THE LONG TERM CURRENT METER RETRIEVAL EFFORT

February 6 - 7, 1975

Scientist, S. C. Daubin, Jr. Lamont-Doherty Geological Laboratory

In April, 1974, four current meter arrays were launched from the R/V MOANA WAVE in 2750 fathoms of water at 8°N and 151°W. This was done as part of a baseline study of the physical, chemical, geological, and biological properties of the water column and ocean bottom in a potential manganese nodule mining area of the Pacific.[1] Each array had an AMF-Sea Link acoustic release which could reply to transponder interrogations from the surface and would release after receiving a coded command. Some of the releases had an enable/disable option which allowed them to remain at low power levels when not in use. Figure 1 shows array No. 4, which is typical of the group.

Two of the arrays were recovered successfully at the end of the cruise in May, 1974. However, the other two remained on the bottom; array No. 2 did not release as commanded and array No. 4 was purposely left down until a retrieval effort could be made later. The release unit for array No. 2 was an AMF-Sea Link, model 324, serial #553. It had a mechanical release, which would cause a double ping reply to transponder interrogations after it had successfully released. The release unit for array No. 4 was a model 322, serial #442. It had a squib fired release which, upon release, would transmit one ping per second for one minute. Furthermore, this release had the enable/disable option. Any time the enable command was sent and understood, this release would transmit one ping per second for one minute.

The shipboard Acoustic Command System used in the second retrieval effort was an AMF model 200[2]. It consisted of a coder, power amplifier, transducer, and receiver. The coder allows the operator to select one of three commands (enable, disable, or release) or to transmit an interrogation ping at either 9, 10 or 11 KHZ. The operator may also select which release unit the commands are being sent to by using one of 10 different channels on the receiver selector of the coder. The amplifier amplifies the signal from the coder and transmits it through the transducer to the release unit. The transducer also acts as a receiver and transmits the return signals to the receiver of the Acoustic Command System, making them audible to the operator.

In early 1975, the R/V ALPHA HELIX, a 133 foot, 294 ton Scripps vessel, was chartered for the second retrieval effort. The deviation of the ALPHA HELIX from its track from San Diego to Honolulu to the current meter site would take no longer than 3 or 4 days. There were no other scientific programs scheduled for this leg other than the current meter retrieval. The author was to be the current meter retrieval technician for this cruise.

The receiver portion of the Acoustic Command System was installed at AMF in Alexandria, Virginia shortly before it arrived in San Diego. At this time, the system operation was checked out by engineers at AMF-Sea Link (Ken Pollock, Peter Moon, and Don Davis), who seemed to be satisfied with its performance. The system was not air tested against a release at this time. However, it had worked successfully on both previous occasions it was used by William McDonald in his Ocean Bottom Tide Gauge work off the coast of Nova Scotia in 2450 fathoms of water.

The ALPHA HELIX left San Diego at 1400Z on January 27, 1975. The navigation was a combination of celestial fixes, Loran fixes, and dead reckoning. In spite of generally overcast weather during the cruise, a sufficient number of star fixes, sun lines, and moon lines were obtained. These compared favorably with the Loran fixes and the accuracy of the ship's position was generally plus or minus 2 miles.

At 1149Z on February 6, the ship hove to at a position approximately 3 nautical miles NE of array No. 4 (Figure 2, position A). The 12 KHZ Giff Depth Recorder (GDR) showed a depth of 2709 corrected fathoms. Fortunately the weather had cleared considerably from that of the previous days. The wind was from 060° true at approximately 6 knots. There was a 3 foot swell from 080° true, the current had a set to the southwest at approximately 1 knot, and the skies had only scattered clouds. The conditions were as close to ideal as possible for the anticipated retrieval.

A faint reply was heard from array No. 2 when it was interrogated from position A at the maximum output level of the Acoustic Command System. The enable command was sent to array No. 4 and, although the minute of timed pings was not positively heard, further interrogations revealed that it had turned itself on.

The ship was then maneuvered to position B on figure 2 and interrogations were continued. The enable command sent to array No. 4 resulted in a minute of clearly heard timed pings. This indicated that the acoustic coupling was good. At 1330Z, a release command was sent to array No. 4 for 30 seconds. However,

no confirmation in the form of a minute of timed pings was heard. This was repeated 4 more times over the next 30 minutes with the same results. Furthermore, continued interrogations seemed to indicate that the arrays were stationary.

Over the next two hours the ship was maneuvered in an attempt to get closer to array No. 4. At 1555Z a star fix was obtained showing the ship to be at position D in Figure 2. Another release command was sent to array No. 4 at this time, but with no confirmation from the release that the command was carried out. However, the enable and disable commands were understood and confirmed by the release unit.

At 1706Z, with the ship very close to array No. 4 at position E on figure 2, the release command was again transmitted to array No. 4, and again with the same results. The interrogations revealed that the arrays were stationary. Several more release commands were sent during the next hour and a half at various frequencies and output levels, but to no avail.

During this time the ship had drifted with the current to position F on figure 2 and was subsequently repositioned at G at 1855Z. A watch was constantly kept throughout this retrieval effort for the current meter arrays, should they unexpectedly appear at the surface. Conditions remained excellent for a possible visual sighting.

Initially at position G, the interrogation replies arrived almost simultaneously. The release command sent to array No. 4 had the same lack of effect as before and it was decided to attempt to release array No. 2, the one which could not be retrieved in May, 1974. This also proved unsuccessful using the

standard release procedures. Next, various combinations of output levels, coder frequencies, receiver selectors, amplifier frequencies, receiver sensitivity settings and audio input levels were tried in an effort to trigger the release of the arrays. The duration of the transmitted signals was generally longer than 30 seconds. The depth of the transducer was varied from its full depth of 200 feet to keel depth. Interrogations were repeated at frequent intervals to determine whether the arrays were released or not. However, at no time during this effort were the confirming signals from the array releases heard, nor did their positions change relative to each other.

The last release command, another all out effort, was sent at 2340Z. For the next 15 minutes the arrays were interrogated to detect any possible movement relative to each other or to the ship. However, the only movement revealed was the steady drift of the ALPHA HELIX toward the southwest. At 2355Z, the disable command was sent to array No. 4. Subsequent interrogations resulted in replies only from array No. 2, indicating that array No. 4 had turned itself off. At 2400Z, the retrieval effort was reluctantly discontinued and the ALPHA HELIX got underway for Honolulu.

A possible explanation for the lack of success would be the failure of the release function of the release unit. Within the release unit, the interrogation and enable/disable functions have a separate battery and electronics from those of the release function. If a failure occurred in the release function and not in the interrogation and enable/disable functions, then the release unit would not release but would give all the appropriate

responses to interrogations and enable/disable commands, as it did in this retrieval effort.

Some other possible causes can be ruled out. Good acoustic coupling between the release unit and the shipboard transponder, as indicated by the responses of the release unit to interrogations and the enable/disable commands, denies the possibility that the release command was not heard. A failure of the shipboard Acoustic Command System is unlikely, since it functioned properly in the interrogation and enable/disable modes during this effort, and the release mode worked properly on both previous occasions it was used by William McDonald. A lack of buoyancy would prevent the meters from returning to the surface, but would not prevent the release units from emitting the appropriate responses when and after they were commanded to release.

Furthermore, it should be pointed out that each release unit was air tested on the deck of the MOANA WAVE, just prior to launch by Bill Henning, an AMF field engineer. At this time they worked properly. Very likely then, the release battery and not the electronics are the culprits in this unsuccessful effort, having lost their power during the first month of their stay on the bottom.

ACKNOWLEDGEMENTS

The author acknowledges the helpful assistance of Dr. Walter Garey, Walter Schneider and Bill Eaton all of Scripps Institute of Oceanography. Captain Wayne Bristol and the mates and crew of the ALPHA HELIX were of great help in this effort. This project is supported under U.S. Department of Commerce, NOAA contracts 03-3-022-144, 03-5-022-27.

