ABSTRACT

THE AEROBIC METABOLISM AND BEHAVIOR

OF THE BURROW-DWELLING MANTIS SHRIMP

HEMISQUILLA ENSIGERA CALIFORNIENSIS

by

Jason Joseph Cassista

The predatory stomatopod *Hemisquilla ensigera californiensis* was captured at 10-20 m depths off California and transferred to an observation tank with artificial burrows similar to those observed in the field. The animals were maintained under a 10L:14D light-dark cycle and monitored on a 24-hour basis using a time-lapse video recorder. Infrared lamps were used for

observations within the burrow and at night. Metabolic rates, as a function of oxygen tension, were measured in respirometry chambers using polarographic oxygen electrodes.

This species is a near complete oxyconformer, so aerobic metabolism is strongly depressed at low oxygen levels, yet the animals remained active within the burrow even under these conditions. *H. ensigera* builds a blind-ended burrow that would be difficult to aerate by pumping oxygenated water into the burrow. The animal further complicates the possibility of water exchange by capping off the burrow for up to three-fourths of the daily cycle. Finally, this mantis shrimp was not observed trying to actively pump oxygenated water into the burrow and often remained at the far end of the burrow with little regard for the better-oxygenated water near the entrance. This is in contrast to other known burrowing shrimp, such as *Upogebia pugettensis* and *Callianassa californiensis*, which also remain in hypoxic burrows but they are oxyregulators and often become dormant in hypoxic conditions or aerate their burrows by pumping oxygenated water into them. *H. ensigera* does not fit well into either the "environmental anaerobiosis" or "facultative anaerobiosis" categories. During times of relative anoxia within the burrow the animal maintains a moderate level of activity instead of the quiescence normally expected under hypoxic conditions.

LOMA LINDA UNIVERSITY

Graduate School

THE AEROBIC METABOLISM AND BEHAVIOR OF THE BURROW-DWELLING MANTIS SHRIMP *Hemisquilla ensigera californiensis*

by

Jason Joseph Cassista

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of the Requirements for the Degree of

Master of Science in Biology

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Each person whose signature appears below certifies that this thesis in his/her opinion is adequate, in scope and quality, as a thesis for the degree Master of Science.

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INTRODUCTION

Hemisquilla ensigera californiensis (Stomatopoda: Hemisquillidae) (Figure 1) is a burrowing mantis shrimp found off the coast of Southern California. It is common from Point Conception south to Baja California, and is found in disjunct populations as far south as Panama (Basch and Engle, 1989). The species is found on gently sloping, soft mud-sand substrate at 4 to 90 meters in depth. These burrows are commonly found in areas of low physical disturbance, high food availability, moderate light and a varying degree of predation pressure (Basch and Engle, 1987). The average flow velocity for the habitats off Southern California ranges from 7 to 10 cms^{-1} and annual temperatures range from 11 to 17 degrees C (Basch and Engle, 1989). Along the coast of California the species live in burrow aggregations that range in size from 150 m^2 to one hectare (Basch and Engle, 1989). *H. ensigera californiensis* has two sister species: *H. ensigera ensigera*, which is found off the coast of Chile and Peru and *H. ensigera australiensis*, which is found off the coasts of Australia and New Zealand (Basch and Engle, 1987).

Behavioral studies of the activity of *H. ensigera californiensis* (hereinafter referred to as *H. ensigera*) in association with the time of day have indicated a distinct time pattern for its activity. This species is said to be a crepuscular animal, being active primarily between dawn and dusk. This activity pattern is probably a response to predation pressures and stimulated by changes in light intensity. These shrimp have excellent vision and may be sensitive to the strong light during the mid-day hours. They also may be so dependent upon their sight that they are incapable of avoiding predators by other means than sight during

Figure 1. *Hemisquilla ensigera californiensis*, 1/2 life size.

the darkness of night. According to one study of a natural population, the mantis shrimp was most active and was found most frequently out of its burrow from 04:30 TO 08:30 h and from 16:00 to 20:00 h (Basch and Engle, 1989).

H. ensigera spends more of its time within the burrow, which it excavates in soft sediment, than outside. This burrow is probably used for protection from predation, for reproduction, and for feeding and molting (Basch and Engle, 1989). The burrow, which generally has only one entrance, extends downward at a 60 degree angle for about two body lengths, (about 1/2 m) then extends about a meter horizontally. The diameter of the entrance to the burrow varies by the size of the animal, and may range from 7 to 95 mm (Basch and Engle, 1989). The mantis shrimp spends most of its time within the burrow when it is not feeding. On occasion another mantis shrimp may attempt to enter and evict the host animal from its burrow. This action is often met with little success unless the aggressor is of significantly greater size than the attacked individual. At times other than its active periods near dawn and dusk, *H. ensigera* often caps off its burrow with a sand and mucus-like secretion that it produces, and remains inside. The cap may be up to 5 cm thick and is apparently used to disguise the location of the burrow from predators and from other individuals of the same species which may seek to evict the occupant and take over the burrow. The most common time period for burrows to be capped is from 08:00 to 16:00 h (Basch and Engle, 1989).

When not foraging, *H. ensigera* remains within the burrow. Much of this time the animal may be found at the entrance, but substantial periods of time are also spent deep within the burrow, leading to possible oxygen limitation. Since the burrow is open at only one end there is little natural bulk flow of water

through the burrow to maintain high oxygen levels. Circulation would be further restricted when the burrow is capped. Preliminary test indicated that *H. ensigera*, in well-oxygenated water, have a rate of O_2 consumption of about 5 μmol O_2 $g^{-1}h^{-1}$. Taking an average animal size to be 50 g and a burrow size to be 1.5 m long by 6 cm in diameter (volume 4.25 liters), a capped burrow should be entirely depleted of oxygen in under three hours by the animal's metabolic demands. However, the species regularly stays in a capped burrow for periods of eight hours or more, and sometimes for up to several days (Basch and Engle, 1987). *H. ensigera* must therefore use some method to actively circulate water through the burrow, even when the cap is present, or alternatively may be able to survive and function in hypoxic to anoxic conditions.

The purpose of this research was to quantify the amount of time *H. ensigera* spends deep within its burrow and its activity while there, and to characterize the species' behavioral and physiological response to oxygen limitation.

MATERIALS AND METHODS

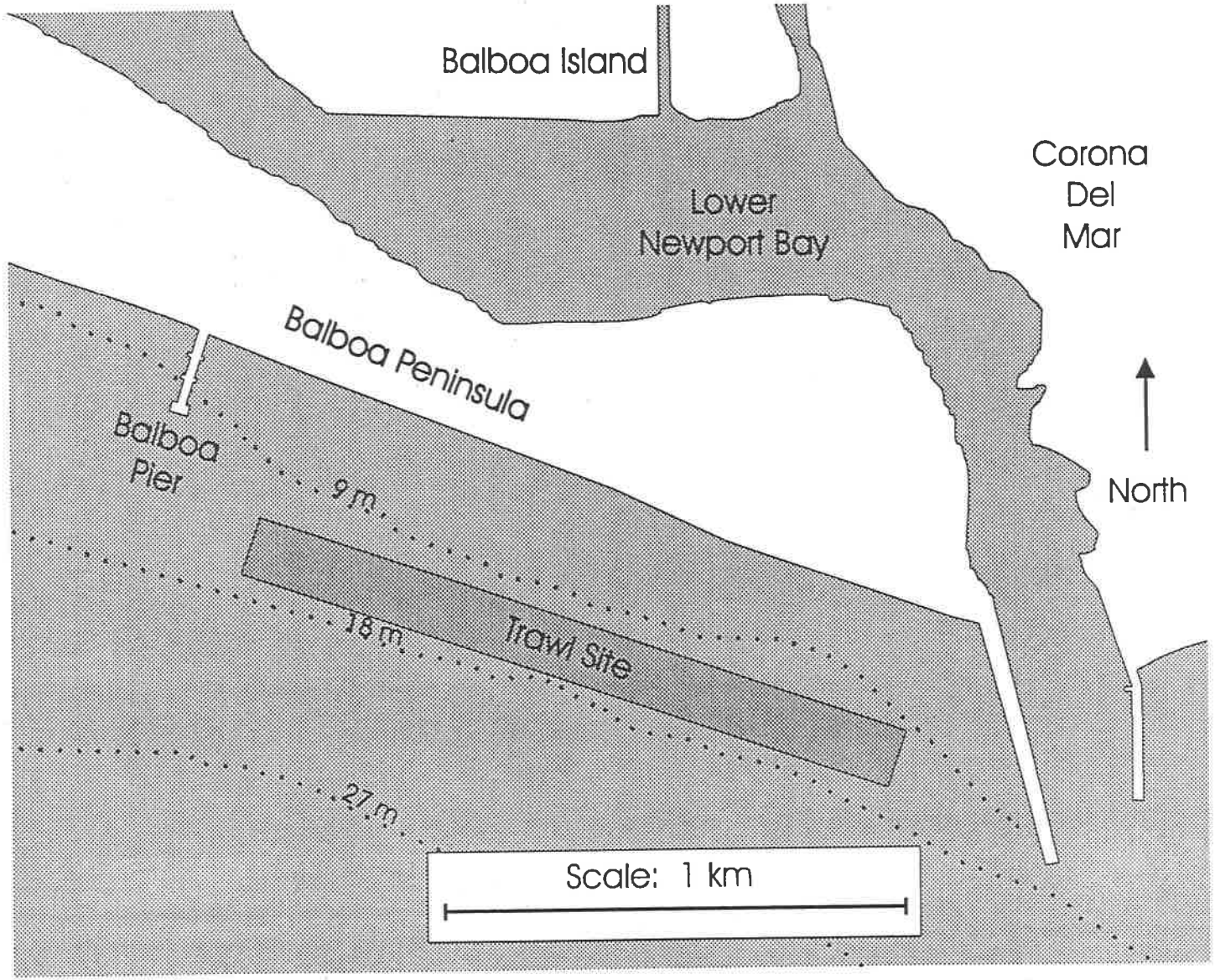
Capture of Specimens:

We tried several techniques for capturing live *H. ensigera*. The first technique used was that of trapping. Three plastic lobster traps were set off the coast of Newport Beach, Corona del Mar and Dana Point: locations reported by local fishermen to have mantis shrimp populations in them. The traps were baited with squid and tuna fish, and were set in depths ranging from 10 to 25 meters of water. Unfortunately this method of capture proved unsuccessful in the collection of *H. ensigera*, though it did prove successful in the capture of several other benthic species.

Most of the specimens for this study were captured by otter trawl from the Conqueror, operated by Newport Institute of Oceanography. During this time period the Conqueror was conducting regular otter trawls along the coast in a 2 km long area south of the Balboa pier off Newport Beach, in 15 to 30 meters of water (Figure 2). Captured animals were transferred to running seawater tanks on shipboard and held there until transported to our laboratory in four-liter aerated containers. With this method of capture the depth could usually be determined, but the actual site of capture could not be determined to greater precision than the nearest kilometer since the net is not inspected until the end of the trawl.

