TIDAL ACTIVITY RHYTHMS IN TWO SPECIES OF INTERTIDAL CLINGFISH (GOBIESOCIDAE) IN THE NORTHERN GULF OF CALIFORNIA

by

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ABSTRACT

Two species of rocky intertidal clingfish exhibited tidal activity rhythms under constant conditions of light, temperature and water level as measured with a photocell apparatus. In *Tomicodon humeralis* the rhythm persisted for at least seven cycles; in *Gobiesox pinniger* this rhythm disappeared after two cycles. Peaks of activity were associated with high tide. Light had a permissive and possibly entraining effect on the tidal rhythm. By direct observation of the fish when a barnacle-covered rock was added to the aquarium, components of activity were broken down into feeding and nonfeeding behavior. A pattern of nonfeeding activity at high tide followed by a rise in feeding activity near the time the fish would normally be emersed in the intertidal environment was exhibited. This pattern disappeared after two tidal cycles. To test whether this pattern observed by direct observation would also disappear in the photocell situation, a barnacle-covered rock was added. Results again showed that the rhythm disappeared after two tide cycles. Food, therefore, suppressed the tidal rhythm. A theory of how *Tomicodon*'s tidal rhythm affects its activities in the environment is proposed, based on activity and behavioral measurements, intertidal distribution and food consumption analyses.

CHAPTER 1

INTRODUCTION

Rhythmic activity associated with the tides has been demonstrated in many intertidal organisms. Animals tested in constant conditions not subject to tidal influences display persistent rhythms of "circatidal" periods. These periods approximate 12.4 hr, 24.8 hr or 14.7 day frequencies. Some investigators (Brown et al., 1953) claim that exact tidal rhythms persist under constant conditions, but these can only be detected after data is analyzed statistically using a method criticized by Cole (1957) and Enright (1965).

Recent studies attempt to define (a) what environmental factors serve to entrain the rhythm displayed in constant conditions, (b) a model for a clock that would control both circatidal and circadian rhythms in a single organism and (c) the function or adaptive significance of the rhythm in relation to environmental demands.

Much research has been directed towards determining the environmental, phase-setting cues which entrain the tidal rhythm. To test one specific environmental factor, arrhythmic animals are placed in constant conditions except for an imposed cycle of light intensity, turbulence, hydrostatic pressure, inundation, temperature, chemical changes or O_2 tension. The animals are then removed to constant conditions and tested for response to the experimental cycle. If activity is random, it is assumed that a different variable sets the rhythm. The regularly changing environmental variable which can entrain a rhythm is called a "zeitgeber". The results of studies on intertidal animals show that only cycles of turbulence and hydrostatic pressure are significant in reentraining animals. Experiments with the isopods *Excirolana* (Enright, 1963, and Klapow, 1972) and *Eurydice* (Jones and Naylor, 1970) indicate that cycles of turbulence can reenstate a rhythm in arrhythmic animals. Pressure cycles reentrained the green crab, *Carcinus* (Naylor and Atkinson, 1972), the amphipod, *Corophium* (Morgan, 1965) and the fish, *Blennius* (Gibson, 1971). Cycles of temperature alone do not reentrain a rhythm but a brief period of cold shock of several hours may reinitiate a rhythm in *Carcinus* (Naylor, 1963), *Eurydice* (Jones and Naylor, 1970) and the gastropod, *Melanerita* (Zann, 1973). Unusual results were obtained by Barnwell (1966) when he found that a 12:12 hr light-dark cycle could phase a precise 12.4 hr *tidal* rhythm while in constant conditions the rhythm became circatidal.

Another focus of research is to define a model for a clock that would control both circadian and circatidal components in rhythms of intertidal organisms. Usually, the basic rhythm is tidal in form but a daily effect is indicated by exaggerated diurnal or nocturnal peaks in swimming crabs, *Callinectes* (Fingerman, 1955), *Carcinus* (Naylor, 1958), snails

(Chandrashekaran, 1965), the crab, Sesarma (Palmer, 1967) and Melanerita (Zann, 1973). That the two rhythms are intimately associated is evidenced by the fact that laboratory reared Carcinus raised under a light-dark cycle have a circadian rhythm, but cold shock of 15 hours induces a 12.4 hr tidal rhythm (Williams and Naylor, 1967). In other experiments with Carcinus Naylor (1960) damped out a tidal rhythm after four weeks in L-D conditions and activity became nocturnal. Similarly, Gibson (1971) found that tidal activity in blennies became circadian after two months in L-D.

Few studies have been directed toward relating the function of a persistent rhythm to the demands placed by environment on the animal. This might be called the ecology of rhythms. Enright (1970) encourages scientists to determine the ecological significance of persistent rhythms. He questions why evolution has selected for a self-sustaining clock rather than a one cycle timer, in view of the regularity of environmental cycles. In the tidal environment what may be important is that the animal is psychologically and physiologically prepared for more than one cycle or a missing one. It would be difficult to test whether an animal is more successful in its niche by being rhythmic. Authors who attempt to make a correlation between rhythm and environmental demands only suggest what the function of a tidal rhythm might be. Zann (1973) felt that predator pressure encouraged low tide activity in a low intertidal snail. Feeding is best for the hogchoker, Trinectes, an estuarine fish at ebb tide when rhythm studies indicate corresponding peaks of activity, and predator avoidance is greatest during the day which accounts for greater nighttime peaks of activity (O'Connor, 1972).

The bulk of rhythm studies on fishes has been accomplished by Gibson working with blennies (1967, 1970, 1971) and flatfish (1973). Early work on oxygen consumption in fishes revealed tidal rhythms in a flatfish, Pleuronectes (Gompel, 1937). Green (1971) found a tidal rhythm of activity in a tidepool cottid that did not relate to field observations of activity. Stahl (1973), also working with blennies, also found a tidal rhythm in the laboratory but a diurnal one in the field. Both Green and Stahl concluded that the tidal rhythm of activity in the laboratory is generated by an escape response. O'Connor (1972) found activity in the hogchoker associated with ebb tide, in contrast to other fish studies in which higher peaks of activity occcurred during the night. A good review of all biological rhythms in fish is provided by Schwassmann (1971), though recent work on tidal rhythms is not included.

The purpose of the present study was to investigate the differences in the tidal activity rhythm of two species of clingfish with overlapping intertidal distributions. Since previous studies indicate that specific environmental cues can reset a tidal rhythm, reentrainment of arrhythmic fish was attempted. To determine the interaction of light and dark with tidal cycles, the rhythm was analyzed for relationship to the tides and the effect of light. Behavioral components of activity peaks were related to the possible function of the rhythm in the field.

CHAPTER 2

DESCRIPTION OF FISH AND THEIR HABITAT

Tomicodon humeralis and Gobiesox pinniger are members of the family Gobiesocidae, order Gobiesociformes. Both are endemic to the Gulf of California.

Clingfish possess a strong sucking disc composed of the fused pelvic fins whose outer rays unite with the pectoral fin base. They use this sucking disc to adhere to rock faces and are frequently found in zones of turbulence. They are deeply compressed and have no scales or swimbladder.

Tomicodon and Gobiesox may be found in the intertidal zone by overturning boulders to which they are attached by their sucking disc. The two species are sometimes found under the same boulder. They can change color easily to fit their background. Males and females are indistinguishable by external features. During breeding season (summer through fall) the males guard eggs adhering to the undersides of boulders.

Both reach the same maximum length according to Briggs (1955), but Gobiesox is more commonly the larger of the two. Of those used in the present experiments, average size of Tomicodon was 31.9 mm S.L. (N=36), while Gobiesox averaged 46.1 mm S.L. (N=18). Data from museum collections indicate that these figures are somewhat low. Maximum sizes of fish used for this study were Gobiesox: 69 mm, Tomicodon: 47 mm.

Extensive work on the physiological adaptations of clingfish demonstrates that both species are well adapted to stresses imposed by exposure at low tide. Clingfish breathe aerially at low tide by taking air into the branchial chamber. Metabolic rate in an aerial medium is similar to that in water. Temperature tolerance data indicate that Tomicodon is less temperature sensitive than Gobiesox and both are more cold tolerant than heat tolerant. During low tide fish lose water through gills and mucus secretion. Mean survival times to exposure in air in 90% relative humidity are 24 hrs for Gobiesox and 33 hrs for Tomicodon. Clingfish integument consists of a single layer of large mucus glands and a thick outer epidermis (Eger, 1971). It is supposed that mucus secretion reduces evaporation as well as protecting the integument from rough treatment among barnacle-covered rocks. Large quantities of mucus are produced when fish are handled, suggesting that this acts as a deterrent to predators.

No natural predators of Tomicodon are known, though octopi will eat them when they are placed in the same aquarium. Stomach analysis of common rocky intertidal piscivores of the area indicate that Gobiesox is occasionally eaten.

Observations of the fish during capture suggests that Tomicodon is much less secretive than Gobiesox. When rocks with Tomicodon underneath are overturned, several seconds may pass before fish are alerted and try to flop into nearby water or under another rock. At night it is not unusual to turn over a rock and have one fall off, seemingly asleep. When a rock harboring

Gobiesox is overturned, the animal immediately seeks other shelter making it more difficult to collect. *Gobiesox* is usually found in wetter habitats, making escape easier by swimming to another hiding place.

Fish were collected from two geologically distinct areas at Puerto Peñasco, Sonora, Mexico, on the Gulf of California. One area, Station Beach Reef, is composed of basaltic boulders overlying a conglomerate sandstone reef. The other site, Norse Beach, is composed of granite boulders overlying granite bedrock and lies about 6 miles west of Station Beach. Puerto Peñasco is the northernmost habitat for clingfishes in the Gulf.

Tides at Puerto Peñasco are of the mixed, semidiurnal type. Tidal amplitude during spring tides can reach a maximum range of 24 ft, with a mean spring range of 17 ft.

CHAPTER 3

MATERIALS AND METHODS

Measurement of Activity Rhythms

Activity of clingfish was measured to determine the nature and duration of a tidal rhythm. I collected clingfish during day and night low tides by handnet and placed them in a 10 l (*Tomicodon*) or 12 l (*Gobiesox*) aquarium at Puerto Peñasco within two hours after collection. The tank was maintained under constant light and temperature conditions and shielded from viewing. Light intensity emitted by three overhead flourescent lamps was 88 lux at the water surface as measured with a Gossen Lunasix light meter. One or two small basalt boulders without attached food organisms (except in one experiment where barnacles were used) were placed on top of sand in the aquarium. Either a subsand filter or an aerator was used.

Activity was monitored by means of one or two photocells (Clairex CL-505) illuminated by lamps giving off a small beam of white light. The fish did not seem disturbed by the light and would sometimes sit directly in the beam for two to three minutes. The photocell was not placed in the same position for every experiment, nor were the same rocks used. However, the photocell was always placed adjacent to a rock so that fish on a rock as well as those passing through the beam on the sides of the aquarium would trigger the relay. A relay connected to the photocell triggered an Esterline-Angus event recorder and activity was measured as the number of marks per hour on the recorder printout. Animals were not fed during any measurement period.

Reentrainment of a tidal rhythm was attempted after which activity was monitored. Four Tomicodon were held for three months in Tucson until they became arrhythmic. They were transported back to Puerto Peñasco and held for a day in nontidal conditions. They were then placed with a basalt boulder and no sand in a semiopaque plastic two liter container punctured with holes. The container with fish was anchored in the intertidal at the +5 level (0 = MLW) for 14 tidal cycles. At this level they were exposed by the tide twice a day. After the seven day period, fish were fed and removed to the photocell apparatus and their activity recorded.

To test for response to an imposed light-dark cycle under otherwise constant conditions, fish in two separate experiments in Tucson were kept under a 12:12 hr L:D regime. In one, a L:D cycle was imposed for one week on arrhythmic fish, after which they were put into constant conditions and tested. In a later experiment, fish brought back from Puerto Peñasco were put directly in a 12:12 L-D cycle for two months then tested. Both groups were subjected to experimental conditions similar to those of previous photocell recordings.

