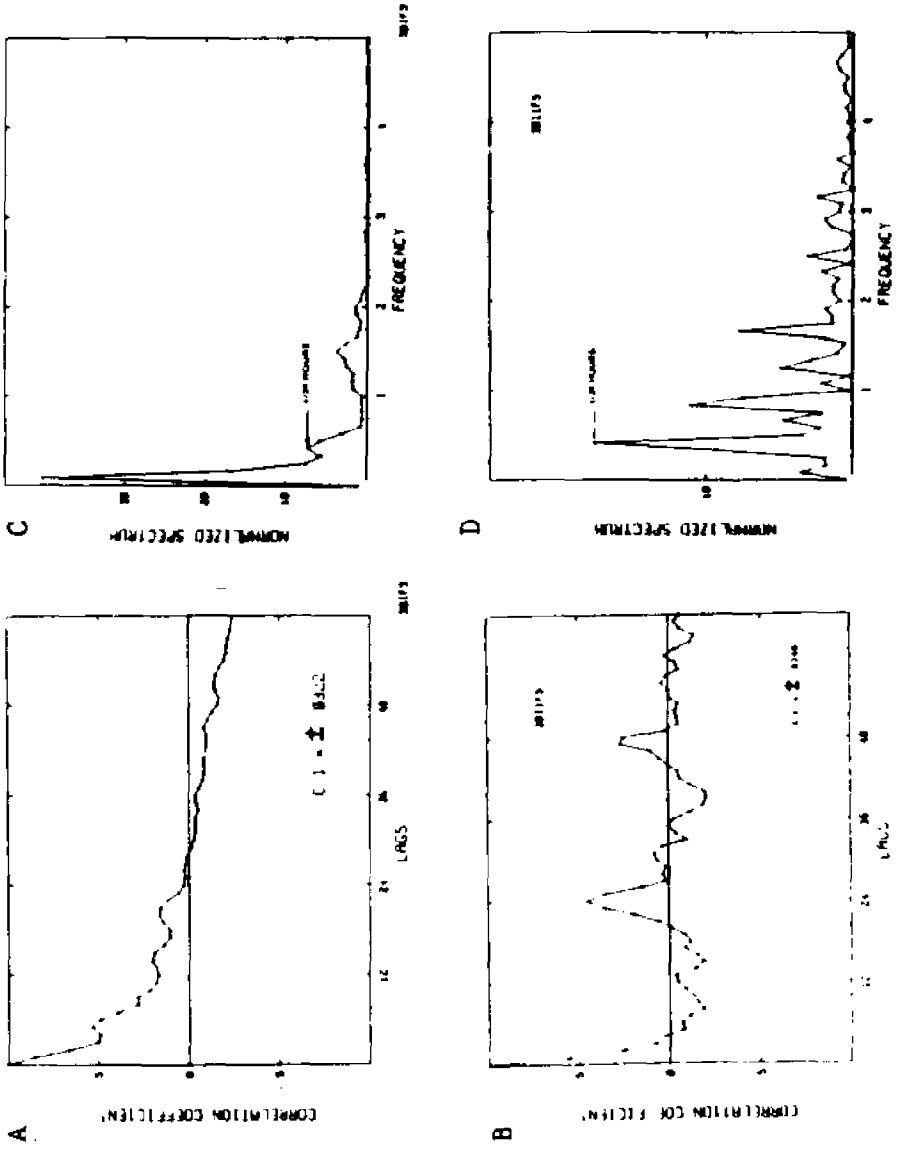
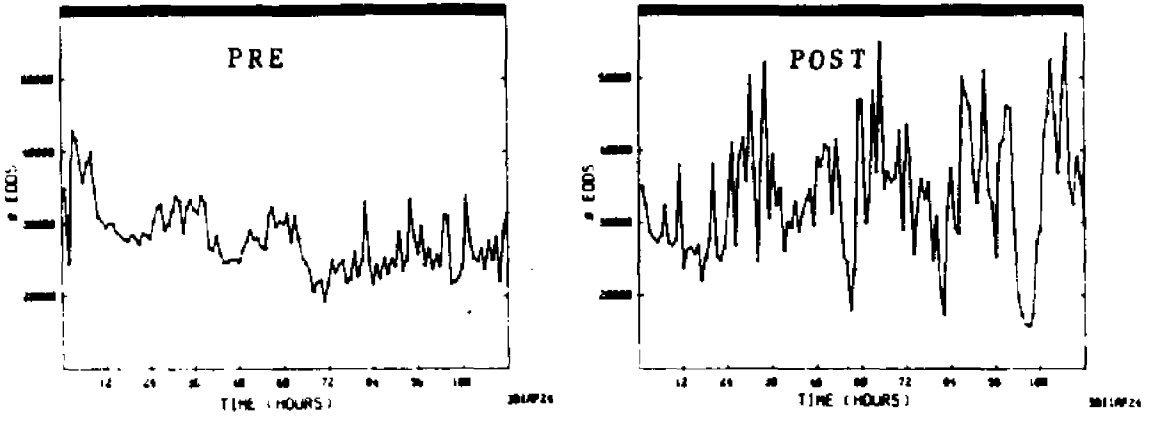

A
B
C
D
FREQUENCY
FREQUENCY

Figure 65. Electric organ discharge rate and locomotor activity, F24, before and during contact. Black bar at tops of graphs constant darkness. Pre is before contact. Post is during contact. A. Electric organ discharge rate. Discharges plotted as function of time in hours. Pre contact data show no obvious rhythmicity. Note decreasing trend. Post contact shows alternating periods of high and low discharge rates. Analysis indicated periodicity during contact was unreliable. Note increasing trend during contact. Variability of discharge rate increased after contact was established. B. Locomotor activity. Activity plotted as function of time in hours. No periodicity of activity before or during contact. Before contact, activity was increasing. During contact activity showed decreasing trend. Activity increased in variability after contact was established.

A


PRE
POST
EODS
TIME (HOURS)

B

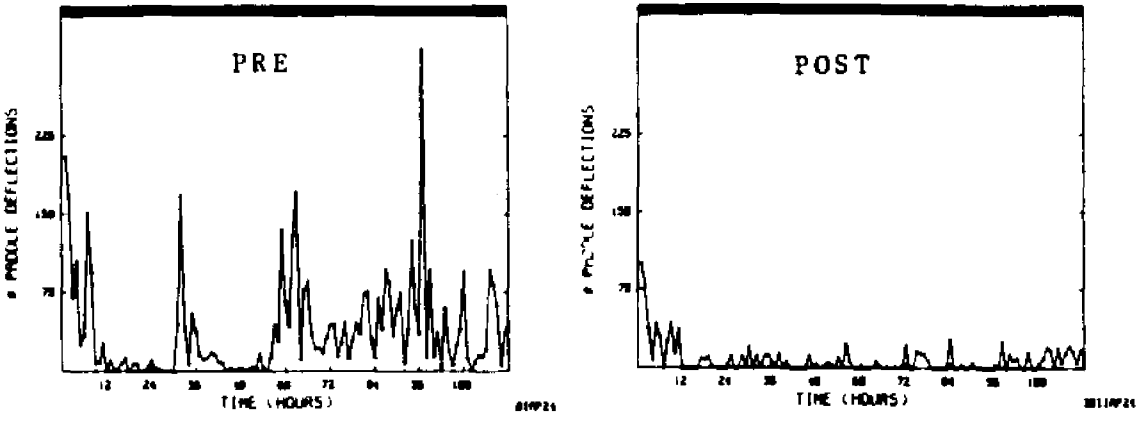
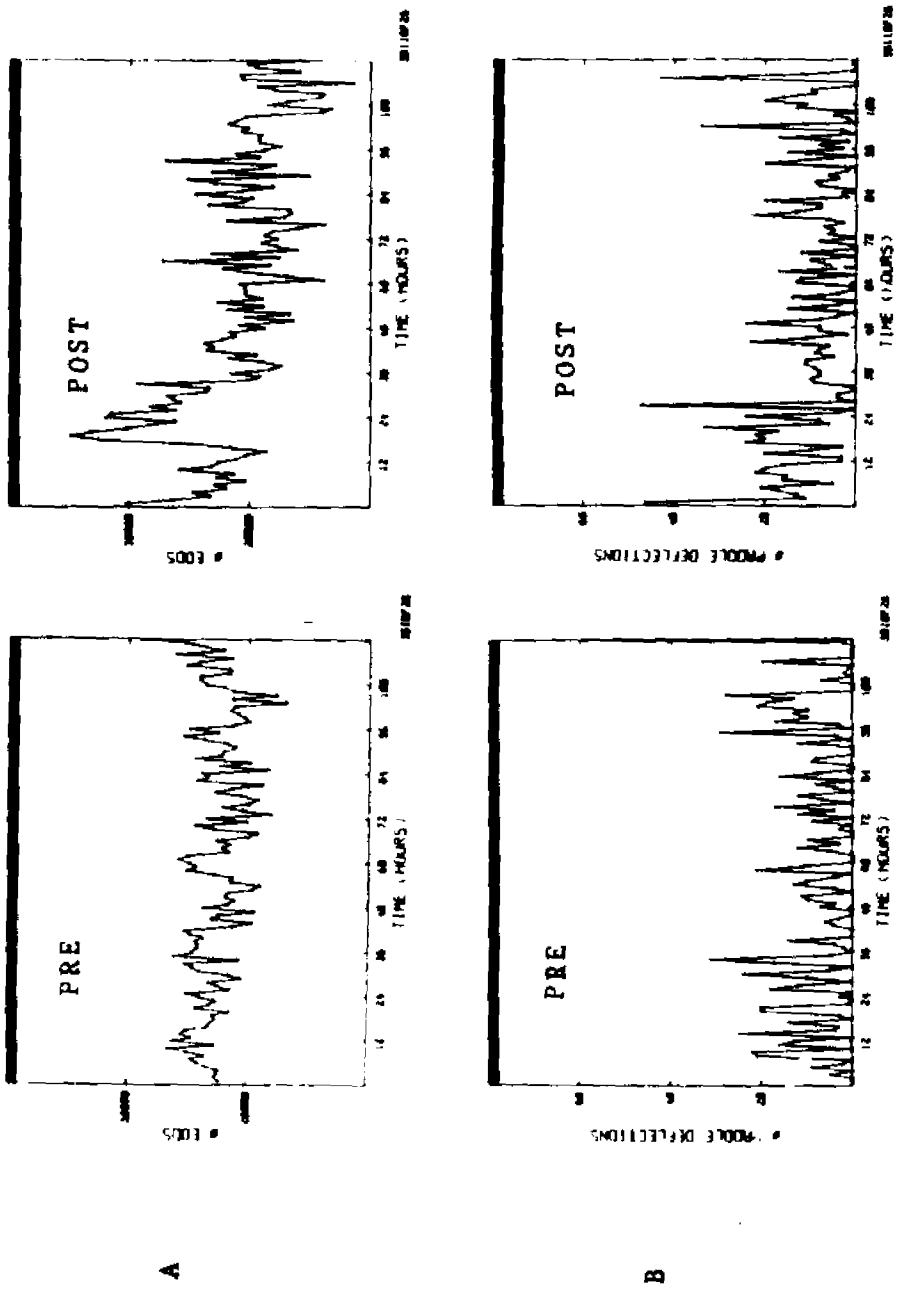

PRE
POST
PROBE DEFLECTIONS
TIME (HOURS)

Figure 66. Electric organ discharge rate and locomotor activity, F26, before and during contact. Black bar at tops of graphs constant darkness. Pre is before contact. Post is during contact. A. Electric organ discharge rate. Discharges plotted detected before and during contact. Note alternating periods of high and low discharge rates. Decrease in electric organ discharge rate before contact reversed itself during contact. Note increase in variabilty during contact. B. Locomotor activity. Activity plotted as function of time in hours. No periodicity of activity before or during contact. Note decrease in variability after contact was established.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For the same three fish, only one showed rhythmic locomotor activity during contact. Figure 63B shows the raw locomotor activity data for F9 before contact. During the first 2 days, more activity was seen in what would be the dark period for this fish. This pattern disappeared in the subsequent days. The lack of periodicity was confirmed by the autocorrelation function and the spectral analysis (Figure 64A). During contact, there were distinct periods of high and low activity (Figure 63B) corresponding to the dark and light periods of its partner, F10. The autocorrelation function oscillated though not consistently, with 24 lags from peak to peak and the largest peak in the spectral analysis occurred at 1/24 hours (Figure 64B).

For the fish entrained to the LD cycles, F10, F25, and F27, each responded differently. The pattern of electric organ discharge rate for F10 reversed itself several times both before and during contact. Before contact, there was more electrical activity during the light part of the cycle on days 1 and 5. On days 2, 3 and 4 there were more discharges during the dark period (Figure 67A). During contact, there were more discharges during the light period on days 1 and 3 and more discharges during the dark period on days 2, 4, and 5 (Figure 67A). The unreliability of entrainment was reflected in the autocorrelation functions and the spectral analyses

Figure 67. Electric organ discharge rate and locomotor activity, F10, before and during contact. Alternating black and open bar at tops of graphs is LD 12:12. Pre is before contact. Post is during contact. A. Electric organ discharge rate. Discharges plotted as function of time in hours. Note reversals of pattern before and during contact. Days 1 and 5, before contact, show more discharges during light period. Days 2,3 and 4 show more discharges during dark period. During contact, more discharges occurred during light on days 1 and 3. More discharges during darkness on days 2,4 and 5. Electric organ discharge rate showed decreasing trend before contact, increasing trend during contact. B. Locomotor activity. Activity plotted as function of time in hours. Note pattern reversals before contact. During contact, entrainment became stable, no reversals. Activity level decreased before and during contact. Activity increased in variability after contact was established.

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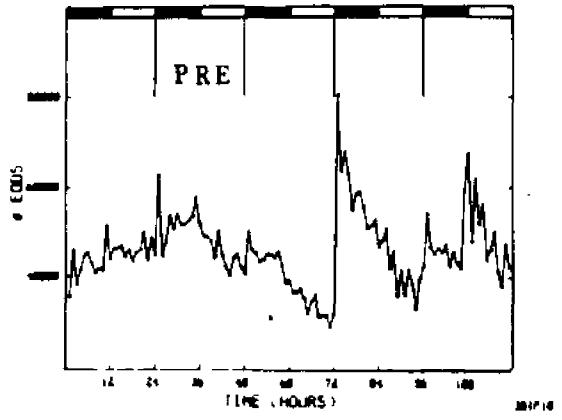
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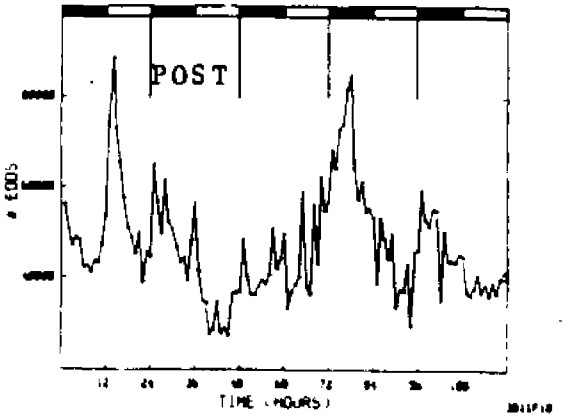
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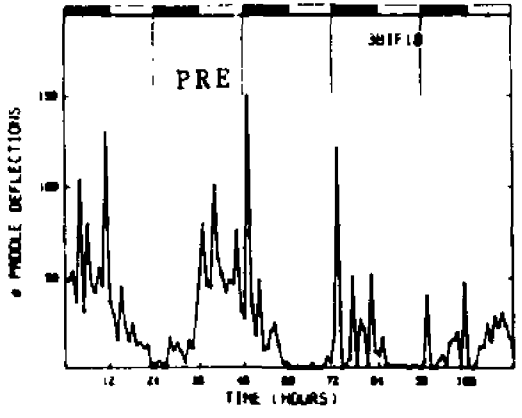
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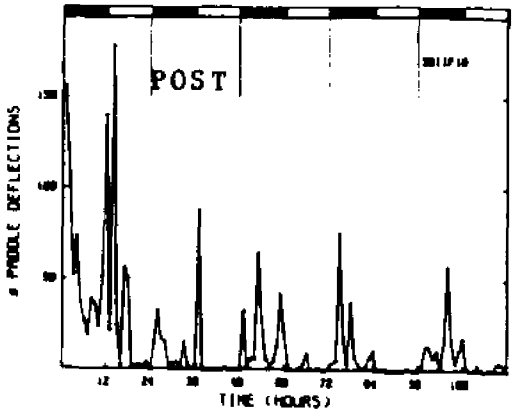
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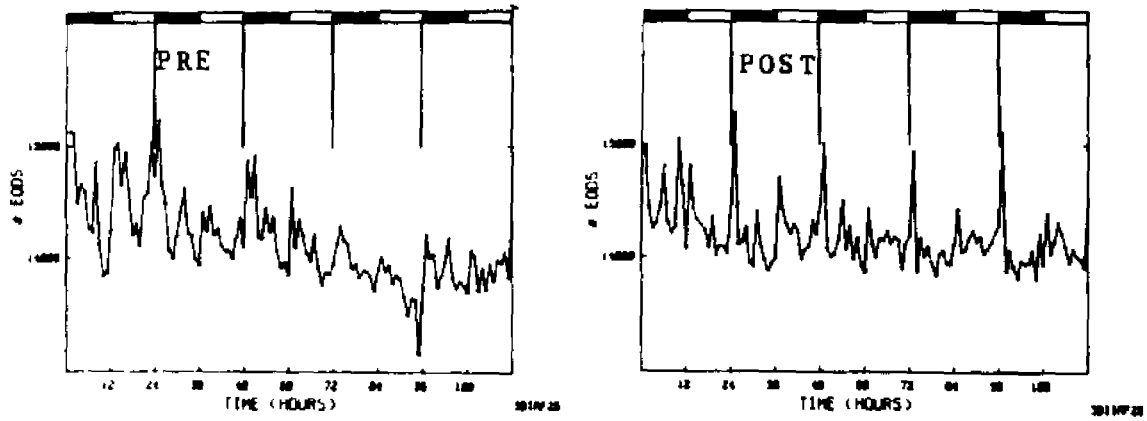
(see Appendix Figure 13). The electric organ discharge rate of F25 entrained to the LD cycle but with an apparent 12 hour periodicity that became most evident during contact. For the 5 days in isolation, there appeared to be alternating periods of high and low electric organ discharge rate (Figure 68A). The autocorrelation function had 12 hours between successive peaks though a smooth oscillation was not seen and the spectral analysis showed a small peak at 1/24 hours and a large peak at 1/12 hours (see Appendix Figure 15A & B). After contact, the 12 hour periodicity was more clearly established. There was a burst of electric organ discharge activity when the light went off and on (Figure 68A). The bursts at light off were always larger than the bursts at light on. There was not more electric organ discharge activity during the dark period as compared with that during the light period. The autocorrelation function oscillated with 12 lags from peak to peak and the spectral analysis had a large peak at 1/12 hours and its submultiples (see Appendix Figure 15C & D). There was no 24 hour periodicity. The electric organ discharge rate of F27 entrained both before and during contact (Figure 69A & Appendix Figure 17) though the pattern became less distinct after contact.