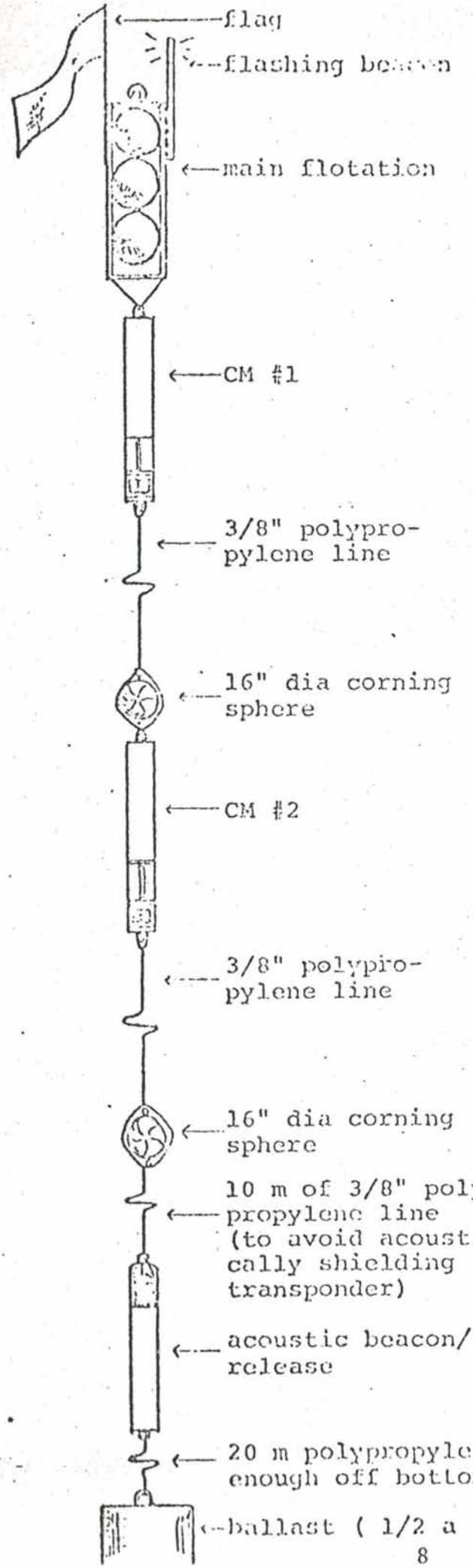
REFERENCES

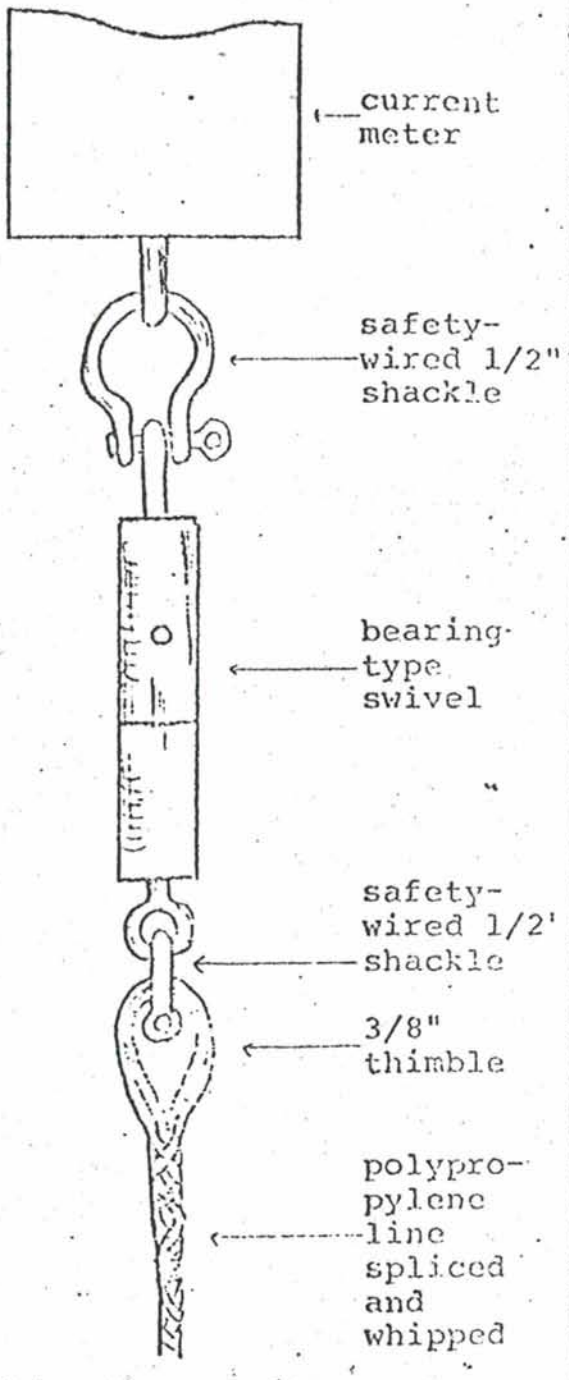
1. "Preliminary Cruise Report, Ships and Cruise R/V MOANA WAVE, MW-72-2", O.A. Roels and A.F. Amos, 1974. Supported by NOAA/ERL, U.S. Department of Commerce, under Contract 03-3-022-144.

2. "The Shipboard Acoustic Command System, Model 200, Instruction Manual", AMF-Sea Link, Alexandria, Va., Ken Pollock, Chief Engineer.

POSTSCRIPT

In March following the unsuccessful recovery attempt from the R/V ALPHA HELIX, the French research vessel MARARA was conducting a manganese nodule study in the current meter sites area. It was hoped that since they were in the same location and since they also had an AMF technician aboard with suitable retrieval equipment, that one more meter retrieval attempt could be made. However, before the ship could do this, it developed trouble with its propulsion system and was forced to divert to Tahiti.





Details of (top and bottom) connections to current meters.

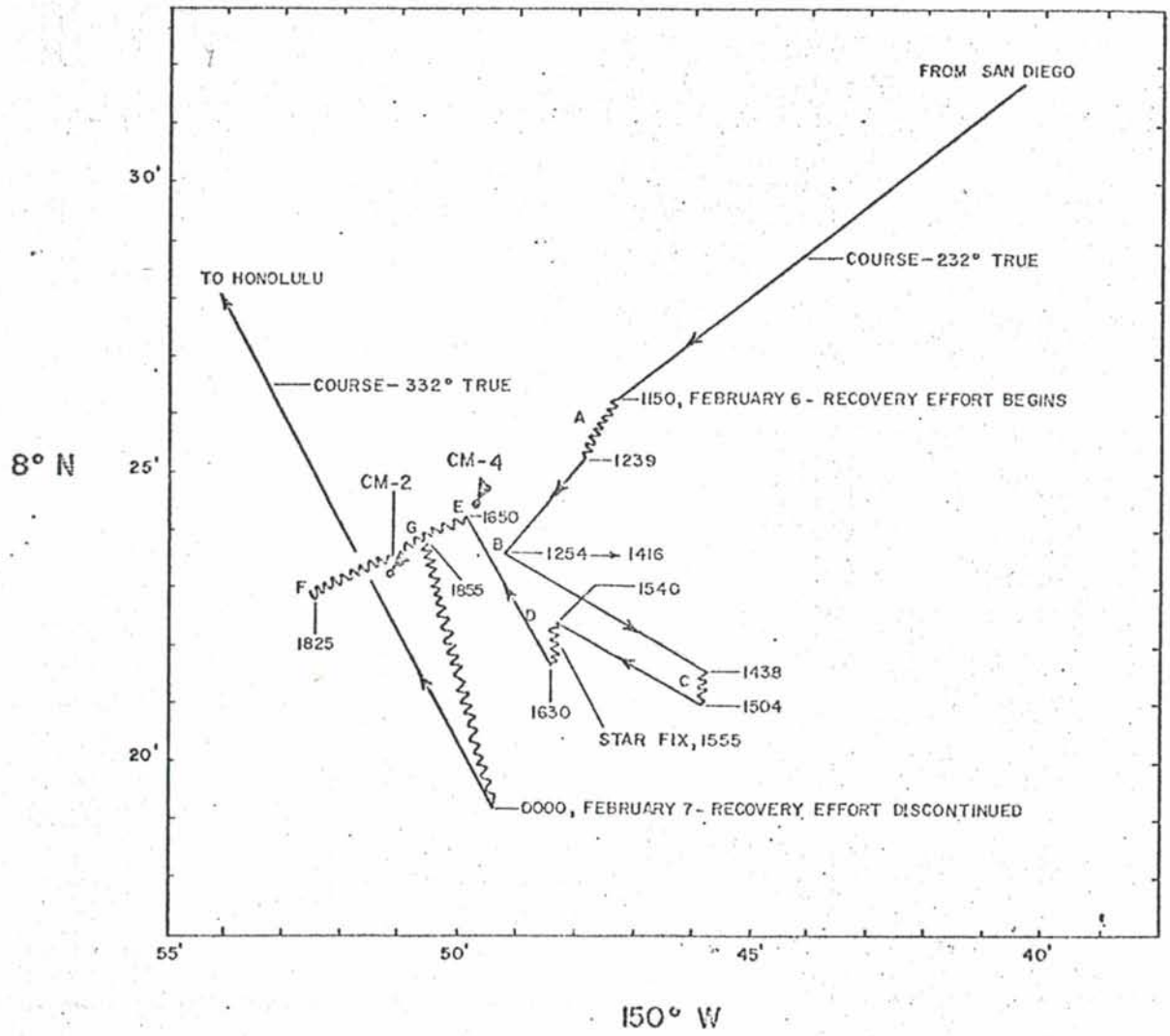

FROM SAN DIEGO
COURSE-232° TRUE
TO HONOLULU
COURSE-332° TRUE
1150, FEBRUARY 6 - RECOVERY EFFORT BEGINS
A
1239
CM-4
CM-2
E
1650
B
1254 → 1416
G
1855
F
1825
1540
D
1438
C
1504
1630
STAR FIX, 1555
0000, FEBRUARY 7 - RECOVERY EFFORT DISCONTINUED
30'
25'
20'
8° N
55'
50'
45'
40'
150° W

Figure 2

ALPHA HELIX Oahu Cruise

February 11 - 13, 1975

Chief Scientist, Richard E. Young, University of Hawaii

On this cruise we collected midwater animals for 2 studies; genetic variability in midwater fishes and bioluminescence in midwater cephalopods.

The first mission was to collect representative species of migratory and non-migratory mid-water fishes so that their genetic variability could be determined. Species of *Lampanyetus, centrobranchus, gonostoma, cyclothone, Myctophum, Ceratoseopelus,* and *Opisthoproctus* were successfully captured in adequate numbers for biochemical and population analyses. Twenty enzyme loci will be determined for each of the species and their degree of genetic variability compared between groups. These data will be correlated with other data determined on Atlantic species with similar habitats.