Each record of activity was subjected to periodogram analysis (Enright, 1965, Williams and Naylor, 1967) to determine the period length in hours. The data (x_1, x_2, x_3 ... x_n) were scanned for possible rhythms of whole hour frequency (f) about the suspected period, 12.4 hr. Data were arranged in columns of 8 to 30 hrs and mean values for each column calculated as:

$$\begin{array}{llll} x_1, & x_2, & \ldots, & x_f \\ x_{f+1}, & x_{f+2}, & \ldots, & x_{2f} \\ x_{2f+1}, & x_{2f+2}, & \ldots, & x_{3f} \\ x_{n-f+1}, & x_{n-f+2}, & \ldots, & x_{nf} \\ \hline \bar{X}_1 & \bar{X}_2 & \ldots & \bar{X}_f \quad \text{(Averages)} \end{array}$$

The standard deviation around the averages for each whole hour frequency was calculated using the formula:

$$s = \sqrt{\frac{1}{f-1}\left(\Sigma X^2 - \frac{1}{f} X^2\right)}$$

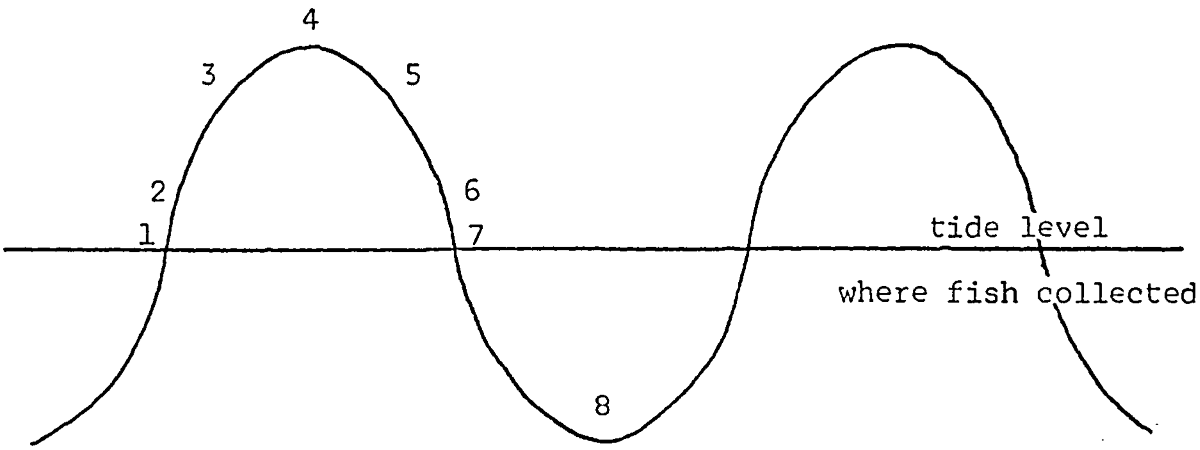
Lack of rhythmicity should give averages approximating the mean for the entire set of data. Rhythmicity is indicated by high values of standard deviation. Standard deviation as a percentage of overall mean hourly activity is defined as the coefficient of variability (c.v.). Coefficient of variability is then plotted against the whole hour frequencies. Data was analyzed on a Control Data Corporation 6400 computer.

Behavioral Observation Experiments

To determine the nature of activity being meausred by the photocell, direct visual observation of fish in a laboratory situation was performed. Ten *Tomicodon* were collected and five each placed in two 120 l aquaria containing one basalt barnacle-covered boulder and sand. In one aquarium a constant flow of sea water was maintained at a constant water level. In the other the aquarium was emptied and filled at times corresponding to the actual low and high tide at Puerto Peñasco. An artificial "tidal" cycle was maintained to see if behavior could be regulated by the same cycle of immersion and emersion as was being experienced by fish in the field. The aquaria were maintained in a semienclosed, outdoor lab so that natural photoperiods were maintained. However, in 2 of 3 experiments lights were turned on in the lab at night to provide constant light conditions. Fish were acclimated for 1 tidal cycle and all subsequent observations made during the second, fourth and sixth cycles after removal from the field. Different sizes of fish were used, sex unknown.

Activity was measured during seven separate periods relative to the ongoing tide (see Fig. 1) each measurement period lasting ten minutes. In the tidal tank fish were observed as the aquarium was filling or emptying, a process which took 15-20 minutes. The aquarium was allowed to empty or fill for several minutes before observations began. Little turbulence was associated with water flow.

Preliminary observations revealed only two pertinent activities; feeding, indicated by simultaneous downward head movement and upward tail movement, usually though not always around a barnacle, or nonfeeding activity, usually not on the



1. tide in
2. one hour after tide in
3. one hour before high tide
4. high tide
5. one hour after high tide
6. one hour before tide out
7. tide out
8. low tide

Fig. 1. Ongoing tide and corresponding observation periods in laboratory aquaria relative to tide level where fish were collected

barnacle-covered rock, but on the sides or bottom of the tank and consisting of either short hops or persistent swimming. When either of these two activities persisted more than 10 to 15 sec, it was recorded. Similar activity lasting 20 to 30 sec, therefore, received two marks for the record. Activity for all five fish was recorded at once. Data collected in these experiments provided behavioral correlates for the quantitative data collected from the photocell experiments.

I maintained the same position in front of the tank for all experiments. The fish did not seem to be much disturbed by my presence. During the first few observation periods (second tide cycle), several minutes elapsed before recording took place to allow fish to settle into normal activity. After that, the fish seemed completely undisturbed by my movements. A stop watch was used to mark ten minute intervals.

Intertidal Distribution

Differences in the nature and duration of tidal rhythms were found to exist between *Tomicodon* and *Gobiesox*. Since the two species are frequently found at the same tide level, a distributional study was necessary to explain differences in strength of environmental stresses, and therefore, differences in strength of zeitgeber ("time-setter" of the rhythm).

Distribution was determined from linear transects, two at Norse Beach and two at Station Beach. Areas at each location were chosen for maximal habitat possibilities, i.e., those with large numbers of boulders. The transect was made by sampling a given level parallel to the water's edge every 15 to 30 min as the tide receded or entered. A given tide level was determined by noting the time (± 5 min) of emersion or immersion of the horizontal area being sampled and checking the corresponding tide level indicated by a tide calendar for Puerto Peñasco (Thomson, 1973, 1974). Conventional methods of checking linear transects every x number of meters proved unsatisfactory because the habitat was uneven and many boulders were too heavy to be lifted. Therefore, distribution data are limited to boulders that I could overturn. Transects were made during several series of spring tides.

At each level 30 boulders of "likely clingfish habitat" were overturned and numbers of fish under each boulder noted. Data obtained from transects was used to determine whether clingfish exhibit a clumped distribution. A "likely clingfish habitat" for Tomicodon was considered to be a boulder lying on a level surface covering little or no standing water. Tomicodon were never found in channels, small tide pools or any spot with more than 10 to 20 mm of standing water. Sampling methods were probably not as accurate for Gobiesox which is found in habitats with more standing water such as tidepools. Thus, only Gobiesox under boulders were included in the transect count, although it is possible that the greater part of the population lives subtidally or in tidepools.

Gut Analyses

To determine where and when fish were eating, they were collected by handnet and placed in plastic bags with quinaldine for no more than 30 min then put into a 10% formalin solution. The entire digestive tract from esophagus to anus was dissected and contents studied under a compound or dissecting microscope. Fish were collected at low tide, usually on the ebb tide. Collections were made either after a daytime high tide or after a nighttime, new moon high tide.

CHAPTER 4

RESULTS

Measurement of Activity Rhythms

Persistent tidal rhythms of activity in Tomicodon can be seen in Figs. 2 to 5. Activity of clingfish is plotted against time. Included in these figures are the corresponding periodograms, which are plots of the coefficient of variability (c.v.) by percent against the period in hours of the rhythm. Large peaks on the periodogram indicate the period. Activity is the number of marks appearing on the recorder as a result of a movement of a fish in front of the photocell.

Peaks of activity correspond roughly to high tide. Nighttime peaks in Figs. 3 to 5 are smaller than daytime peaks indicating a diurnal effect. These differences in amplitude are not related to differences in amplitude of tides at time of capture. The period of the peaks is circatidal as periodogram analysis of data in Figs. 2 and 3 indicates periods other than 12 and 24 hrs. Activity expressed by the peaks decreases with time. In Fig. 3 activity of a single Tomicodon gradually decreases and by the eighth and ninth tidal periods, the tidal pattern becomes blurred and possibly damps out. Fig. 4, the rhythm of four Tomicodon, demonstrates the clearest record of activity in which the tidal rhythm is still strong in its sixth cycle.

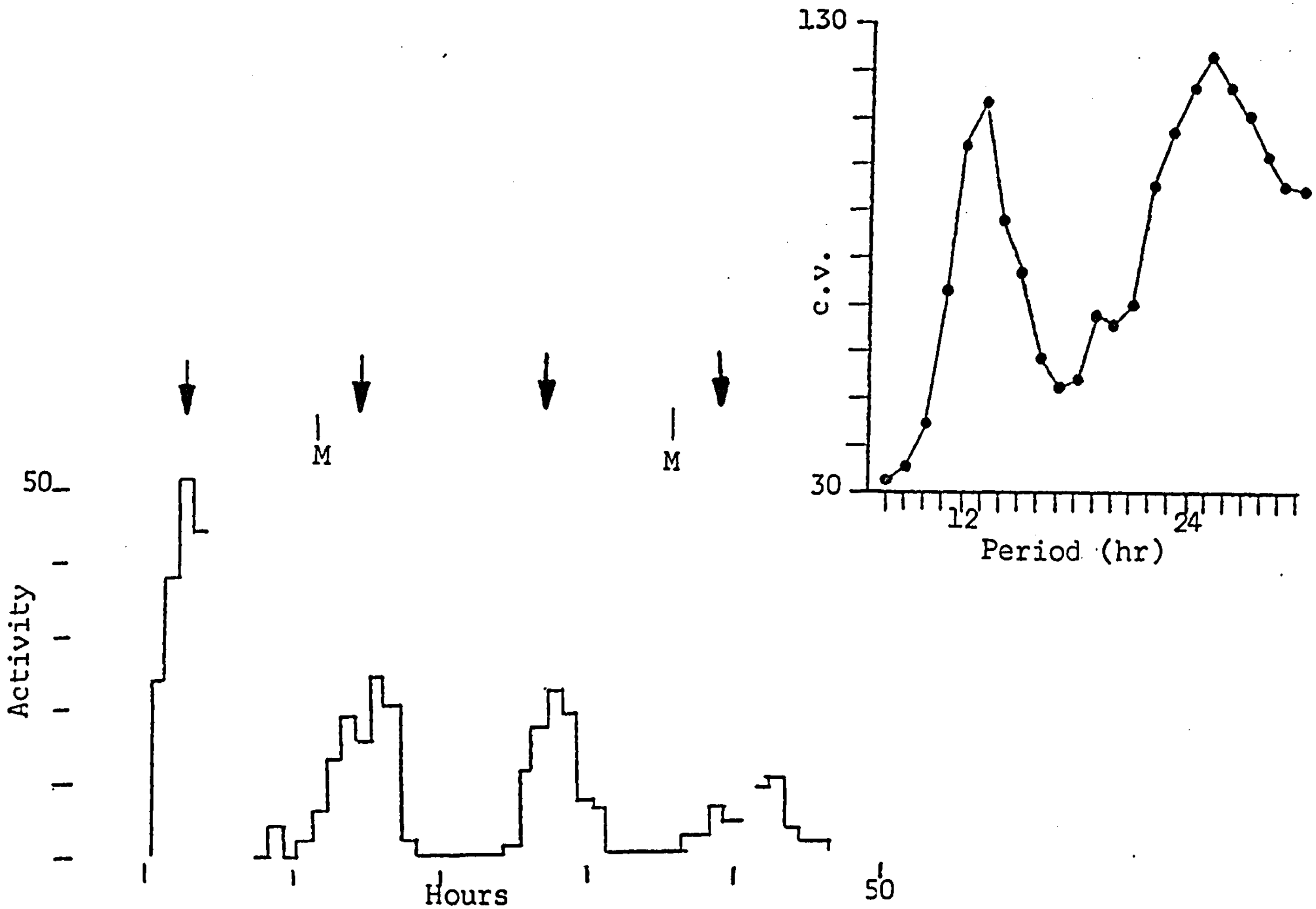


Fig. 2. Hourly activity of four freshly caught Tomicodon in constant light, July 17 to 19, 1973.--Arrows indicate time of high tide; M: midnight; c.v.: coefficient of variability by percent. Peaks on the periodogram indicate the period of the rhythm.