Locomotor activity showed a similar variability of entrainment. F10 showed the same kind of pattern reversals of

Figure 68. Electric organ discharge rate and locomotor activity, F25, before and during contact. Alternating black and open bar at tops of graphs is LD 12:12. Pre is before contact. Post is during contact. A. Electric organ discharge rate. Discharges plotted as function of time in hours. Before contact, note burst of discharges during hours of light-on and light-off, every 12 hours. After contact, 12 hour entrainment became most evident. Large burst at every light-off. Smaller burst at every light on. No 24 hour periodicity detected. Twelve hour periodicity confirmed by statistical analysis. Note decrease in electric organ discharge rate and variability of discharge rate during contact. B. Locomotor activity. Activity plotted as function of time in hours. Before contact, no activity for first 4 days. More activity during dark period of day 5. After contact was established activity entrained though level remained very low. Increase in activity level reversed after contact was established. Note decrease in variability during contact.

A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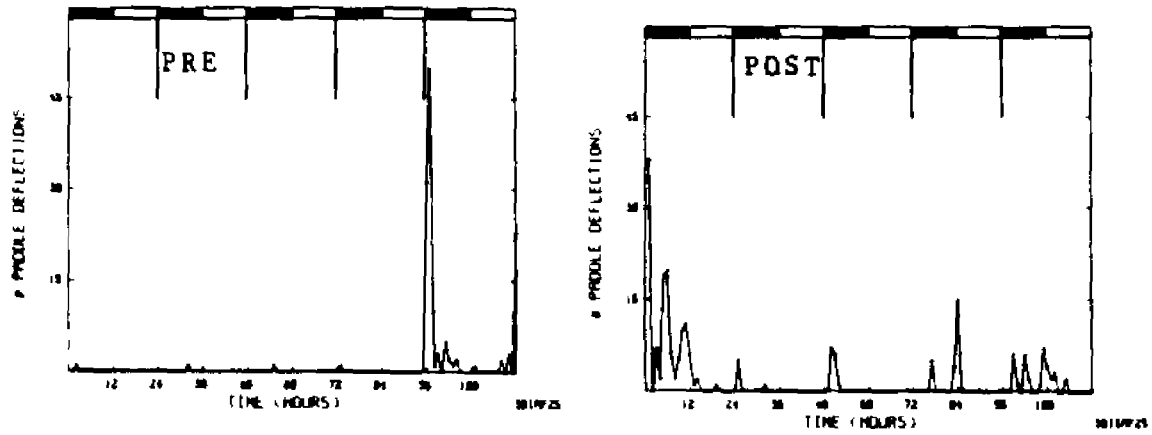
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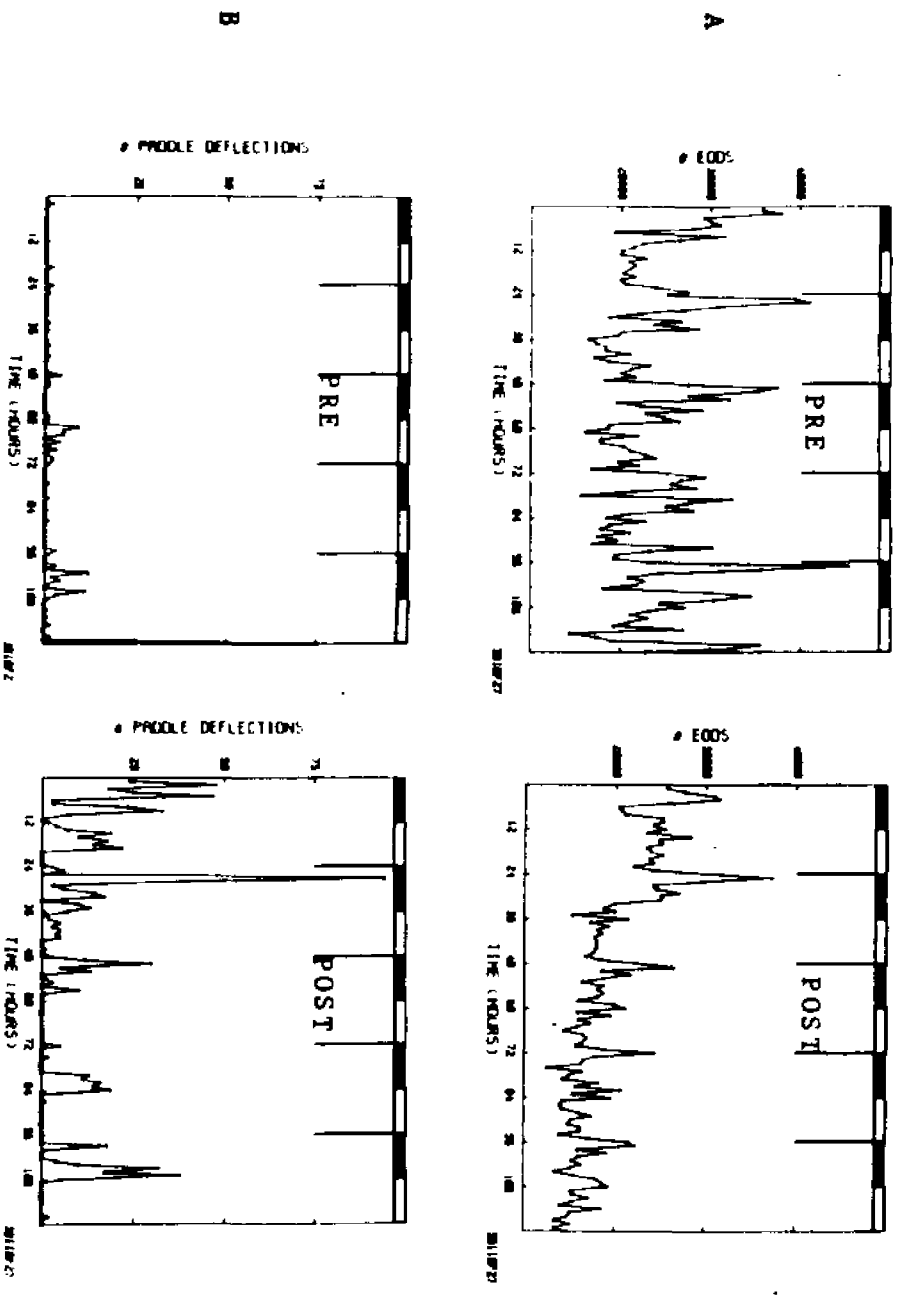
Figure 69. Electric organ discharge rate and locomotor activity, F27, before and during contact. Alternating black and open bar at tops of graphs is LD 12:12. Pre is before contact. Post is during contact. A. Electric organ discharge rate. Discharges plotted as function of time in hours. Reliable 24 hour entrainment of electric organ discharge rate both before and during contact. Note alternating periods of high and low discharge rates and decreasing trend during contact. Variability of electric organ discharge rate decreased during contact. More discharges during darkness on days 2,4 and 5. Electric organ discharge rate showed decreasing trend before B. Locomotor activity. Activity plotted as function of time in hours. No entrainment of activity before contact. Note 24 hour periodicity after contact was established. Increase in activity level before contact reversed after contact was established. Note decrease in variability during contact.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TIME (HOURS)

locomotor activity as it did for electric organ discharge rate. Before contact, entrainment was unreliable. After contact, locomotor activity entrained (Figure 67B & Appendix Figure 14). F25 was almost completely inactive for the first 4 days but was more active during the dark period on the fifth day (Figure 68B). A small peak at 1/24 hours in the spectral analysis confirmed the last day's entrainment (see Appendix Figure 16A & D). After contact, locomotor activity entrained (Figure 68B). Though the autocorrelation function did not confirm this, the spectral analysis did (see Appendix Figure 16C & D). F27 did not show entrainment of locomotor activity prior to contact but a clear pattern developed during contact (Figure 69B & Appendix Figure 18).

2. Trend. Before contact, there was an overall increase in the number of electric organ discharges for F9. During contact, there was a general decrease (Figure 63A). The number of discharges of F24 decreased before contact but reversed, after contact was established (Figure 65A). F26 showed no changes (Figure 66A).

For both F9 and F24, locomotor activity showed an overall increase before contact (Figures 63B & 65B). This tendency reversed itself during contact. F26 showed no change (Figure 66B).

Before contact, F10 showed no trend in electric organ discharge rate. Locomotor activity decreased. After contact was established, both decreased (Figure 67B). F25 showed a decrease in discharge rate both before and during contact. An increase in locomotor activity before contact, reversed itself after contact was established (Figure 68B). F27 showed no trend in electric organ discharge rate before contact while locomotor activity increased. After contact was established both decreased.

3. Variability. For all three pairs, the electric organ discharge rate of the fish in DD increased in variability after contact (Figures 63A, 65A & 66A & Table 1). One LD fish, F10, also increased its electric organ discharge variability, while the remaining two LD fish decreased variability during contact (Figures 67A, 68A & 69A & Table 1).

Variability of locomotor activity decreased for two of the fish in DD, F9 and F26, and increased for the third, F24, (Figures 63B, 65B & 66B & Table 1). For the LD partners, locomotor activity variability decreased for two, F25 and F27, and increased for the third, F10, during contact (Figures 67B, 69B & 67B & Table 1).

For the LD fish, variability changes in locomotor activity and electric organ discharge rate tended to move in the same direction, i.e., within the same fish locomotor activity and electric organ discharge rate both either increased or decreased in variability during contact. This was not the case for DD fish.

c. Experiment C (LD vs LD 90 out of phase)

1. Periodicity. Of the two fish entrained to the LD cycles, only one, F12, showed stable entrainment of electric organ discharge rate before contact (Figure 70A & Appendix 19A & B). The locomotor activity of F12 did not entrain (Figure 70B & Appendix 20A & B). F11 entrained to the LD cycle during days 2, 3, and 4 but showed a reversed pattern on days 1 and 5 (Figure 71A & Appendix Figure 21A & B). The locomotor activity of F11 showed a similar pattern (Figure 71B & Appendix Figure 22A & B). After contact was established, the electric organ discharge pattern of F12 changed (Figure 70A). On day 1, the pattern was reversed, with more discharges occurring during the light period than during the dark period. The autocorrelation function no longer oscillated and the large peak at 1/24 hours that appeared before contact was much smaller (see Appendix Figure 19C & D). Visual inspection of the raw data suggested that the locomotor activity of F12

Figure 70. Electric organ discharge rate and locomotor activity, F12, before and during contact. Alternating black and open bar at tops of graphs is LD 12:12. Pre is before contact. Post is during contact. A. Electric organ discharge rate. Discharges plotted as function of time in hours. Note obvious 24 hour periodicity before contact. After contact was established pattern reversal occurred on first day—more discharges during light period. Then more discharges during dark period for remainder of contact. Variability of electric organ discharge rate increased during contact. B. Locomotor activity. Activity plotted as function of time in hours. Note more activity during dark period for days 1, 2 and 3. Pattern was reversed on day 5. After contact was established entrainment occurred after first day. Entrainment was not confirmed by statistical analysis. Variability decreased during contact.

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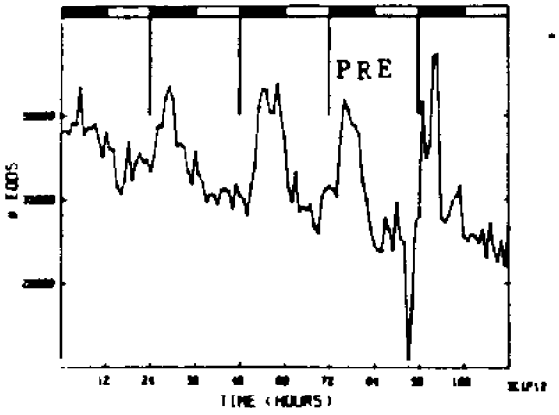
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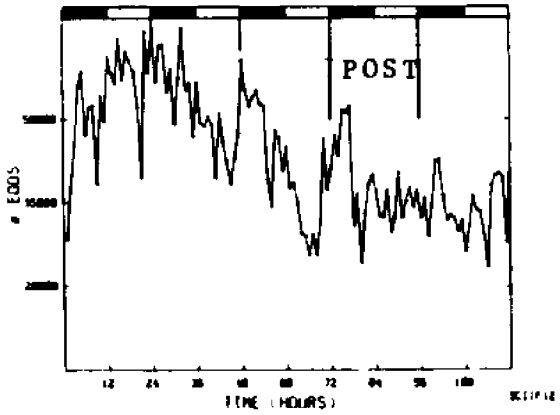
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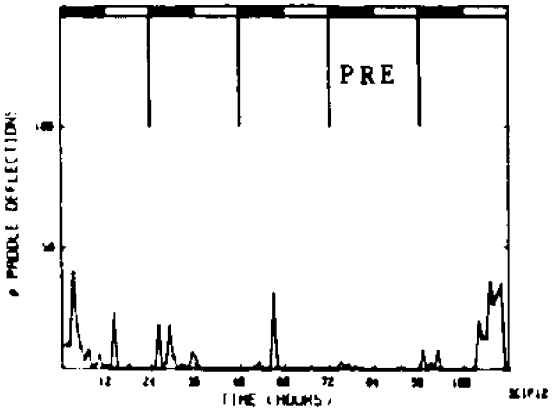
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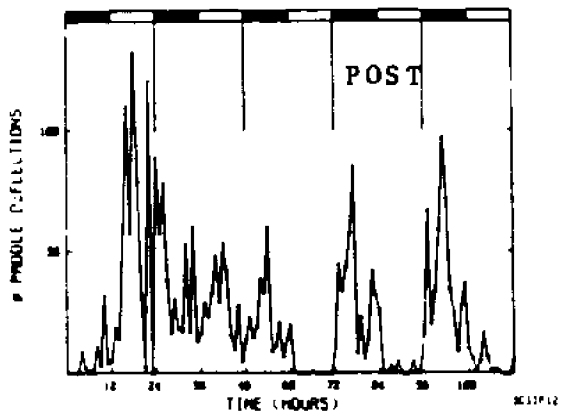
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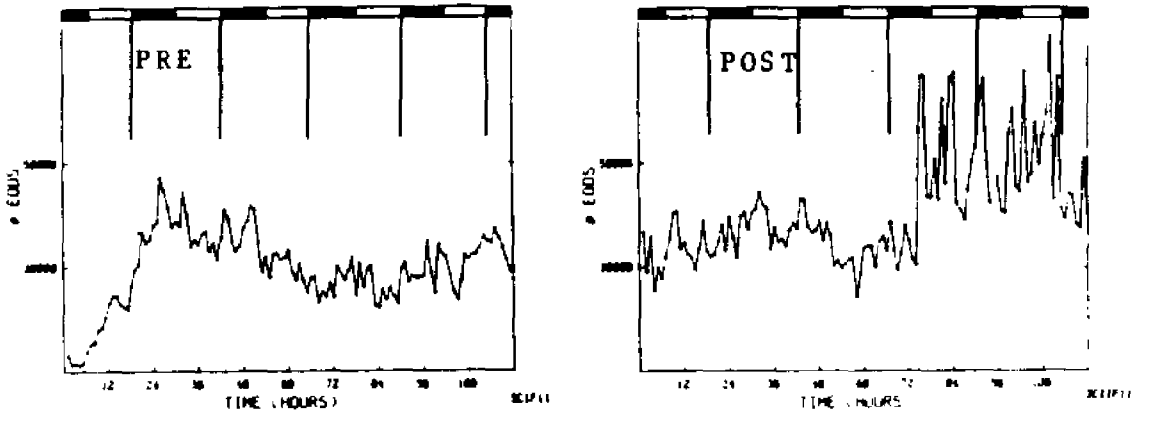
TIME (HOURS)

Figure 71. Electric organ discharge rate and locomotor activity, F11, before and during contact. Alternating black and open bar at tops of graphs is LD 12:12. * indicates 6 hour phase difference relative to F12. Pre is before contact. Post is during contact. A. Electric organ discharge rate. Discharges plotted as function of time in hours. Note unstable entrainment. On days 2,3 and 4, more discharges occurred in dark period. Pattern was reversed on days 1 and 5. After contact was established, entrainment remained unstable. Note general decrease in electric organ discharge rate before contact and increase after contact was established. Increase in variability occurred during contact. B. Locomotor activity. Activity plotted as function of time in hours. Activity shoed similar pattern as electric organ discharge rate. More activity during dark period for days 3,4 and 5. More activity during light period on days 1 and 2. After contact was established activity entrained to LD cycle.

A



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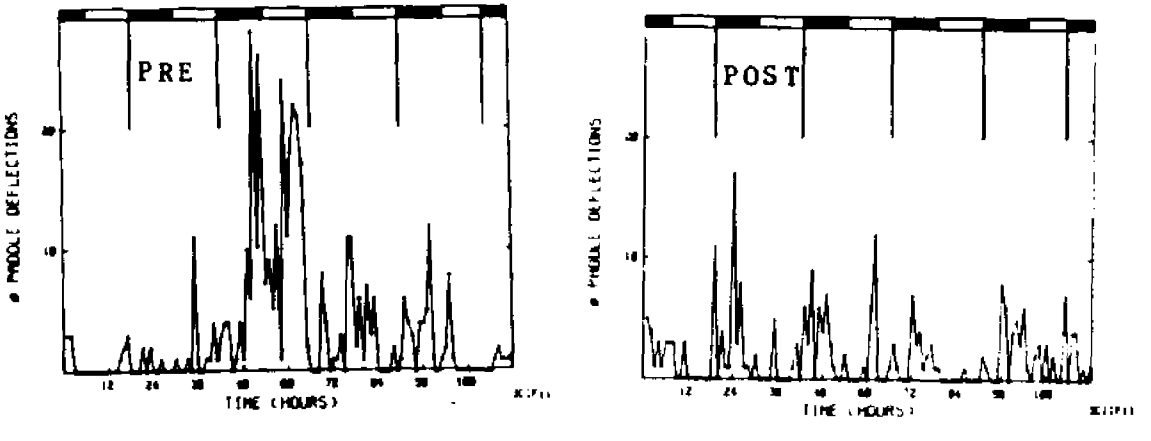


entrained after the first day of contact (Figure 70B) though this was not confirmed by the spectral analysis (see Appendix Figure 20C & D). There was still no stable entrainment of electric organ discharge rate for F11 (Figure 71A & Appendix Figure 21C & D) though locomotor activity did develop a 24 hour periodicity (Figure 71B & Appendix Figure 22C & D).

2. Trend. One fish, F11, showed an overall decrease in the number of electric organ discharges before contact. This tendency was reversed during contact (Figure 67A). No other changes were found.

3. Variability. For both fish, the electric organ discharge rate increased in variability during contact while locomotor activity decreased in variability (Table 1).

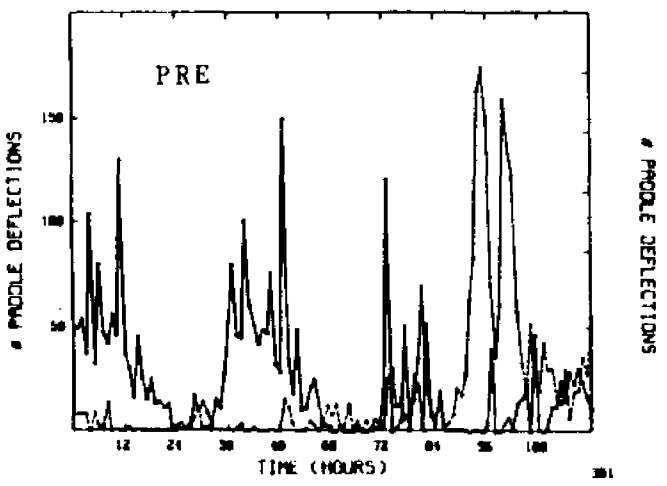
Discussion

The results of this experiment demonstrated that social contact, through the electrosensory channel, may be an entraining cue for locomotor activity, but not for electric organ discharge activity, in G. petersii. However, changes in pattern, trend and variability in electric organ discharge rate during contact were observed.