The third capture technique was that of luring *H. ensigera* directly from their burrow entrances; a procedure which requires using SCUBA and other appropriate tools for capture. This procedure was developed by Roy Caldwell of U.C. Berkeley and Jack Engle of the Tatman Foundation. SCUBA divers visit

Figure 2. The Newport Beach trawl site. At this location the Mantis Shrimp were captured by otter trawl in 40 to 80 feet of water.

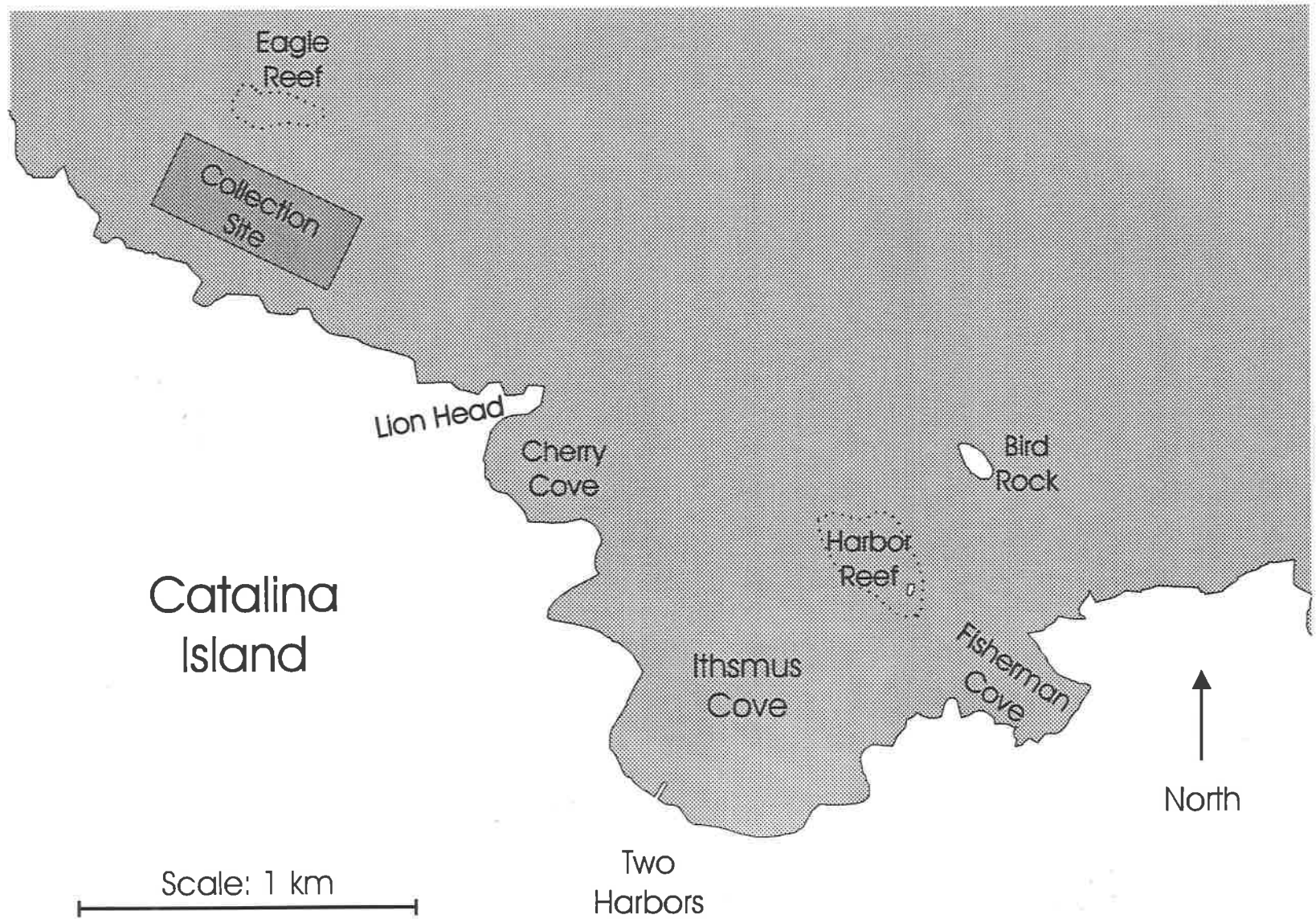

Balboa Island
Lower
Newport Bay
Corona
Del
Mar
Balboa Peninsula
Balboa
Pier
9 m
18 m
27 m
Trawl Site
North
Scale: 1 km

known sites of *H. ensigera* populations. At the sites one can recognize the dwellings of the mantis shrimp by the visible burrow openings scattered across the substrate. Two persons swim down to the burrow entrance without disturbing the sediment or alerting the inhabitant. It is best to stay down current if possible. One person has a pole with bait, (such as squid), attached to the end. The animal is lured completely out of the burrow by suspending the bait near the entrance. The second person holds a pole with a clear acrylic square fastened to the end. This plastic shield is then quickly placed over the vacant hole. The mantis shrimp immediately tries to get back into the burrow, but becomes confused; for it can see the entrance but cannot get there. The first diver then quickly nets the animal and places it in a mesh collection bag. Several mantis shrimp were caught and a few were used for this study by using this technique in Willow Cove, off Catalina Island (Figure 3) during a cruise on the Cormorant directed by Jack Engle.

The shrimp were maintained either in three large holding tanks or in the observation tank (to be described later). The seawater in these tanks was changed once a week. The tank was kept on a 10:14 light/dark cycle and a temperature of 17 degrees C, conditions similar to those of their springtime natural habitat. The animals were fed with frozen halibut and shrimp, twice a week.

The laboratory study consisted of two basic types of analysis. First; the behavioral analysis utilized an artificial habitat within an observation tank. The shrimp were kept and observed in a square observation tank with burrows extending down beneath it. The tank was created out of plywood and sealed with a waterproof fiberglass finish and silicone. The dimensions of the tank

Figure 3. The Catalina Island capture site. At this location the Mantis Shrimp were captured by hand using SCUBA in 30 to 40 feet of water.


Eagle Reef
Collection Site
Lion Head
Cherry Cove
Bird Rock
Harbor Reef
Catalina Island
Ithsmus Cove
Fisherman Cove
North
Scale: 1 km
Two Harbors

were: 1.3 m X 1.3 m square with 0.3 m high sides. The floor of the tank was covered with 3 centimeters of light colored sand. The seawater within the tank was about 15 centimeters in depth. The area of the tank was separated into four equal quadrants, each of which contained a single burrow, by plastic mesh barriers (Figure 4).

Within each quadrant there was an entrance hole to an artificial burrow. The burrow consisted of an acrylic tube 6.3 cm in diameter and 1 m or more long. The first section of the burrow descended at a 30 degree angle from the vertical for 0.35 m. The final section of the burrow extended horizontally for 0.7 m ending in a dead end. The clear acrylic burrows were normally wrapped in opaque, black plastic covers. These covers were removable so any individual could be observed at any one time while not disturbing the others in nearby burrows. Sand had been secured to the inside lip of each burrow entrance to aid the shrimp in securing the sand-mucus cap they create.

The area below the tank, where the burrows were, was illuminated by infrared lamps. An infrared-sensitive video camera was secured beneath the tank to record the burrow activity of the animals. These lights provided enough infrared light for the infrared-sensitive video camera to record images, but not enough visible light to disturb the animals within the burrows. The animals' behavior within the burrow was recorded by video on a time-lapse VCR. Each recording session was 48 hours long.

For the video analysis from the "below-burrow" perspective, the burrows were divided into three separate zones (Figure 4). These zones were indicated with fluorescent yellow tape. The first zone included the 0.33 m section that descended at a 60 degree angle from the entrance to the elbow of the burrow.

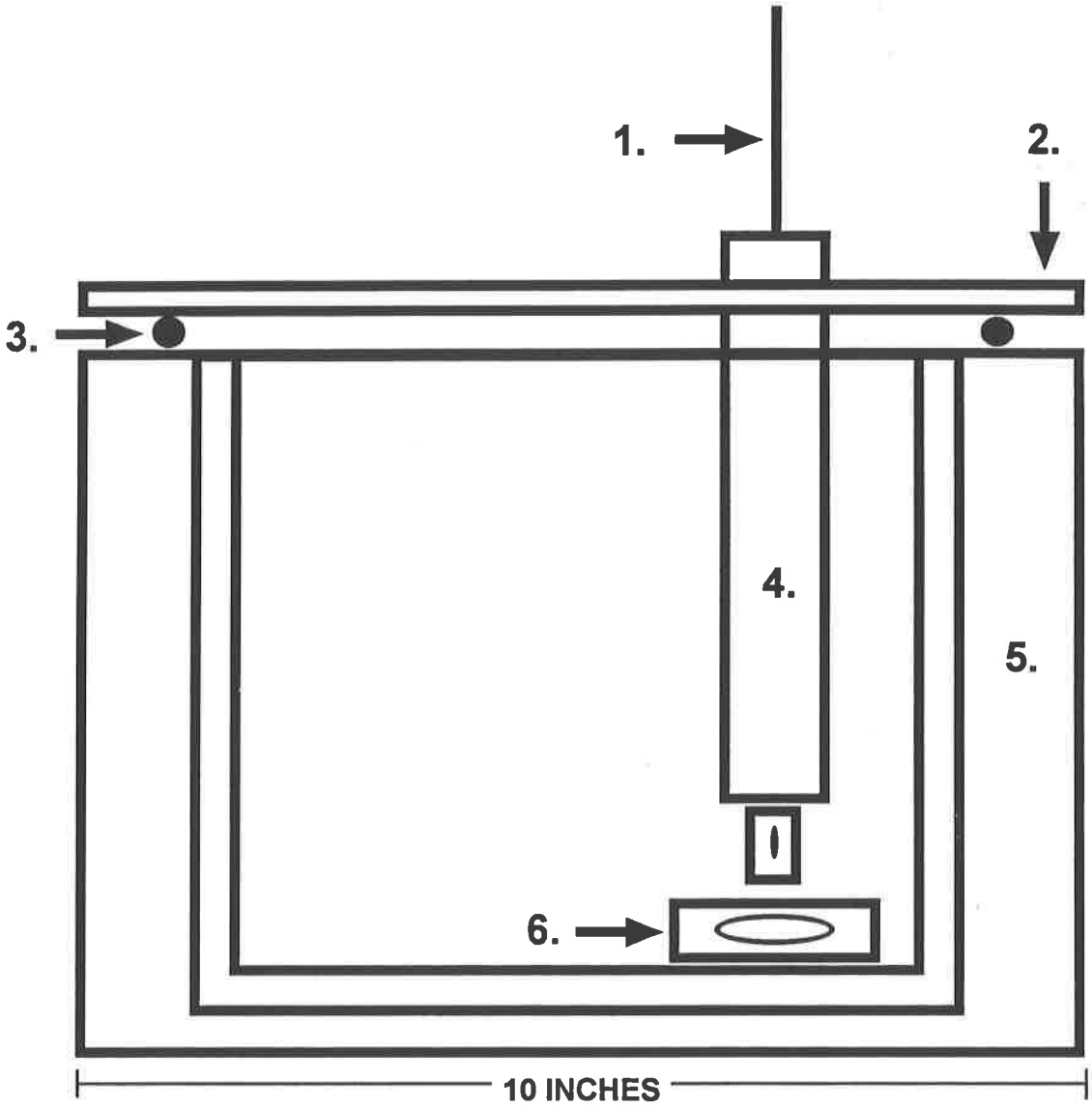


Figure 4. The respirometry chamber. The animal was sealed within the chamber and the consumption of oxygen was monitored by an oxygen meter and the information was communicated to the probes program on the computer. 1. The line to the oxygen meter and computer. 2. The clear acrylic cover. 3. The rubber o-ring used for sealing. 4. The YSI oxygen probe. 5. The water jacket for temperature regulation. 6. The magnetic stir bar.