For the bioluminescence study live cephalopods were captured and their photophores fixed for electron microscopy. To accomplish this 34 midwater tows were taken with a 10 ft Isaacs-Kidd midwater trawl during a 3-day period. Photophores from various species of *Heteroteuthis, Pterygiotheuthis, Abralia, Abraleopsis, Onchoteuthis, Bathyteuthis, Tiocranchia, Histioteuthis* and *Sandalops* were preserved. Most photophores were fixed in 2% collidine-buffered gluteraldehyde and post-fixed in 1% OsO_4 in seawater. The initial fixation period was varied between 1 and 10 hr. Duplicate sets of photophores from *Pterygioteuthis* and *Heteroteuthis* were fixed in gluteraldehyde-osmium and Palade's fixative for comparative purposes

We thank Captain Bristol, Walter Schneider and the crew of the ALPHA HELIX for a very pleasant productive cruise.

ALPHA HELIX SOUTHEAST ASIAN EXPEDITION

I Indonesia (Bioluminescence)

March 22 - June 10, 1975

Compiled By:
ALPHA HELIX Program
Scripps Institution of Oceanography
University of California, San Diego
La Jolla, California

ALPHA HELIX SOUTHEAST ASIAN EXPEDITION

I Indonesia (Bioluminescence)

March 22 - June 10, 1975

Compiled By:
ALPHA HELIX Program
Scripps Institution of Oceanography
University of California, San Diego
La Jolla, California

ALPHA HELIX SOUTHEAST ASIAN BIOLUMINESCENCE EXPEDITION

March 22 - June 10, 1975

Chief Scientist, James F. Case, University of California, Santa Barbara

Introduction

For approximately 16 weeks, beginning in early April, a total of 25 American, Australian and Indonesian scientists conducted a variety of investigations on bioluminescence, visual physiology, ecological physiology and related problems in terrestrial environments, on coral reefs, and in subtital and deep sea environments in Eastern Indonesia. A shore station and laboratory in the Banda Islands was established from which members of the group conducted investigations on the inshore fauna of the Banda area and on Ambon, Ceram, Bali, and in Malaysia. The ship ALPHA HELIX was in direct support of the shore station and also conducted a closely related program of study of the midwater fauna of the Banda, Halmahera and Sulu Seas, making a total of 165 midwater trawls of which 90 were discrete depth samples.

This particular region of the world was chosen for study because it contains one of the most interesting and diverse faunas on earth, in terms not only of marine organisms but in terms of terrestrial life. Although this great diversity has been known since the days of the early naturalists, and particularly since the long sojourn there of A. R. Wallace, co-discoverer with Charles Darwin of the Natural Selection Theory, it has yet to be well studied, especially by the techniques of modern physiology, biochemistry and systematics.

The primary investigative focus of the expedition was bioluminescence because this phenomenon represents many of the great problems of modern biology in a particularly accessible form, and because there never before has been a

concerted program of the study of bioluminescence of this magnitude conducted with facilities for study in such a rich and varied environment. The expedition, however, follows and was conceptually rooted in the earlier ALPHA HELIX bioluminescence program in New Guinea for which the ship itself was largely unavailable. Bioluminescence is of interest in terms of such basic questions as how the cell or organism controls utilization of energy and how such energy utilization is directed into processes of value to the organism. Bioluminescence is also of great interest in terms of the behavioral uses to which it is put, and nowhere is the great variety of such uses more apparent than in the tropical seas.

Investigators on the expedition discovered a number of bioluminescent organisms new to science and conducted many biochemical, physiological, and behavioral studies on bioluminescent systems. Biochemists obtained over 90 new luminescent spectra which will be of use in understanding the chemistry of light production while physiologists investigated the means by which light production is controlled by the nervous system. Behaviorists examined two particularly fascinating luminescent organisms of widely divergent nature, synchronous flashing fireflies and two species of shallow water luminescent fishes which use the light generated by symbiotic luminescent bacteria.

Synchronous flashing fireflies are peculiar to Southeast Asia where in appropriate sites males gather in very large numbers of certain trees where, night after night they flash synchronously, virtually every insect among the hundreds on a tree turning its light on and off in exact synchrony with all the others on the tree. This form of communal timekeeping is so precise that it has few parallels in the Animal Kingdom, and possibly is equalled only in man. Hence the mechanism of synchrony was under intensive study on the expedition as was the biological significance of the process. With respect to the

latter, synchrony appears to be a communal device of attracting mates while the synchronizing mechanism appears to be an elaboration of the well-known phenomenon of photic inhibition known to occur in many non-synchronous fireflies.

One of the reasons for locating the shore station at Banda was the presence there of the reef-dwelling fishes *Photoblepharon* and *Anomalops* which carry beneath their eyes organs which harbor cultures of luminous bacteria. While the bacterial light cannot be turned on and off, the fish has structures able to conceal the luminous bacteria and thus effectively turn them on and off. Studies made by scuba diving at night as well as laboratory work on captured specimens found these fishes using their lights for a variety of purposes which probably included aggressive displays, communication and feeding. A third luminescent fish of shallow waters which was studied, the pony fish (*Gazza, Leiognathus* and *Secutor*) uses its luminescence for still another purpose, obliterating its silhouette by matching the color and intensity of the surrounding light. This is a most remarkable adaptation because the light used for this purpose is generated by bacteria living deep within the body, requiring some unusual adaptations of structure and function to make it externally visible.

The bacteria which produce light in these fish were and are still under study with regard to such questions as the mode of transfer of bacteria to the newly hatched fish and whether the same or different species are found in the various species of bacterially luminescent fishes. The relationship between the fish and the bacteria produce a highly selective antibiotic to insure that the bacteria exist in the fish as a pure culture since the bacteria

containing organs are open either to the gut or to the sea.

The visual systems of several luminescent organisms were examined for the purpose of determining if they were specially modified to detect luminescence. Work on characterization of the visual pigments of several species of deep sea luminescent fishes as well as of the shallow water forms is still underway, but it is already evident that there is a shift in the wavelength of greatest sensitivity towards the peak emission wavelength for bioluminescence in some fishes. Other workers collected material and conducted experiments on the optics of various systems and of reflector tissues. Some of the crustacean material collected promises to be unusually suitable for the analysis of the visual process in the compound eye.

Since the expedition was centered in a relatively unexplored area it was necessary to provide an ecological and systematic back-up to the experimental work by conducting a large series of discrete depth samples in order to be able to know where desired material could be located for research, to know the conditions under which it lives, and to provide material for taxonomic evaluation. This very large collection of fishes, crustaceans and other invertebrates which was made in the course of this work is itself proving to be of great scientific value. As might be expected from the fact that the sampling was done in three relatively isolated deeps (Banda, Halmahera and Sulu Seas) lying between major oceans, much new material has been obtained and is now in the course of being described by specialists throughout the world.

Since the ALPHA HELIX is a National Oceanographic Facility, use of it is potentially open to any competent scientist who has a project appropriate to its facilities and locale of operations. In keeping with this philosophy, and

because establishment of a shore station permitted an expanded scientific party, the expedition supported a number of workers in complementary fields. There was, for example, a geologist along who studied the biology of crinoids, which abound in that part of the world, in order to clarify his studies of fossil crinoids. Not only was he successful in attaining his own goals, his expertise substantially enriched the work of the biologists. In like vein the expedition was able to support the work of an expert invertebrate zoologist whose research interest is in parasitic copepods and who otherwise would have been unlikely to have been able to visit such an out of the way place with sufficient equipment to permit effective work. We were also accompanied by two neurophysiologists who worked on the insects which are unique to Indonesia and which particularly well served their interest in the correlation of structure and function in muscles capable of extremely high contraction frequencies. Peter Anderson took advantage of the remarkable variety of corals present at Banda to undertake an otherwise impossible study of the most interesting problem of neuro-interactions between the individuals comprising the colony in certain soft corals, which may achieve their truly stupendous colony size.

Acknowledgements

The expedition was greatly facilitated by the cooperation of the Indonesian Academy of Sciences. Mr. Wirosuhardjo Kartomo, then Educational and Cultural Attache of the Indonesian Embassy, Washington, was most helpful in establishing initial contacts in Indonesia. Miss Sjamsiah Achmad, Chief of the Bureau of International Relations of the Indonesian Institute of Science, and her associates expertly conducted us through the process of obtaining clearances and obtained for us the cooperation of the staff of the Ambon Oceanology Institute. Indonesian

participants in the expedition were Mr. M. Hutomo, of the National Aquarium in Jakarta and Mr. Ono Sumadhihnarga, of the Ambon Oceanology Institute. Their efforts considerably smoothed our way in Ambon and the Bandas and they were themselves able to advance their own research interests during the expedition and to add specimens to the national marine collection.

The U.S. State Department was most helpful and the expedition is indebted to staff members of the Embassy in Jakarta for innumerable forms of assistance and particularly of the use of their radio facilities during the difficult start-up period. We are expecially indebted to Mr. Richard T. Whistler and Mr. Donald S. Harris and to their secretary, Mrs. Julia Kuowenhoeven.

Miss Fran Ciluaga, Administrative Assistant of the UCSB Marine Science Institute handled many of the details of the expedition and did a spectacularly effective job of coping with continually changing travel arrangements.

Considering the remarkable efforts of all scientific members of the expedition, it is unfair to single out individuals, but all members of the expedition will agree that Eldon Ball and Peter Anderson were prime movers in making the shore operation survivable. It goes without saying that the unfailing efforts of the ALPHA HELIX crew, particularly Captain Bristol, Tom Forhan and the untiring engineers were the essential substrate of success.

Preliminary Estimate of Research Accomplishments

As of 17 March, 1976, it is estimated that the minimum number of publications to be expected from the 25 participants (not counting the two Indonesians) is 28 and that the most probable number is about 45.