In all the DD manipulations of Experiments 1 and 2, no fish free ran with respect to locomotor activity. When the activity data for F9 are examined (Experiment 3B) it can be seen that the activity level was very low. It began increasing during the fourth day of DD in isolation (Figure 63B). No periodicity was detected when the data were statistically analyzed. During contact (Figure 63B) a pattern of alternating periods of high and low activity rates occurred, and the 24 hour periodicity of this pattern was confirmed by statistical analysis. When locomotor activity of F9 was compared with that of its LD partner, F10, some correspondence of pattern was seen during contact. Figure 72A shows the locomotor activity for both fish before and during contact. Before contact, there was very little similarity of their patterns. During contact, in general, low rates of activity of F9 corresponded to low rates of F10 and the patterns of activity were similar. Since the effects of constant darkness on locomotor activity included loss of rhythmicity and damping out of activity over time (Experiment 1), social contact must have played some role in the development of 24 hour periodicity of the locomotor activity of F9. Furthermore, since the fish in Experiment 3A (DD vs DD) did not develop periodic locomotor activity during contact, F10 in Experiment 3B must have provided cues about its environment and/or its activity level to F9, possibly

Figure 72. Comparison of locomotor activity of F9 in constant darkness with locomotor activity and electric organ discharge rate of F10, its LD 12:12 partner. Pre is before contact. Post is during contact. A. Number paddle deflections of F10, left ordinate, plotted as function of time in hours. Solid line is F10. Number paddle deflections of F9, right ordinate, plotted as function of time in hours. Dashed line in F9. Note little similarity of patterns before contact. After contact was established, activity of F9 corresponded with that of its LD partner. Peaks corresponded with peaks and trough with troughs. B. Number of electric organ discharges of F10, on left ordinate, plotted as function of time in hours. Solid line is F10. Number of paddle deflections of F9, right ordinate, plotted as function of time in hours. Dashed line is F9. Before contact no similarity of patterns. During contact, activity pattern of F9 became similar to electric organ discharge pattern of F10. Peaks corresponded to peaks and troughs to troughs.

A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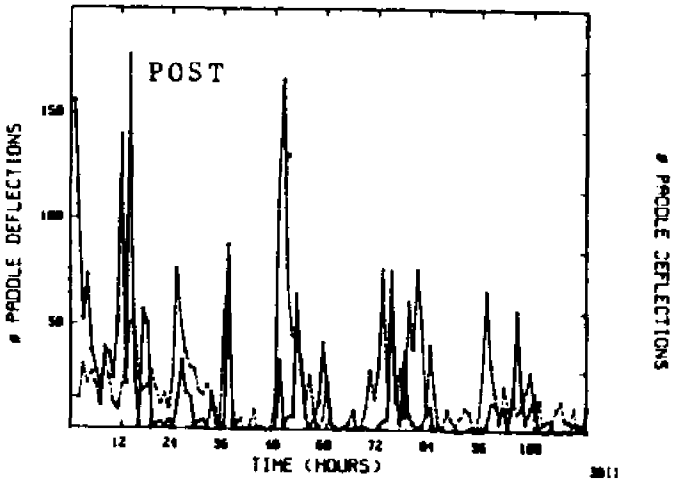
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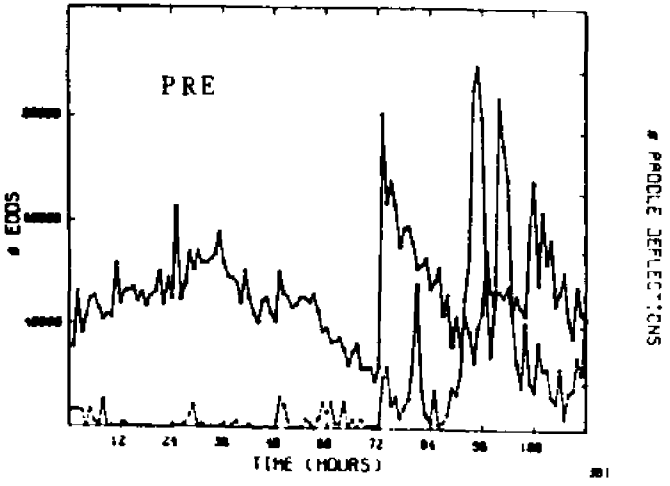
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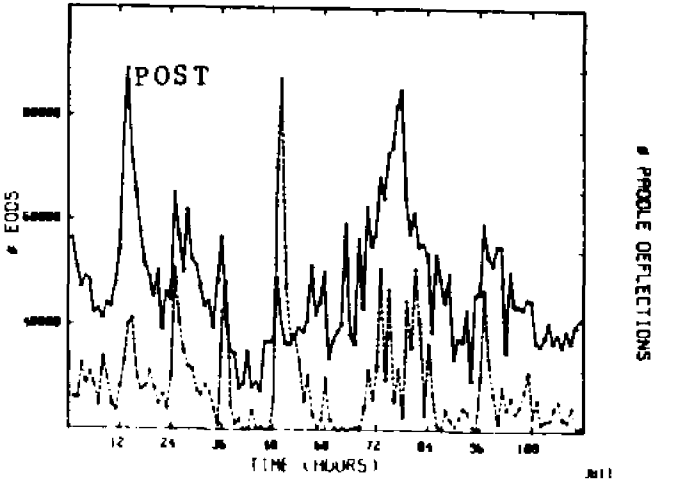
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through its electric organ discharges. To examine this, locomotor activity of F9 was compared with the electric organ discharge rate of F10 both before and during contact. Figure 72B shows the data of both fish. Before contact, there was no similarity of pattern. After contact, however, the locomotor activity pattern of F9 became similar to the electric organ discharge pattern of F10.

Social contact also appeared to have some effect on electric organ discharge rate. No fish developed reliable periodicity of electric organ discharge rate following contact. It should be noted that one fish, F22, prior to contact, showed periodicity of electric organ discharge rate. The peak at 1/24 hours found in the spectral analysis (Appendix Figure 3c) appeared in all three sequential segments, however, in progressively decreasing size. After contact was established, no 24 hour periodicity was detected. This could again suggest the disappearance of rhythmicity during the course of the experiment. If the previously discussed definition of circadian rhythmicity is applied (see Discussion-Experiment 2), i.e., the persistence of a rhythm in constant conditions for a 'reasonable' number of cycles without major changes in period and without attenuation, then it is still not possible to conclude that circadian rhythmicity of electric organ discharge rate was demonstrated.

The evidence from Experiment 2 and from this experiment indicates that the electric organ discharge activity may exhibit a circadian rhythm if the fish are observed for longer recording periods. Future experiments should consider this.

Trend and variability changes in electric organ discharge rate did occur. A systematic relationship was not found, but a change in the direction of a trend was seen following contact for four of the six fish in Experiment 3B. In Experiment 3A, when all fish were in DD, there was no change in the direction of trend in electric organ discharge rate. The same was true of Experiment 3C. It appears that in the conflict manipulation with the greatest difference in environmental information, the DD fish were more likely to change the direction of trend in electric organ discharge rate.

A change in the variability of electric organ discharge rate during contact was also noted. Across manipulations, five of the seven DD fish increased the variability of electric organ discharge rate during contact. One decreased variability and one did not change. For the five LD fish, three increased variability and two decreased variability during contact. The increase in variability of electric organ discharge rate suggested that the fish were interacting

electrically after contact. Both mormyrids (Moller, 1970a, 1970b) and gymnotids (Hopkins, 1974, 1977; Westby, 1975, 1981) change their discharge patterns when presented with artificial pulses (Moller, 1970a), taped recorded natural discharges (Moller, 1970b) or intact, electrically active conspecifics (Moller & Bauer, 1973). A change in the observed hourly electric organ discharge patterns could reflect any combination of periods of highly variable, regularized (Moller, 1970a) discharges and periods of electric 'silence' (Moller & Bauer, 1973). The raw data of the DD fish in Experiment 3B (Figures 65-70) indicated that during contact there were many more peaks and troughs as compared with the data of isolated fish. These peaks and troughs may be a reflection of the changes in patterning described by Moller and Bauer (1973). The present data are based on hourly totals of electric organ discharges while Moller and Bauer used a much finer average time resolution (500 ms). Thus, a further step in the analysis of the data would include looking at much smaller sampling intervals, minute or second totals, for example, to establish the interval-to-interval interactions between the fish.

General Discussion

Circadian rhythms share several general characteristics: they free-run under constant conditions, entrain to environmental cycles, and phase shift in response to phase shifts of a zeitgeber. Typically, a circadian rhythm will show one or more transient cycles when a short light pulse is presented during constant darkness or a zeitgeber, to which it is entrained, is phase shifted. A transient is a cycle with a rapidly changing period that occurs between two steady states. A steady state refers to a rhythm with an unchanging or stable period length. A steady state may not occur for several months following a manipulation (Aschoff, 1979). There may be anywhere from one to ten transient cycles. If a zeitgeber is phase advanced, that is, if light onset and offset occur earlier than on the previous day, a phase shift of the behavior rhythm will occur through one or more transient cycles whose periods are shorter then the period of its original steady state. A phase delay of a rhythm in response to a phase delay of a zeitgeber will occur through one or more transients that have periods longer than the original steady state period (Pittendrigh & Daan, 1976a).

The presence of transients suggests two possibilities regarding the nature of an organism's underlying circadian

organization. First, transients may reflect the gradual shift of an internal pacemaker. If this is the case, then it is likely that an oscillator and its overt periodicities are tightly coupled. The second possibility proposes that an underlying pacemaker shifts immediately and that transients reflect a gradual shift of an overt rhythm until it re-couples with its pacemaker(s). This type of system is weakly coupled. Regardless of the type of system, however, "The overt transient cycles...reflect the behavior of the underlying oscillator." (p.124, Bünning, 1973).

In all my experiments, none of the fish exposed to constant conditions exhibited a free running activity or electric organ discharge rate rhythm. However, entrainment to a light-dark cycle did occur. Four fish subjected to a 6 hour phase shift (delay) of the light dark cycle shifted their locomotor activity and electric organ discharge rhythms. One fish, F1, (Experiment 1) exhibited transient cycles (Figure 15a). Over a 3 day period locomotor activity was delayed 2 hours per day until it re-entrained. The presence of the three transient cycles suggests either a gradual shift of a pacemaker that times locomotor activity or a shift of the overt behavior to re-couple with the already shifted pacemaker.

If transients reflect the existence of an internal timing system, then the fact that three phase shifted fish did not show transients should be explained. In general, within individuals and across individuals and species, great variability of periodicity, rhythm amplitude, stability and responsiveness of rhythms to changes in environmental cues is found. For example, Eriksson & van Veen (1980) found a circadian activity rhythm in 75% of the brown bullheads they studied. Still, though, 25% did not show a rhythm. Among humans, 10-16% tested showed a dissociation of body temperature and activity rhythms in DD (Pittendrigh, 1974). Consequently, there is no reason to assume electric organ discharge rate and locomotor activity of all G. petersii will respond the same way in all experimental manipulations. Instead, much individual variability was found. The fact that one of the four fish did show transients is significant. The remaining fish may have been less sensitive to the 6 hour delay than F1. Perhaps an 8 hour delay would have resulted in the demonstration of transients in these fish.

The most obvious example of the variability found in the fish tested occurred in the three replications of Experiment 3B (DD vs LD). Of the three fish entrained to the LD cycle, each appeared to entrain differently (Experiment 3B). When the experimental situation was analyzed in an attempt to

discover reasons for the variability no reliable cyclic cues in the environment outside the experimental chamber could be shown to have influenced the fish. For example, the data were re-examined for possible effects of weekday activity as opposed to weekend activity in the laboratory. No systematic effects were seen. It was concluded that, barring some unknown influence, the variability of response must be due to some factor or factors in the fish, perhaps their rhythmic organization.

Fish may possess a multi-oscillator circadian system (Eriksson, 1978; Eriksson & van Veen, 1980; Kavaliers, 1981a, b). Under synchronized or entrained conditions, it has been found that some fish will alternate between nocturnalism and diurnalism in relation to seasons and migratory behavior (Godin, cited in Eriksson & van Veen, 1980; Osterdahl, 1969) and food availability (Eriksson & van Veen, 1980). The dualism of behavior synchronization (Eriksson, 1978) allows for ecological adaptibility. This kind of system is probably controlled by several circadian oscillators that are loosely coupled. If an organism with this type of circadian organization is placed under constant conditions, the oscillators will un-couple and each will free run with its own spontaneous frequency. With each oscillator free running, arhythmic activity results (Eriksson & van Veen, 1980). In

the field it is most likely that the oscillators and overt activity rhythms are synchronized by the light-dark cycle.

So far, little is known of the circadian organization of behavior in African mormyrids and South American gymnotids, and the ecological factors related to behavior. However, several points should be made. Studies made in the Swashi River, a tributary of Lake Kainji, Nigeria revealed a daily migratory pattern of mormyrid fish and a nocturnal increase in electric organ discharge rate and locomotor activity. The fish remained in an inlet during the day and moved out of the inlet at night to feed (Moller et al., 1979). Gymnotid fish are also nocturnal and feed at night (Lissmann & Schwassmann, 1965, Hopkins, 1974). Observations in our lab have shown that both mormyrids and gymnotids will feed during daylight hours if food is made available. Rusak (1981) has suggested that the flexibility of feeding behavior found in many organisms may reflect the adaptive flexibility of an underlying oscillator system. It is not simply a behavioral response to environmental stimuli that is independent of the controlling oscillators.

In Experiment 1, during the first free run condition, both fish showed small bursts of activity during the hour when the light would have gone off if an entrainment schedule had

been in effect. During entrainment, there was almost always a reliable burst of activity at lights off (Figures 14A & 15A). This behavior was not seen in other free run conditions. The small bursts seen for the first two fish suggest the presence of a time keeping mechanism that may have still been running even though the overt behavior rhythm disappeared. An overt rhythm may disappear, the behavior becomes arhythmic or it may damp out completely, but the internal pacemakers may actually be free running. In this situation the internal pacemakers and the overt behavior rhythm are un-coupled (Bunning, 1973; Enright, 1981). Furthermore, no fish demonstrated reliable free running rhythmicity of either electric organ discharge rate or locomotor activity. Fish commonly become arhythmic in constant conditions (Eriksson & van Veen, 1980). The explanation for arhythmicity may be due to the flexibility of a multi-oscillator system and not to the absence of a pacemaker (Eriksson & van Veen, 1980).

Another piece of evidence for several oscillators in G. petersii was found. The first entrainment schedule occurred after the fish had been unintentionally exposed to constant dim light followed by the alternation of two constant light intensities. The second entrainment occurred after 16 days of constant darkness with only one 15-minute light pulse on day 7 that had no obvious effect on the overt pattern of activity.

It cannot be determined what effect, if any, the light pulse may have had on the internal oscillators. If the data from these two entrainments are compared (Figures 14, 15, 28 & 29) it can be seen that the entrainment to the light-dark cycle was immediate, following the alternation of light intensities while it is clearly more irregular following constant darkness. It is possible that the alternation of constant light intensities was sufficient to entrain one or more of the internal oscillators in a multi-oscillator system. However, alternating constant light intensities was not sufficient for the entrainment of the overt behavior rhythm. Once a light-dark cycle occurred the overt activity rhythm immediately recoupled with the already entrained oscillators. Entrainment following constant darkness may have been irregular because each oscillator in the system was free running with its own natural frequency, producing an arhythmic behavior pattern. Once the light-dark cycle was introduced it may have taken several days for all the oscillators to resynchronize and for the overt behavior to become rhythmic.

Results from Experiments 2 and 3 also provide more evidence for a multioscillator system controlling behavior in G. petersii. Locomotor activity was arhythmic under constant conditions for all fish. However, electric organ discharge rate consistently showed unreliable 24 hour periodicity in DD. As mentioned earlier this unreliability may have been due to

the appearance, disappearance or change in a rhythmic component during the recording period. The exact nature of this change can only be determined with more data. During entrainment and phase shifting of the LD cycle, electric organ discharge rate for F18 entrained immediately. Locomotor activity did not establish a stable pattern until 2-1/2 days after introduction of the LD cycle (Figure 44). When F18 was released into DD again, electric organ discharge rate was high and locomotor activity was low. However, locomotor activity increased over time until it 'caught up' with electric organ discharge rate. Then both decreased. For F19, locomotor activity remained arhythmic even when electric organ discharge rate established a 24 hour period. What these results suggest is the presence of a separate oscillator (or oscillators) controlling each behavior. Under natural conditions, an LD cycle, electric organ discharge rate and locomotor activity show a high degree of correspondence. Under experimental conditions, the two rhythms responded differently. Whereas electric organ discharge rate readily entrained, locomotor activity did not. If the two periodic activities were controlled by the same oscillator or oscillators, one would expect their responses to changes in the LD cycle to be the same. They were not. Looking at the relation between locomotor activity and electric organ discharge rate, for both fish, the results suggest that the oscillator(s) controlling

electric organ discharge rate responded to the light manipulations faster than the oscillator(s) controlling locomotor activity or recoupling of electric organ discharge rate with its oscillator(s) occurred first.

Finally, one fish demonstrated the presence of a pre-emergence electric organ discharge rate increase. During entrainment, electric organ discharge rate was higher during the second 6 hours of the light period as compared with the first 6 hours. Following the a 6 hour phase shift (delay) this 'anticipatory' increase disappeared. Perhaps the mechanism controlling the pre-emergence increase was slower in its shifting then that actually controlling the overall electric organ discharge rate rhythm.

It is hoped that the present investigation has set some of the groundwork for a more extensive analysis of the environmental control of rhythmic behavior in G. petersii. Light is certainly an important cue for both locomotor activity and electric organ discharge rate. The effectiveness of temperature and limited social contact cannot be ruled out. The results do suggest the possible existence of an internal timing system but more subjects must be studied for longer periods of time before clear cut conclusions and generalizations may be made about behavioral rhythmicity in G. petersii.

Appendix 1

Glossary

Circarhythm: a rhythm that is capable of free running in constant conditions, e. g., constant darkness, constant temperature. The period of a circarhythm closely resembles the period of the environmental cycle to which it normally entrains. Circadian rhythms have periods equalling 24 hours, the duration of the daily light-dark cycle. Circarhythms are entrained by zeitgebers. They are typically found only under laboratory conditions.

DD: constant darkness or dim light.

Diurnal rhythm: Event or behavior that occurs during the light phase of a light-dark period.

Endogenous rhythm: A rhythm requiring no periodic environmental input to maintain its periodicity; a self-sustaining rhythm or an active system (cf. free running).

Entrainment: the coupling of a rhythm to an environmental cue such that both display the same period, also called synchronization.