Animals at the entrance, within the sloping section, or that left the burrow were counted as being in zone 1 for the purpose of measurement within the burrow. The second and third sections included the horizontal extension of the burrow. This horizontal section was divided into two equal parts of 0.33 m and labeled zone 2 and zone 3, respectively. Zone 2 included the midsection of the burrow that extended from the elbow to the middle of the horizontal section of the burrow. Zone 3 was the deepest section of the burrow.

The mantis shrimp's behavior was also analyzed by video in a similar fashion from above the burrow. When analyzing the data from the "above burrow" perspective, the shrimp was counted as being at the entrance if it was visible through the entrance opening or if it was partly out of the burrow but some part of its body was still touching the burrow entrance. If the animal was within the burrow and could not be seen the animal was classified as inside the burrow. If the animal was clearly outside of the entrance and away from the mouth of the burrow it was classified as outside the burrow.

The area above the observation tank was illuminated with incandescent white light from 08:00 to 18:00 hrs giving the animals a 10L:14D cycle. During the dark cycle an infrared lamp was turned on, giving the camera sufficient illumination to continue recording while not disturbing the shrimp. The filming of the behavior above the tank was also conducted in 48 hour blocks.

The tank was completely surrounded by a light-proof tarp. This allowed the "below-burrow" area to remain completely dark while work on the "above-burrow" area was being done, and also allowed physical movement in the laboratory without disturbing the animals. The entire tank was also elevated so

that even when the light-proof chamber was opened and changes were being made to the setup, human activity was not visible to the animals.

The second experimental thrust used a metabolic approach to determine the pattern of changes in *H. ensigera's* aerobic metabolism under hypoxic conditions. This approach utilized respirometry chambers (Figure 5). The specimens were set in the 2000 ml respirometry chambers and their rates of oxygen consumption were measured as the oxygen in the chamber decreased. The chambers had an outer jacket connected to a recirculating water bath to allow constant control of the temperature of the water inside the chamber. The oxygen electrodes used were YSI BOD Oxygen Probes, which are Clark-type electrodes with a gold cathode and a silver anode within a tube filled with half-saturated KCL solution. An oxygen permeable membrane covered the end and maintained the solution. The probe was polarized and placed near a stirrer within the respirometry chamber. Output from the probe, which is proportional to the oxygen level in the chamber water, was monitored first by a YSI Oxygen meter model 57 and then communicated to a computer and recorded using the custom computer program PROBES (Cowles et al., 1991).

This metabolic study had two purposes. The first was to determine the average rate of aerobic metabolism for this species, both for the purposes of comparing it to other species and for determining the rate at which it is likely to deplete oxygen in the burrow. The second purpose was to determine the pattern by which *H. ensigera*'s aerobic metabolism changed with the changes in oxygen level; i.e. whether the species is an oxyconformer or an oxyregulator. Special interest was paid to the species' aerobic metabolism at the low oxygen levels likely to be encountered within the burrow.

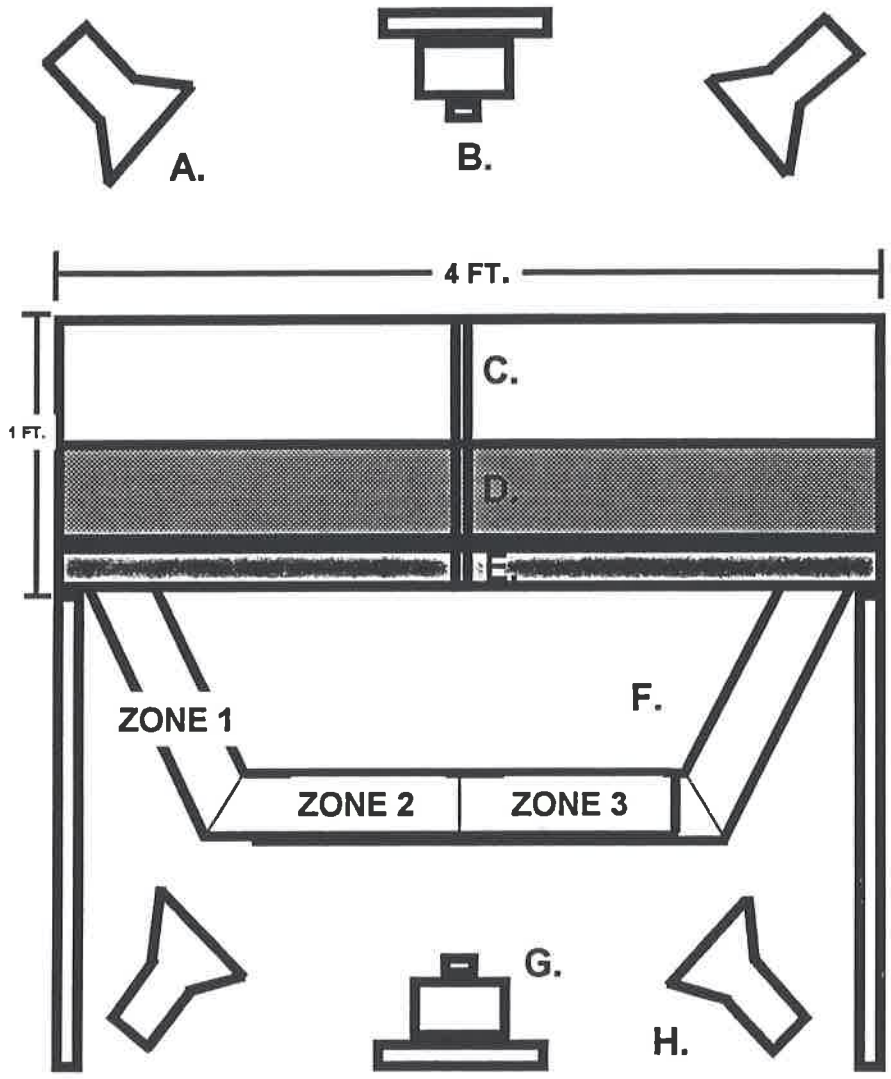


Figure 5. The observation tank with the artificial burrows from which the activity of the Mantis shrimp was observed and recorded. This entire tank was enshrouded with an opaque plastic covering. A. The visible light source. B. The "Above-Burrow" camera. C. The quadrant border. D. The seawater. E. The fine-grain sand. F. The artificial burrows.G. The "Below-Burrow" camera. H. The infrared light source.

The time spent in different regions in and around the burrow at different times of the day were compared using Analysis of Variance and the Scheffe post-test. The extent of oxyregulation or oxyconformity were assessed by linear regression of aerobic metabolism as a function of oxygen pressure at all oxygen pressures greater than 10 mm Hg.

RESULTS

General

Every subject placed within the observation tank entered readily into the artificial burrows and took up residence there. Three of the subjects introduced to the tank actually fled to the burrow openings and into the burrows immediately upon release from the transfer containers. There were two instances when the animal would slowly approach the burrow and quickly retreat, as if wary of an occupant. These were times when another individual had recently been removed from the burrow and there had not been a complete water exchange in the tank.

The subjects in the burrow did not show any obvious response or flee from the burrows when exposed to the infrared lights, which were used for illumination in the dark below the tanks, and at night above the tanks. These observations agree with physiological measurements (Marshall and Cronin, 1991) of the spectral sensitivity of the pigments in the mantis shrimp' eyes. Their eyes are sensitive to light up to 615 nm wavelength, and would not be expected to perceive the 660+ nm wavelengths of infrared.

The subjects foraged little, and in fact spent 98.5% of their time within the burrow or at the entrance with just their eyestalks protruding out of the opening. Forays out of the burrow were mostly brief. In a foray the animal would typically leave the burrow entrance and walk quickly to some specific spot in the tank; then turn, walk directly back to the burrow, and take up station just inside the entrance again.

Activity around the burrow entrance

Three of the animals were videotaped for 48 hour periods from a vantage point above the tank, in order to observe the activity outside of the burrow and around the entrance. Contrary to my initial expectations the subjects did not show a strong crepuscular behavior pattern. All three showed a bimodal activity pattern, but the peaks didn't necessarily fall at dawn (08:00 h) and dusk (18:00 h). Rather, they were most active shortly before first light and in the early afternoon, well before dark (Figures 6,7). Though there was variability among the subjects, all three followed the same general activity pattern. Each spent the majority of time (49.6 minutes or 82.7% of each hour on average, s.e. = 1.4), inside the burrow and out of sight from above. The next most common position was at the burrow entrance, where an average of 9.5 minutes of each hour was spent (s.e. = 1.3). Only a little time was spent foraging outside the burrow (average of 0.6 minutes per hour, s.e. = 0.1). These times were all highly significantly different from one another (ANOVA, $F = 545.345$, $p = 0.0001$, Scheffe post-test).