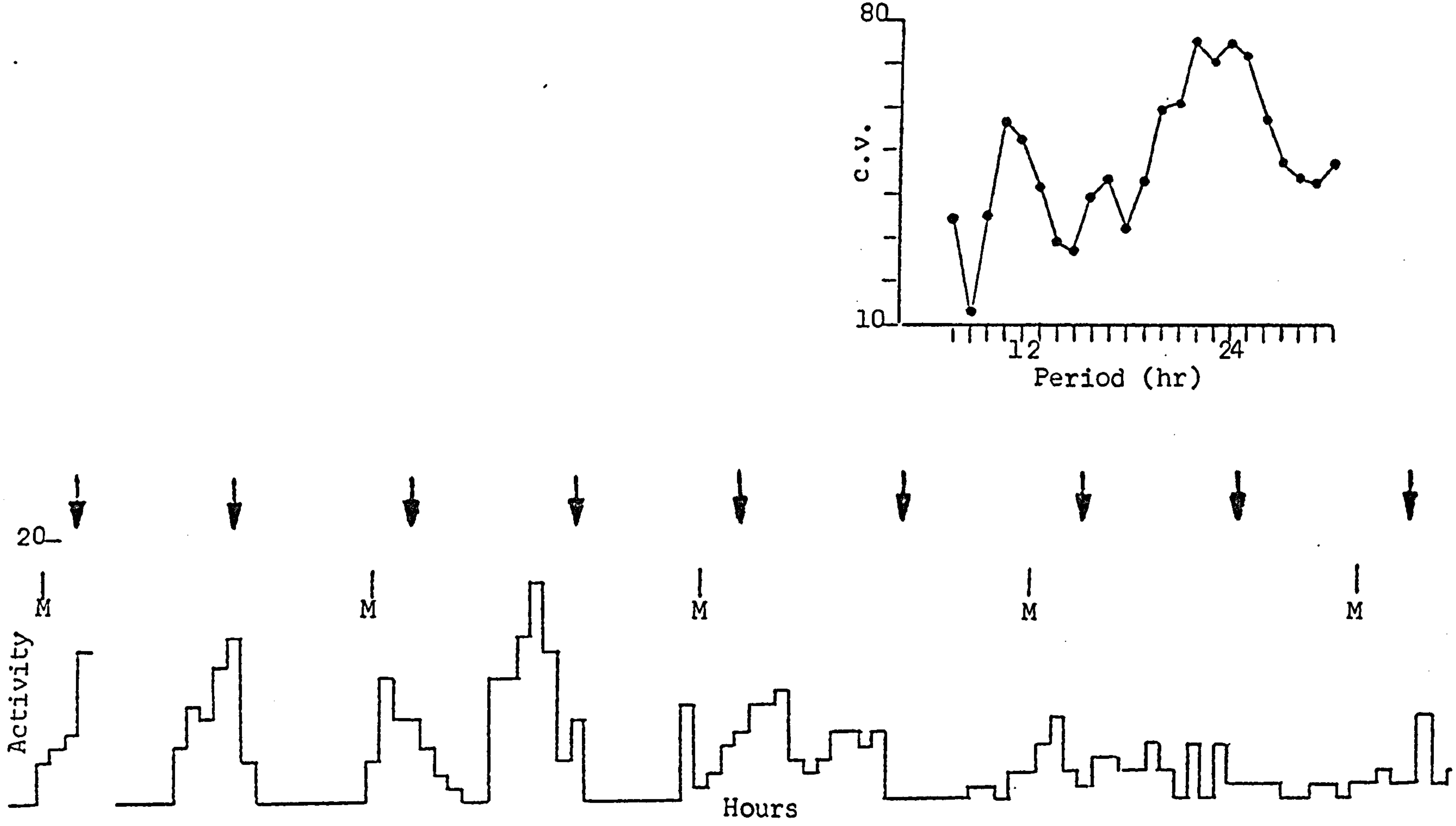


Fig. 3. Hourly activity of a freshly caught Tomicodon in constant light, Dec. 24 to 29, 1973. -- Arrows indicate time of high tide; M: midnight.

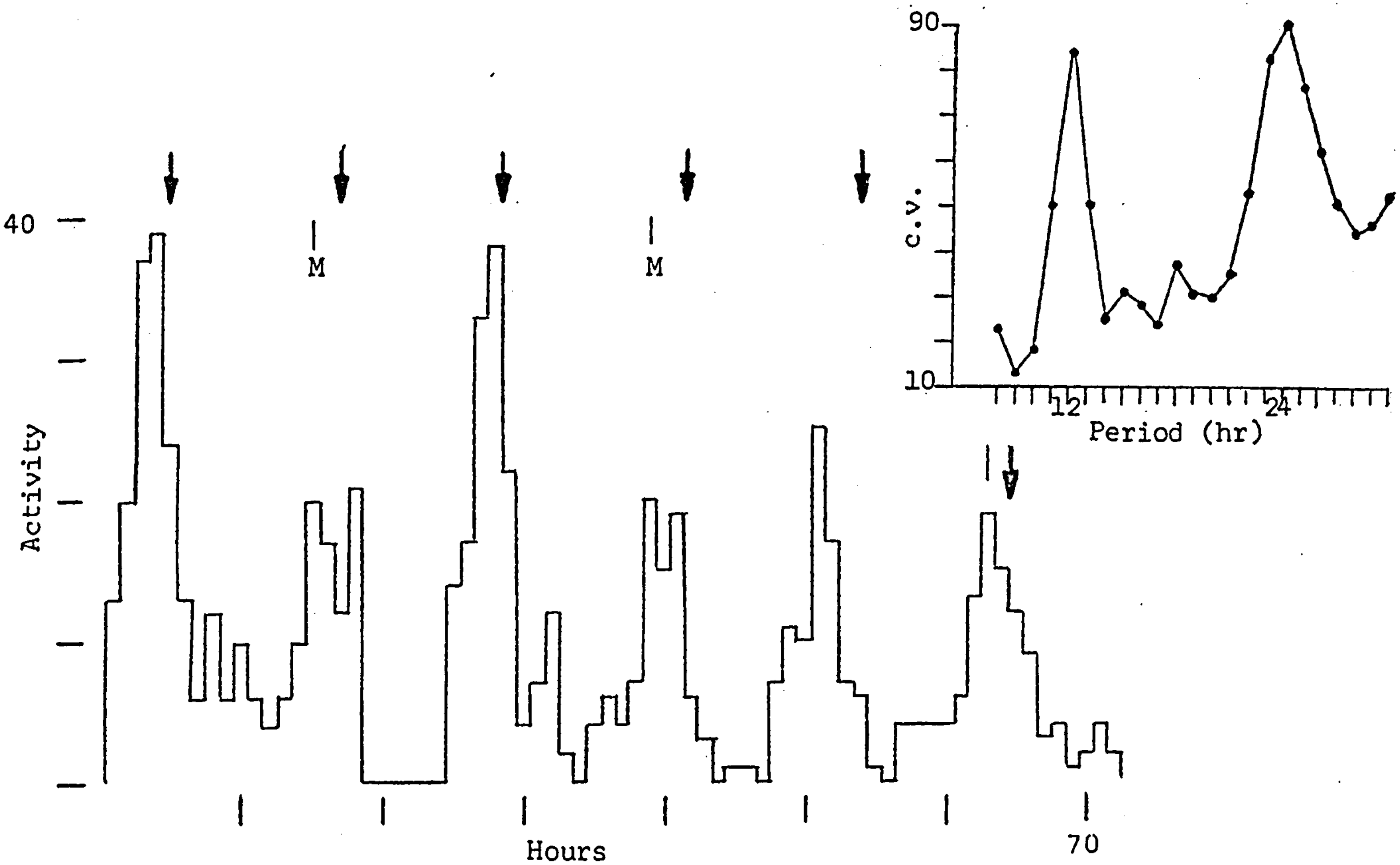


Fig. 4. Hourly activity of four freshly caught Tomicodon in constant light, Feb. 21 to 24, 1974. -- Arrows indicate time of high tide; M: midnight.

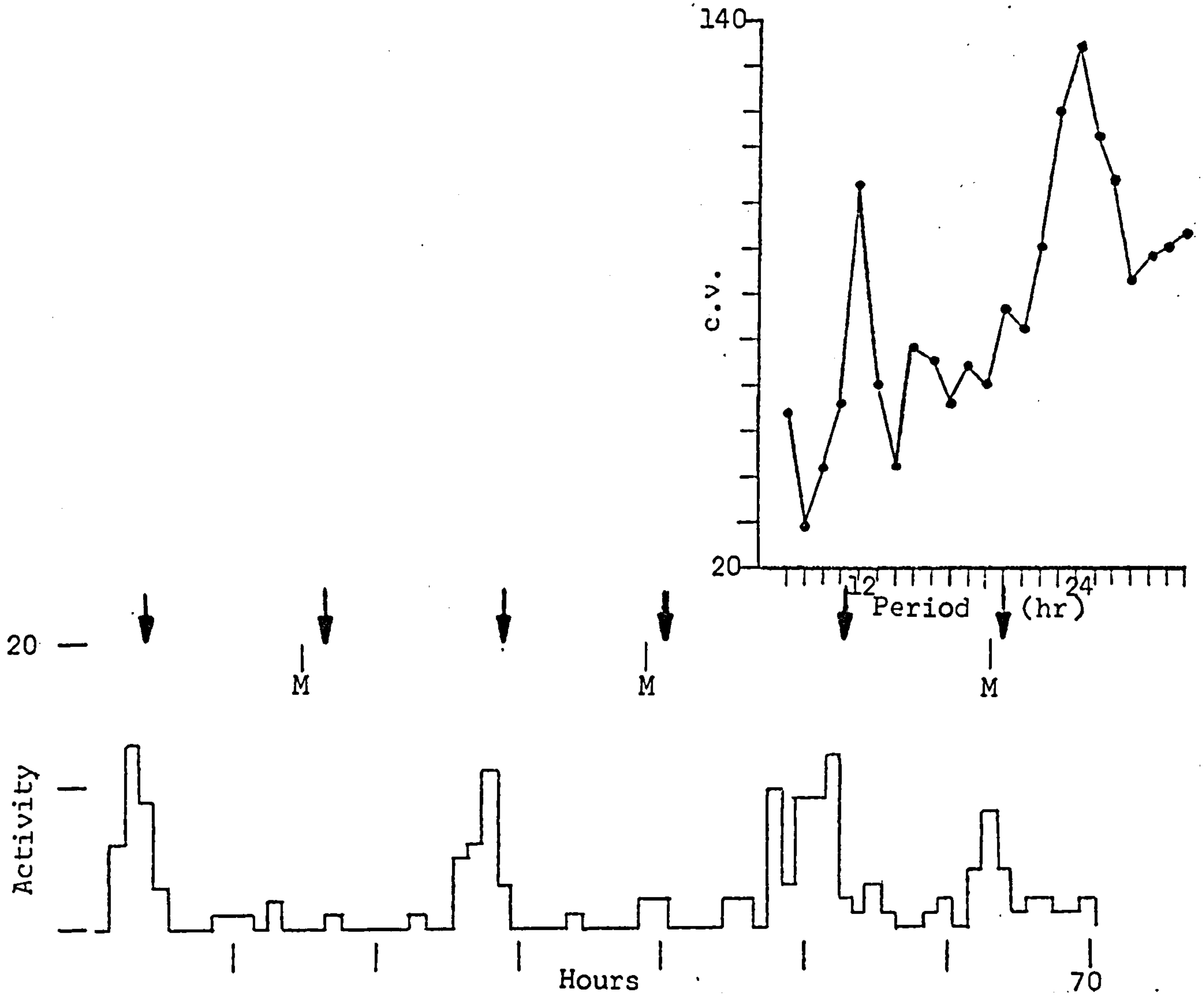


Fig. 5. Hourly activity of four freshly caught *Tomicodon* in constant light, Mar. 21 to 24, 1974. -- Arrows indicate time of high tide;M: midnight.

Evident in these records is a point of no activity for most of the low tide troughs. Brief observation of fish in the experimental aquarium usually indicated that fish were under a boulder, or adhering to the dark corners of the tank during low tide. Thus, they were behaving in a manner similar to that which they would in the field.

Figs. 6 to 8 show the activity of Gobiesox. Gobiesox has a brief tidal rhythm of activity which disappears after two cycles (Fig. 8). Activity peaks occur at time of high tide and decrease in amplitude as do those found in Tomicodon.

Since tidal rhythms show sensitivity to time of day, light entrainment was attempted. Tomicodon maintained in Tucson for three weeks were subjected to a 12:12 hr light-dark schedule for one week (Fig. 9). Results of periodogram analysis reveal periods at 19 and 25 hrs. The meaning of the 19 hr period cannot be explained and the periodogram is perhaps too "rough" to assume that entrainment has occurred. The same treatment was given to fish for two months (Fig. 10) after which a strong 23 hr period is evident as well as a 12 hr period. A weak peak at 12 hrs was also present in the periodogram of Fig. 9 which might indicate a tidal rather than circadian rhythm. In the light-dark experiment of Fig. 10, activity is minimal between the hours of 9 pm and 2 am when lights were off in the entraining conditions. This lull in activity does not occur between the same daytime hours, suggesting that a diurnal rhythm had developed.