Exogenous rhythm: a response to a periodic input coming from outside the biological unit in which it is observed. Analogous to forced oscillations of a system that shows rhythmicity only under the influence of external signals. Rhythmicity damps out under constant environmental conditions.

Free-running rhythm: a self-sustaining rhythm not entrained to any zeitgeber and therefore displaying its spontaneous or natural period. This occurs under constant conditions.

Frequency: the reciprocal of the period, for example, if the period of a rhythm equals 24 hours, the frequency equals 1/24 hours or .0147.

LD cycle: alternating periods of light and darkness (or brighter and dimmer periods). An LD 14:10 cycle has 14 hours of light followed by 10 hours of darkness.

LL: constant light

Nocturnal rhythm: Event or behavior that occurs during the dark phase of a light-dark period.

Period: the duration of a single cycle, i.e., the time between two successive recurrences of a specified phase of a cycle.

Phase: the instantaneous state of an oscillation. Any point within the cycle.

Phase angle: the value on the abscissa corresponding to a particular phase of a cycle; this is given as some fraction of the total cycle (hours, degrees, radians).

Phase angle difference: the difference between corresponding phase angles of two coupled oscillators.

Phase shift: a displacement of an oscillation along the time axis. A shortening of the period is a phase advance while a lengthening of the period is a phase delay.

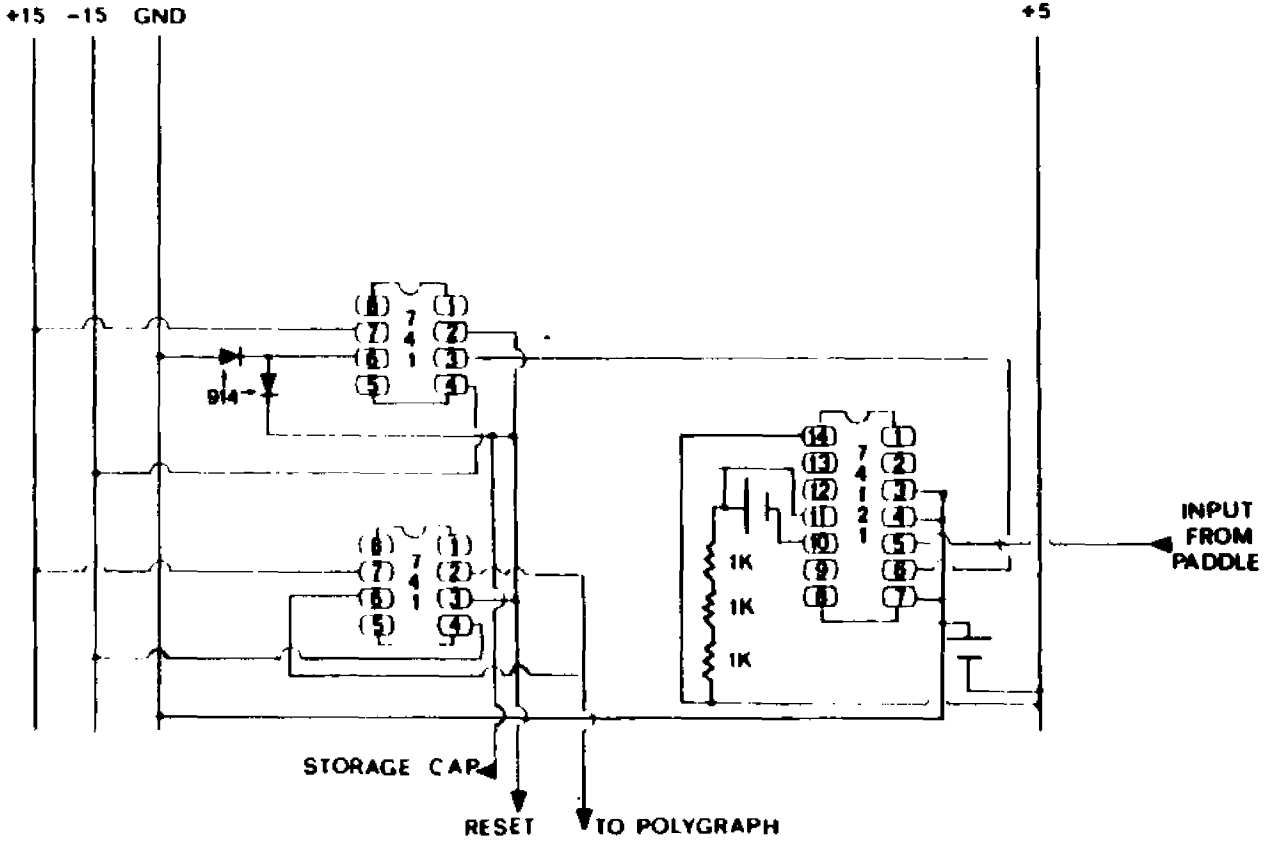
Zeitgeber: a periodic environmental factor that entrains a circadian rhythm; the LD cycle is a zeitgeber.

Definitions modified from Aschoff (1965, 1981).

Appendix 2

Circuit diagram- Activity counter

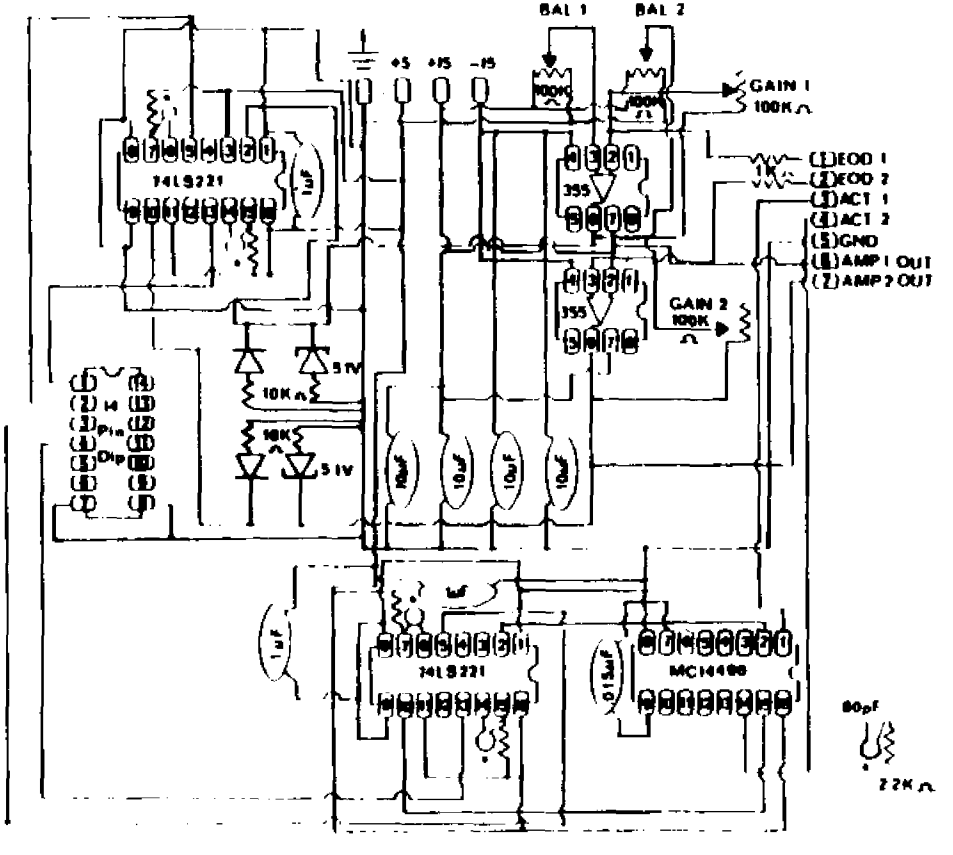
Experiment 1


+15
-15
GND
+5
914
1K
1K
1K
INPUT
FROM
PADDLE
STORAGE CAP
RESET
TO POLYGRAPH

Appendix 3

Schmidt trigger interface

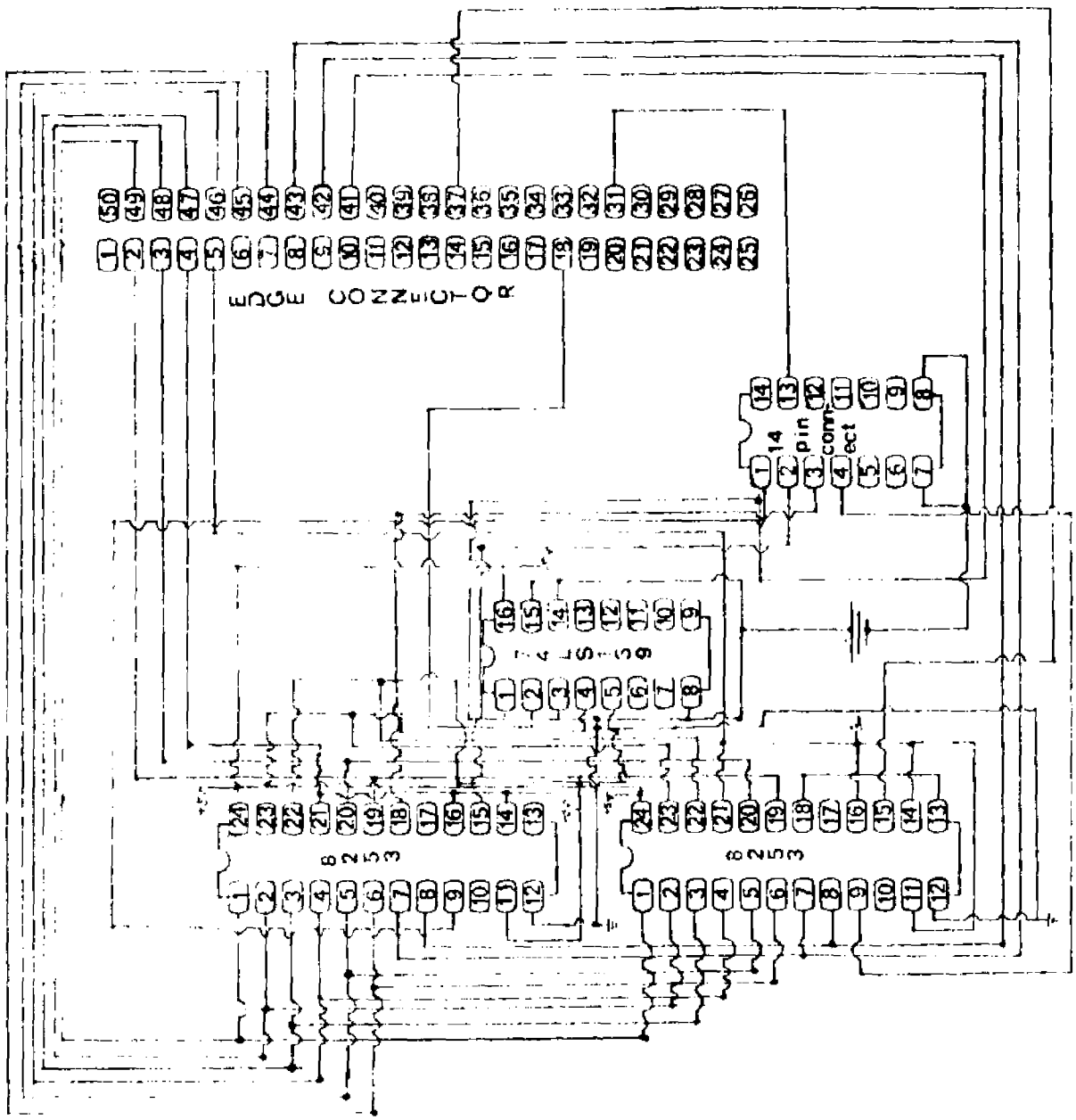
Experiments 2 and 3


BAL 1
BAL 2
+5
+15
-15
GAIN 1
100K Ω
74LS221
355
(1) EOD 1
(2) EOD 2
(3) ACT 1
(4) ACT 2
(5) GND
(6) AMP 1 OUT
(7) AMP 2 OUT
GAIN 2
100K
5.1V
10K Ω
10µF
1µF
74LS221
MC14490
80pF
22K Ω

Appendix 4

Pulse former interface

Experiments 2 and 3


14 pin connect
8253

Appendix 5

Autocorrelations and spectral analyses of locomotor activity and electric organ discharge rate data.

Experiment 3

List of Figures

Figure 1. Electric organ discharge rate, F20.

Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. Note autocorrelation function did not oscillate (A) and there is no peak at 1/24 hours before contact (C). After contact was established the autocorrelation function oscillated (B) but not with 24 lags peak to peak. Note peak at 1/24 hours in spectral analysis (D). Reanalysis of data by sequential segments suggested the 24 hour periodicity was unreliable.

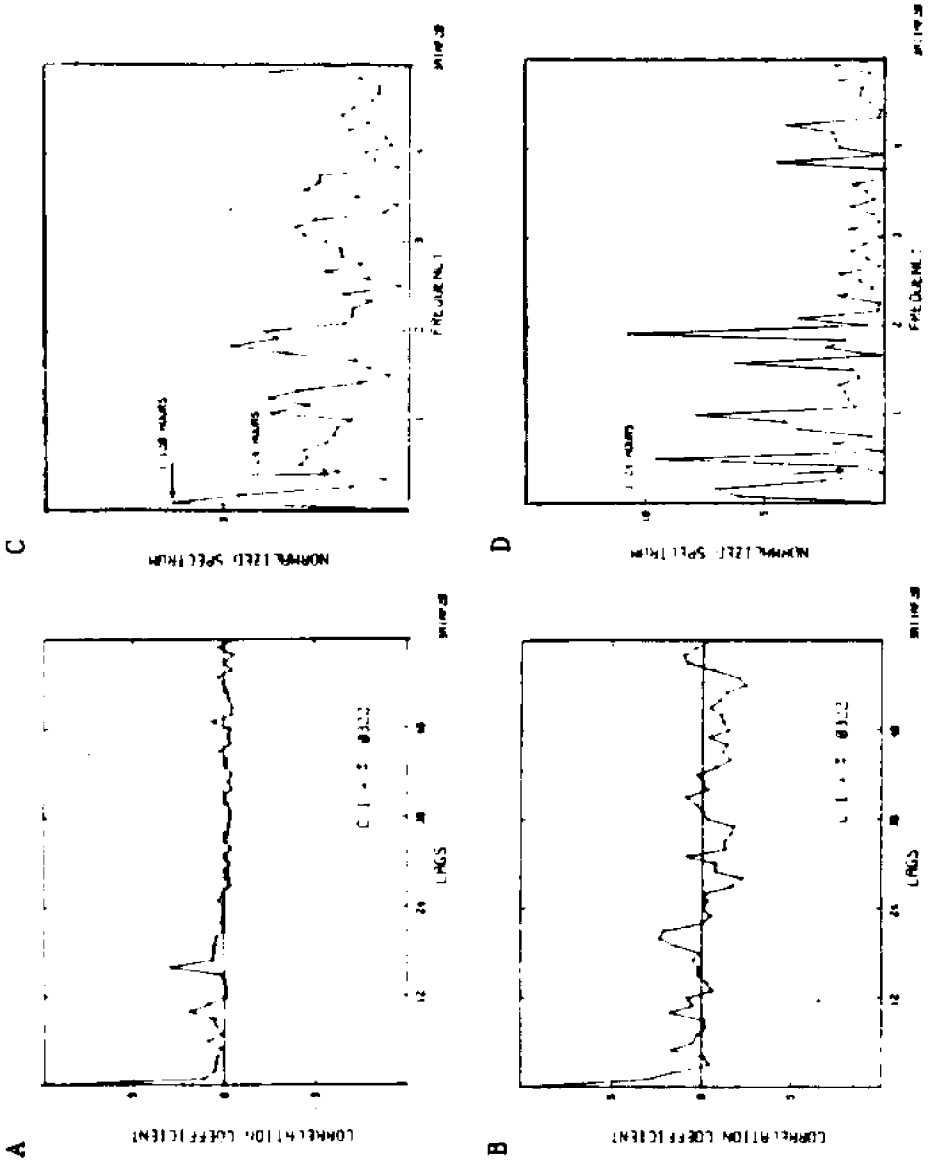

A
B
C
D
CORRELATION COEFFICIENT
LAGS
NORMALIZED SPECTRUM
FREQUENCY

Figure 2. Locomotor activity, F20. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours.
No oscillation of autocorrelation functions before (A) or during contact (B). No peak at 1/24 hours in spectral analysis before (B) or during contact (D).

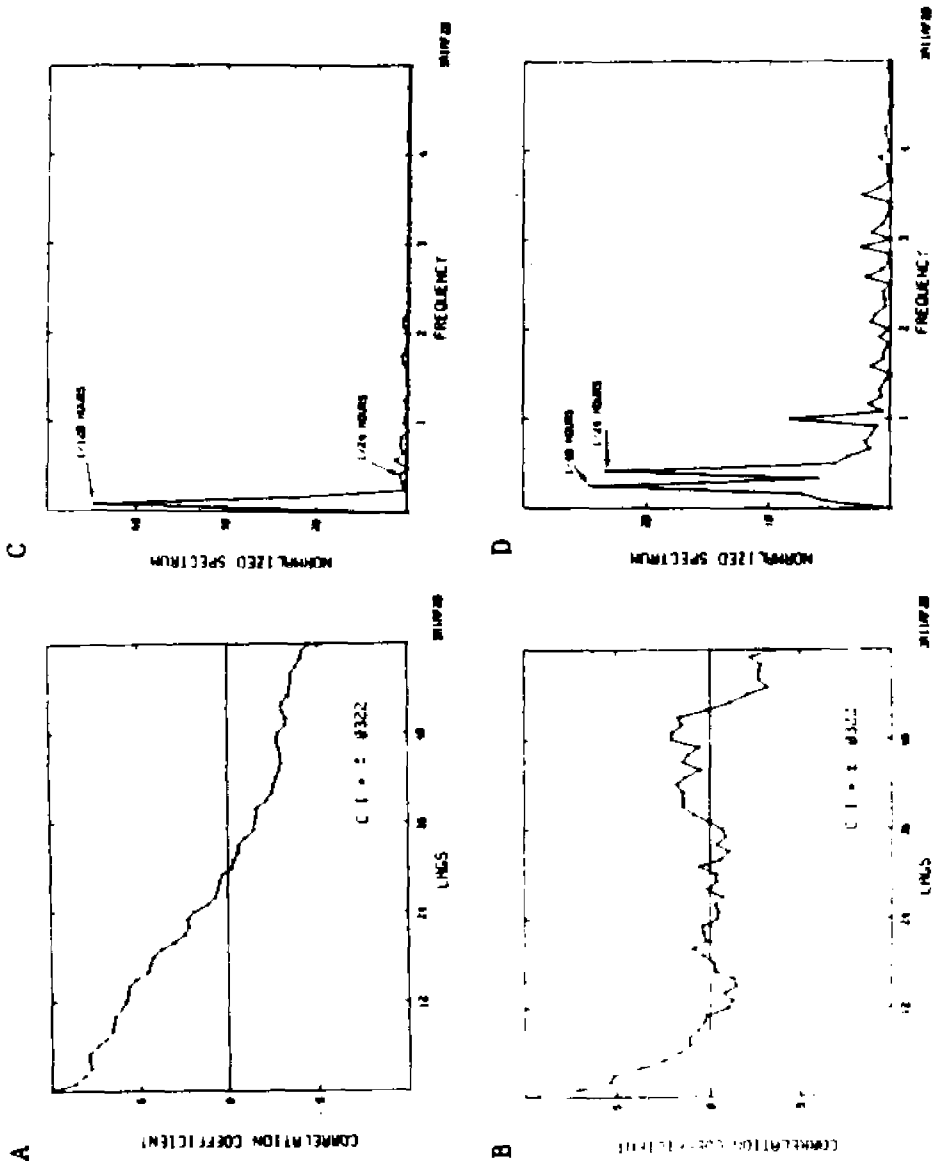

A
B
C
D
CORRELATION COEFFICIENT
LAGS
NORMALIZED SPECTRUM
FREQUENCY

Figure 3. Electric organ discharge rate, F22. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. Note no oscillation of autocorrelation function before contact (A) but small peak at 1/24 hours in spectral analysis (C). After contact was established, autocorrelation function still did not oscillate (B) and peak at 1/24 hours in spectral analysis disappeared (D). F22 showed rhythmicity of electric organ discharge rate before contact but not during contact.