Time spent inside the burrow

The shrimp spent a large majority of their time, 82.7%, completely inside the burrow (Figure 6). Most of the occasions that they went down into the burrow involved a fairly short stay (median = 35.8 minutes). Around once every

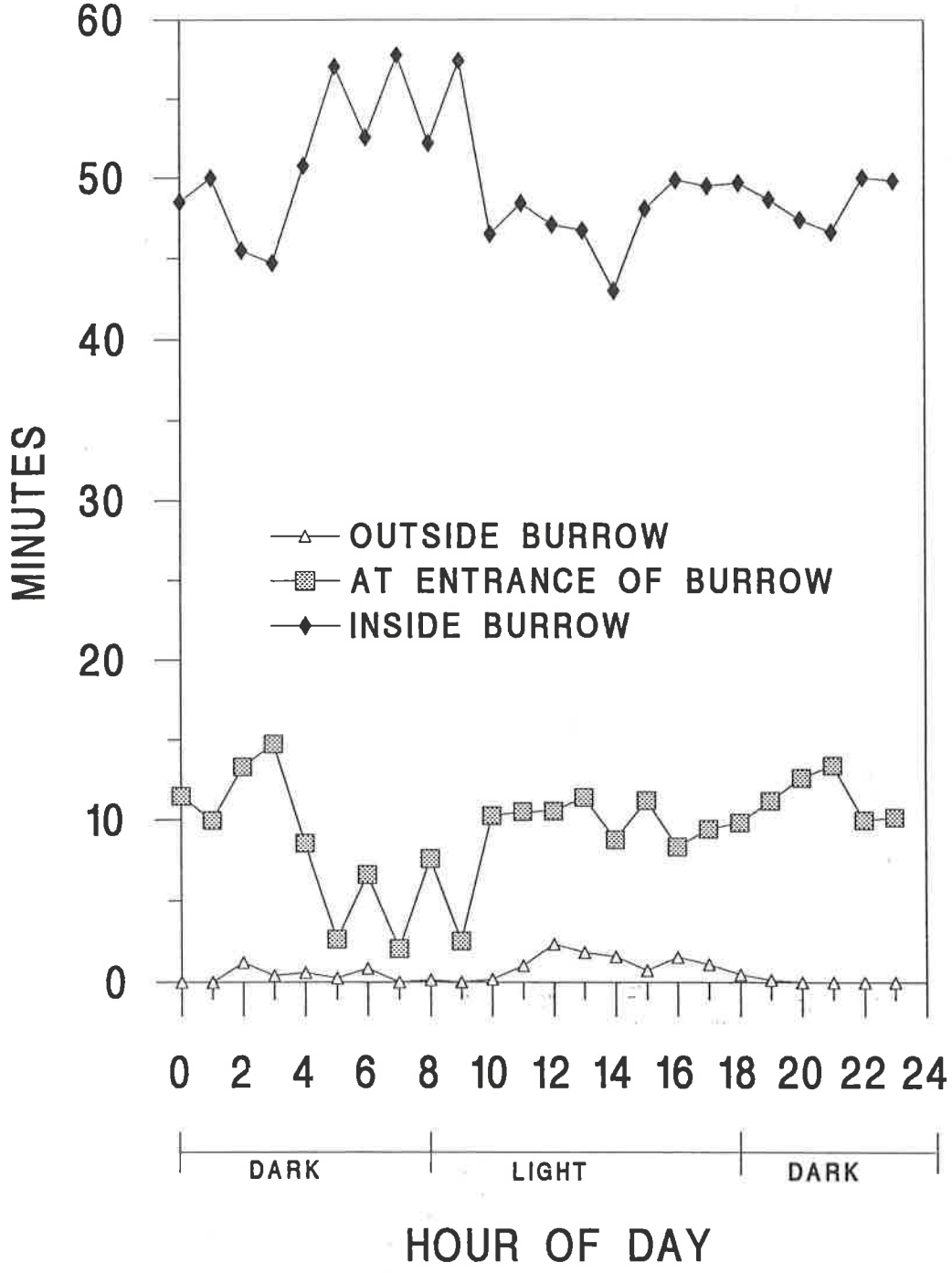
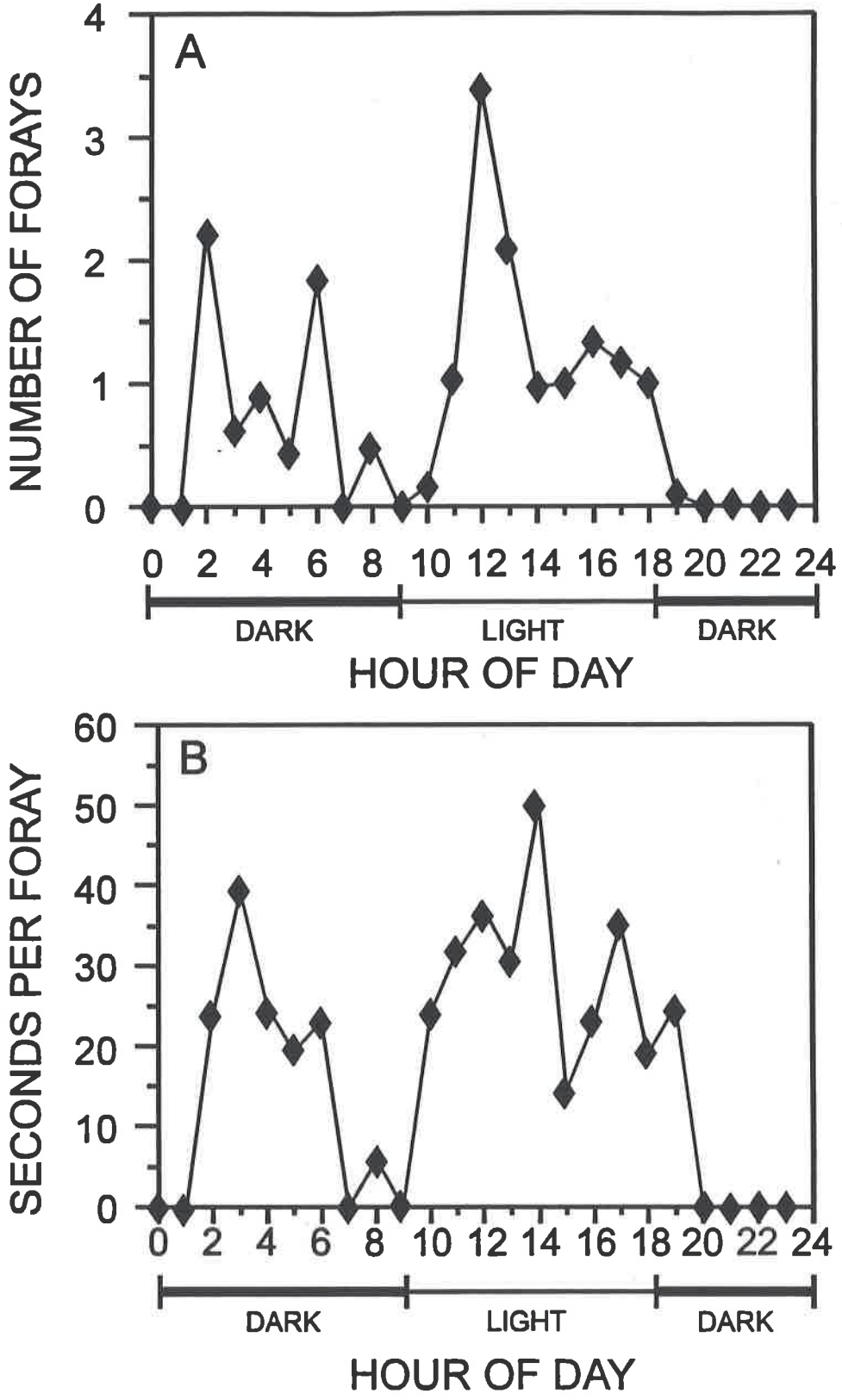


Figure 6. Activity at and near the burrow entrance by all 3 animals.

Figure 7. A. The average number of forays per hour made by all the animals, by hour of the day. B. The average number of seconds a foray lasted, by hour of the day.


A
NUMBER OF FORAYS
4
3
2
1
0
0 2 4 6 8 10 12 14 16 18 20 22 24
DARK
LIGHT
DARK
HOUR OF DAY
B
SECONDS PER FORAY
60
50
40
30
20
10
0
0 2 4 6 8 10 12 14 16 18 20 22 24
DARK
LIGHT
DARK
HOUR OF DAY

24 hours, however, they went down and stayed for a substantial amount of time (range = 7.2 to 14.4 hours).

Time spent at the entrance

The animals spent 15.8% of their time at the mouth of the burrow (Figure 6). For much of the time spent at the entrance the animal was just inside the burrow with only its eyestalks and antennae protruding. They appeared to be either watching for any activity around the entrance, looking for food or just getting more oxygen. The greatest percent of time spent at the entrance was at night, between the hours of 20:00 h and 03:00 h. The subjects spent 25% of their time at the entrance at 03:00 h. The least time spent at the entrance was between 05:00 h and 09:00 h when they tended to remain in the burrow without many forays. Activity at the entrance seemed to be important in and of itself and not just a preparation for a foray. It appeared to be either a vigilant guarding behavior or positioning for better oxygen uptake.

Forays outside the burrow

On average, the animals spent less than 1% of their time outside of the burrow, and most of this time was concentrated in the early morning hours and in mid to late afternoon (Figure 6). Though the subjects' forays were distributed throughout much of the day, the greatest activity outside the burrow was between the hours of 11:00 to 18:00. The hour of greatest activity was at 12:00 during which they spent an average of 140 seconds in the hour foraging outside

the burrow. The subjects did not venture outside the burrow in the late evening (20:00 to midnight), even though they could often be seen at the entrance during this time.

Forays tended to be brief. The average foray length was only 45.6 seconds. In a typical foray the animal left the burrow entrance and walked rapidly to some distal point in the aquarium. The maximum distance available for them to travel in the aquarium was 0.7 m. After a brief examination of whatever was at the location the subject would return directly to the burrow to either take up station at the entrance or remain inside. Other times they traveled briefly around the perimeter of the aquarium and returned to the burrow. Rarely were they outside of the burrow for more than a minute (Figure 7). They foray length did not change through the day (Figure 8). This is the primary reason for the very small proportion of time that has been recorded for the animals being outside of the burrow. The fact that most of the forays were in the hours leading up to dawn and in the afternoon means that, at least in this experiment, their activity could better be called crepuscular-diurnal than purely crepuscular.