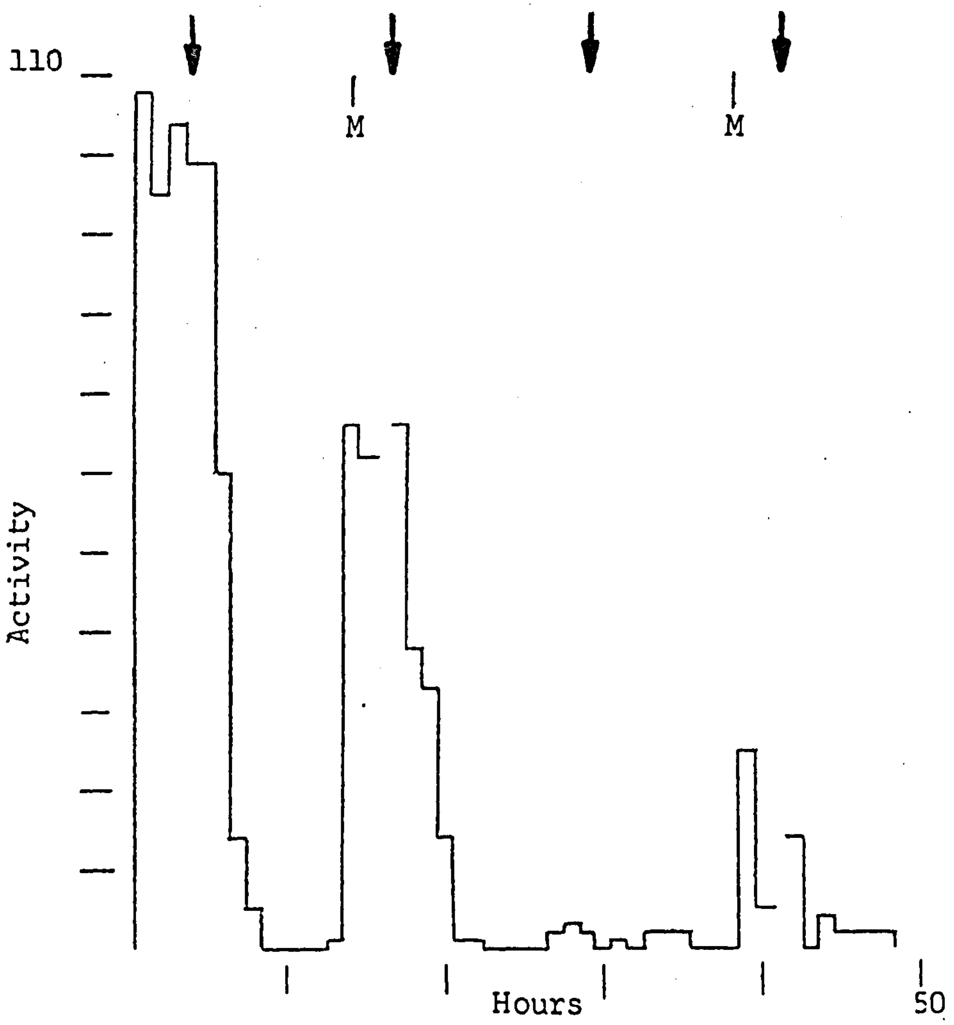


Fig. 6. Hourly activity of four freshly caught *Gobiesox* in constant light, July 17 to 19, 1973. -- Arrows indicate time of high tide; M: midnight.

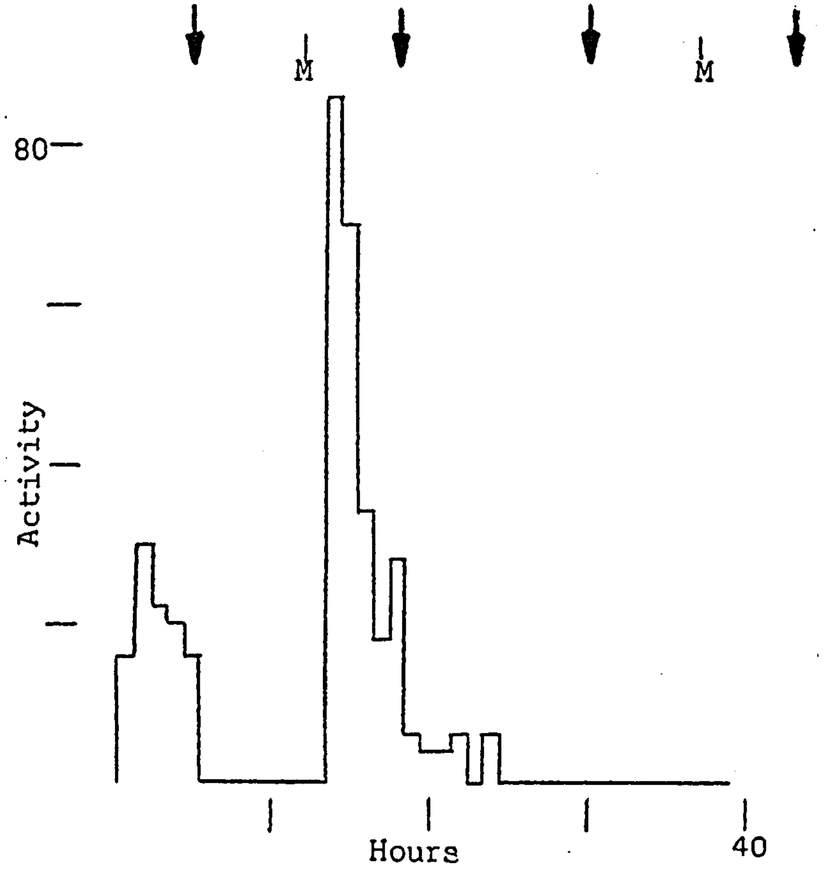


Fig. 7. Hourly activity of one freshly caught *Gobiesox* in constant light, July 20 to 22, 1973. -- Arrows indicate time of high tide; M: midnight.

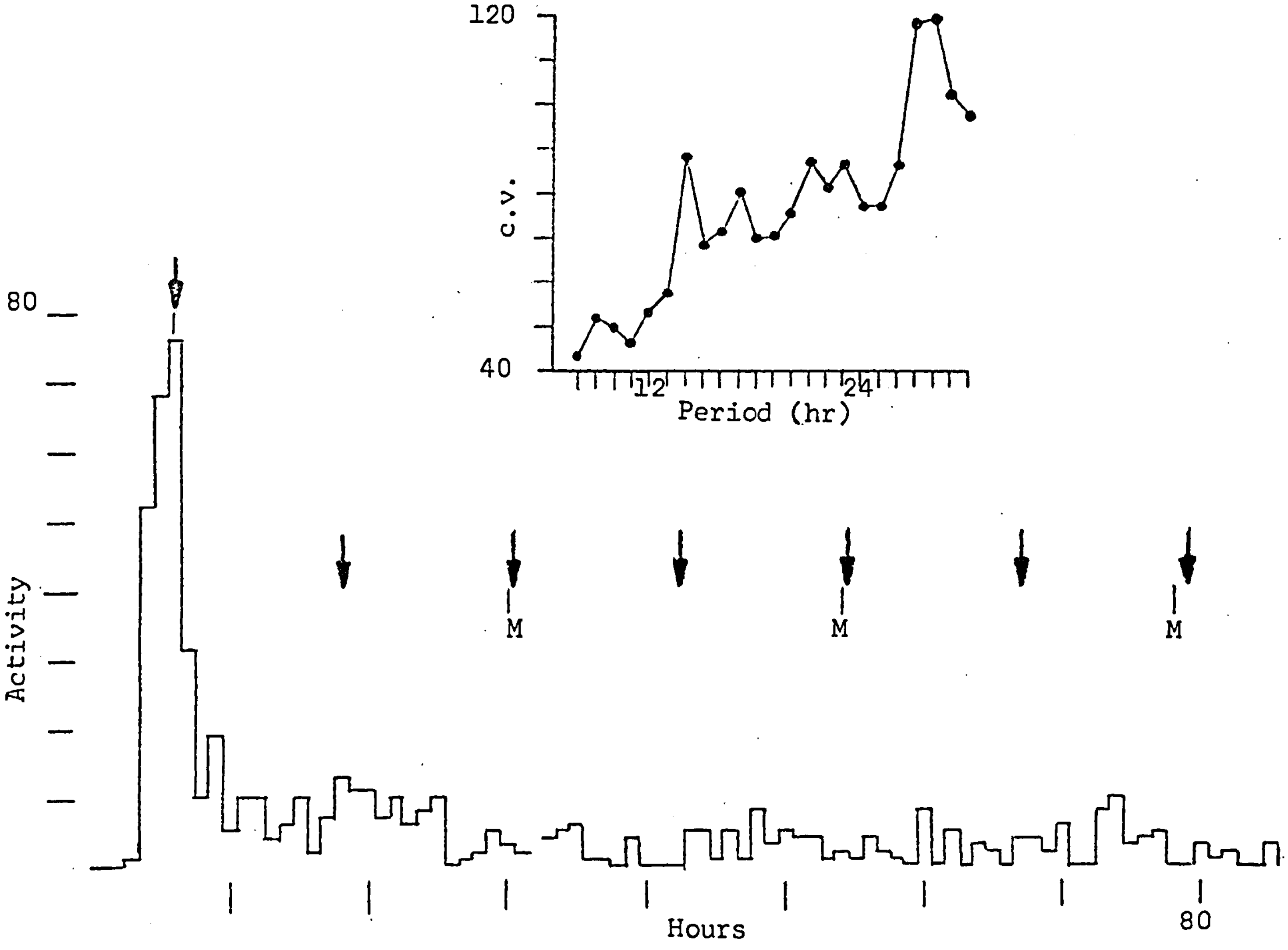


Fig. 8. Hourly activity of four freshly caught *Gobiesox* in constant light, April 17 to 21, 1974.-- Arrows indicate time of high tide; M:midnight.

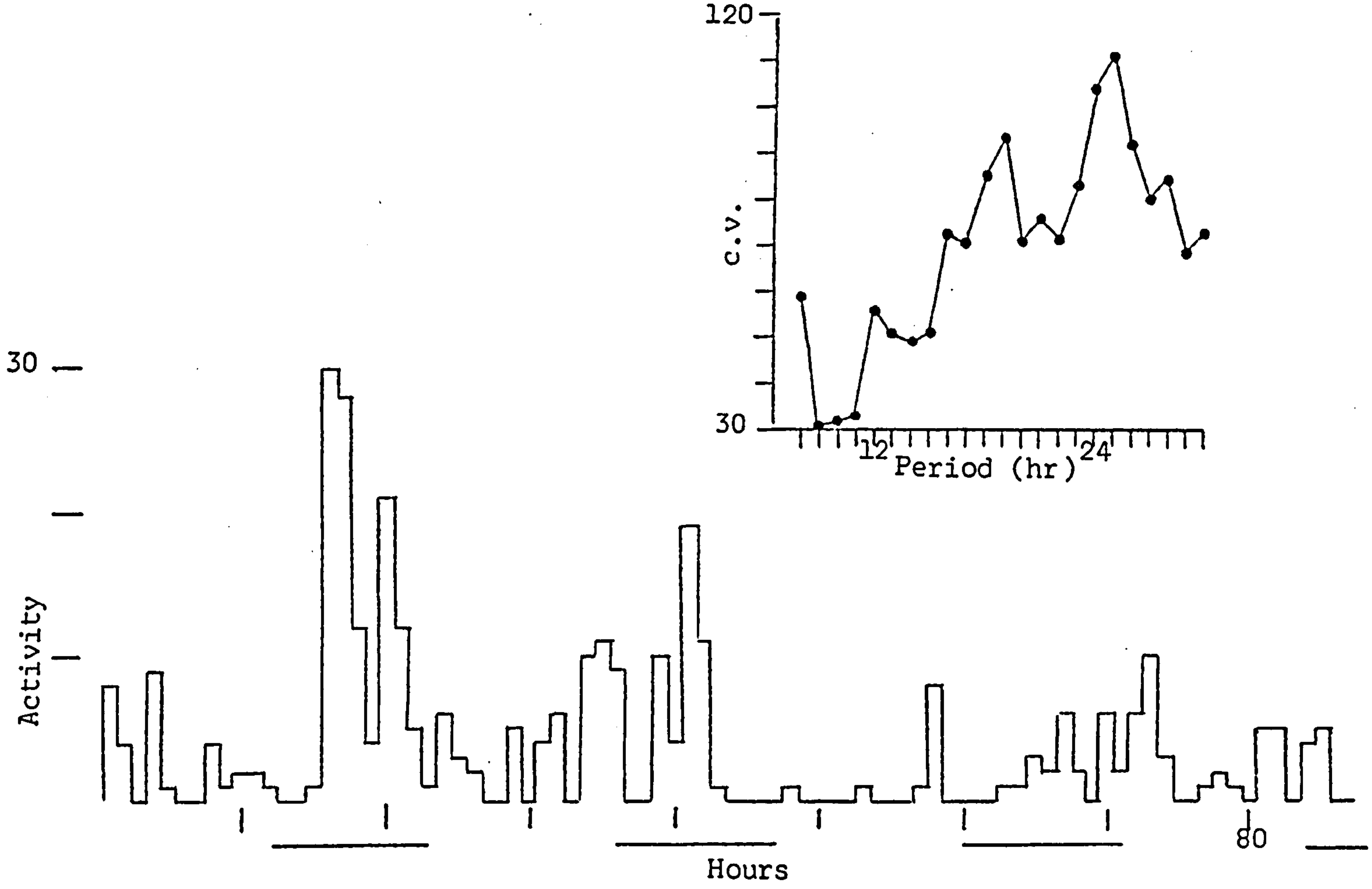


Fig. 9. The effect of seven days in 12:12 hr light-dark on seven *Tomicodon*. -- Activity recorded in Tucson, Oct. 18 to 21, 1973 in constant light. Lines under record indicate when light was on for entrainment period.