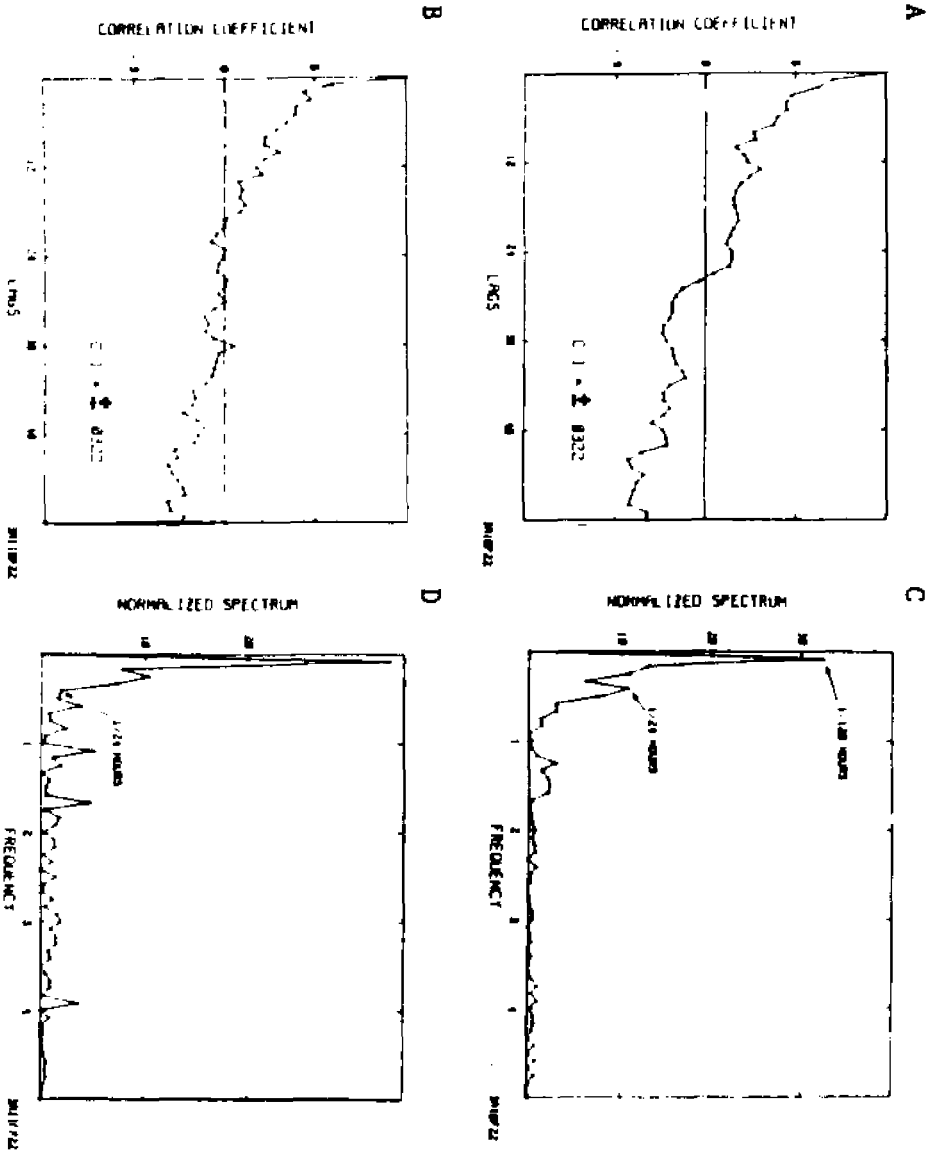

A
CORRELATION COEFFICIENT
B
CORRELATION COEFFICIENT
C
NORMALIZED SPECTRUM
FREQUENCY
D
NORMALIZED SPECTRUM
FREQUENCY

Figure 4. Locomotor activity, F22. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. Note lack of oscillation of autocorrelation functions both before (A) and during (B) contact. No peak at 1/24 hours in spectral analysis before (C) or during (D) contact. No periodicity of locomotor activity before or during contact.

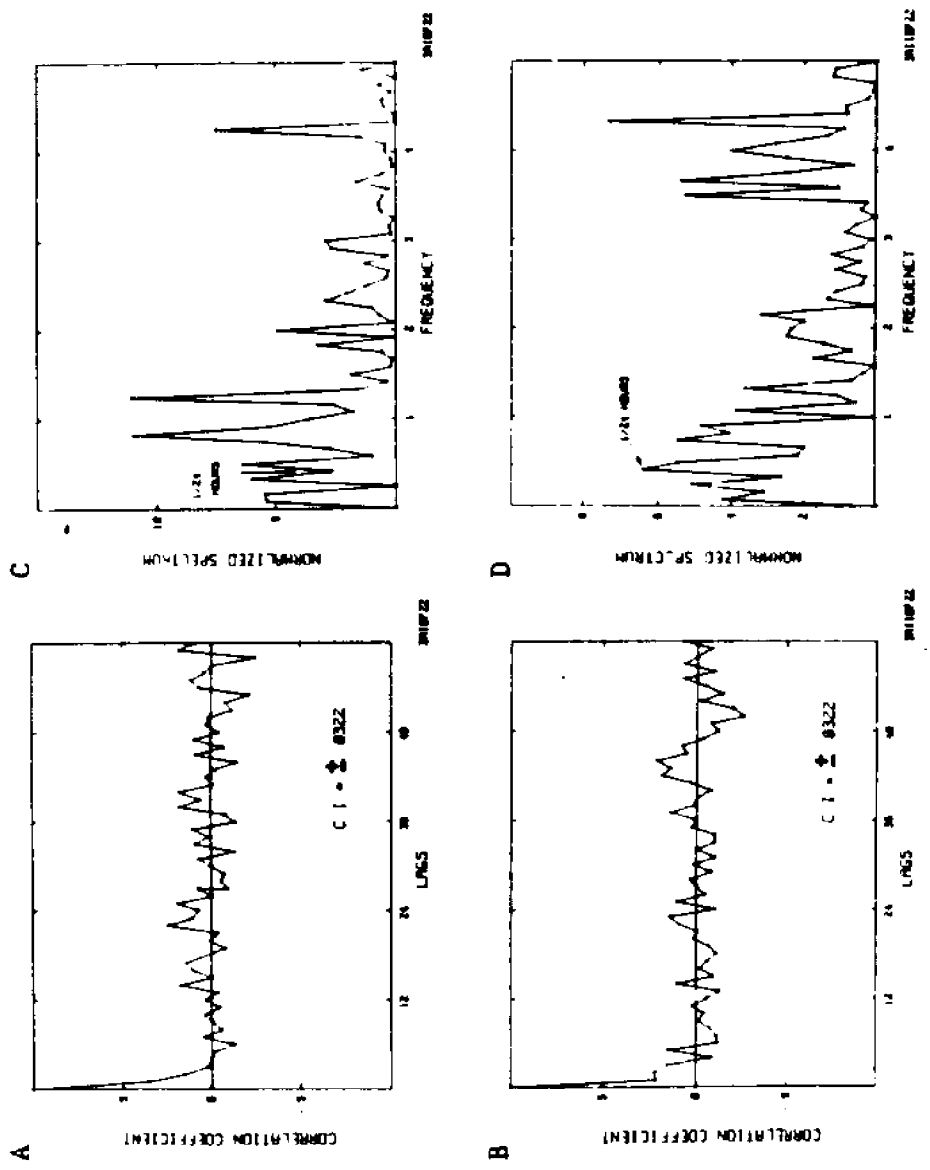

A
B
C
D
CORRELATION COEFFICIENT
NORMALIZED SPECTRUM
LAGS
FREQUENCY
C I = ± 0322

Figure 5. Electric organ discharge rate, F23. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. No periodicity of electric organ discharge rate before or during contact. Autocorrelation functions did not oscillate (A,B) and there were no peaks at 1/24 hours in spectral analyses (C,D).

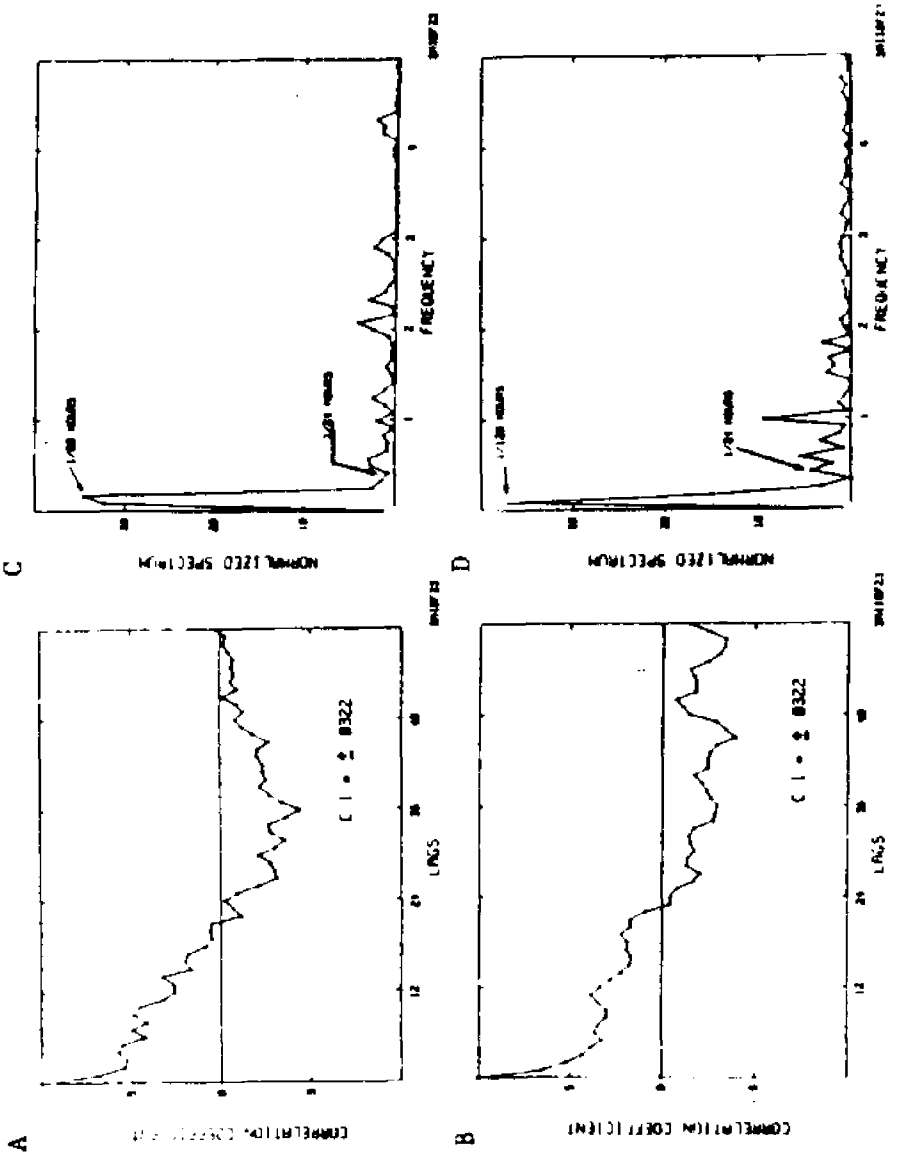

A
B
C
D
FREQUENCY
LAGS
95% CI
NORMALIZED SPECTRUM

Figure 6. Locomotor activity, F23. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. No periodicity of locomotor activity before or during contact. Note absence of oscillations of autocorrelation functions and absence of peaks at 1/24 hours in spectral analyses both before (A,C) and during (B,D) contact.

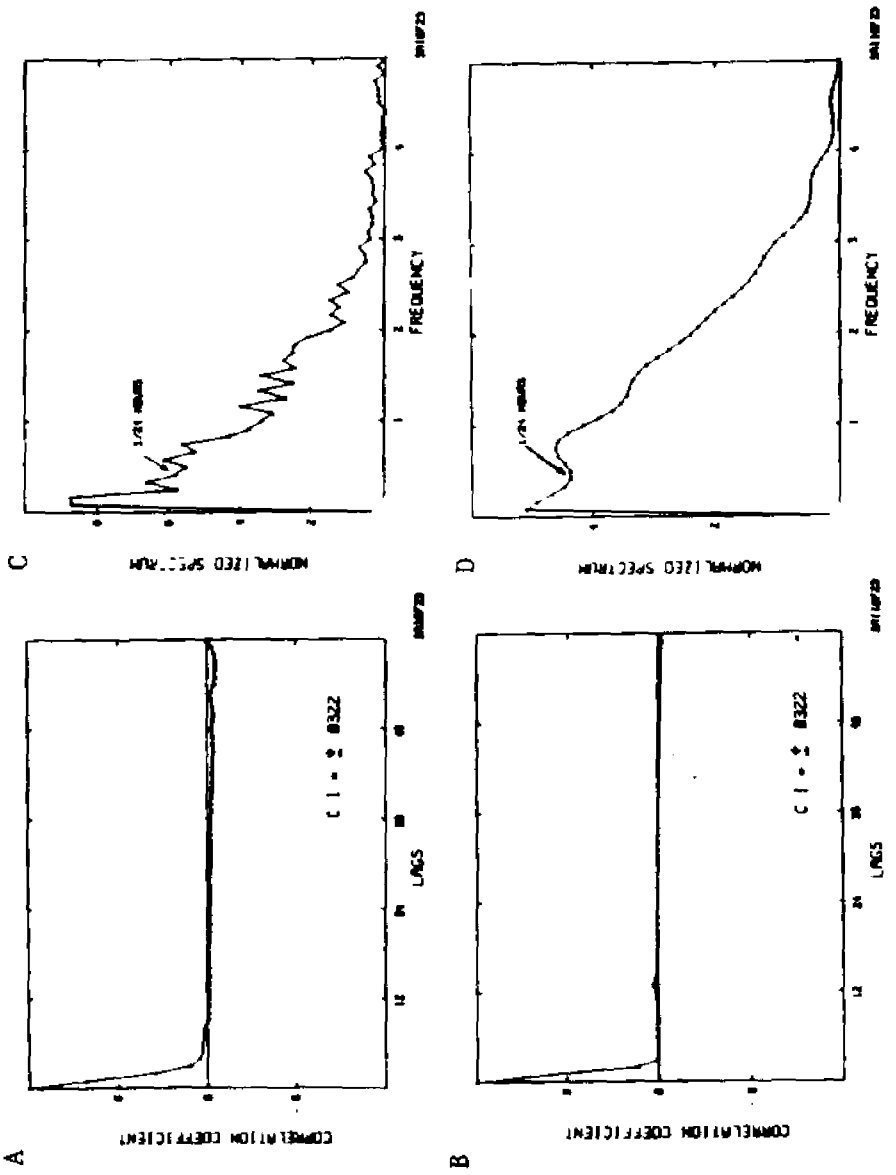

A
B
C
D
CORRELATION COEFFICIENT
LAGS
NORMALIZED SPECTRUM
FREQUENCY

Figure 7. Electric organ discharge rate, F21. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. No periodicity of electric organ discharge rate before or during contact. Note lack of oscillation of autocorrelation functions before (A) and during (B) contact. No peak at 1/24 hours in spectral analyses before (C) and during (D) contact.

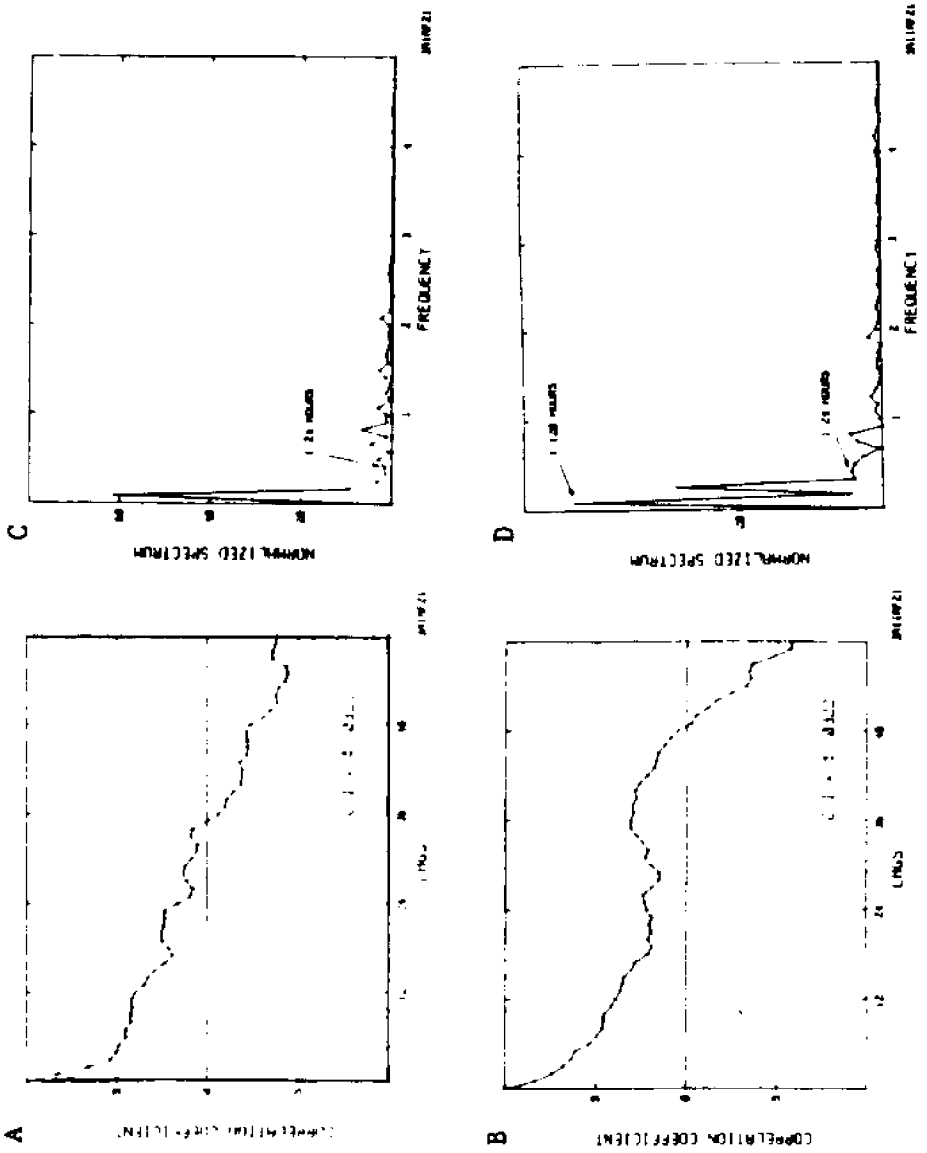

A
B
C
D
FREQUENCY
FREQUENCY
NORMALIZED SPECTRUM
NORMALIZED SPECTRUM

Figure 8. Electric organ discharge rate, F9. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. Note absence of oscillation of autocorrelation functions before (A) and during (B) contact. Note small peaks at 1/24 hours in spectral analyses both before (C) and during (D) contact.