Observations made within the burrow

The analysis of the behavior within the burrow itself involved eight runs of 48 hour length using three animals. All subjects spent considerable time in all zones throughout all the days. The only significant difference in the times spent in the different zones is that significantly more time was spent in zone 2 than in zone 1 (ANOVA with Scheffe post-test, $F = 7.3$, $p = 0.0008$). The average time

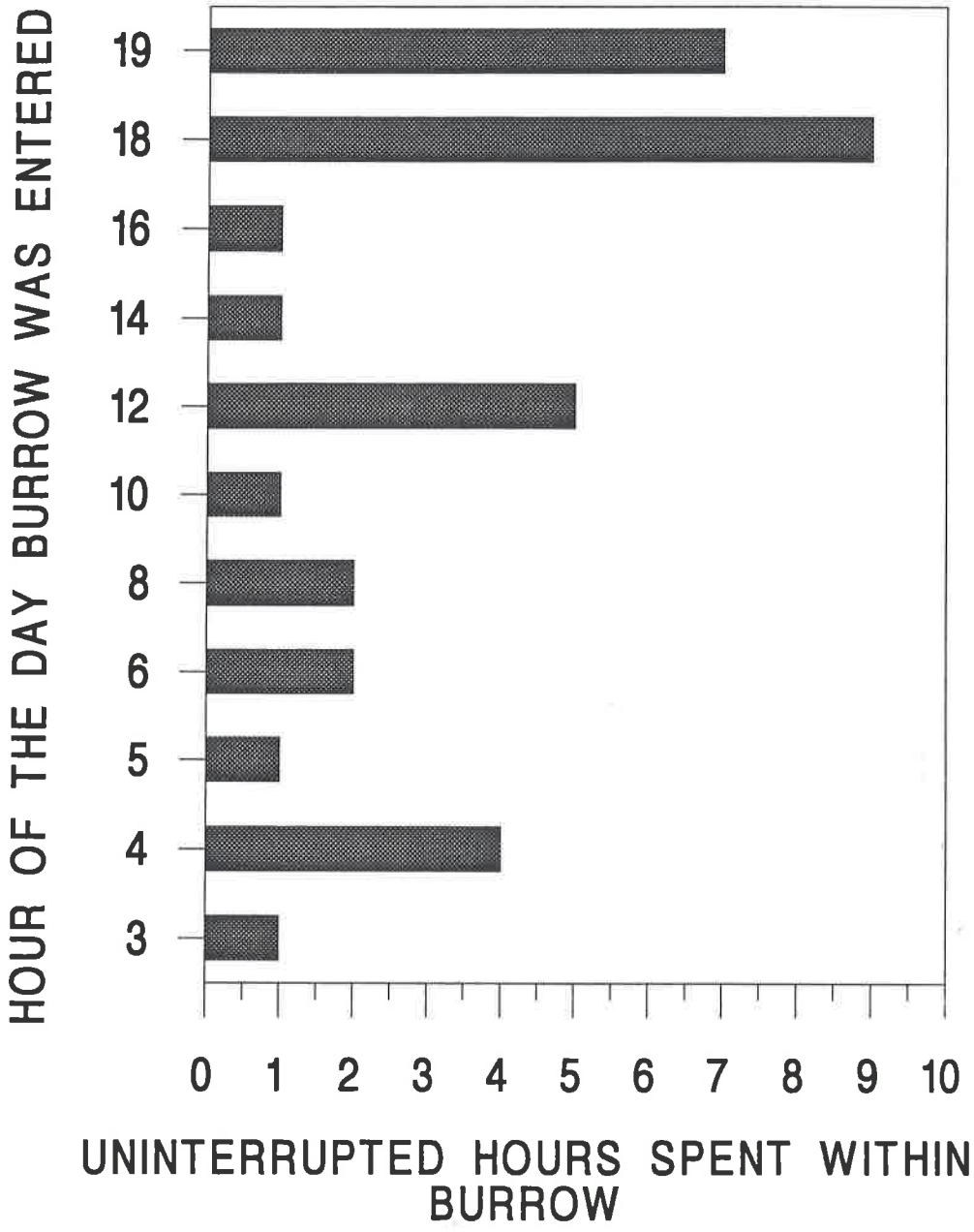


Figure 8. The mean length of uninterrupted time the subject spent within the burrow beginning at the indicated hour as a function of the day they entered the burrow.

spent in zone 1 was 16.3 minutes per hour, the average time spent in zone 2 was 23.5 minutes per hour and the average time spent in zone 3 was 19.9 minutes per hour (Figure 9). The animals did not seem to have a particular time of day in which they would predictably rest or remain inactive. Instead they made transitions from one zone to another throughout the day and within each hour. There were more transitions between zones in the evening than in the morning and at midday (Figure 10). The average number of transitions occurring per hour was 7.69. There were significantly more transitions between zone 1 and 2 than between zone 2 and 3 (ANOVA $F = 7.7$, $p = 0.0001$).

Among the experimental subjects there were two sharply different groups in terms of activity levels, based on the number of transitions between the different zones. One specimen, which was filmed in four 48-hour runs, was very inactive and made very few transitions (around 2/hour), while the other two animals seemed to traverse from zone to zone much more actively (more than 5/hour). Due to this dissimilarity in activity level the subjects were divided into two groups, active and inactive. There was a highly significant difference between the active and inactive subjects when comparing the time spent at the different zones (ANOVA $F = 224.5$, $p < 0.0001$).

There was a highly significant difference in the amount of time the active animals spent in the different zones (Figure 11, ANOVA $F = 60.5$, $p = 0.0001$). The active animals tended to remain about the entrance of the burrow in zone 1 during much of the day, averaging 29.9 minutes per hour. Here the animals would either stand looking out the burrow entrance, or would travel back and forth between zones 1 and 2. The subjects spent 20.4 minutes per hour in zone 2 and the least amount of time, averaging only 8.5 minutes per hour, in zone 3 at

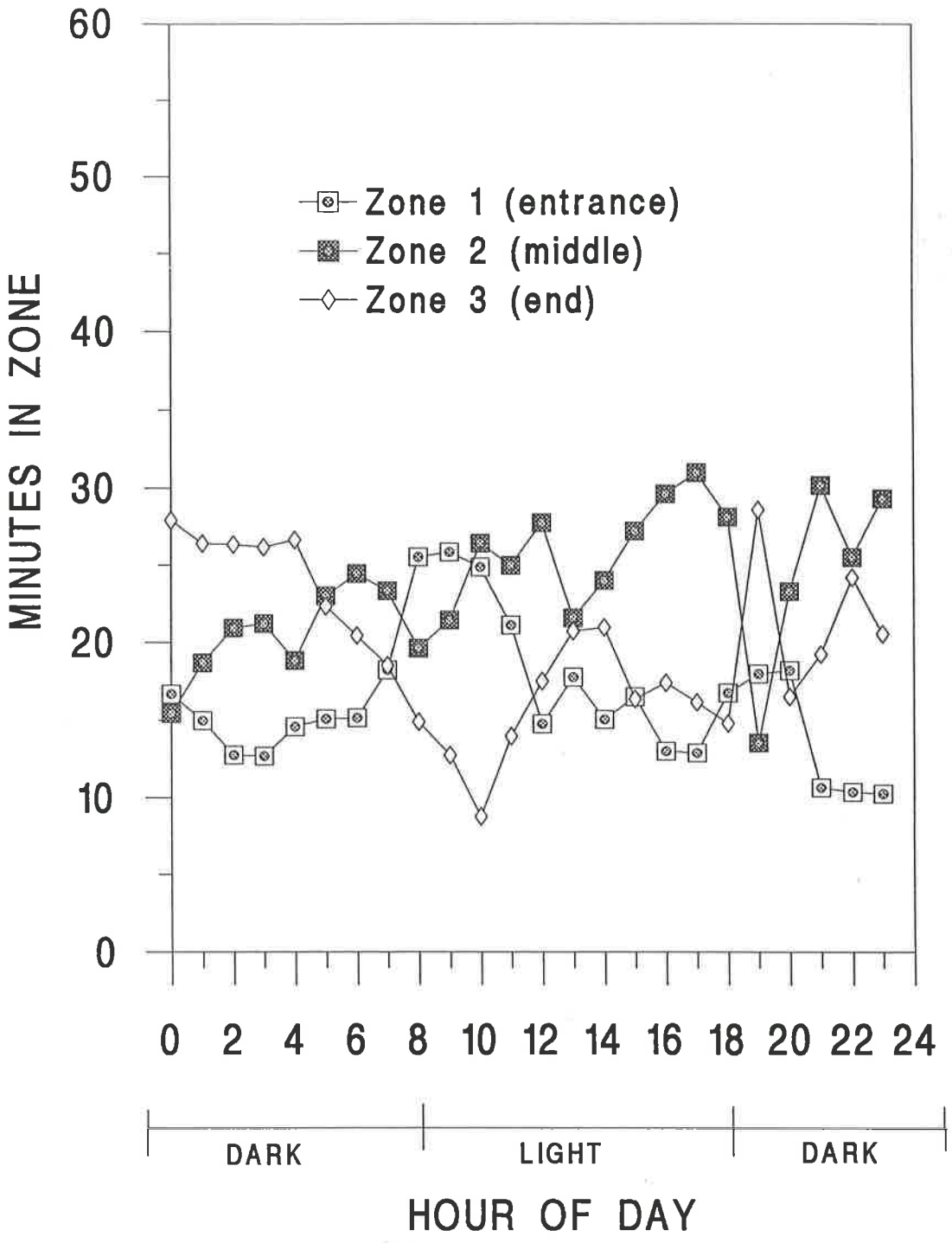


Figure 9. The average time spent in each zone of the burrow by all animals, by hour of the day.

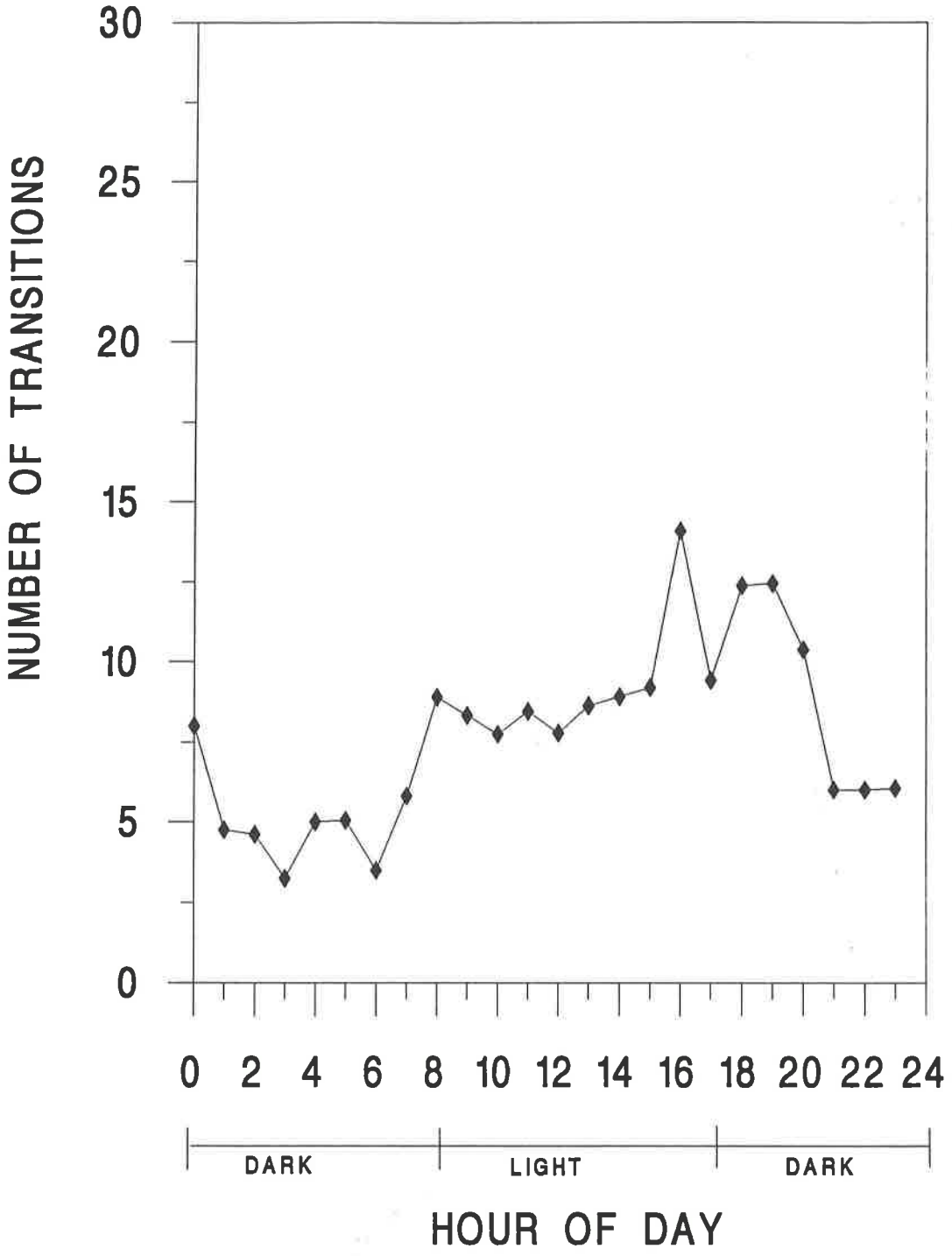


Figure 10. Average number of transitions between burrow regions by all 3 animals.