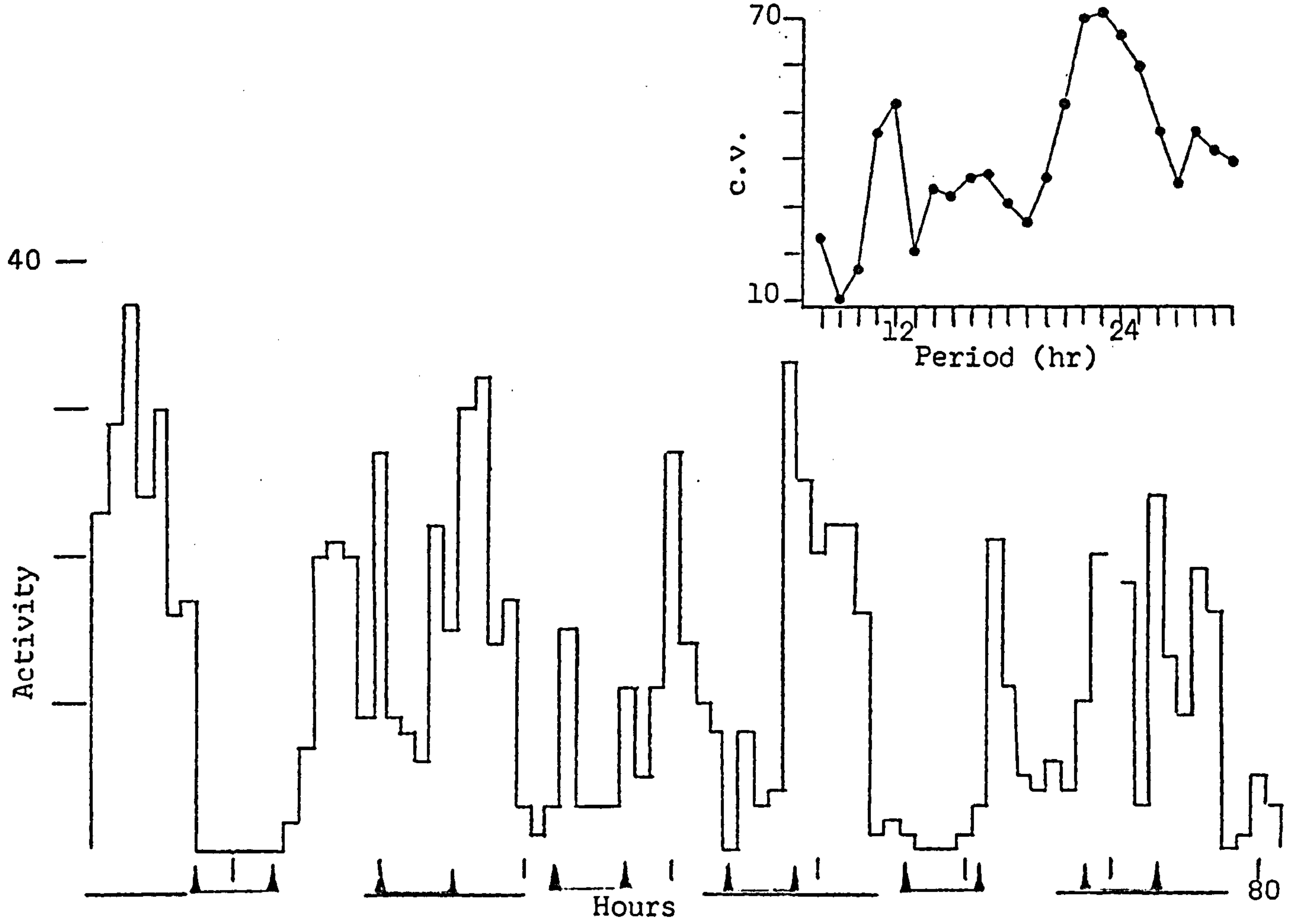


Fig. 10. The effect of two months in 12:12 hr light-dark on six Tomicodon. -- Activity recorded in Tucson, May 21 to 24, 1974 in constant light. Lines under record indicate when light was on for entrainment period. (▲—▲) indicates hours of 9 to 2 (see text).

A similar response was observed by Gibson (1971) when blennies were subjected to a light-dark cycle. It is difficult to determine from these results whether a tidal or a bimodal diurnal rhythm actually exists in Tomicodon after light entrainment. Since periods of 12 and 24 hrs are indicated, as appeared with fish taken freshly from the field, it seems that the light cycle has entrained a circatidal rhythm. Most bimodal circadian rhythms involve peaks at subjective sunrise and sunset (Aschoff, 1966, Livingston, 1968) which may or may not be the case here. The peaks here seem to occur less in the nighttime than daytime, but whether this is just the influence of nighttime suppression of an innate tidal rhythm is hard to say. Unfortunately, a photocell apparatus is unsatisfactory for measuring activity in the dark.

In Fig. 11 activity of four Tomicodon is shown after removal from a potentially entraining situation. The fish were anchored for seven days at the +5 level of the intertidal. They had previously been held until they became arrhythmic. Such treatment failed to reentrain the animals. Gibson (1971) found weak entrainment after five days with blennies in a similar situation. Clingfish in this experiment were subjected to potentially entraining cycles of turbulence and wave action, hydrostatic pressure, chemical cycles, temperature and light intensity, so that the lack of results is surprising. It is likely that a longer period on the bottom may have reentrained these fish.

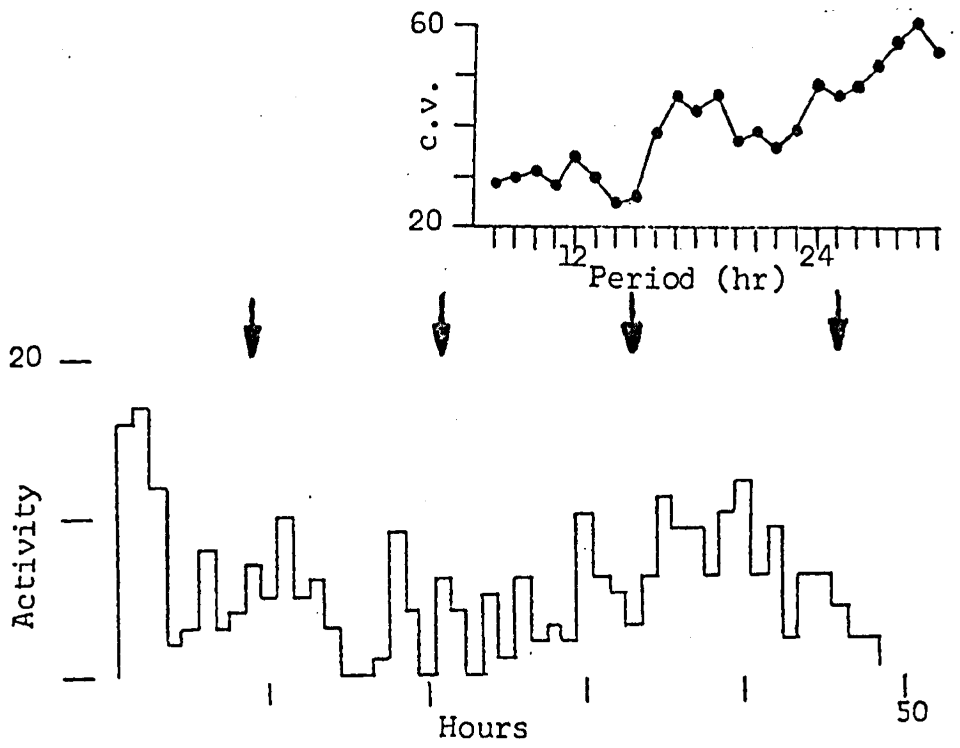


Fig. 11. The effect of anchoring four <u>Tomicodon</u> in the intertidal for seven days. -- Fish were previously arrhythmic. Activity measured in constant light, Dec. 30, 1973 to Jan. 1, 1974. Arrows indicate high tide.

Fig. 12 shows the records of four Tomicodon placed in the same photocell recording situation as previous experiments with the exception that one of the two rocks in the tank was barnacle-covered. This experiment duplicated the situation in behavioral experiments (Figs.13 to 15) but allowed continuous recording and comparison between the photocell apparatus and behavioral observations. Periodogram analysis reveals no rhythm, even if data prior to 3 p.m., April 18 is ignored, and the remaining numbers only are used because peaks seem to occur consistently at low tide. Thus, presence of the barnacles suppressed the tidal rhythm of activity usually seen in previous, similar experiments. The rhythm disappears after the second high tide (12 p.m., April 18) which corresponds to observations made during concurrent behavioral experiments in Fig. 15. These results are discussed further in the section on behavioral experiments.

Behavioral Observation Experiments

Clingfish were placed in two aquaria and observed over a period of two to three days. The water in each aquaria was either kept constant or lowered and raised to simulate the tides. The set of data that made up a "run" in either tidal or nontidal situations was composed of consecutive second, fourth and sixth tidal cycle observations. For five of the six runs (Tables 1 to 3) the F/NF (feeding to nonfeeding) ratio increased with successive tidal periods. Furthermore, total feeding increased from the second to the fourth tide cycle in five of six runs. In all