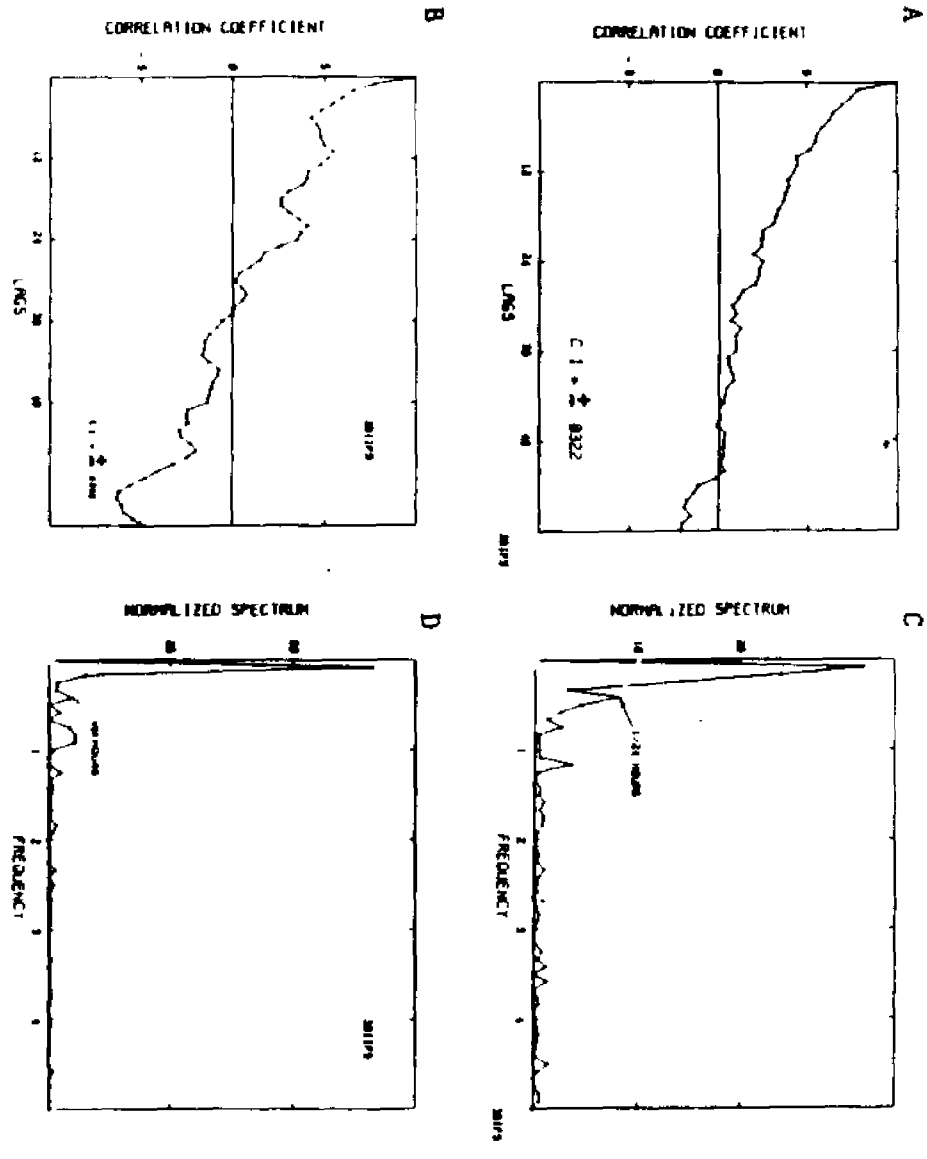

A
CORRELATION COEFFICIENT
LAGS
B
CORRELATION COEFFICIENT
LAGS
C
NORMALIZED SPECTRUM
FREQUENCY
D
NORMALIZED SPECTRUM
FREQUENCY

Figure 9. Electric organ discharge rate, F24. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. Note oscillation of autocorrelation function around zero both before (A) and during (B) contact. Peak at 1/24 hours before contact (C) disappeared after (D) contact was established indicating presence of unreliable periodicity.

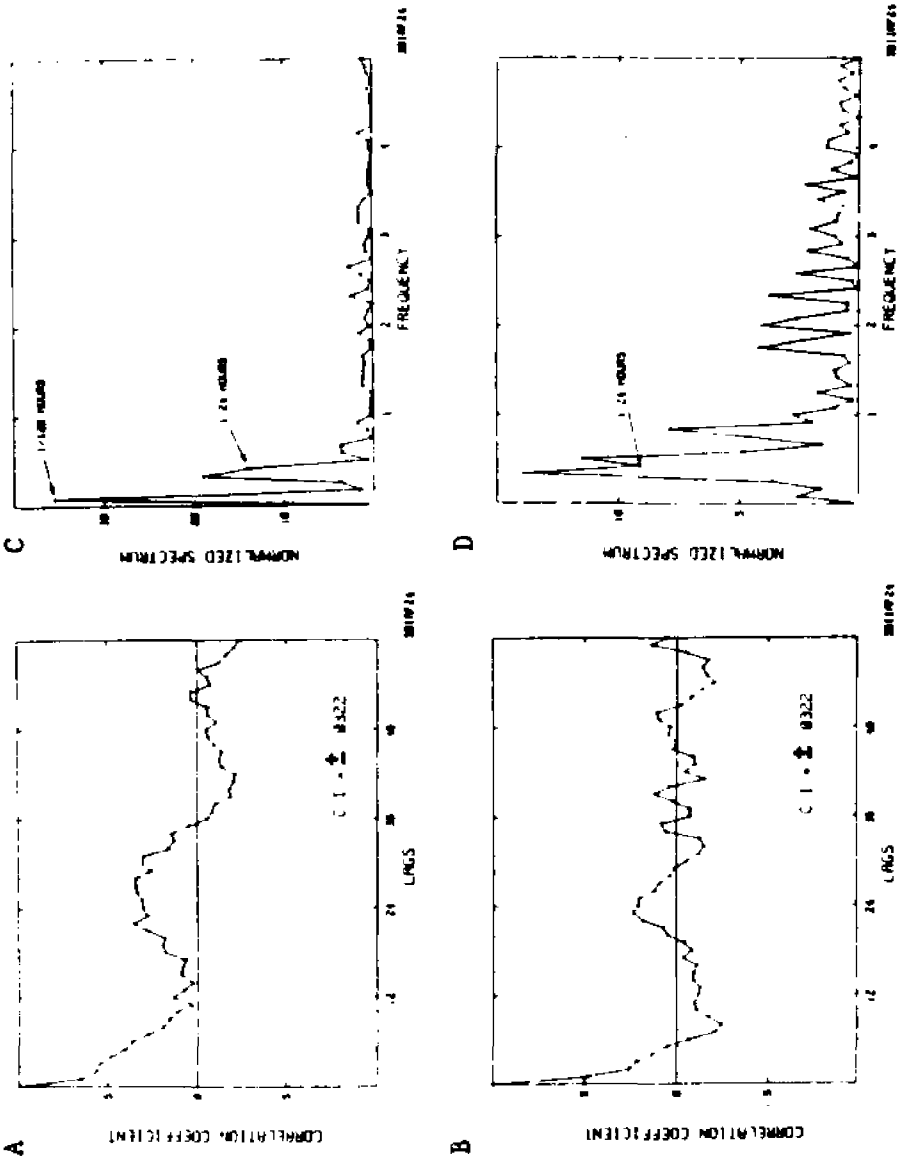

A
B
C
D
CORRELATION COEFFICIENT
LAGS
NORMALIZED SPECTRUM
FREQUENCY

Figure 10. Locomotor activity, F24. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. Note oscillation of autocorrelation function before contact (A) but no peak at 1/24 hours in the spectral analysis (C). After contact was established autocorrelation function did not oscillate (B) and a very small peak appeared at 1/24 hours (D).

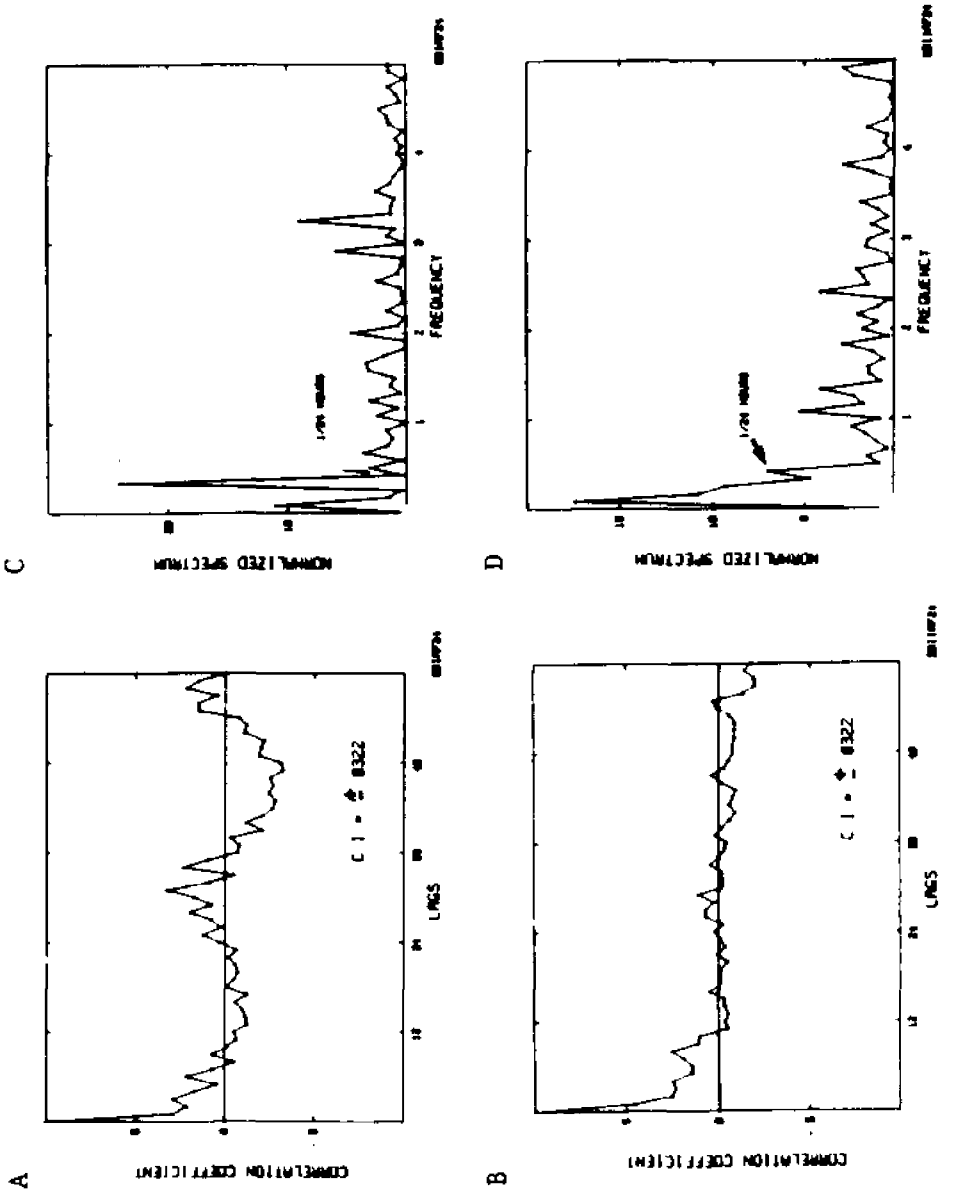

A
B
C
D
FREQUENCY
LAGS

Figure 11. Electric organ discharge rate, F26. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. Note oscillation of autocorrelation function before contact (A) and small peak at 1/24 hours in spectral analysis (C). After contact was established the autocorrelation function did not oscillate (B) and there was no peak at 1/24 hours in the spectral analysis (D). Periodicity of electric organ discharge rate was unreliable.

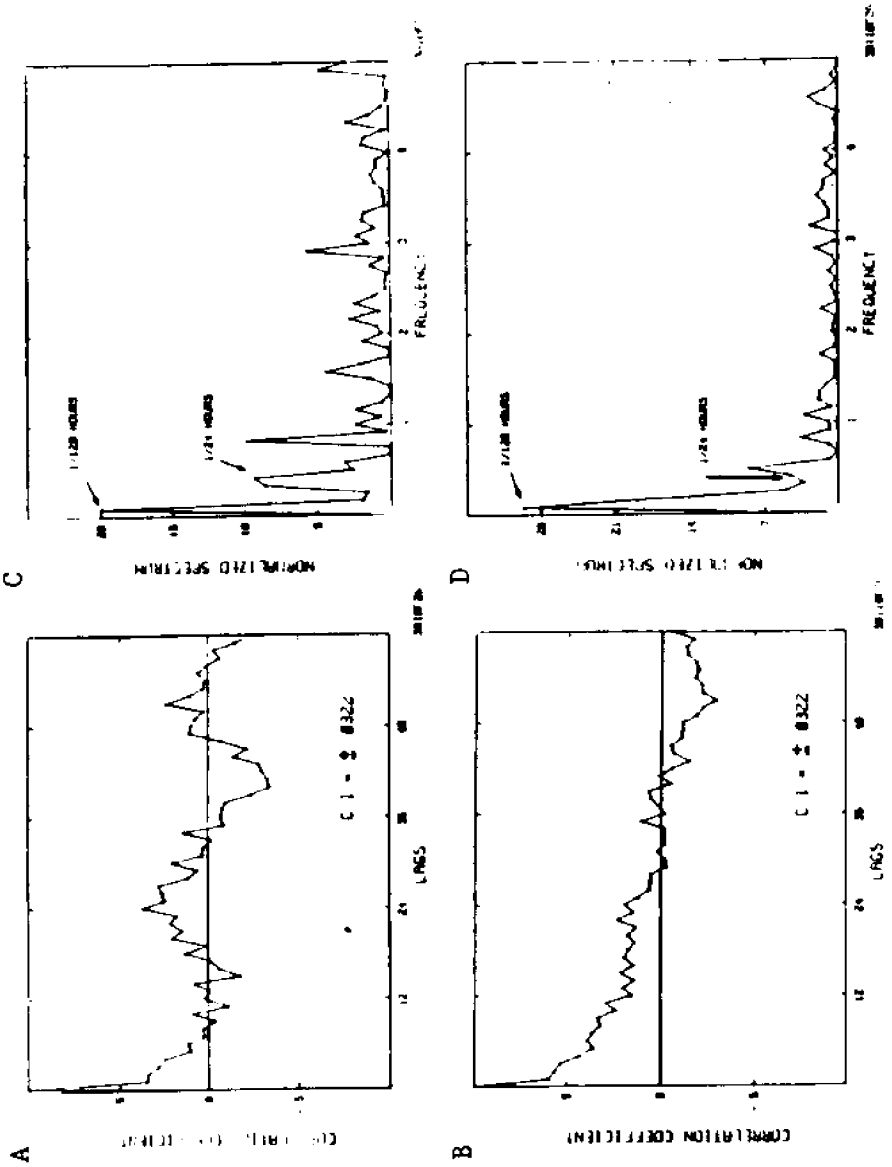

A
B
C
D
LAGS
FREQUENCY

Figure 12. Locomotor activity, F26. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. No periodicity of locomotor activity before or during contact. Note absence of oscillation of autocorrelation functions (A,B), small peak at 1/24 hours in spectral analysis before contact (C) and no peak after contact was established (D).

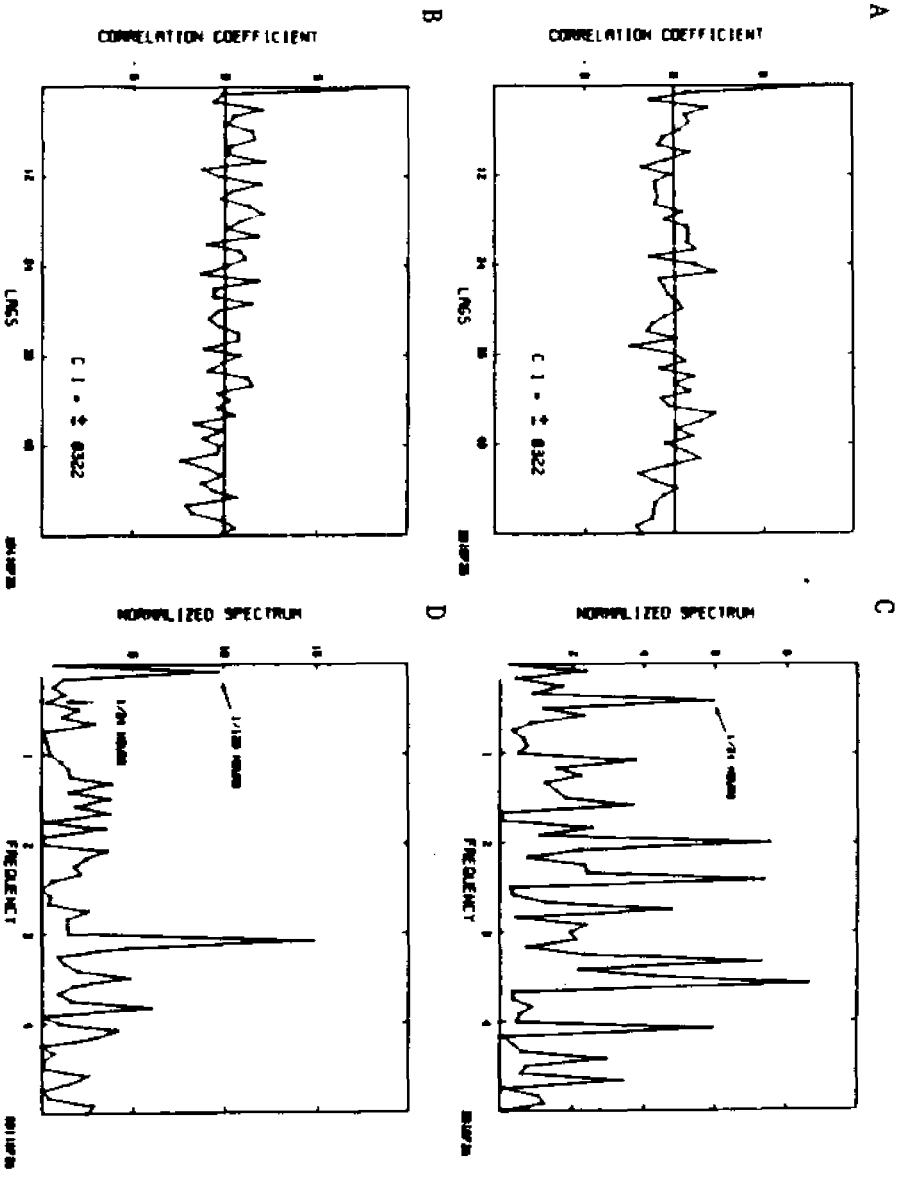

A
CORRELATION COEFFICIENT
LAGS
B
CORRELATION COEFFICIENT
LAGS
C
NORMALIZED SPECTRUM
FREQUENCY
D
NORMALIZED SPECTRUM
FREQUENCY

Figure 13. Electric organ discharge rate, F10. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. Note unstable oscillation of autocorrelation functions before (A) and during contact (B). No peak at 1/24 hours before contact (C) but small peak appears in spectral analysis after contact was established (D). This reflects instability of entrainment.