The midwater trawling program, organized by Childress and Robison provided living material for some 15 workers in biochemistry and physiology and itself

constituted a major accomplishment, providing information on the midwater fauna and its vertical distribution in the poorly known Banda, Halmahera and Sulu Seas. The development of a thermally protected, closing cod-end for the trawl by Childress, Barnes and Robison was essential to getting quantities of living material from the depths to the surface in good condition since the surface water temperature was a uniform 28°C. Childress and Robison have obtained NSF support to work up the systematics of this material.

Investigations by Childress and Robison on respiratory physiology and chemical composition of midwater organisms are detailed in the attached abstracts.

On the way to Ambon from Cairns Paxton and Ho (not a member of this expedition) obtained excellent material by otter trawls. Paxton also collected material relative to his interests in bioluminescence in fishes using the midwater gear and is returning this to the Australian Museum for study.

Peter Herring and A. T. Barnes were primarily interested in the physiology of luminescence in midwater organisms. They recorded flashing behavior with an image intensifier-TV system, making observations on several lampanyctids, *Triphoturus niger* and on *Ceratoscopelus*. Excitational characteristics of some of these were spontaneous and electrically stimulated luminescence in several Astronesthids and Stomiatoids. In these, sub- and postocular organs capable of rotation from view flashed at species specific rates and could be controlled "intrinsically," without the necessity of organ rotation. Their videotape records of luminous secretions from searsiids will allow documentation of decay rates in the secretions of several forms and also documentation of a surprising phenomenon, the "rescintillation" of recently spent luminous exudates. A new

form of luminous secretion was documented from the cheilodipterid fish *Florenciella* in which luminous material is released from paired esophageal glands and dispersed by way of the gills. Another interesting discovery was the transparent mouth of the fish *Neoscopelus* which permits display of light from photophores borne on the tongue.

Herring and Barnes were able to clear up a minor mystery concerning the identity of a common but elusive organism of shallow waters that responds to a flashlight by spewing out a luminous secretion. It proved to be *Thalassocaris crinita*, which was known only to have internal light organs and not secretory glands. They have a report regarding this in press.

Meyer-Rochow has submitted a paper on spawning and fecundity in *Photoblepharon* and other work is in progress on parasitic fish in holothurians, and the eyes of deep sea crustaceans and whip scorpions.

The Georgians, Wampler and James Anderson, were involved in almost every project by reason of their fast spectrometer. They examined 120 luminescent spectra in detail. These included 12 fish species, 7 crustaceans, 5 coelenterates, 5 echinoderms (including a mesopelagic holothurian), 3 squids, 2 polychaetes, 2 fireflies, a centipede, several pyrosomids and various fungi. Wampler is publishing about 80 of these spectra in a catalog of luminous spectra, part of which will appear in *Photochemistry and Photobiology*. It was possible to arrange for these two investigators to visit New Zealand and Australia on the way to meet the ship in Cairns to allow them to collect luminous earthworms for biochemical study. Their Australian collections represent the first luminous earthworms know from there. Finally, they undertook biochemical studies of several of the fish systems and Anderson made an intensive investigation of the biochemistry of the luminous millipede found on Banda.

Fernandez substantially expanded his continuing investigation of the correlations between spectral properties of visual pigments and the bioluminescent emission spectra of their possessors with material from 9 species of fish.

A signal experiment bearing on the biological role of luminescence was performed by Morin, Harrington and Hastings who showed experimentally that the pony fish, *Gazza*, employs its luminescence in countershading.

Moring and Harrington were able to conduct a series of field and laboratory investigations on *Anomalops* which almost uniquely abounds in the Banda Islands. They are continuing these studies in the U.S. on specimens returned alive to the Steinhardt Aquarium in San Francisco. Interestingly, when these fish arrived in San Francisco they were nonluminous. Hastings and Nealson are inquiring into whether the bacteria have been lost or rendered nonluminous while Morin and Harrington have seized this unique opportunity to observe behavior in a luminous fish that is unable to luminesce.

Hastings, Harrington and Morin made a remarkable large and varied series of bacterial isolations from the luminous organs of pony fish. Their observations bear on the interesting problem of how cultures of these bacteria are maintained in the luminous organs of these fish, their origins and upon the problem of how the bacteria are maintained in pure culture. These workers, with Nealson, already have indications that the bacteria produce antibiotics and such matters can now be pursued more effectively with these very large collections.

Harrington undertook, and is continuing on preserved material, an investigation of the anatomical basis of control of light emission in pony fish.

Firefly research was impossible at the Banda site. Useful collections with data on flashing behavior were collected from one synchronizing species on Ceram (Barnes), and on non-synchronizing forms on Ambon (Lloyd, Case), Bali (Case), Sumatra (Morin, Harrington) and peninsular Malaysia and Thailand (Case). Case investigated population biology and synchronizing behavior of a remarkable population of *Pteroptyx tener* on the Kuala Selangor river, N.W. of Kuala Lumpur, Malaysia, and was able to collect useful material for laboratory investigations on the Benui River in Johore and on the Chao Praya River, west of Bangkok. Josephson joined Case during his work with the Kuala Selanger populations and together they undertook a study of the necessity of visual feedback during synchronization. Material was preserved for EM studies.

The Josephson-Elder team accomplished measurements of contraction kinetics on 9 different insect muscles and examples of each were prepared for EM study. Elder was able to gather data on coelomic pressures in holothurians, part of his long continuing investigation of invertebrate connective tissues and their role in movement. Josephson added to our knowledge of the problem of propagation of luminescence among the individuals of the colonial tunicate, *Pyrosoma*, during the transit to Manila.

Sweeney undertook a number of problems. Her primary objective, to obtain cultures of a *Noctiluca* (luminescent protozoan) with endosymbiotic algae, was successful and this and other dinoflagellates are now in culture at UCSB. She also made micro-plankton collections and examined those in the collections of the "Marine Laboratory" at Poka, Ambon. With very primitive facilities she undertook an investigation of diurnal cycles in several organisms including corals.

Reports by Humes and Macurda are attached indicating that their goals were

substantially attained.

Peter Anderson continued his investigations of the colonial nervous system in corals and now has one paper on this subject in press in the *Journal of Experimental Biology* and another ready for submission.

SUMMARY OF SHIP TIME USE

March 19-June 9, 1975 (SEAL)

	@ Time in Hours				Total		
	March	April	May	June	Hours	%	Days
Total	288	720	744	204	1956		82
Midwater trawling	46	240	264	124	674	34	28
Transit to trawling		24	8		32	1.6	1½
Transit to trawling & diving		70			70	3.5	3
Ambon (Including transit between Banda and Ambon)	~116	~216	~140		472	24	20½
Banda		~192	~140		332	17	14¼
Exploration for shore site	~106	~48			154	8	6½
Transit to Manila			54	80	134	6.8	6
Scuba dives+	5 dives	13 dives	9 dives				27 dives
Total time of shore camp		20 days	22 days				42 days

@ The times given were derived as follows: the midwater trawl times were taken from the trawling log sheets maintained by the bridge and from those maintained by J. Childress and B. Robison. Trawl times were rounded to the nearest 0.5 hr. The aggregate times are accurate within a matter of an hour or so. I arrived on the other times by filling in the holes on the trawling logs and relying on my memory. Thus the trawling times are extremely accurate and the other times are less so. Vertical columns do not always equal the total time because I was unable to estimate many of the times with sufficient accuracy.

\+ Scuba dives made by B. Robison, from his diving log.

FIELD REPORTS

Field Reports - Continued Page

Chemistry of Bioluminescence of a Centipede Found on Banda Island, Indonesia

James M. Anderson, University of Georgia

A bioluminescent centipede, in the order *Geophilomorpha* (probably *Orphaneus brevilabiatus* as described by Haneda), was collected on Banda Neira, Indonesia by the local children. The centipede exudes an intensely luminescent slime when disturbed. The color of the bioluminescence is green (corrected spectral distribution maximum at 510 nm). The intensity of the bioluminescence dies quickly but can be reinitiated by adding water to the exudate.

For chemical studies the centipedes were electrically stimulated in anaerobic buffer solutions. The luminescent material proved to be soluble when excited in this fashion. The reaction was initiated by introduction of air to the solublized exudate. Luminescence was directly dependent on oxygen introduction as H_2O_2 or catalase addition had no effect. The light producing reaction showed a pH optimum of 4.6. This is probably the lowest pH optimum known for a bioluminescent reaction. Light production was completely extinguished by raising the pH above 5.5 or lowering it below 3.2 with only partial recovery of luminescence being obtained by going to high pH and back to the optimum.

The bioluminescent reaction can be divided into two separate components. This first component was found to be labile in oxygen but stable to anaerobic boiling for several minutes, and therefore, has some of the properties of a classical luciferin. The second was found to be labile to the conditions used to keep the system anaerobic, namely argon aeration, but was stable to aerobic conditions. When the two components were added to each other light was produced.

If either a light producing or spent reaction was made anaerobic with argon, the addition of 'luciferin' no longer elicited luminescence. It is interesting that the 'argon-labile' component built up slowly after the animal exuded the luminescent material but only if the solution was kept aerobic.

Centipede luminescence had some of the properties of an enzyme requiring reaction. An enzyme, however, was not demonstrated. The luminescent reaction would not diffuse through a dialysis bag, but no active components were obtained by chromatography on G75 Sephadex. That the light emission could be extinguished permanently by heat or temporarily by cold treatment, also suggests an enzyme system. Cross reactivity with firefly luciferin was tested and found lacking even in the presence of Mg-ATP. Cross reactivity was also tested against Cypridina and Coelenterate luciferins, again with negative results.