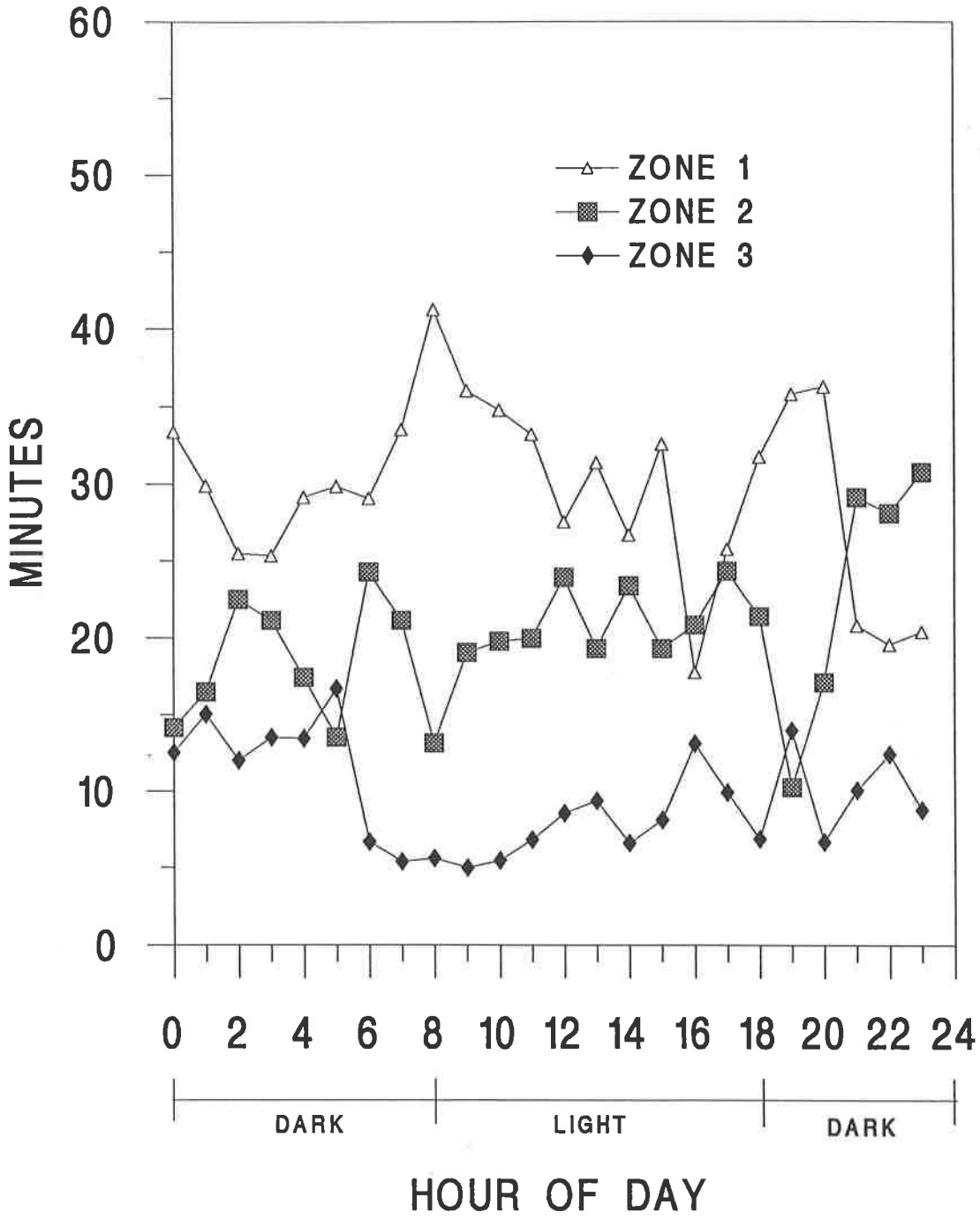


Figure 11. The average time spent in each zone of the burrow by the 2 active animals, by hour of the day.

the end of the burrow (Figure 11). The active subjects made significantly more transitions between the first two zones (zone 1-2, 9.0 per hour) than deeper in the burrow (zones 2-3, 4.4 per hour)(ANOVA F = 34.9, p = 0.0001, Figure 12). The greatest amount of movement within the burrow occurred between the hours of 17:00 and 20:00 in the evening. The animals were therefore quite active within the burrow during this evening time period even though they rarely left the burrow, and would have appeared, from a surface examination, to have been resting during this time.

The times spent in the different zones by the inactive animal were also significantly different from one another (ANOVA F = 55.0, p = 0.0001). The inactive animal spent 95.5% of its time away from the entrance and zone 1 (Figure 13). The time spent at zone 1 (4.4 minutes per hour) was much less than the time spent at either of the other zones, and a highly significant difference. The animal spent significantly more time, a total of 55.6 minutes per hour, in zones 2 and 3. The total number of transitions made by the inactive animal was very small, averaging only 1.98 per hour. Most of the activity was centered deep within the burrow, in zones 2 and 3 (Figure 13). There was a highly significant difference among the transitions made between zones 2 and 3 (1.52 per hour) and those made between zones 1 and 2 (only 0.5 per hour)(ANOVA F = 26.7, p = 0.0001)(Figure 14). The majority of the transitions occurred in the late morning and evening.

There was no obvious pumping activity by any of the subjects that would indicate that the animals were pumping oxygenated water into the burrows. All the subjects nearly always oscillated their platelike pleopods regularly (when they were not walking), but appeared to make no attempt to position themselves

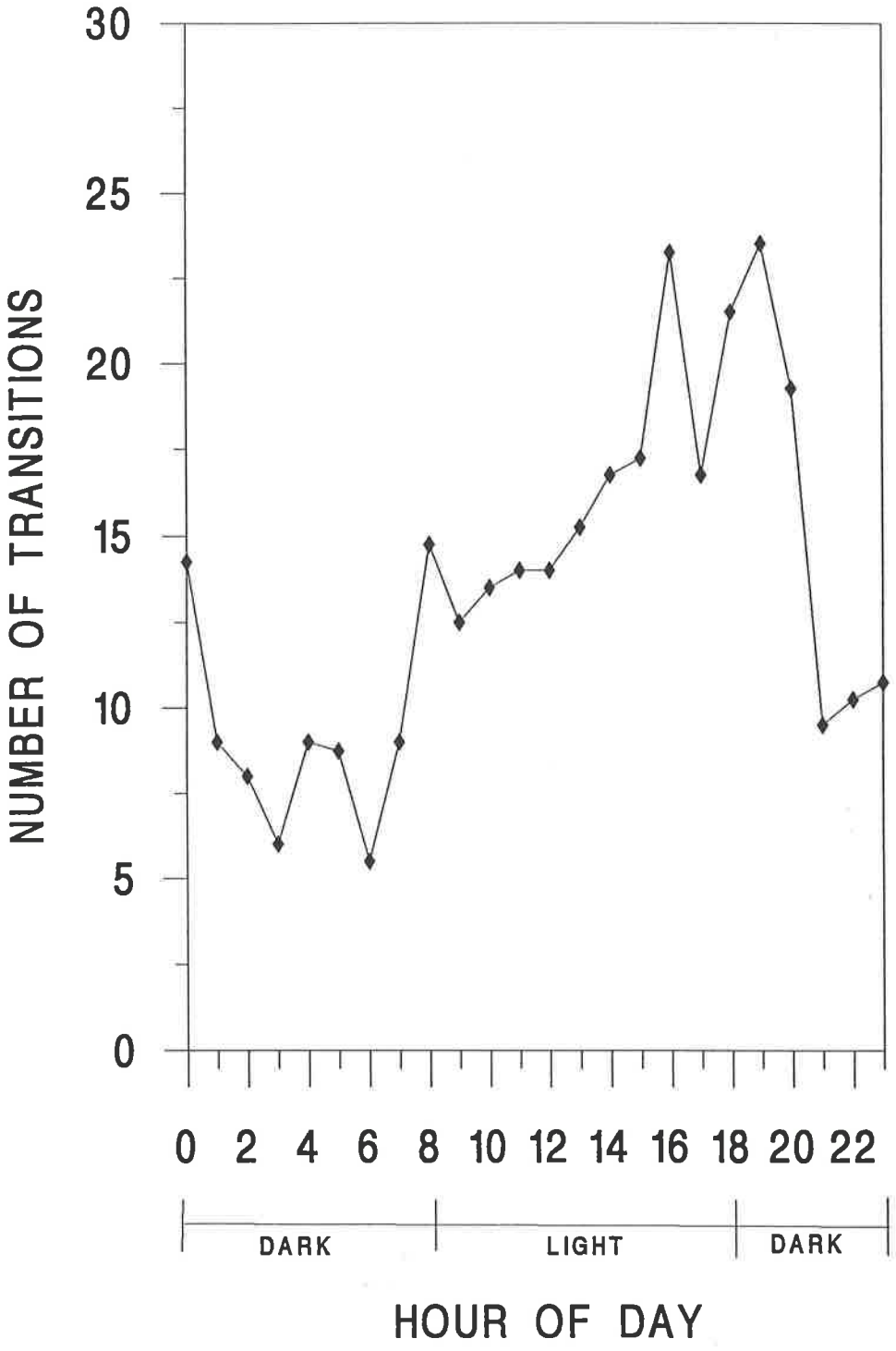


Figure 12. The average number of transitions between burrow regions by the 2 active animals, by hour of the day.