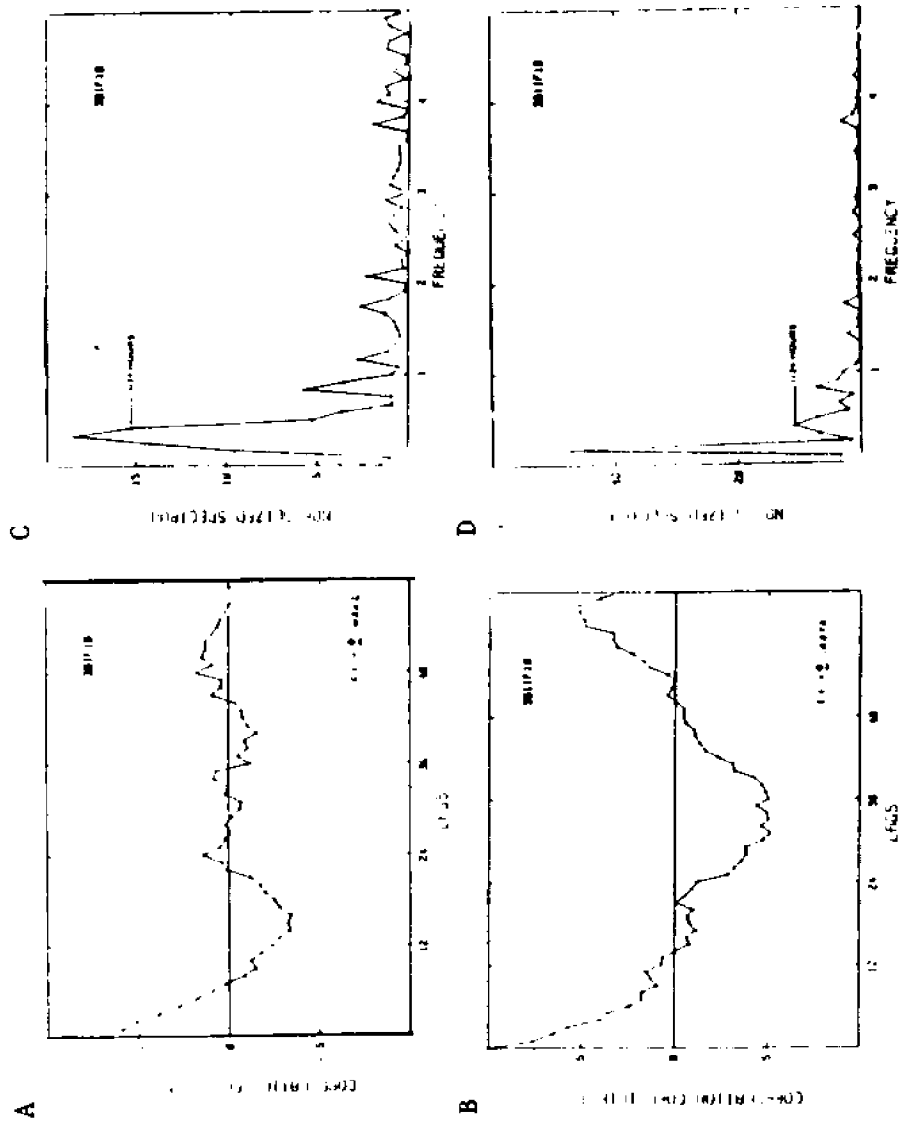

A
B
C
D
FREQUENCY
FREQUENCY

Figure 14. Locomotor activity, F10. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. Analysis reflects instability of entrainment. Autocorrelation function oscillated before contact (A) without 24 lags peak to peak and no peak appeared at 1/24 hours in the spectral analysis (C). After contact was established the autocorrelation function did not oscillate (B) but a small peak appeared in the spectral analysis at 1/24 hours (D).

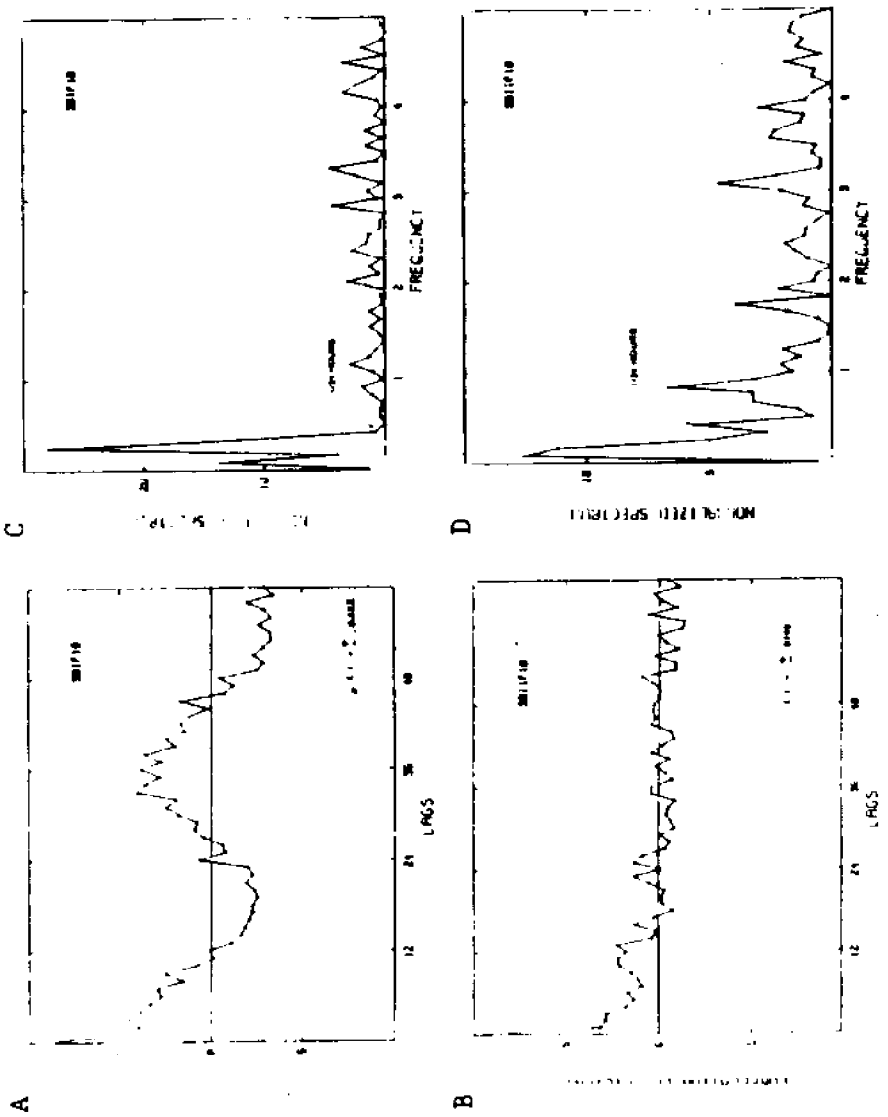

A
B
C
D
LAGS
LAGS
FREQUENCY
FREQUENCY

Figure 15. Electric organ discharge rate, F25. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. Before contact (A) the autocorrelation function did not oscillate around zero though a peak occurred every 12th lag. Spectral analysis showed small peak at 1/24 hours and a larger peak at 1/12 hours (C). After contact was established, the autocorrelation fucntion oscillated with 12 lags peak to peak (B). No peak appeared at 1/24 hours in the spectral analysis but a large peak appeared at 1/12 hours suggesting entrainment with a 12 hour periodicity.

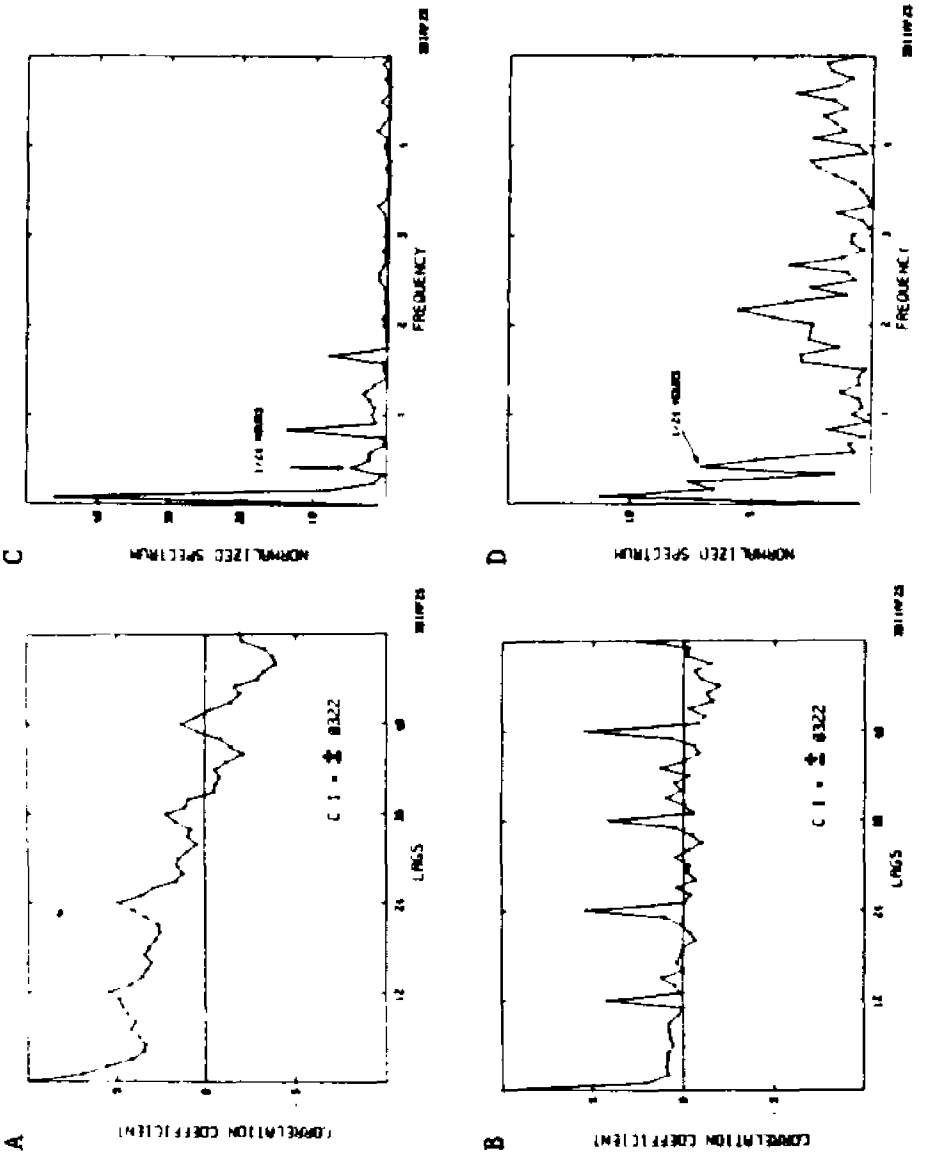

A
B
C
D
LAGS
FREQUENCY
NORMALIZED SPECTRUM

Figure 16. Locomotor activity, F25. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. Note lack of oscillation of autocorrelation function both before (A) and during (B) contact. Small peak in spectral analysis appears at 1/24 hours both before (C) and during (D) contact indicating entrainment of locomotor activity.

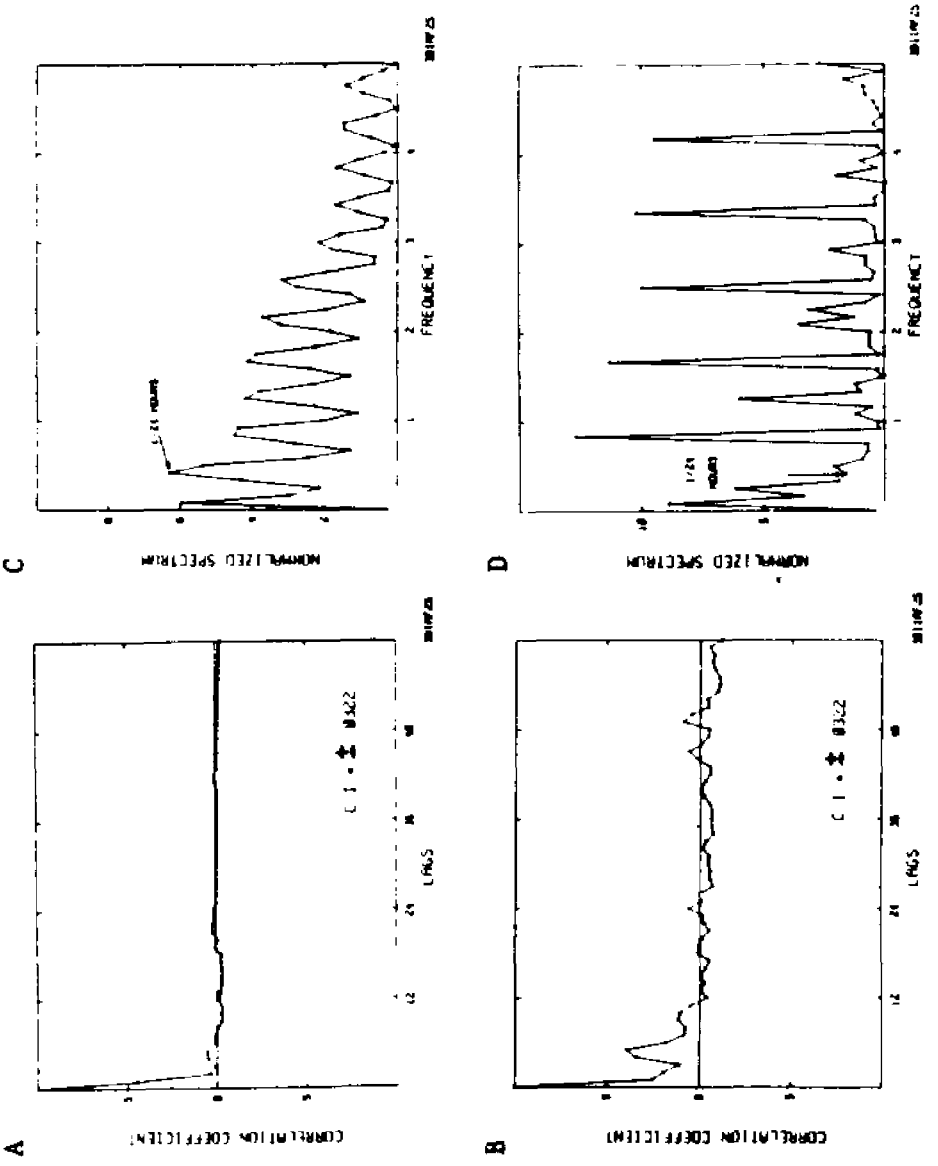

A
B
C
D
LAGS
FREQUENCY

Figure 17. Electric organ discharge rate, F27. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. Electric organ discharge rate entrained to LD cycle both before and during contact. Note oscillation of autocorrelation function before contact (A) with 24 lags peak to peak and large peak at 1/24 hours in spectral analysis (C). After contact was established, autocorrelation function did not oscillate (B) but peak at 1/24 hours appeared in spectral analysis (D).

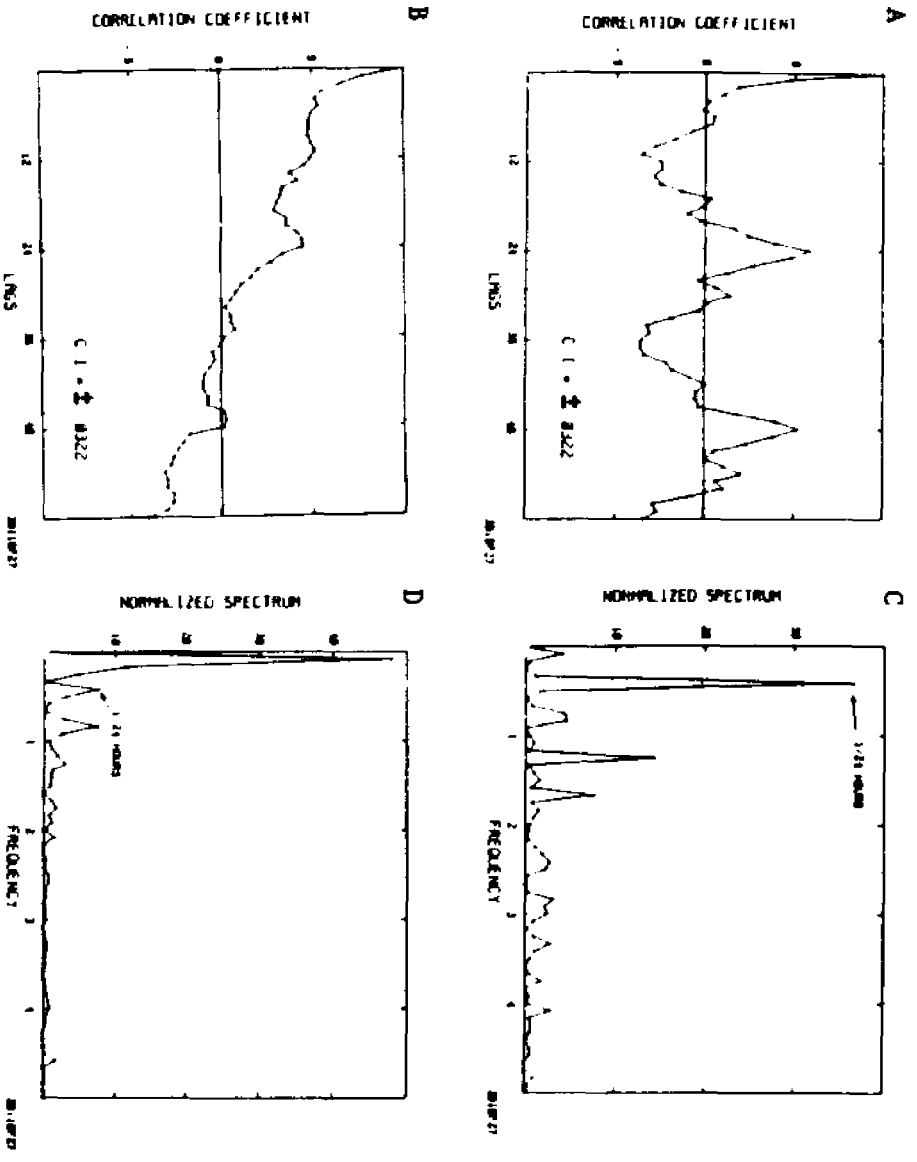

A
CORRELATION COEFFICIENT
LAGS
B
CORRELATION COEFFICIENT
LAGS
C
NORMALIZED SPECTRUM
FREQUENCY
D
NORMALIZED SPECTRUM
FREQUENCY

Figure 18. Locomotor activity, F27. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. Locomotor activity entrained after contact was established. No oscillation of autocorrelation function before (A) or during (B) contact. No peak at 1/24 hours before contact was established (C) but large peak at 1/24 hours appeared during contact (D).