Cross Reactivity of the Luminescent Systems of Several Midwater Animals with *Cypridina* and Coelenterates

James M. Anderson, University of Georgia

As the chemistry involved in producing bioluminescence is elucidated in differing groups of animals, the results can be extended to other groups by testing the cross reactivity of the components of the known systems with the unknown. *Cyprindina* was the first of the marine bioluminescent systems to be understood chemically and some effort has been made to show cross reactivity with the *Cypridina* system and others. Recently the chemistry of *Renilla* bioluminescence has also been elucidated and further, the luciferin involved has been shown to be the same throughout the Coelenterates and the Ctenophores. The cross reactivity of the two groups, the Myctophid fish and the spewing shrimp, were investigated using both the *Renilla* and *Cypridina* systems. Both the Myctophids and shrimp have been shown in the past to have some cross reactivity with *Cypridina*.

A partially purified luciferase preparation and an acid-methanol extract, for the luciferin, were used from *Cypridina*. A fully active, synthetic analog of *Renilla* luciferin, and a highly purified preparation of *Renilla reniformis* luciferase were used for the coelenterate system. It can be noted that *Renilla* luciferin shows no cross reactivity with *Cypridina* luciferase and *Cypridina* luciferin shows less than 0.01% cross reactivity with *Renilla* luciferase. Both *Renilla* and *Cypridina* luciferins are imadazole pyrazines with differing substituent R groups.

The results of the cross reactions attempted for this report can be summarized in the following table:

	Renilla luc	*Renilla* lase	*Cypridina* luc	*Cypridina* lase
Myctophids				
Symbolophorus ventral photophores	-	+	-	-
Lampanyctus ventral photophores	-	+	sl	sl
Diaphus head light organ	sl	+	-	-
Shrimp				
Oplophorus typus spew in buffer	+	+	-	+
Acanthephyra liver extract	a	+	a	-
Systellaspis affinis liver extract	a	+	a	-

sl=slight cross reaction
- =no cross reaction
+ =strong positive cross reaction
a =cross reaction not measurable because of methanol stimulation of liver extracts
luc=luficerin
lase=luciferase

All of the tested extracts showed only one strong cross reaction, that with *Renilla* luciferase. The indication is that both the fish and shrimp use an imadazaol pyrazine type luciferin. That there was some cross reaction with *Cypridina* luciferase, in some extracts, as well, indicates that the substituant R groups on the imadazole pyrazine ring are not identical to *Renilla* luciferin but are more like *Renilla* than *Cypridina*. Enzyme free extracts of *Diaphus*, *Acanthephyra*, and *Oplophorus* were prepared allowing conclusive evidence for cross reactivity with the luciferases.

An interesting problem was discovered in testing the cross reactions of the luciferins. The luciferin is usually dissolved in anaerobic methanol and dispensed in small volumes into the reaction mixture. In the case of the shrimp extracts, methanol in small amounts was found to directly stimulate the luminescence over 10 fold in both the spew and liver extracts. The liver

extract of Acanthephyra was chromatographed of G75 Sephadex. All of the activity, both luciferin and luciferase were found in the void volume as it came off the column. If the luminescent system is a high molecular weight complex, as the column data indicates, then the methanol stimulation of the reaction may be caused by solubilization of part of the complex.

The data presented in this report are preliminary as extracts are being transported to Georgia for further analysis. It is still significant, however, that the luciferin of the spewing shrimp and the myctophid fish cross react with the luciferase of *Renilla reniformis* which is a coelenterate.

Electrophysiological Studies of Coordination in Coral Colonies

Peter A. Anderson, University of California, Santa Barbara

The problem of how individual polyps are coordinated into the behavior of the entire colony has received little attention despite the many advances in the field of coelenterate physiology. Behavioral studies (Horridge, 1956) have indicated that the manner of spread of polyp withdrawal falls into two categories; through-conduction over the entire colony, and incremental spread, the latter being further subdivided into three groups on the basis of the relative increase in incremental area.

For this project an example of each of the main catagories was studied *Tubipora musica* as an example of a through-conducting colony and *Goniopora* (species undetermined at this stage) as an example of a colony in which the spread is incremental but in which there is a finite maximum area of response.

Observations and recordings from *Tubipora* confirm that this colony is through-conducting. Each stimulus produces a through-conducted pulse. The first pulse has no observable effect on the colony but each subsequent one is associated with a twitch of all polyps and a series of such pulses leads to withdrawal.

The pulses are bimodal, consisting of an initial fast invarient phase that appears after every stimulus and a second slower facilitating phase that follows the second and subsequent stimuli, suggesting that both activity in a conduction system and the resultant muscle action potential are being recorded. The effect of excess magnesium suggests that the conduction system is not neuronal.

The conduction velocity of the recorded pulses decays from approximately 18-10 cm/sec over a series of 5 pulses, the rate of decay and the final value being determined by the frequency of stimulation.

A further problem associated with colonial systems is how the individuals can withdraw without affecting the remainder of the colony. Recordings suggest that this is effected by some polarized connections between the conduction system in the polyps and that in the colony, such that colonially conducted pulses pass into the polyps easily whereas those originating in the polyps pass out only with great difficulty.

The behaviour of *Goniopora* proved very different from that of *Tubipora*. One electrical stimulus causes withdrawal of one or two polyps immediately surrounding the point of stimulation. Subsequent stimuli increase the area of response with the number of additional polyps responding decreasing until a maximum area of withdrawal is reached. This area varies with different species of *Goniopora*; the species studied electrophysiologically in this project having a maximum spread of 1.5 cm. Behaviour such as this has been interpreted as being a consequence of a need for interneural facilitation within the colonial conduction system.

Electrical recordings indicate that the spread of polyp withdrawal reflects the spread of activity in some conduction system. The recorded pulses are not through-conducting. Instead, several stimuli are required before activity can be recorded from a point within the maximum area of response, the number being a function of the distance involved and the stimulus frequency.

In order to determine what limits the spread of polyp withdrawal, various parameters were considered. Changes in conduction velocity were observed but

were found to be insufficient to account for the limited area. The facilitatory period of the colony was determined and the refractory period of the colony measured as 75-80 msec. It was found that this value of refractory period is misleading. Occasionally, the first stimulus of a series produced a pulse that could be recorded at points beyond the normal response area for a single stimulus. No further pulses would be observed until the appropriate number of stimuli normally required to spread activity the distance involved had been applied. A system involving only interneural facilitation would not operate in this manner and instead it is suggested that individual pathways in the conduction system involved have a far longer refractory period than the entire conduction system. Such a long refractory period combined with the requirement of interneural facilitation would reduce the probability that any one unit will pass activity on to another. If this probability is less than unity, the incremental spread would decrease as is observed with *Goniopora*.

Electrophysiological Studies of Conducting Systems in Corals

Peter A. Anderson, University of California, Santa Barbara

My research centered around an investigation of conduction systems in corals. Two corals, *Tubipora musica* and *Goniopora* (species undetermined at this time) were studied. It was possible to make electrophysiological recordings from both corals that could be closely correlated with their behaviour.

The recorded potentials in *Tubipora* are through-conducted over the entire length of the colony. They resemble the neuroeffector potentials recorded from a variety of other coelenterates, but the pathway for the propagation of this activity may not be neural but epithelial. It was possible to propose a mechanism by which the behaviour of the polyps can remain separate from that of the remainder of the colony, as previous behavioural studies have observed.

The behaviour of *Goniopora* was very different from that of *Tubipora* and this difference was reflected in the manner of propagation of the recorded potentials. It was found that activity spreads in an incremental fashion, each stimulus serving to produce a single potential which only travels a short distance. Each potential increases the total area of response, but the relative increase in size decreases after the first stimulus. The net effect is a maximum area of response. Behaviour such as this had been previously described but the mechanism by which it is effected not proved. This study indicated that it is achieved by some interneural facilitating process coupled with long refractory periods in the conducting units responsible for propagating the potentials. This conduction system seems to be neuronal.

During the latter part of the expedition, I worked in conjunction with Dr. R. K. Josephson on the salp *Pyrosoma*. Although we were able to observe and explain some facets of the behaviour of this luminescent organism, the very limited supply of specimens during the short voyage to Manila prevented a complete study from being made.

Summary of Research Activities

E. E. Ball, Australian National University

Midwater crustaceans were preserved for anatomical study of their mechanoreceptors.

Portions of colonies of the pelagic tunicate *Pyrosoma* were fixed and embedded for electron microscopy of the presumed photoreceptor, of the light organ, and of the connection between the two.

The eyes of the pelagic amphipod *Phronima* were fixed in a wide variety of fixes for light microscopy and scanning and transmission electron microcopy. Some experiments on light and optomotor responses of *Phronima* were carried out in collaboration with G. A. Horridge.

Hermit crabs were collected, and information on their natural history was recorded at 15 stations in the Torres Strait and on the islands of Ambon, Saparua, and Ceram. On the Banda Islands a much more thorough investigation of the hermit crab fauna was possible with 25 stations in an area of less than 100 km^2. Physical data collected at each station included exposure, bottom type, and depth of occurrence. Relative abundance, commensals and color in life were also recorded for each species. Approximately 35 species of hermit crabs were collected in the Banda Islands; a surprising diversity probably reflecting the diversity of habitats present in the Islands.

Luminescent Responses in Midwater Teleosts

A. T. Barnes, University of California, Santa Barbara

Although the extreme variety of luminous tissues among midwater organisms has prompted much speculation as to mechanisms and functions of light thus produced, much of this speculation has stemmed from work on preserved rather than live specimens. The extensive midwater sampling conducted during this cruise (see Childress, Robison) provided unique access to a wide range of living midwater fish, thereby allowing me to compare several radically different luminous systems.