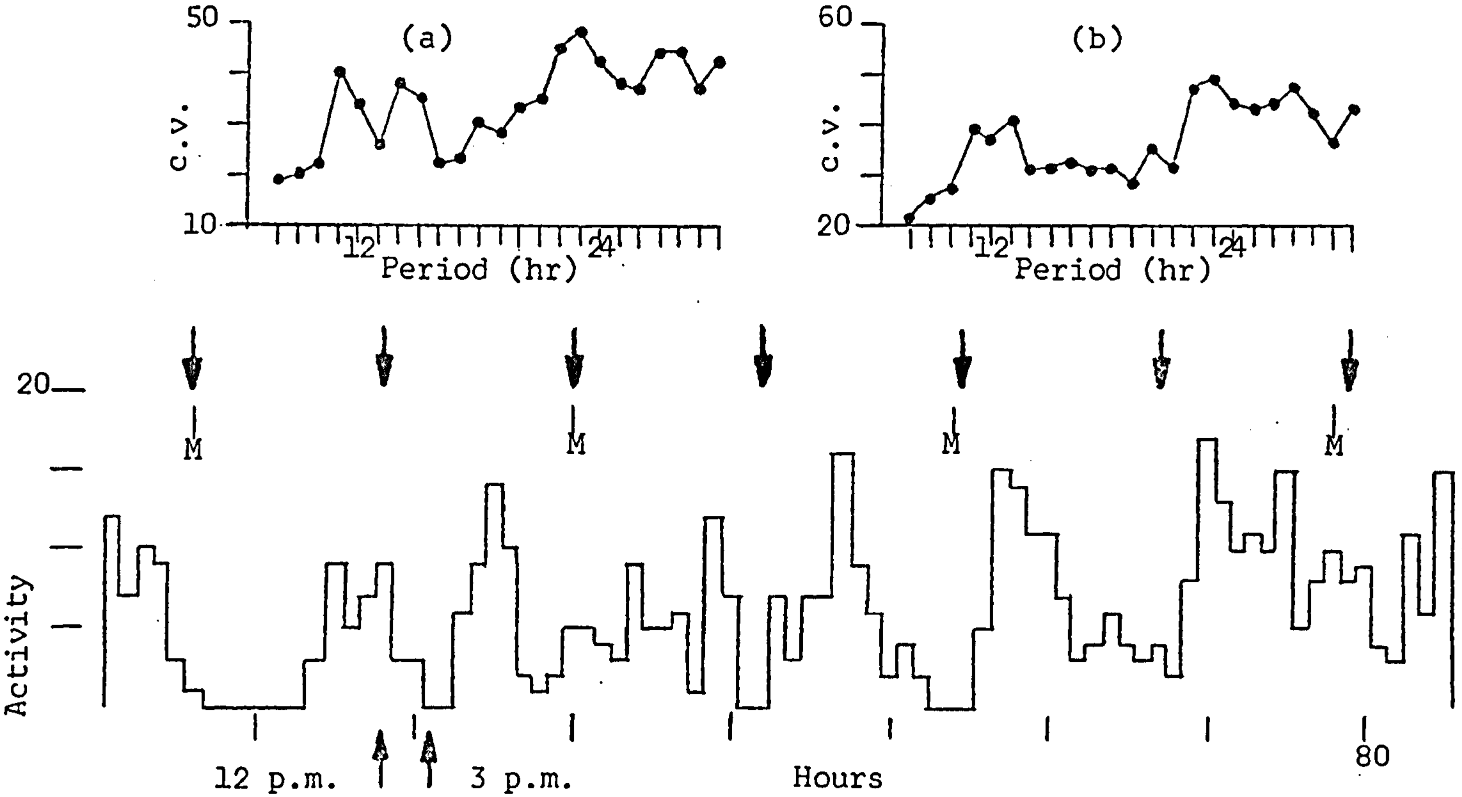


Fig. 12. The effect on the tidal rhythm of five freshly caught <u>Tomicodon</u> of placing a barnacle-covered boulder in the aquarium, April 17 to 21, 1974.--Arrows indicate high tide; M: midnight. The two periodograms analyze (a) the entire set of data and (b) data beginning 3 p.m., April 18.

Table 1. Feeding (F) and nonfeeding (NF) behavior in *Tomicodon humeralis*, Mar. 8 to 9, 1974. -- Eight different periods of the tide relative to point of collection and ongoing tide were measured (see Fig. 1). Two tanks, one maintained in constant conditions, one with simulated tide, contained five fish each. Observations were made on the second and fourth tidal cycle after collection. Data indicates relative activity (see text).

	Constant				Tidal			
	2nd		4th		2nd		4th	
Tidal period	F	NF	F	NF	F	NF	F	NF
8			15	12				
1	27	4	16	17	75	14	64	7
2	0	1	0	0	36	27	70	18
3	17	31	0	60	47	49	62	15
4	8	38	0	4	20	81	95	7
5	0	4	20	0	25	20	89	9
6	30	2	42	0	40	0	56	0
7	79	4	54	0	55	4	64	1
8	100	0			0	0		
	$\frac{F}{NF} = 3.1$		$\frac{F}{NF} = 1.6$		$\frac{F}{NF} = 1.5$		$\frac{F}{NF} = 8.8$	

Table 2. Feeding (F) and nonfeeding (NF) behavior in *Tomicodon humeralis*, Mar. 21 to 23, 1974.

	Constant						Tidal					
	2nd		4th		6th		2nd		4th		6th	
Tidal period	F	NF	F	NF	F	NF	F	NF	F	NF	F	NF
1	3	7	11	10	26	28	2	0	55	3	14	35
2	13	16	10	45	49	19	13	14	8	75	8	54
3	23	29	37	38	61	7	3	75	47	25	29	18
4	11	78	33	34	41	10	4	90	16	59	31	6
5	22	59	26	46	47	13	24	33	23	8	20	3
6	57	17	71	9	29	25	12	3	7	0	37	0
7	16	12	46	14	33	12	24	0	36	0	23	0
8	15	11	23	21			0	0	0	0		
	$\frac{F}{NF} = .70$		$\frac{F}{NF} = 1.1$		$\frac{F}{NF} = 2.5$		$\frac{F}{NF} = .38$		$\frac{F}{NF} = 1.1$		$\frac{F}{NF} = 1.4$	

Table 3. Feeding (F) and nonfeeding (NF) behavior in *Tomicodon humeralis*, Apr. 18 to 20, 1974.

	Constant						Tidal					
	2nd		4th		6th		2nd		4th		6th	
Tidal period	F	NF	F	NF	F	NF	F	NF	F	NF	F	NF
1	0	1	10	37	38	34	8	15	35	16	36	17
2	1	4	17	34	39	31	17	52	15	54	18	47
3	9	25	34	40	37	25	15	42	14	66	26	35
4	8	22	48	31	33	26	11	52	19	53	33	26
5	1	93	30	35	23	30	2	93	13	55	31	43
6	50	36	51	19	28	30	63	13	60	28	37	44
7	57	23	31	27	29	16	74	0	47	6	62	11
8	39	0	17	0			0	0	0	0		
	$\frac{F}{NF} = .81$		$\frac{F}{NF} = 1.1$		$\frac{F}{NF} = 1.2$		$\frac{F}{NF} = .71$		$\frac{F}{NF} = .73$		$\frac{F}{NF} = .93$	

six experiments a pattern occurred during the second tidal cycle. This pattern was composed of a nonfeeding peak of activity followed by a rise of feeding activity toward the end of the observation period. This pattern, which was the only consistent behavioral rhythm observed, disappears except in Fig. 15b where it is maintained throughout all three test periods.

Peaks of activity as measured by photocell occur at high tide. Observations of behavior at high tide reveal that these are peaks of nonfeeding activity. The experiment whose results are shown in Fig. 12 was approximately the same as that which produced the results in Figs. 13 and 14, in that in both situations there was constant light (although natural daylight was followed by artificial light), a constant water level and the presence of a barnacle-covered rock. The two situations can be equated in that in each, the second cycles after capture are rhythmic while succeeding cycles are not.

After 3 p.m. on April 19 in the same experiment (Fig. 12) there are successive peaks and troughs which at first glance may look like a tidal rhythm with peaks at low tide and troughs at high, but periodogram analysis indicates that this is not so. It is possible that a rhythm still exists which is constantly changing in period length and therefore would appear arrhythmic in periodogram analysis. In any case the rhythm in both observational and photocell experiments has been changed dramatically

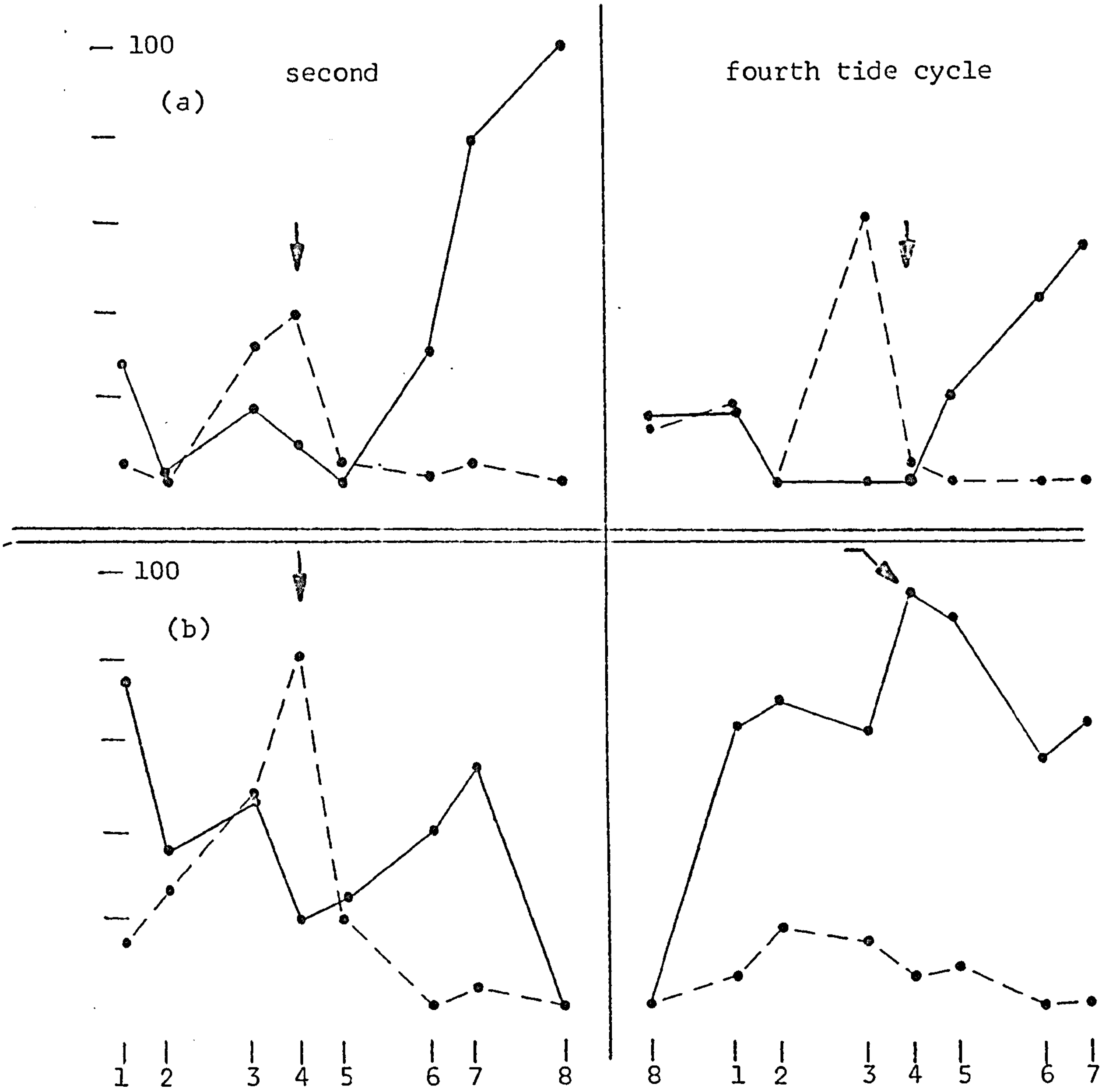


Fig. 13. Graphical analysis of data in Table 1. -- Experiments of Mar. 8 to 8, 1974. Feeding (——) and nonfeeding (– –) activity. Arrows indicate high tide. For explanation of abscissa, see Fig. 1. (a) constant and (b) tidal conditions.