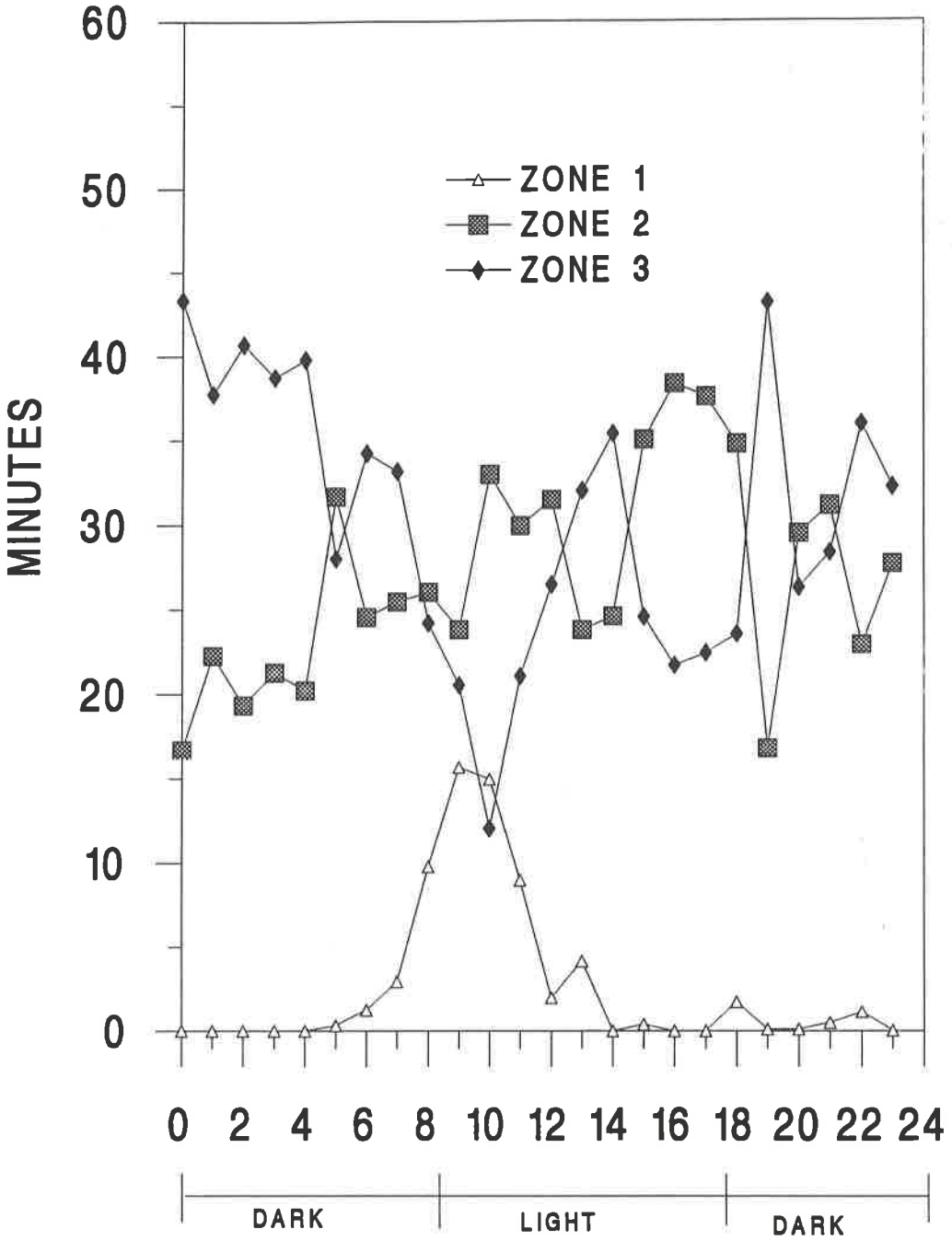


Figure 13. The average time spent in each zone of the burrow by the inactive animal, by hour of the day.

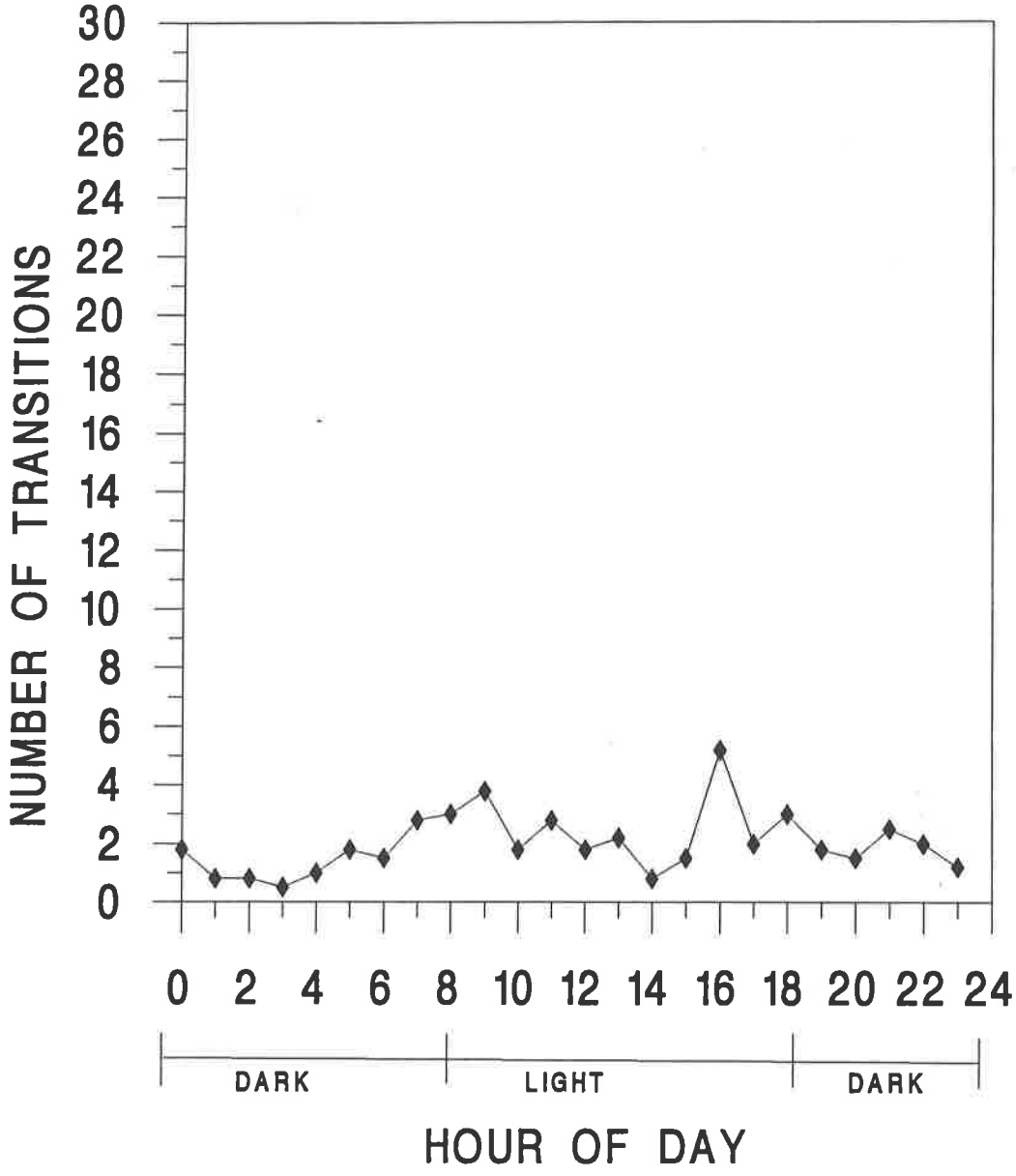


Figure 14. The average number of transitions between burrow regions by the inactive animal, by hour of the day. The scale is the same as Figure 13 for comparison.

at the entrance or to assume a position that would make this activity effective at oxygenating the burrow.

Capping

The animals capped the burrows frequently, as was also noted in the field. The caps the animals created in the laboratory, however, were generally not complete. They would often create a half-cap, or a cap that was almost but not quite complete. Even the most complete caps generally still had a small hole. The caps were also not as thick as has been described for caps in the field (Basch and Engle, 1989). When the cap was only half-way complete the animal tended to remain at the entrance peering out of the incomplete hole. When the caps were nearly complete, on the other hand, the subject would extend its antennae through the small hole while remaining at the entrance, or alternatively would frequently be found at the end of the burrow in zone 3. There was no obvious ventilating activity of the burrow when there were caps present.

Physically, a capping of the one ended burrows such as they inhabit would strongly inhibit water exchange. There would consequently be an almost complete restriction on the aeration of the burrow. In a blind burrow, and in the absence of obvious pumping activity, we can safely assume that any time the subject is in zones 2 or 3 it is so far from the entrance that it must obtain its oxygen almost entirely from the water inside the burrow since bulk flow would have little role and the distance to the entrance is far too long for diffusion to have any significant effect. All subjects spent at least one long, uninterrupted session in zones 2 or 3 of the burrow without approaching the entrance for

oxygen; ranging from 50 minutes for the most active animal up to 18.5 hours for the most inactive animal. In the absence of oxygenation of the burrow they would have experienced moderate to extreme oxygen limitation during this time.

Aerobic metabolism

The aerobic metabolic studies utilized 11 different runs using 6 animals weighing from 25 to 135 grams. Although there was variability among the aerobic metabolism of individual animals, all the subjects appeared to be oxyconformers (Figure 15). This conclusion was verified by a linear regression of the aerobic metabolism (MO_2, μ mol O_2 g^{-1} h^{-1}) as a function of O_2 pressure (MO_2, mm Hg O_2):

$$MO_2 = 0.027^* pO_2 + 0.385 \text{ +/- } 0.008,\ R^2 = 0.8$$

Since the slope of this regression was highly significantly different from zero ($p < 0.0001$), meaning that oxygen consumption declines significantly with decreasing oxygen levels, my conclusion that these animals are oxyconformers was strongly supported.