With the relatively abundant lantern fish (Myctophidae) I concentrated on the rapidly flashing luminous patches typically arrayed as supra- or infra caudal organs. I.I.-V.T.R. records indicated that spontaneous flash rates in caudal organs of *Lampanyctus nobilis, L. niger, Triphoturus turner, and Ceratoscopelus warmingi* were 8-10 per second, but these organs could follow electrical stimulation (1 m sec square pulses *via* fine wire electrodes) at rates up to 20 per second. Similar responses were obtained from the luminous scales of several *Diaphus* species. The precise neural control of these effectors was underscored by not only their rapid flash characteristics and synchronization of infra- and supra caudal flashing, but often total coordination of the multiple luminous patches arrayed virtually from head to tail along the ventrum of *Ceratoscopelus*. Two sets of each type of ventral patch of *Ceratoscopelus* were fixed and embedded for E. M. examination, their ideally flattened scale-like shape providing a particularly favorable preparation for examining nerve-photogenic tissue relationships.

Both spontaneous and electrically stimulated luminous responses were

General Biology of Fireflies

James F. Case, University of California, Santa Barbara, James E. Lloyd, University of Florida, A. T. Barnes and Anne Harrington, University of California, Los Angeles

Specimens were collected and natural history and flashing behavior observations were made on at least 11 species of fireflies from Ceram, Ambon, Sumatra, Bali, Peninsular Malaysia and Thailand. Barnes' observations of synchronizing forms on Ceram promise to be helpful in filling the "gap" between well-known synchronizing forms from New Guinea and the mainland of S. E. Asia and thus perhaps giving us some suggestions as to the evolution of synchrony. On Bali Case was able to work with a "rice-field" firefly that exhibits a lovely follow-the-leader loose synchrony based on male-male interactions.

Light Organ Ultrastructure

James F. Case, University of California, Santa Barbara

Structure-function relationships in the firefly light organ are far from fully understood despite some recent advances in knowledge (Oertel and Case, 1975, *Cell and Tissue*, in press). We are currently taking a comparative approach to the problem, relating light organ structure to function in fireflies of widely spread taxonomic affinities having markedly different light organ functional capabilities. Living fireflies of four species were returned to Santa Barbara and prepared for EM. Two of these have been examined in a preliminary way. In synchronizers the photocyte granules are found to be approximately four times the volume of those in non-synchronizers. This and other characteristics of the tissues seem appropriate to the differences in light organ activity cycles seen in synchronous and non-synchronous forms. Synchronizers are far more steadily active than non-synchronizers, the species under study usually performing at a continuous rate of 3 flashes/sec for perhaps 6 hours/night for several months.

Population Biology of a Synchronizer, *Pteroptyx tener*

James F. Case, University of California, Santa Barbara

A remarkable population of *P. tener* occupying a ten-mile stretch of the Kuala Sleangor River in NW Malaysia was visited on six occasions ver a period of two months. Groups of synchronizers were marked at night to permit relocation and study during the day. Random collections were made as a function of time and position. General behavior including the activity of predators was observed. Such data are expected to elucidate such matters as population stability and are important to ultimate assignments of biological significance of the synchronization phenomenon.

Experiments on the Synchronization Process in *P. lepe* and *P. malaccae*

James F. Case, University of California, Santa Barbara and Robert K. Josephson, University of California, Irvine

A. Normal behavior and pacing experiments

By using a portable 4-channel plus voice recorder with portable photometers and pulsed light sources it proved feasible to conduct an exhaustive series of experiments in the field, in a laboratory in Kuala Lumpur and in Santa Barbara. Experiments include establishment of normal synchrony by undisturbed groups, pacing with artificial light sources at various frequencies, pacer phase changes and random addition of flashes during pacing, determination of temperature relations of synchrony and the free-running period of flashing, and the effects of the number of participants upon synchronizing frequency. All of these data are essential to development of an adequate model of the sunchronization process.

B. Visual feedback and synchrony

Obviously one firefly must see another before synchronizing. However, it is not known if it must view the light from its own light organ, which is a question analogous to those regarding auditory feedback in speech and so forth. In a test of this, experiments were done in which fireflies were caught by their middles in a transparent membrane of common usage, allowing them to see their own lights although just as tightly restrained as later in the experiment when, with black plastic and lampblack saturated silicone grease, each firefly was prevented from seeing its own luminescence without impediment of its view of others. Under these conditions some degree of synchrony, including forced phase shifting, was possible. A careful study of the data is necessary because there are indications that feedbackless synchrony can occur only at the natural synchronizing frequency.

recorded from sub- and post-ocular organs in a variety of Astronesthids, Stomiatoids, and their allies. These organs, which can be rotated out of view, were found to flash at apparently species specific rates and could be controlled intrinsically, without relying on organ closure. Many of these fish responded to low level photic stimulation by flashing.

Videotapes of the post-cleithral luminous ducts in Searsiids yielded comparisons of decay rates in *Searsia* and *Platytroctes* as well as demonstrating the 'rescintillation' of recently spent luminous exudate. Peter Herring and I documented an analogous spewing mechanism in a Cheilodipterid, *Florenciella*, the luminous material being released from paired esophageal glands into the esophagus out the gills. Comparable esophageal bulbs were not found in other morphologically similar, but non-luminous Cheilodipterids.

These investigations represent a continuation of work centered not only on establishing the behavioral capabilities of these unique effector systems but also on using them as means of examining neuroeffector mechanics directly. I am, therefore, grateful for the considerable assistance extended me by Captain W. Bristol and the entire crew of the ALPHA HELIX.

Oxygen Consumption of Midwater Crustaceans and Fishes as a Function of Depth of Occurrence and Oxygen Partial Pressure

J. J. Childress, University of California, Santa Barbara

Previous studies off California have shown that the midwater crustaceans there exhibit lower oxygen consumption rates the deeper their species lives. Furthermore, the species which live in the oxygen minimum layer at partial pressures of 6mmHg O_2 are able to regulate their oxygen consumption down to this extremely low partial pressure. In an effort to understand the reasons for the decline in O_2 consumption with depth and to find out how general the phenomenon of exceptional O_2 consumption regulation is in midwater animals, I have investigated these parameters in three areas in this region. These areas are very different hydrographically from each other and all are very different from California. The Banda Sea has a minimum of about 50mmHg O_2 and has a very deep sill giving a rather usual Pacific style temperature profile. The Halmahera Sea has a sill at about 700m, is isothermal below this depth (8.7° C) and has a minimum of about 60mmHg O_2. The Sulu Sea has a sill at 400m, is isothermal (10.5°C) below this depth and has a minimum of about 30mmHg O_2.

Deeper animals from all of these areas had much lower O_2 consumption rates than did shallower ones. This indicates that factors other than low temperature and low O_2 are behind the decreased O_2 consumption of deeper living animals. In addition it was found that the midwater species studied in this area could not regulate their O_2 consumption below about 20 to 40 mmHg O_2 and further had virtually no tolerance for oxygen lack. This was true even of 8 species which are also found off California and which exhibit

considerable regulatory ability there. In general, however, these cosmopolitan species were better regulators than the species which do not occur in minimum layer regions. Those species and individuals from the relatively low O_2 Sulu Sea exhibited considerably greater regulatory ability and low O_2 tolerance than did species and individuals of the same species from the higher O_2 Banda and Halmahera seas. Clearly, outstanding O_2 regulatory abilities are not a general characteristic of midwater animals, but are developed in individuals and species only in low O_2 regions.

Use and Development of a Thermally-Protecting Cod-End for Midwater Trawls

J. J. Childress and A. T. Barnes, University of California, Santa Barbara

The most severe problem which one encounters in trying to do physiological studies on midwater animals is that they are generally dead on arrival at the surface. To a large extent this mortality is due to the high temperatures of the surface waters as compared to temperatures at greater depths. In regions with relatively low surface temperatures, animals from depths as great as 1200m can be brought to the surface alive. However, in regions where the surface temperatures are above 25° C, virtually no animals come alive to the surface except those which have minimum depths of occurrence of less than 200m.

In anticipation of this problem, we designed and constructed a closing cod end for this cruise. The cod-end is based on 6" nominal PVC pipe with a slider valve at each end. The closing of the valves is triggered by a messenger trip mechanism. In practice, this device proved to be very reliable and was used successfully on about 60 hauls. It was used with both the RMT-8 and the IKMT midwater trawls. When bringing water from temperatures of 4-7° C at depth to a surface temperature of 28° C, the enclosed water was between 10 and 13° C on arrival on deck. This degree of thermal protection was dramatically effective in bringing animals to the surface alive. Many species, some with minimum depths as great as 1500m, which never came up alive normally, were always alive when they came up in the cod end. This device provided material for a variety of physiological studies.

Chemical Composition and Buoyancy of Midwater Crustaceans and Fishes

J. J. Childress and B. H. Robison, University of California, Santa Barbara

As has previously been shown, the composition and buoyance relations of midwater animals can vary greatly even within a taxon as narrow as a genus. Further the composition of animals from a given depth is generally more similar to that of other animals from the same depth than to closely related ones from greater or lesser depths. In an attempt to isolate some of the variables which might be responsible for the observed variations with depth, we have collected and frozen for later analysis about 450 crustaceans and fishes from three very different seas. These are the Banda, Halmahera and Sulu. In addition, buoyancy measurements were made on about 150 crustaceans from these areas. These analyses will also be important in understanding the structure of the midwater communities sampled.