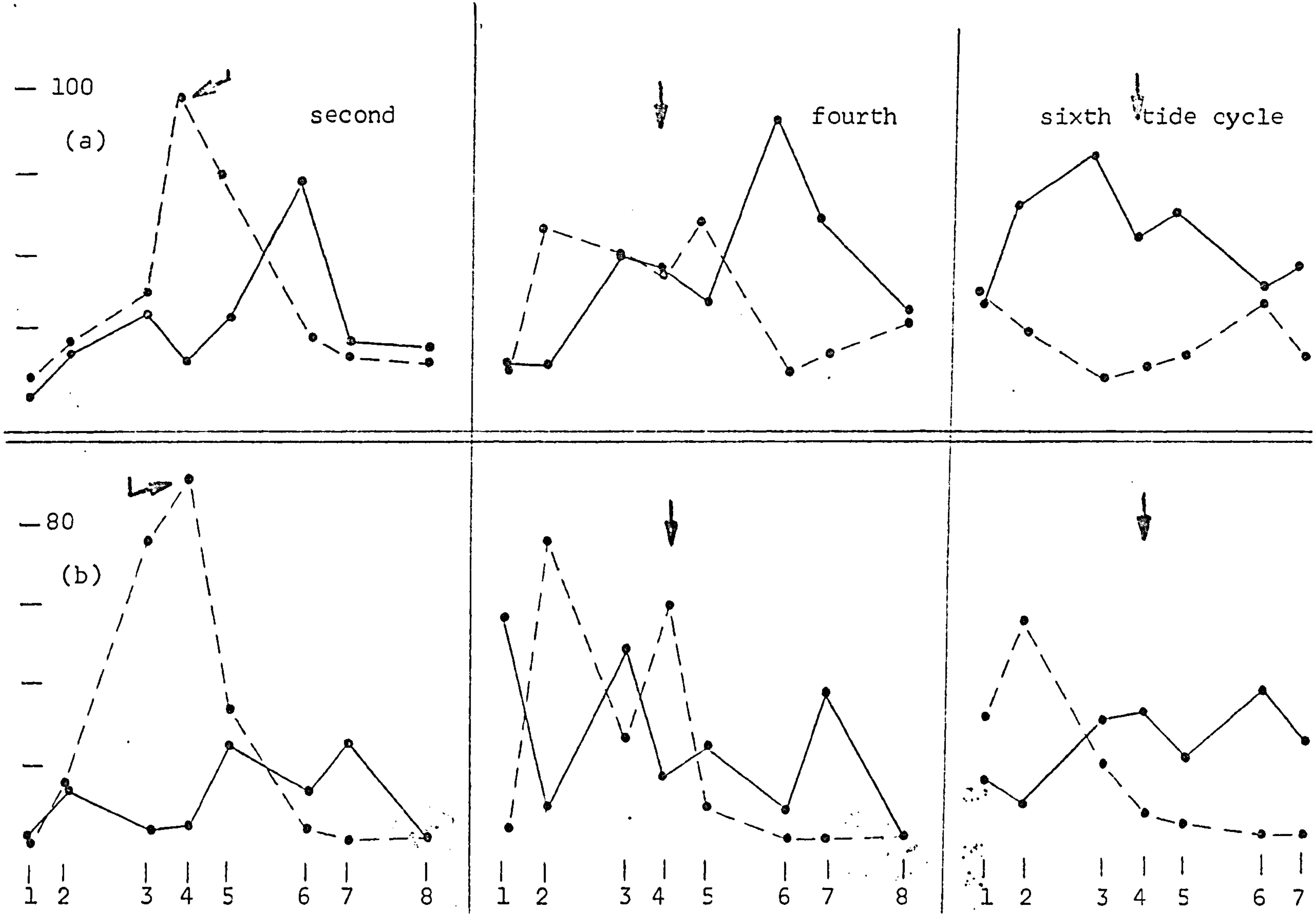


Fig. 14. Graphical analysis of data in Table 2.--Experiments of Mar. 21 to 23, 1974. Feeding (——) and nonfeeding (– –) activity. Arrows indicate high tide. For explanation of abscissa, see Fig. 1. (a) constant and (b) tidal conditions.

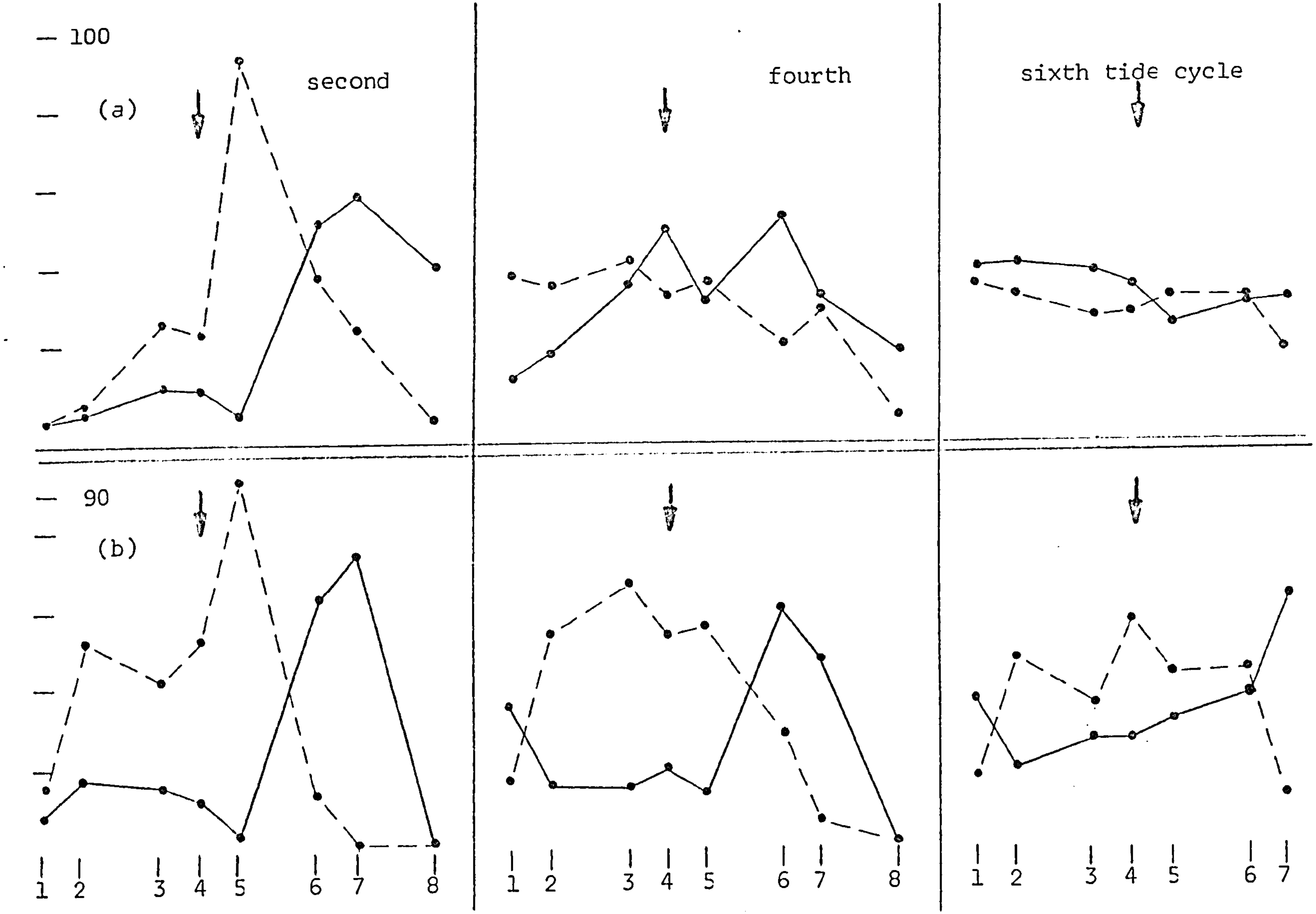


Fig. 15 Graphical analysis of data in Table 3. -- Experiments of Apr. 18 to 20, 1974. Feeding (——) and nonfeeding (– –) activity. Arrows indicate high tide. For explanation of abscissa, see Fig. 1. (a) constant and (b) tidal conditions.

by presence of the barnacles, where previous tests without food had indicated a very clear-cut tidal rhythm.

Data in Fig. 15b, in which the pattern of the second cycle is repeated in the fourth and sixth, could be interpreted as demonstrating a persistent rhythm. Whether this is so can only be confirmed by repeated experiments. This was the only experiment where diurnal as well as tidal effect were introduced, and it may be that the effect of a light-dark cycle concurrent with an imposed "tide" was enough to maintain the rhythm.

Observations on the nights of April 17 to 21, 1974, of clingfish being observed during the day, revealed that no fish could be seen in any part of the tank. Since fish were checked at a time when photocell results indicated they should have been active, it can be assumed that they hide in the dark.

Intertidal Distribution

Distribution under boulders at Norse and Station Beaches were nearly identical for *Tomicodon* while *Gobiesox* showed a wider vertical distribution at Norse Beach (Fig.16). *Tomicodon* is found in greatest abundance from the +3 to -1 tide levels, while *Gobiesox* is not abundant until the -3 level although there is overlap. The lower end of the *Gobiesox* distribution was not determined. At Station Beach a third species of clingfish, *Pherallodiscus funebris*, was found at the same lower levels as *Gobiesox* and may compete with *Gobiesox* for resources. Eger (1971) found many *Gobiesox* in tide pool collections at Norse Beach, and

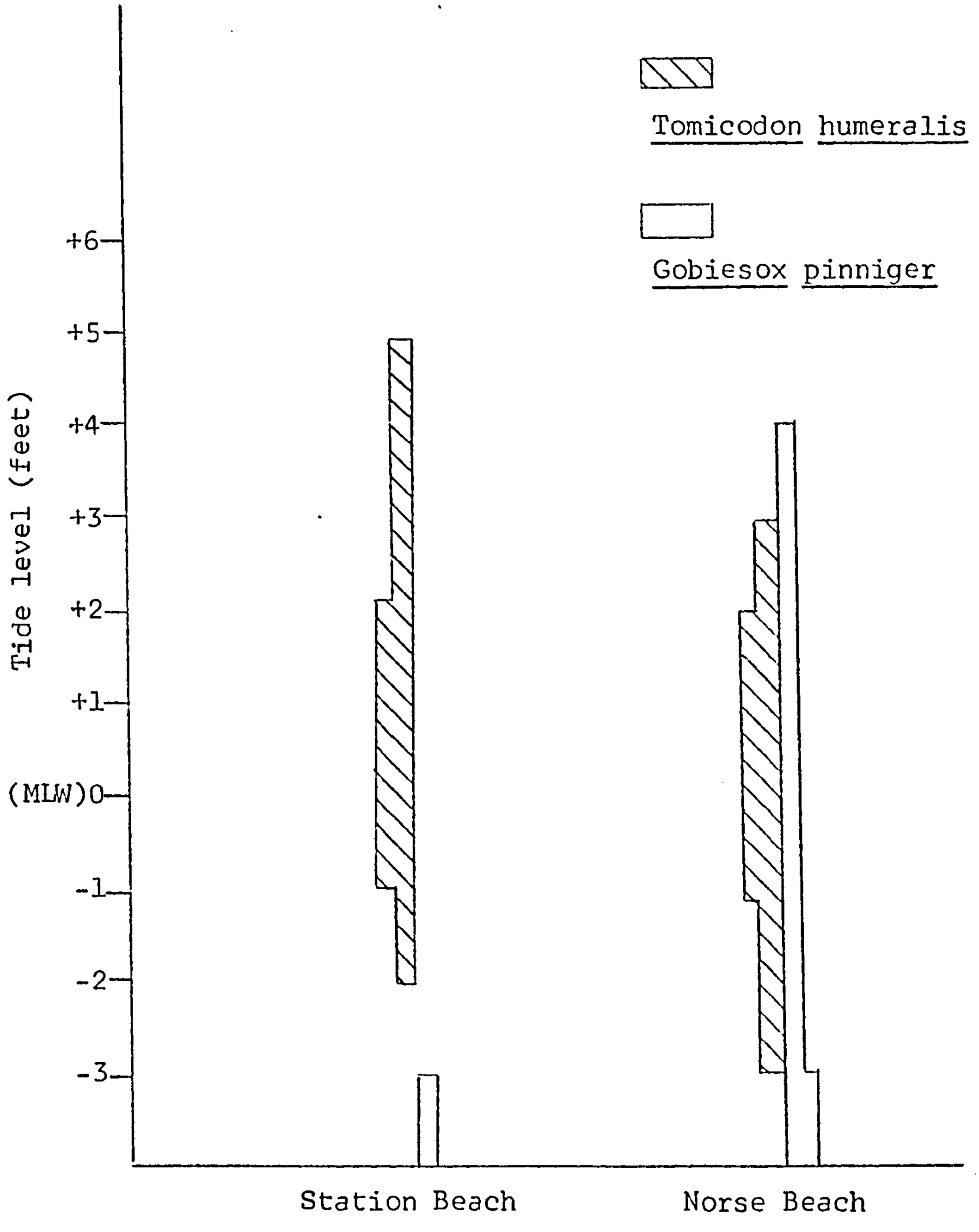


Fig.16. Distribution of clingfish on transects at Station Beach (basalt boulder) and Norse Beach (granite boulder). -- Width of bars indicates relative abundance; wider areas include greater numbers of fish.

they have been found in tide pools at Station Beach while *Tomicodon* has only rarely been found, so that different habitat preferences may exist with regard to standing water during low tide exposures. *Tomicodon* inhabits the same tide levels as *Balanus* and distribution usually stops at the lower limit of *Tetraclita*, a larger barnacle living only in the upper intertidal starting at about +4. The lower levels of *Tomicodon* distribution may be limited by competition with and predation by *Gobiesox*, although the two species are sometimes found together under the same rock. The upper limits of the distribution of *Gobiesox* are determined by its ranges of physiological tolerances, which are narrower than those of *Tomicodon* (Eger, 1971). The lower distribution of *Gobiesox* indicates an environment less reinforcing of a tidal rhythm. Since the physiological demands to regulate activity are less as a result of less exposure, the need for a precise, enduring rhythm is less than that of *Tomicodon*.