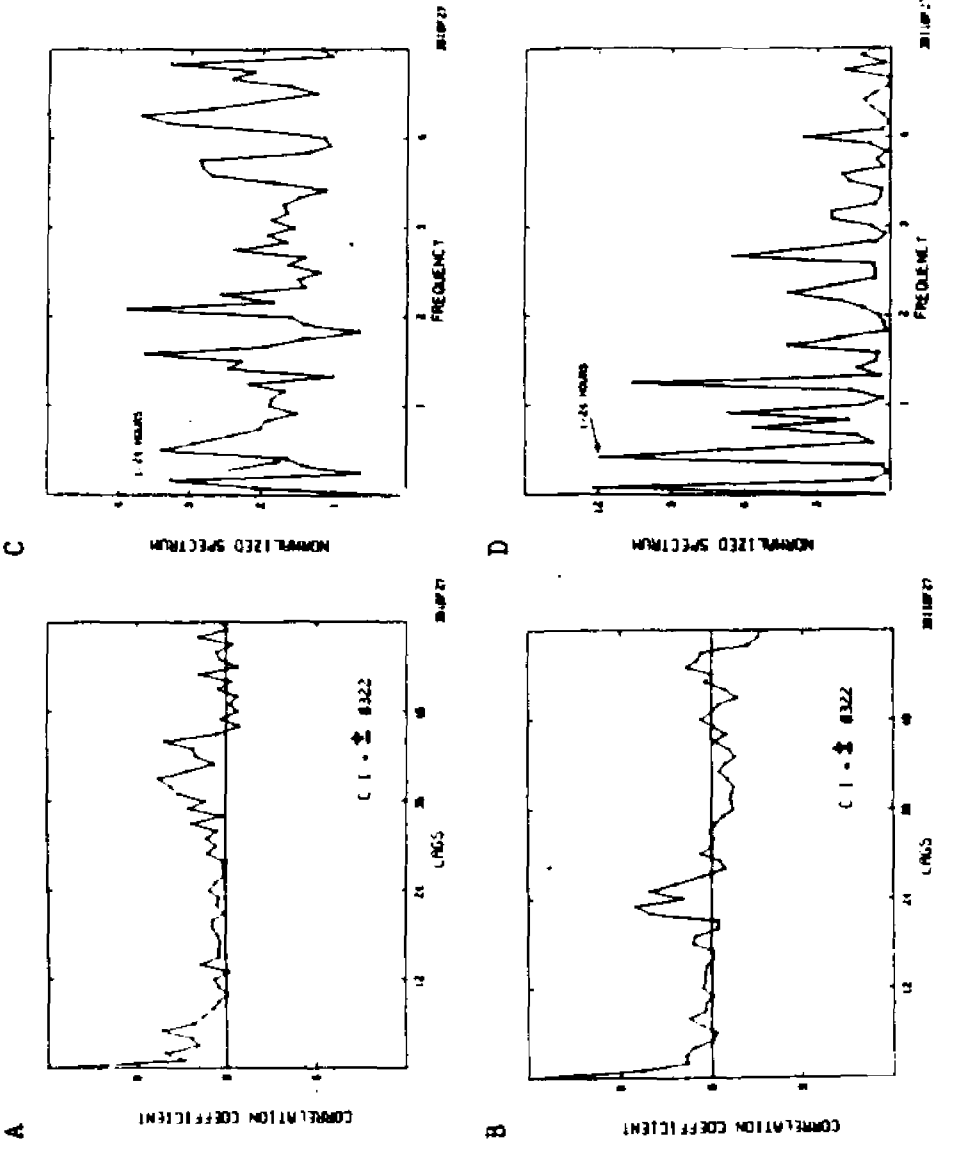

A
B
C
D
CORRELATION COEFFICIENT
NORMALIZED SPECTRUM
LAGS
FREQUENCY
1-24 HOURS

Figure 19. Electric organ discharge rate, F12. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. Before contact autocorrelation function oscillated with 24 lags peak to peak (A) and large peak at 1/24 hours appeared in spectral analysis (C). After contact was established, autocorrelation function did not oscillate (B) but a small peak at 1/24 hours appeared in the spectral analysis (D).

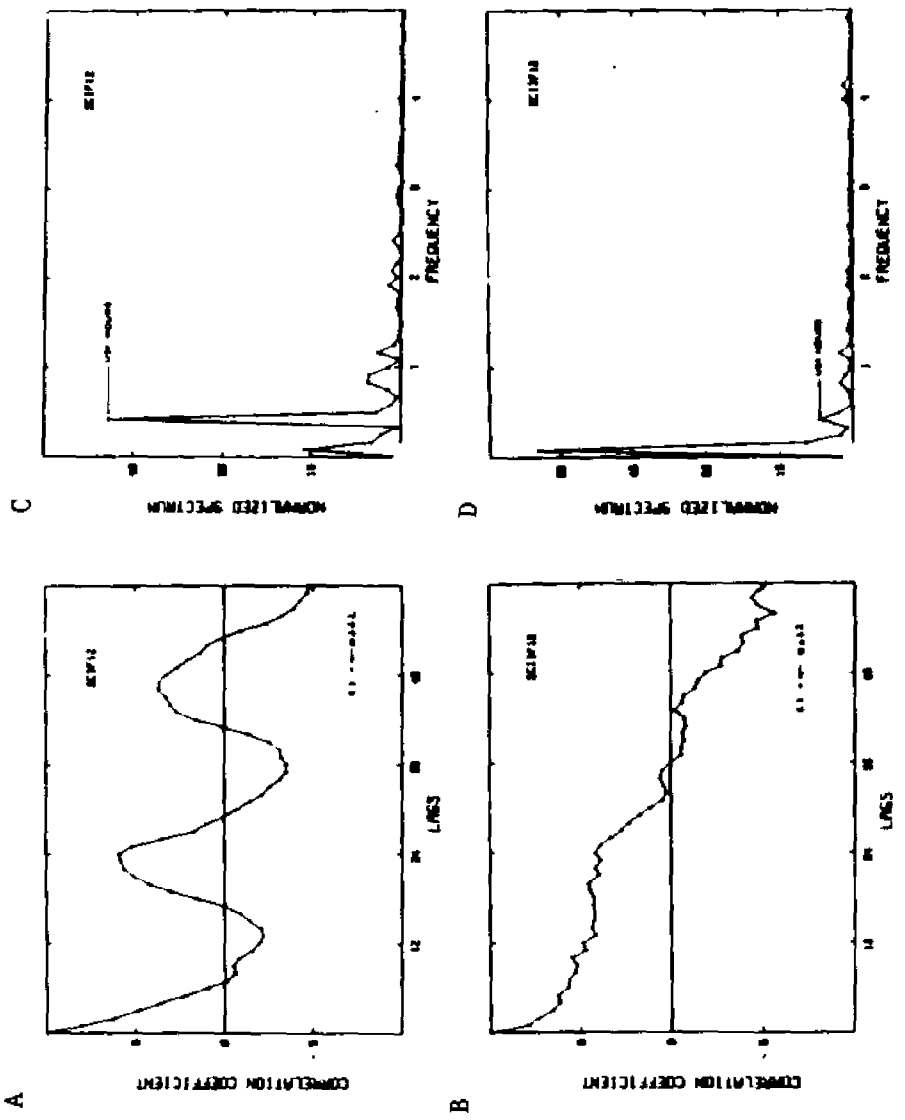

A
B
C
D
CORRELATION COEFFICIENT
LAGS
NORMALIZED SPECTRUM
FREQUENCY

Figure 20. Locomotor activity, F12. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. Locomotor activity did not entrain. Before contact was established the autocorrelation function did not oscillate (A) and no peak at 1/24 hours appeared in the spectral analysis (C). During contact the autocorrelation function oscillated but not with 24 lags peak to peak (B). No peak at 1/24 hours appeared in the spectral analysis (D).

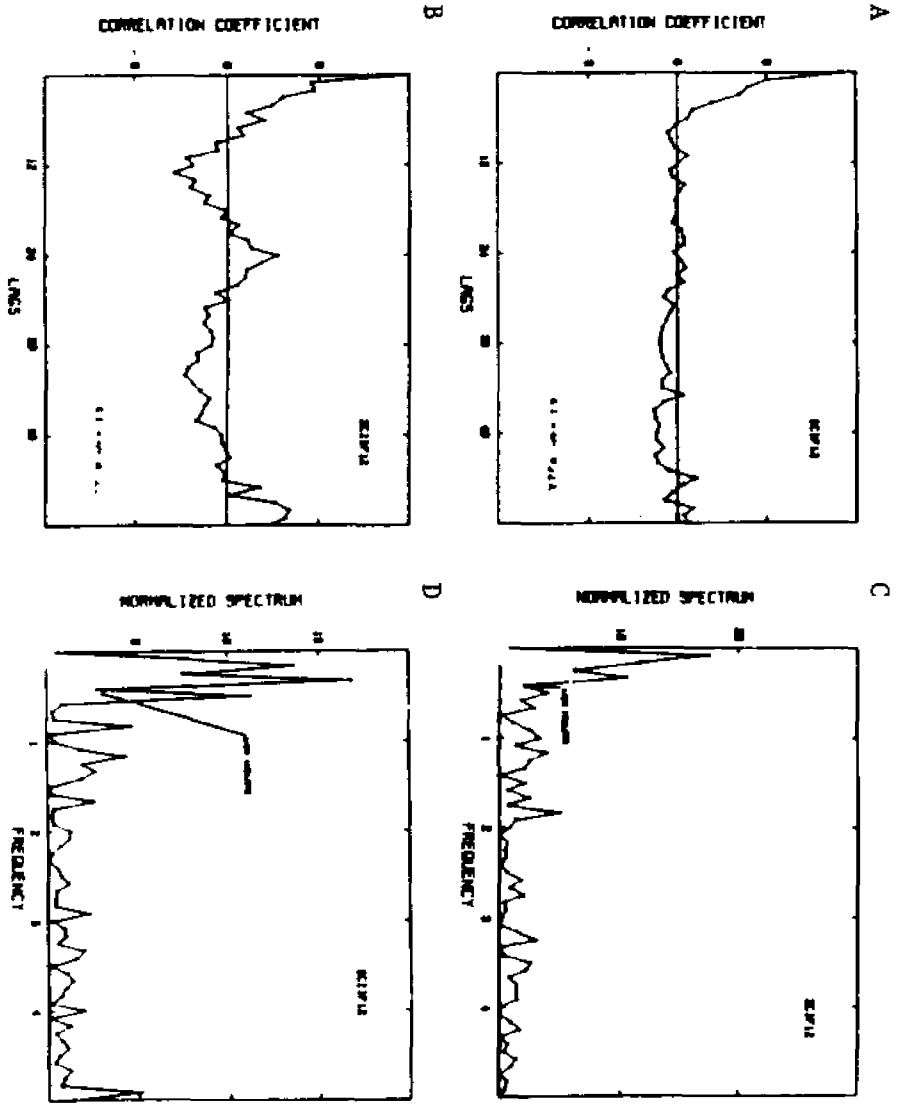

A
CORRELATION COEFFICIENT
LAGS
B
CORRELATION COEFFICIENT
LAGS
C
NORMALIZED SPECTRUM
FREQUENCY
D
NORMALIZED SPECTRUM
FREQUENCY

Figure 21. Electric organ discharge rate, F11. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. Instability of entrainment was reflected by statistical analysis. Autocorrelation functions did not oscillate before (A) or during (B) contact. No peak at 1/24 hours appeared in spectral analyses (C,D).

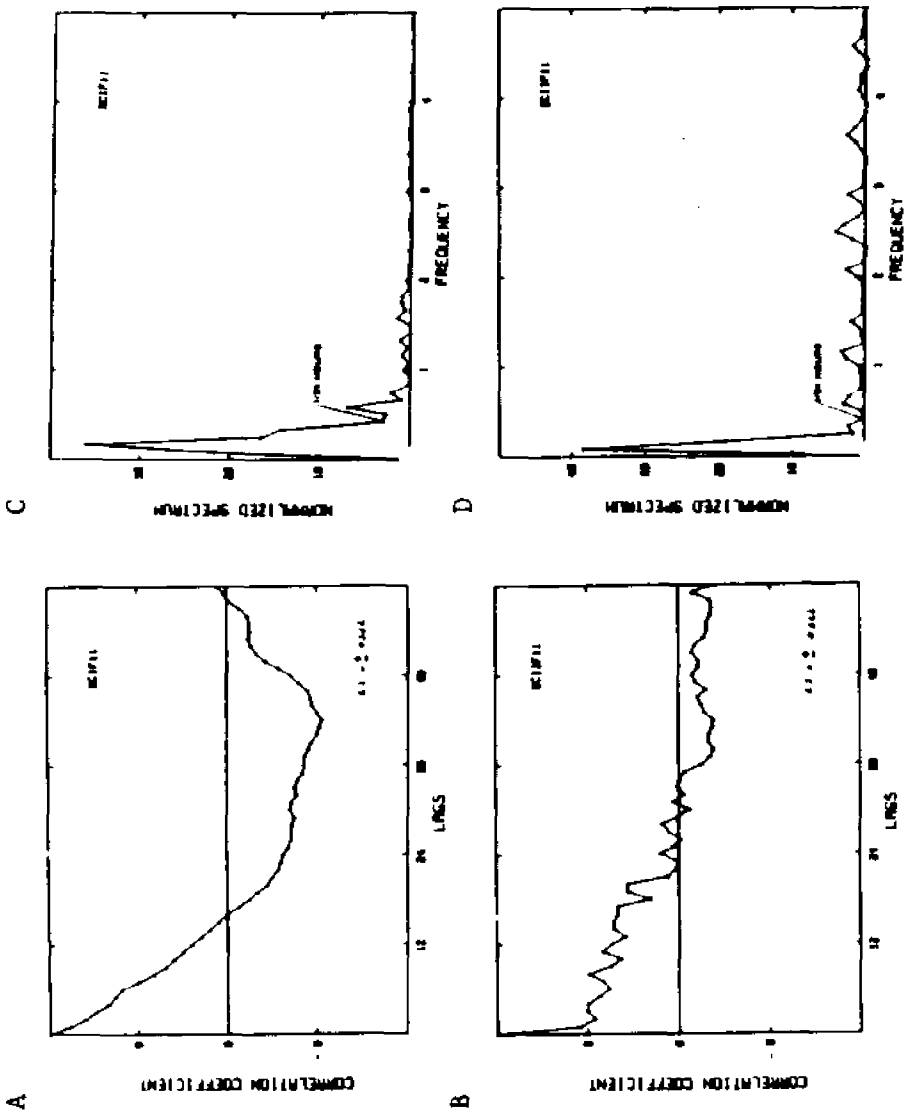
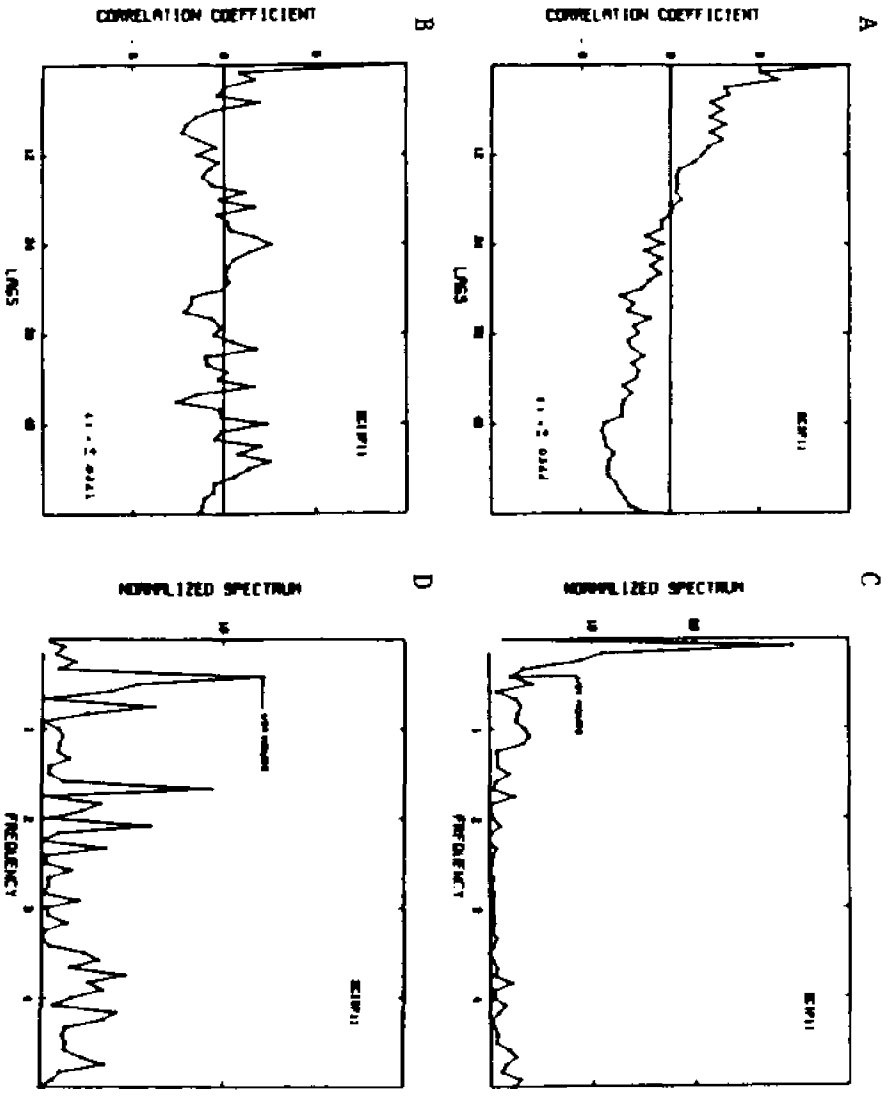

A
B
C
D
CORRELATION COEFFICIENT
LAGS
NORMALIZED SPECTRUM
FREQUENCY

Figure 22. Locomotor activity, F11. Autocorrelation functions and spectral analyses of data before contact (A,C) and during contact (B,D). For autocorrelation function, correlation coefficient is plotted as a function of lags. C.I. for 0 appear in lower right corner. For spectral analysis, normalized spectrum is plotted as function of frequency (1/period in hours). Arrows indicate 1/24 hours. Before contact, autocorrelation function did not oscillate (A) and no peak appeared at 1/24 hours in the spectral analysis (C). After contact was established, the autocorrelation function showed an unstable oscillation around zero (B) and a peak at 1/24 hours appeared in the spectral analysis (D) confirming unstable entrainment of locomotor activity.


A
CORRELATION COEFFICIENT
LAGS
B
CORRELATION COEFFICIENT
LAGS
C
NORMALIZED SPECTRUM
FREQUENCY
D
NORMALIZED SPECTRUM
FREQUENCY

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