Studies on the Gastropod Parasite of the Common Blue Starfish, *Linckia laevigata*

Hugh Y. Elder, University of Glasgow

A sample of 100 Linckia were examined from several collections sites around Banda. Approximately 70% were infested with a small gastropod parasite and up to 18 parasites were found on one starfish. The smaller gastropods (0.5-3.0mm) occur exclusively on the tops and sides of the arms, while the bigger specimens (3-8mm) are found on the under surface of the arms. The small specimens are mobile and in some instances may become reattached if dislodged but the large specimens are permanently attached, with a proboscis several millimetres into the host tissue. The large individuals are probably all females and in the case of mature individuals (over 6mm length) tiny males (less than 1 mm) are found under the mantle of the female, possibly permanently attached. Data were collected for a more detailed statistical analysis of gastropod distribution on the arms and material was fixed for histological examination.

Ultrastructural Studies on Selected Orthopteran Flight Muscles

Hugh Y. Elder, University of Glasgow

Previous work has shown that there is a good correlation between the mechanical properties and certain utrastructural characteristics of different tettigoniid flight muscles, e.g. those used for flight alone and those used for stridulation and flight (Josephson and Halverson, 1971. *Biol. Bull. 141*; Elder, 1971. *Biol. Bull. 141*.). As a part of a collaborative project with R. K. Josephson, aimed at examining mechanical and ultrastructural properties of the muscles of a wide range of stridulation tettigoniids, specimens of several species were collected and their songs and flight frequencies recorded. Analysis of the sound patterns suggested that three longhorns in particular, with sound frequencies ranging from about 5 per second to 60 Hz, would be worth investigating.

The first tergo-coxal, and in some instances, the tergo-trochanteral and the pleuro-tergal muscles, were taken from the meso- and meta-thoracic segments in the males of the three species and also from the femlaes (which do not stridulate) of two. These were fixed in 2% glutaraldehyde in a 50:50 mixture of locust saline and 0.2M cacodylate buffer at pH 6.8-6.9, buffer washed and post fixed in 1% OsO_4. Quantitative estimations of the amount of sarcoplasmic reticulum, mitochondria and contractile myofilaments will be made for correlation with the physiological parameters, contraction frequency, stamina, force per unit cross sectional area, etc., examined by R. K. Josephson. These data, and in particular the lower frequencies, will greatly extend the range on which we have data from other sources.

Studies on Holothurian Locomotor Mechanisms

Hugh Y. Elder, University of Glasgow

Previous work (Hunter and Elder, 1967. *Biol. Bull. 133*; Elder, 1973. *Biol. Bull. 144)* has shown that the synaptid holothuria progress by an unusual form of locomotion utilizing direct peristaltic waves in which, at some phases, there is simultaneous contraction of circular and longitudinal muscles. This form of locomotion differs from the better known caterpillar-like locomotion of other holothuria. Locomotion was studied in three species, a large brown mottled Aspidochirote, metre long Synapta from the eel grass beds and a small synaptid found on encrusting sponges on vertical rock faces 15-25 ft below low water level. From flash photographs and slow motion cine films it will be possible to calculate the sequence and amount of both circular and longitudinal muscle contraction. Coelomic pressure records, made by canulating through the body wall with a hypodermic needle connected to a pressure transducer, showed that the large synapta operates for most of the time with remarkably low pressures (less than 1 cm of water) and that there are no pressure waves associated with any phase of direct peristaltic progression.

Three types of locomotion were distinguished in the synaptas. One form is intermediate between the caterpillar-type locomotion (exhibited by the Aspidochirote) and the direct peristaltic mode. This suggests the way in which the latter may have evolved from the former. Synaptid tissues were also fixed for light and electron microscopical examination of body wall connective tissues.

Visual Pigments of Bioluminescent Marine Teleosts

Hector R. C. Fernandez, University of California, Los Angeles

Upwards of 10 different species of bioluminescent marine fishes were collected either by means of midwater trawls or in shore by means of nets. Adequate numbers of eyes from dark adapted specimens were first enucleated, the retinas removed and placed in potassium aluminum sulfate. After a minimum of twenty-four hours they were washed three times in M/15 phosphate buffer (pH 7.0) at 3° C. After each wash, the tissues were centrifuged for 15 minutes at 27,000 x g. The pellet was extracted with 1% digitonin in M/15 phosphate buffer. The retinas were repeatedly extracted until the resulting extract was devoid of visual pigment. All extracts from each species were pooled and stored at -70° C in light-tight containers. Adequate amounts of extract were obtained from all the species examined. All procedures were carried out either in dim red light (Kodak Filter No. 1) or in complete darkness to protect the visual pigments. The extracts will be sent to the University of Southern California where they will be analyzed during the course of this summer.

Although most species that were examined were pelagic, deep sea forms, two inshore specimens (*Photoblepharon* and *Anomalops*) were also studied. While on the shore camp, a chemical darkroom was set up equipped with a spectrophotometer (Beckman DB) in order to carry out preliminary observations on the characteristics of the visual photopigments of the two inshore species. The difference spectra of *Anomalops* extracts indicate that the wave length of maximum absorbance is 496 nanometers (nm), very close to the maximum of the bioluminescence spectrum which Dr. J. Wampler measured during this expedition.

(λmax 490 nm). Further analysis of the data revealed the following points. The final product of bleaching is retinal, as revealed by the peak of the oxime which forms when retinal combines with hydroxylamine. Fitting the absorbance spectrum from Dartnall's nomogram of similar λ_{max} to the experimental results strongly suggests that the extract contains not one but two visual pigments one of which being in greater concentration. If this latter observation is confirmed by subsequent experiments carried out under more favorable conditions, there is a high probability that the component of the mixture which absorbs at shorter wave lengths will be very similar in spectral properties to the emission spectrum of the photophores.

Similar experiments have been performed on samples from extracts of *Photoblepharon* retinae. The results are also quite similar. The main difference is that the λ_{max} of the difference sprectrum is about 502 nm. This extract also appears to be a mixture of at least two different photopigments. Careful examination of these extracts will be undertaken in my laboratory at U.S.C. and it is expected that all the work will be completed by the end of the coming summer.

Behavioral and Anatomical Observations on Leiognathid Luminescence

Anne Harrington, University of California, Los Angeles

Members of the family Leiognathidae are known to possess bacterial luminescent organs. We purchased at least seven species of this family (including members of the genera *Gazza, Leiognathus and Secutor*) in the fish market in Ambon. Most of these specimens were preserved for later anatomical study. Some live *Gazza minuta* were obtained from fishermen in Ambon and maintained on board ship on the way to Manila. With these fish, I did some behavorial observations and preliminary dissections on fresh material to determine the nature and control of light emission. Most of the work reported here was done on *Gazza minuta* and *Leiognathus bindus*.

In the living fish light emission was seen in two situations: when the fish is exposed to ambient light and when the fish is handled. Control in these situations appears to be different. The ventral luminescence displayed when the fish is exposed to ambient light has a slow rise and fall (see report for Morin, Harrington and Hastings), but the response when the fish is handled is very rapid and intermittent. Occasionally the fish can be seen to flicker the light. Light emission in response to ambient light exposure is highly directed toward the ventral surface of the fish (very little light can be seen laterally) and the entire ventral surface is illuminated from the caudal fin to the mouth. When the fish is handled, the luminescence covers the entire ventral and ventral-lateral surface of the fish with a bright glow in a triangular area of the breast. The organ can be

seen as a bright spot just behind the pectoral fin. Sometimes only the triangular area on the breast will luminese.

Fish that died with the light off were found to have a shade drawn over the organ. This shade is a thin white tissue that is attached on the posterior side of the organ and moves to the anterior. Continuous with the shade is a clear jelly-like tissue which covers the organ when the shade is withdrawn and light is being emitted from the fish.

The organ forms a ring around the esophagus. On the basis of my dissections I conclude that the light is emitted in three general areas. (1) The dorsal part of the organ enters the swim bladder just anterior to the rete. The inside of the bladder is highly silvered except for a clear curved bone at the posterior end. Using light guides I determined that light entering the bladder at the level of the organ is seen as a diffuse glow over the posterior-ventral and ventral-lateral part of the fish from the caudal fin to the vent. (2) The lateral aspect of the organ emits light into the ventral musculature just posterior to the pectoral girdle. (3) The ventral part of the organ appears to emit light into the mouth and my preliminary conclusion is that the light is gathered by the gill arches and bones at the back of the tongue and is piped foreward to the fishes mouth and lower jaw. In living fish light was not seen emitted from the mouth except when the mouth was forced open; however, in one fish that died with the light on, light could be seen along the gill arches (which are silvered on their posterior edges) and along the tissues lining the back of the mouth.

One function suggested for the luminescence in Leiognathid fishes is

that it is used to countershade the animal and new evidence to support that hypothysis has been obtained on this expedition (see scientific report Morin, Harrington and Hastings). From the observations I feel that these fish have the ability to use the luminescence in alternate ways, possibly for feeding, interspecific signaling or defense against predators.

Endosymbiotic Luminous Bacteria of the Pony Fish

J. W. Hastings, Harvard University, Anne Harrington and J. C. Morin, University of California, Los Angeles

The pony fish (Leiognathidae) of the IndoPacific region derive their ability to bioluminesce from bacteria which are cultured within an internal organ, donut shaped, which surrounds and communicates with the esophagus. The fact that these bacteria are 100% luminous and 100% viable was shown earlier (*ALPHA HELIX New Guinea*, 1969; *Biol. Bull. 141*, 1971). Luminous bacteria are abundant in sea water itself, and it may be assumed, though it has not been shown, that this provides an innoculum for the original infection. The questions which we wish to investigate here, in collaboration with Drs. K. Nealson and J. Reichelt at the Scripps Institution of Oceanography are: (1) in a given organ, are all bacteria taxonomically identical? (2) do all members of that host species always cultivate the same bacterial species and (3) do different species of pony fish have different species of bacteria as symbionts? In addition to giving the information on specificity itself, these experiments will help to understand the mode of infection.