Table 4 gives the frequency distribution of *Tomicodon* under boulders. Patchiness may be determined by using the method of "mean crowding" of Lloyd (1967). Mean crowding is defined as the mean number per individual of other individuals in a quadrat (boulder). $\overset{*}{m}$ is calculated by counting, for each individual in a total population of N individuals, the number of co-occupants x_i that share the boulder:

$$\overset{*}{m} = \frac{1}{N} {}_{i=1}^{N} x_i$$

Table 4. Distribution of Tomicodon humeralis under boulders in the intertidal zone. -- Data collected from transect studies July 31, 1973 to Feb. 9, 1974.

No. fish/boulder	No. boulders	No. fish
0	543	0
1	26	26
2	15	30
3	8	24
4	5	20
5	2	10
6	2	12
7	0	0
8	1	8
9	1	9
10	1	10
-	-	-
23	1	23
Totals:	605	172

mean (m) = .28

If distribution is random, $\overset{*}{m}$ should equal the mean density, m. Where distribution is patchy, $\overset{*}{m}$ should be larger than m and in this case,

$$\frac{\overset{*}{m}}{m} = \frac{5.59}{0.28} = 19.7$$

These numbers are based on an average using the number 543 for 0 number of fish under boulders. This figure represents the number of boulders overturned that were considered "likely clingfish habitats" but where no fish were found. What is a likely clingfish habitat to the experimenter may not be to the fish, however. By ignoring this figure the average of rocks with clingfish under them alone is 2.6 and this still gives a ratio $\frac{\overset{*}{m}}{m}$ greater than 2. Thus distribution of clingfish under rocks is a patchy one.

An attempt to test for the presence of homing behavior in *Tomicodon* was made. However, results were unsatisfactory as only two fish of 44 were recovered after moving them only five meters from where they were found. No conclusion, therefore, could be made about homing in clingfish.

Gut Analyses

Both *Tomicodon* and *Gobiesox* were captured for gut analyses. Gut analyses were performed with the intention of discovering differences in feeding habits such as method, where and when they feed so that sample sizes are not large. Seven *Tomicodon* were collected after daytime high tides, six after nighttime high tides. Five Gobiesox were collected after daytime high tides (Tables 5 and 6).

Tomicodon gut contents in daytime collections always had large percentages of encrusting algae and barnacle cirri. Most gut material was undigested and easily identified. Other prey organisms occurring less consistently and in smaller numbers show that Tomicodon has a varied diet of mostly rock surface associated forms. Diet and behavior indicate that Tomicodon is a sight feeder.

Analysis of Gobiesox guts revealed no algae, lower percentages of barnacle cirri and larger and faster prey than were found in Tomicodon. Its gut contents were in a much more digested state. There is overlap in prey types between the two species, but their diets indicate differences in feeding method. Tomicodon is more of a substrate grazer while Gobiesox captures its prey off the substrate or in the water column.

Collections of Tomicodon after nighttime high tides during a new moon revealed that four of the six fish collected had not taken any food. Of the two fish which had food neither had full stomachs and neither had much algae, which was so abundant in the daytime collections. When and how these fish capture food without light is difficult to predict as sight is very important to the daytime diet.

Since algae found in the guts of Tomicodon was in unusually good condition, (chloroplasts visible), there was some

Table 5. Gut analyses of *Tomicodon humeralis*.

Organism	Percent by Volume	
Composite of seven fish collected after daytime high tide. 21-40 mm S.L.		
Algae	70%	Gut condition: full in all
Barnacles (cirri only)	12%	
Amphipods	6%	
Dipteran larvae	1%	
Snails	1%	
Copepods	1%	
Other (Polychaetes, Ostracods, gravel)	1%	
Unidentified	8%	
Six fish collected after new moon, nighttime high tide. 25-44 mm S.L.		
25 mm S.L.		Stomach and intestine half full
Ostracods	92%	
Algae	5%	
Protozoa	2%	
Barnacles (cirri only)	1%	
44 mm S.L.		Stomach full only
Isopods	75%	
Nematode	10%	
Unidentified	15%	
Four others, gut empty		

Table 6. Gut analyses of Gobiesox pinniger.

Composite of five fish collected after daytime high tide.
38-58 mm S.L.

Organism	Percent by Volume
Crabs (hermit)	20%
Crabs (other)	3%
Snails	5%
Amphipods	5%
Fish*	4%
Isopods	1%
Dipteran larvae	1%
Shrimp	1%
Barnacles (cirri only)	1%
Other (Copepods, Ostracods, Malacostracans)	1%
Unidentified	58%

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* Two fish (Gobiidae) were found in the same container with Gobiesox after collection in partly digested form and most likely were spit out at time of capture.

question as to whether it is as important to the diet as percentages indicate. The algae may just pass through the digestive tract, having been consumed at the same time as the prey species according to the fish's feeding habits. A long intestine would indicate that the fish was primarily an herbivore, carnivores usually having short, straight intestines. Tomicodon has a short, thick, straight intestine and this, along with the evidence of algae in near perfect condition indicates that Tomicodon is not an herbivore, although some algae may be digested.

CHAPTER 5

DISCUSSION

A persistent tidal rhythm of activity which is suppressed by darkness and the presence of food has been shown to occur in Tomicodon. A nonpersistent tidal rhythm of activity exists in Gobiesox.

A clue to what may be timing the rhythm can be obtained from an analysis of the only behavioral experiment in which a rhythm persisted throughout the experiment. Two important possible zeitgebers (time-setters) that were not present together in any other behavioral experiments were operating at the same time, these being a natural light-dark regime and a cycle of inundation. Presence of food suppressed the rhythm in other experiments. Perhaps the interaction of both circadian and tidal synchronizers ensured that the rhythm was maintained when barnacles were present. No matter what the entraining zeitgeber may be, it requires longer than seven days to effectively reentrain a tidal rhythm in arrhythmic animals. Fish held in the intertidal zone for that length of time with both natural and tidal light-dark cycles present remained arrhythmic. Rhythms in Tomicodon have thus been shown to exist in two situations; under constant conditions in the absence of food, and during natural light-dark and tidal cycles with food being present. It would be interesting to test the animals in the presence of food, and then remove it to see if the tidal rhythm reappeared.

An undetermined threshold of light "permits" the tidal rhythm in Tomicodon to appear. Although clingfish in constant diffuse light are less active during the hours corresponding to night in the environment than during the day, observation of fish held in darkness shows that they are completely inactive. To the animal in the field this means that activity can continue during a high tide as long or as soon as light is available. Perhaps nighttime activity has been eliminated because food objects and predators cannot be sighted.

After light-dark entrainment experiments in Tomicodon, a bimodal circadian rhythm appeared when the fish was returned to constant conditions. Bimodal circadian rhythms are not unusual and have been observed in finches and non-intertidal fish (Aschoff, 1966 and Livingston, 1968). Juvenile plaice reared in the laboratory had a bimodal circadian rhythm while adults of the same species collected from the field showed a tidal rhythm (Gibson, 1973). It seems difficult to me to differentiate between a free-running, approximately 12 hr bimodal circadian rhythm and a tidal rhythm. Perhaps the definition of the nature of the rhythm (tidal or circadian) depends on the environment from which the animal is taken.

There is an extraordinarily close and subtle relationship between tidal and circadian rhythms within a single organism. The questions may be asked: which arose first in the organism (or in evolution)? did they both arise concurrently? and is one only a slight "adjustment" of the other? That the two exist as two separate rhythms cannot be disputed. By controlling the environment, one of the rhythms can be made to appear while the other is damped out (Zann, 1973). Perhaps what is fundamental is a basic bimodal clock which can become either tidal or circadian when environment demands.

One could postulate a basic bimodal timer which couples by unknown mechanisms, a circadian and tidal rhythm. Palmer (1973) and Zann (1973) propose such a multiple clock theory to explain the existence of two, slightly different rhythms. It is most interesting that Barnwell (1966) could phase the tidal rhythm in fiddler crabs by entraining the diurnal one to an external zeitgeber. Such a basic bimodal timer whose rhythm is set by environmental cues is highly adaptable to the activity and ecology of many species of intertidal organisms.

Perhaps the most significant result in this study was suppression of the rhythm in <u>Tomicodon</u> by the presence of food. This immediately suggests that the rhythm is related to food seeking activity. If this were true, observations of fish before the rhythm was suppressed would indicate high tide associated peaks of feeding activity. However, fish in aquaria with barnacles present did not engage in feeding behavior at high tide. Also, distributional studies as well as stomach analyses show that fish don't have to go far to seek food, since their low tide habitat is under barnacle-covered boulders. Only with caution can the function of the rhythm in the animal's niche be related to these peaks of activity as measured in the laboratory. The expression of the rhythm, the activity peaks, may not be related to either the zeitgeber, in this case some element of tidal fluctuation, or to its function in the field which may or may not have anything to do with activity at high tide. The peaks, however, are the expression of the rhythm and demonstrate how the animal behaves when its clock is engaged.

A few investigators have attempted to relate environmental demands to the overt rhythm of their animals by noting peaks of high or low activity and associating these peaks with a survival-related response to ongoing conditions. These theories would be difficult to prove experimentally. Morgan, Nelson-Smith and Knight-Jones (1964) note that increased activity of pycnogonids during low and ebb tide, and less or no activity during high tides prevents the animals from being carried over the upper shore and being stranded as the tide recedes. Strangely, just the opposite mechanism ensures that isopods persist in swimming at high and ebb tides so that they maintain themselves in the water column and don't settle until low tide (Morgan, 1965).

The question to ask of <u>Tomicodon</u>'s rhythm is since peaks of activity are not related to feeding, why does the presence of food damp out the expression of the rhythm (although most likely the clock is still engaged)? Would an animal whose food source is always available need a clock to tell it when to feed? Most likely it wouldn't, but it might need a clock to tell it when it could not feed, especially when feeding is limited by the tide cycle. The clock, then, might function to tell it when to begin or stop feeding, since food is not available at low tide.

This theory suggests another theory. The animal must in some way "remember" his vertical distribution in the intertidal. Since Tomicodon does not follow the tide out but settles in a specific vertical intertidal area, it must know when the time for low tide approaches so that a suitable location can be found instead of swimming into the subtidal. There is no lack of boulders in the subtidal. These fish are mobile enough to move with the tide. However, unlike many other fish which do move, they must choose a suitable spot prior to low tide.

How does the animal recognize its limits of vertical distribution? Presumably, if it were too high up, it would be subjected during low tide to physiological stresses beyond its limits. If it moves too low in the intertidal, its range overlaps with that of large numbers of Gobiesox. One result of this could be competition for space. For example, one Gobiesox was held in the same aquaria with 15 Tomicodon and was observed to dominate the only rock in the tank which was of considerable size. All Tomicodon were found high on the sides of the tank, mostly clumped together. Another consequence may be that Gobiesox eat Tomicodon eggs, as well as Tomicodon.

Unlike many of the invertebrates with which it is found under rocks, Tomicodon is mobile enough to range over the entire intertidal zone during one high tide. However, it is not likely to for the most efficient use of its time and energy, but should stay close to a suitable low tide food source near its habitat, since feeding activity is maximal just before low tide. Clumped distribution indicates that the same habitat is chosen by more than one fish at a time. Therefore, it is possible that just prior to a low tide a single fish might initiate feeding activity and thus become associated with other fish. Should one fish find a suitable location, other fish could join it under that boulder rather than searching for another. The presence of barnacles may serve as a cue for a suitable location, but they do not provide the only one as clingfish are not found under barnacle-covered rocks in deeper pools.

Such a theory whereby the animal seeks his low tide distribution before low tide would work well for animals such as blennies that have low tide habitats in one area and feed elsewhere. The time sense of the animal could then be used to gauge when the approach of a low tide would dictate that he feed or return immediately to the home area. The animal, instead of swimming out with the tide, remains in an area that will be exposed within 15-20 minutes. If it were not prepared, it might find itself stranded and separated from its territory.

Why the rhythm of Gobiesox is nonpersistent has not been determined by this study. To fit in with the proposed theory, Gobiesox would not need a "knowledge" of tides in preparation for low tide exposure as it is found only in subtidal and lower intertidal areas. At low tide levels the animal is exposed briefly and the physiological adjustments required are not as great as for Tomicodon. A more pertinent question, one posed by Enright (1970), might be why does Tomicodon's rhythm persist, i.e., why does an organism in a regularly changing environment need more than a two or three cycle timer? Tomicodon's rhythm might be better understood by a thorough investigation of what factors serve to entrain it, and why these factors are not as demanding of Gobiesox to produce a persistent rhythm.

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