This species also has considerable potential for remaining alive in almost completely anaerobic conditions. A number of the animals were left in the chamber till virtually all the O_2 was depleted. Their rates of aerobic metabolism dropped to a level indistinguishable from zero, yet these animals survived up to 16.9 hours at well below 1 mm Hg of O_2 under these conditions and none of them died. At the conclusion of the anaerobic periods these animals readily

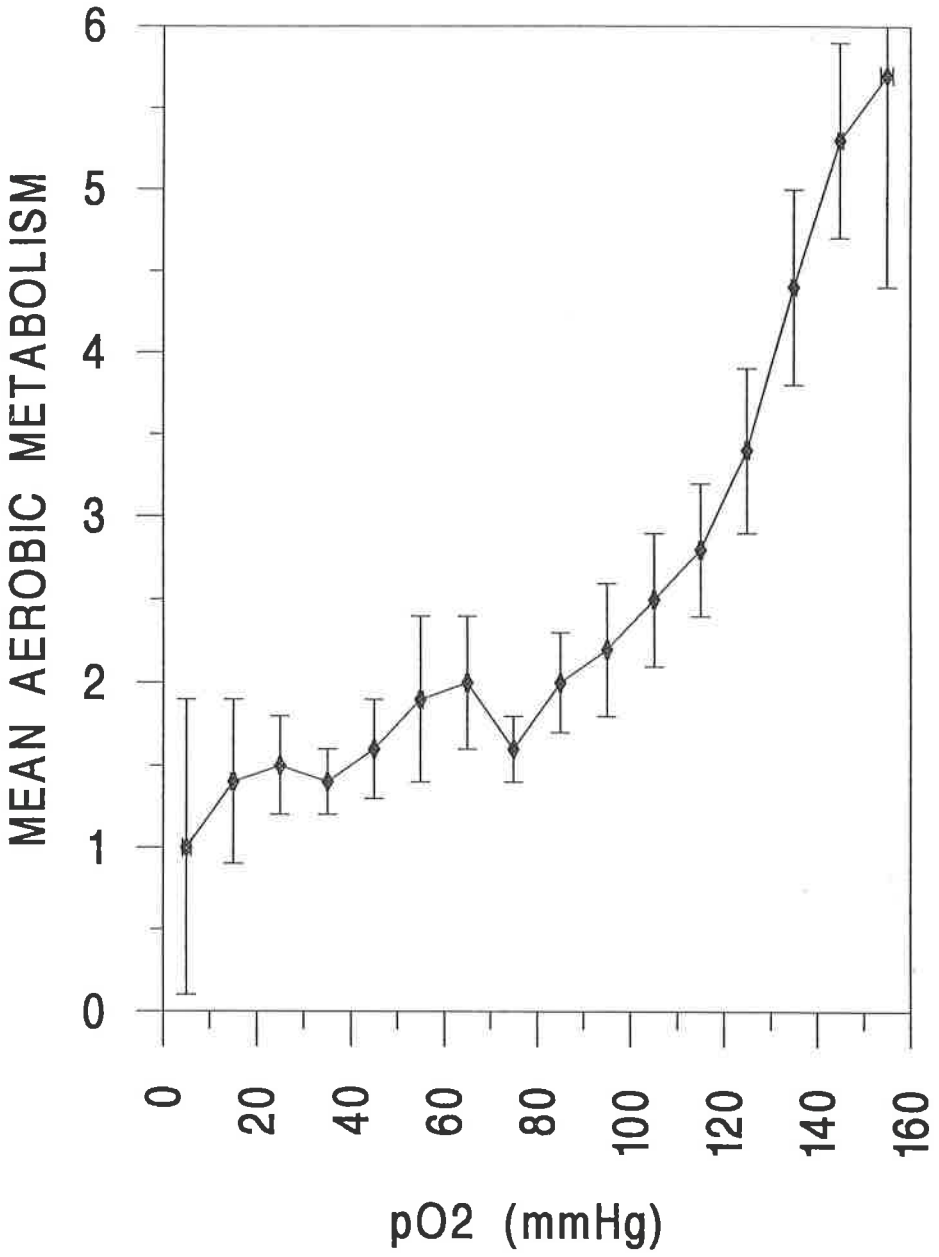


Figure 15. Rate of aerobic metabolism of all 9 *Hemisquilla ensigera californiensis* as a function of ambient oxygen pressure. Error bars = standard error. The slope of the relationship is highly significantly different from zero.

became highly active, striking and jumping at the capture net when the chambers were opened. The animals were often fed and observed carefully for a period after the anaerobic tests. Their activity level and behavior after experiencing anaerobiosis appeared to be veery similar to that before. They exhibited no clear pattern of the inactivity which is often associated with repayment of oxygen debt..

DISCUSSION

Observations of the natural burrows and the environment of *H. ensigera* in the sea were made in December 1992, while assisting Jack Engle from the Tatman Foundation in his population research off Catalina Island, California. The behavior of the shrimp around the burrows in the holding tank at the Loma Linda laboratory was similar in many respects to that observed around their natural habitat. In their natural environment mantis shrimp often remained at the entrance of their burrow during the morning and evening periods. Otherwise there was little indication of movement or even the location of the burrows as the shrimp would cap off the entrances to the burrows thereby concealing their location. During the mornings and evenings some shrimp could be found foraging away from their burrows. When the shrimp became aware of the observers they would immediately return to their burrow and dive in. When the burrow was not capped the shrimp were commonly at the entrance with only their head and thorax region visible. Presumably they were watching for food or for other mantis shrimp which could threaten to take over their burrow. The mantis shrimp colony was situated on a plain, soft sediment, in a gently sloping environment just off the shore in about ten meters of water with little wave action.

In this study a number of contemporary ideas of how a burrow dwelling species generally functions were challenged. Behaviorally, this species did not show the strong crepuscular activity pattern that was expected from the previous research (Basch and Engle, 1987). It is not known, however, whether this species is more active during the day than previously thought or whether the behavior was an artifact of the artificial environment simulated in the laboratory.

The species' lack of any obvious attempt to aerate the burrow was also a surprise. Active animals such as this species have relatively high metabolic rates. The closed-ended burrow that the shrimp lives in is composed of moderately fine-grained sand which would not allow diffusion of sufficient oxygen to support the animal's high activity. Capping the burrow would only compound the problem. With these factors I expected a high degree of pumping to aerate the burrow to support the animal's oxygen demands. None, however, was observed. This is in contrast to what has been observed for other burrow-dwelling shrimp species. *Callianassa californiensis*, for example, clearly pumps oxygenated water into the burrow, even though to a limited degree (Childress, Torres and Gluck, 1977). *Upogebia pugettensis* is another burrow dwelling crustacean that aerates the burrow by pumping. Both of these species are often found near the burrow entrance under low oxygen conditions. Both species use their pleopods, which are paddle-like plates, as the mechanical device to pump water into the burrow. *H. ensigera* also has platelike pleopods which could potentially be very effective for burrow aeration, but did not seem to use them in this function.

H. ensigera's metabolic pattern was a surprise as well. Most species which experience oxygen limitation tend to be strong oxyregulators, and maintain their aerobic metabolism down to quite low oxygen pressures (Gade, 1983). The other burrow dwelling crustacean species studied, *Upogebia* and *Callianassa*, match this pattern (Thompson and Pritchard, 1969). This is to be expected since oxygen limitation and the consequent shift to anaerobic metabolism sharply limit metabolic efficiency and the scope of activity available to the organism. Indeed, it has been suggested that no metazoans are even

facultatively anaerobic (Hammen, 1976), although some can tolerate anoxia for a limited time period. *H. ensigera's* known status as a predator which roves around the bottom near the burrow and can make rapid attacks had led me to expect that the species would have well-developed oxyregulatory abilities to support its active metabolism. The very clear oxyconformity of the species was therefore quite unexpected.

In sharp contrast to its poor oxyregulatory abilities was its substantial capacity for survival under anaerobic, or at least extremely hypoxic, conditions. Crustaceans in general do not usually have well-developed anaerobic capabilities (Zebe, 1991). Anaerobic metabolism is not only notoriously inefficient, but it also produces by-products which are toxic to cellular metabolism (Prosser, 1991). Some other invertebrate species, such as mollusks, frequently utilize alternate metabolic pathways which increase the efficiency of anaerobic metabolism while decreasing the amount and toxicity of by-products produced (De Zwaan and Putzer, 1985). Crustaceans, however, do not appear to use these pathways, and oxygen limitation generally leads to their death within a few hours or less (Hammen, 1976). The *H. ensigera* in this study, while not exhibiting the longest period of survival known for a crustacean, did survive periods of many hours with virtually no oxygen with no apparent ill effects. It should also be noted that none of these animals had reached their limits to anaerobiosis in this study. Their actual capacity to withstand anaerobic conditions may well considerably exceed the maximum times recorded here. The species' anaerobic capacities must therefore be unusually large for a crustacean. This discovery calls for further investigation of *H. ensigera's* full capacities for anaerobic survival and into what metabolic adaptions it is using to

accomplish this, such as increased glycolytic capacity, buffering its hemolymph, or whether alternate pathways are possibly being used.

Metazoan animals appear to encounter hypoxia under two different conditions, and to deal with them differently. The first condition, often called environmental hypoxia, occurs when the organism remains for extended periods of time in environments low in oxygen. One of the most common examples of this is that of the aquatic sediment dwellers (Gade, 1983). The bacterial breakdown of organic material within the sediment rapidly depletes it of oxygen. The fine sediment structure at the same time limits the diffusion of oxygen into the sediments, resulting in anaerobic conditions just millimeters below the surface. Metazoans living within the sediments must deal with this anoxia, either by ventilating their burrows with oxygenated water or by tolerating the lack of oxygen. Among those species which tolerate environmental hypoxia, several characteristic metabolic and behavioral adaptions are seen. One of the most characteristic is that the species tend to remain quite inactive during the period of hypoxia. For example, the animal may nearly cease feeding, moving about, and pumping water over its gills (Gade, 1983). Another characteristic alternative is to switch to alternative metabolic pathways, as occurs with mollusks. Neither of these responses seem to occur with *H. ensigera*, however. Crustaceans up to this time have not been found to use alternate metabolic pathways such as glycolysis to any appreciable extent, so it is unlikely that *H. ensigera* does this. The animals did not remain quiescent, but continued to move about within their burrows for many hours at times with no attempts at ventilating the burrow or trips to the entrance for oxygen.

The second situation in which an animal experiences anaerobiosis, called "functional anaerobiosis" (Zebe et al., 1981), occurs when an animal is performing a burst of activity at such a high rate that the oxygen supply is depleted within the muscle tissues and they become temporarily anaerobic. Such activity generally only occurs for a brief period of time, and is followed by an extended period of increased aerobic metabolism to pay off the "oxygen debt".

H. ensigera's anaerobic metabolism does not seem to fit well into either of these two classical categories. The extended periods of time for which the water in the burrow would be anaerobic would seem to place it within the "environmental anaerobiosis" group. Yet the animals did not become quiescent, as it is classically seen in this situation. On the other hand, the species' activity was not so high as to be classified as "burst" activity, and the duration was far too long to fit into this category. *H. ensigera* maintains a moderate routine activity level for long periods of time under presumably anaerobic conditions. I know that even if the burrow was not entirely anaerobic, but only hypoxic, that the species must be utilizing substantial anaerobic metabolism because it is such a poor oxyregulator and cannot sustain significant levels of aerobic metabolism under these conditions.

In summary, *H. ensigera's* approach to dealing with the reduced oxygen tensions within its burrow is surprising in several ways. Though, being a crustacean, it presumably does not have well-developed anaerobic capacities, it builds a blind-ended burrow and makes no apparent attempt to ventilate it, a situation sure to result in hypoxia in the burrow within a short time. Yet, unlike other similar burrow-dwelling crustaceans, *H. ensigera* has very poorly

developed oxyregulatory capabilities and cannot extract appreciable oxygen from the water under hypoxic conditions. Further, the animal, from time to time, spends extended periods of time deep within the burrow with no attempt to come to the entrance to breathe oxygenated water. During this time of relative anoxia within the burrow the animal maintains a moderate level of activity instead of the quiescence normally expected under hypoxic conditions. A better understanding of these unexpected phenomena will require a closer look at the conditions within the burrow and a detailed analysis of this species' mode of anaerobic metabolism.

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