Pony fish (*Gazza minuta*) were obtained live from a fisherman in Ambon, Indonesia. Five other species (*Leiognathus* and *Secutor*) were obtained from the fish market and found to be sufficiently fresh to permit isolation of the bacteria from the organ. Organs were dissected and sterilized externally, and then homogenized in sterile sea water. From several dilutions, platings were made to permit the isolation of single colonies, which were then examined for luminescence and transferred to vials. These will be taken to Scripps immediately for taxonomic studies. From 8 fish 100 single colony isolates were made and from an additional 12 between 10 and 50 isolates each.

Observations of Bioluminescence in Oceanic Animals

Peter J. Herring, Institute of Oceanographic Sciences, Wormley, U.K.

The occurrence of hepatic luminescence in caridean decapods, previously investigated in Atlantic species, has been extended to include a number of additional oplophorid and pandalid species, together with observations on the natural emission of the luminescence. Most species examined have been meso- or bathypelagic in habit, but, in collaboration with Dr. A. T. Barnes, a small shallow living pandalid was found to be responsible for flash-light-stimulated responses in Banda harbor.

Observations of luminescent responses, fluorescence, and photopore anatomy have been made on a number of cephalopods. Fluorescence changes follow luminescence in cranchiids such as *Liocranchia* and *Phasmatopsis*, and induced luminescence has additionally been observed in species of *Chiroteuthis*, *Ctenopteryx*, *Octopoteuthis*, *Onychoteuthis*, *Symplectoteuthis*, *Spirula* and certain enoploteuthids, confirming the function of putative luminous tissues in some of these species.

Luminescence has been observed for the first time in evermanellid fish, and in joint work with Dr. Barnes a new luminescent emission system has been found in certain cheilodipterids. This has some functional resemblance to the post-cleithral system in searsids. Other work with Dr. Barnes on the flash characteristics of some stomiatoid fishes post-orbital organs has shown significant differences in the flash parameters in different species. Further examples of barbel luminescence in the ceratioid *Linophryne* have been noted, as well as escal luminescence of *Gigantactis*. The undescribed

oral light organs in *Sternoptyx* have been investigated and compared with the serial ventral organs. Earlier observations on the red and blue emitting systems of *Aristostomias* from the Atlantic have been supplemented by the capture of additional species in the Halmahera area, and Dr. Barnes has obtained videotaped image intensifier records of one of these. A species of *Astronesthes* has been taken whose post-orbital organ appears capable of emitting light of an orange-yellow color rather than blue.

The angular distribution of light emitted by specimens of *Anomalops* and *Photoblepharon* has been determined in all three planes.

Material has been prepared for light and electron microscopy from some of the above species, and emission spectra determined in many instances (see Wampler, Anderson and Herring).

Copepoda Associated with Marine Invertebrates

A. G. Humes, Boston University Marine Program, Woods Hole

Previous investigations over the past twenty years in the West Indies, Madagascar, New Caledonia, and Eniwetok Atoll have shown that copepods, particularly Cyclopoida, are common associates of most of the major metazoan phyla. They range from unmodified species loosely associated with the host and often having weak host specificity (as in certain species of *Acanthomolgus* from nephthyid alcyonaceans) to those species which are highly modified and endoparasitic with strong host specificity (as in many species of *Xarifia* from madreorarians). Often a single host may harbor large numbers of copepods, for example, several thousand from one basket star.

In the Moluccas, where very few copepods from invertebrates have been reported, as many different copepods as possible were collected from a wide array of intertidal and subtidal (to 20 M) invertebrates. Collections were made at Ambon, Banda, Marsegoe Island off western Ceram, Karanh Mie in Weda Bay in eastern central Halmahera, Parang Island off northeastern Ceram, and Gomumu Island south of Obi Island. The 260 collections were primarily from the Madreporaria (63 collections). Alcyonacea (47), Holothuroidea (29), Asteroidea (27), Crinoidea (23), and Gorgonacea (18), with the remainder from other groups. Samples of the hosts have been preserved for future identification.

From gross examination it is estimated that approximately 80 new forms are represented in the collection. About 60 lots of copepods represent already described species. Further detailed study of dissected specimens will be necessary in order to confirm these identifications.

The significance of this research lies in two areas. In the first place, the abundant new material collected will provide much information for the understanding of phylogenetic relationships within the groups of Copepoda concerned. Hopefully, collections such as these will make possible eventual revisionary studies. Secondly, the Moluccan collections will expand knowledge of the geographical ranges and host preferences of these diversified associates in the Indo-Pacific area.

Contraction Kinetics of Insect Wing Muscles

Robert K. Josephson, University of California, Irvine

There are clear correlations between the ultrastructure of a striated muscle and its contractile properties: muscles able to contract and relax rapidly have extensive sarcoplasmic reticulum (SR) and relatively short sarcomeres while slower muscles have less well-developed SR and, in arthropods but not vertebrates, long sarcomeres. The question to which I addressed myself during the ALPHA HELIX expedition to Banda is, "Can anyone quantitatively relate a muscle's ultrastructure to its contraction kinetics, i.e. can one accurately predict a muscle's contractile properties from its ultrastructure, or conversely, its ultrastructure from physiological data?" To answer this, contraction kinetics were determined for homalogous wing muscles of three species of tettigonids; a large species with low wing frequency during flight (12 Hz) and a low wing frequency during stridulation (less than 5 Hz), a species of intermediate size with a flight frequency of 19 Hz and a stridulation frequency of 60 Hz, and a small species with a flight frequency of 22 Hz and a stridulation frequency of 50 Hz. Nine muscles were examined from these animals, including muscles used solely for flight (male metathorasic muscles and female muscles) and muscles used for both stridulation and flight (male mesothorasic muscles). For each muscle data were obtained on: (1) the time course of twitch contractions, (2) maximum isometric tension during twitches and tetany, and (3) the fatigue resistance during continued stimulation at 100 Hz. Data were obtained from five replicates for each muscle, and each parameter was measured at both 30° C and 35° C. At this time the species used have not yet been identified nor have the records collected been quantitatively analyzed, so only qualitative statements can now be given.

As expected, twitch contractions were consierably slower in purely flight muscles than in muscles used for high-frequency stridulation; and slower in the flight muscles of the species with the low wing frequency than in the two species with higher flight frequency. Dr. H. Elder fixed examples of each of the muscles studied for later electron microscopic examination. Micrographs from these muscles will be analyzed, using stereological techniques, to determine the relative abundance of SR, mitrochondria, and contractile filaments. When the physiological records have been analyzed and the ultrastructural data is available, it will be possible to determine how well the contraction kinetics and ultrastructure of these muscles are related.

Bioluminescent Patterns of Anomalopid Fishes

James G. Morin and Anne Harrington, University of California, Los Angeles

The functional role of bioluminescence in fish has been difficult to assess because of their relative inaccessability. In the Banda Islands, Indonesia, we had the unique opportunity to study two species of closely related luminescent fish that live in shallow water, *Photoblepharon palpebratus* and *Anomolops kaptoptron*. Our approach was to study the normal activity patterns and luminescent displays in the field and to observe the luminescent reactions in the fish to controlled situations in the laboratory.

Both *Photoblepharon* and *Anomolops* emerge from caves after sunset (or moonset) and return before sunrise (or moonrise). During the night *Photoblepharon* remains in a fairly circumscribed area (a couple of meters square) in pairs or occasionally singles or small groups of 3 to 60 fish in depths of 0 to over 100 feet. The light remains on most of the time except when the fish is disturbed or during aggressive displays. If disturbed *Photoblepharon* will swim under a near-by rock, blinking rapidly.

Anomolops ranges widely over several hundred meters of reef in pairs, loose assemblages of pairs or tightly knit groups of up to several hundred individuals. The fish blink their lights rapidly. In the pairs one member almost always blinked 100% more rapidly than the other. In the large groups there was a distinctive disruptive luminescent display - the light from the group appeared to pulsate and sometimes a kind of synchrony of blinking can be seen. We collected both fish species from different times, locations, depths and groupings for later gut content, sex, pairings, age and histological analyses.

Plankton tows were also made to determine food availability and food selectivity by the fish.

Laboratory studies were made by isolating two fish at a time in a dark room and recording from photomultipliers suspended above their tanks. We first looked at day-night activity in the fish and established that there is a rhythm in the luminescent display. At night *Photoblepharon* has the light ON most of the time and during the day it blinks with an equal ON-OFF period about 30 times per minute. *Anomolops* blinks at a rapid equal ON-OFF period at about 60 to 80 times a minute at night and was mostly OFF during the day. Both showed an increase blink frequency when disturbed and showed a decreased blink frequency in the presence of food (small crustaceans). Communications experiments between fish isolated in tanks and then allowed to see each other were photometrically recorded but have not yet been carefully analyzed. There are, however, apparent changes in the luminescent patterns of the fish.

At this time we feel that the luminescence is used by the fish to see with; probably to detect prey and in the case of *Photoblepharon* possibly to attract positively phototatic prey; to deter predation by a disruptive blinking pattern; and in communication, certainly as an aggressive display and possibly in other ways. About 20 of these fish were shipped to the United States from Manila where we hope to perform more behavorial studies.

Protective Illumination by the Pony Fish: A Confirmation of the Countershading Hypothesis

J. G. Morin and A. Harrington, University of California, Los Angeles
and J. W. Hastings, Harvard University

Over fifteen years ago it was suggested that organisms which are capable of emitting bioluminescence ventrally might use it for protective illumination, allowing them to obscure their silhouette by matching the intensity and color of the background light. Although the hypothesis is applicable to quite a number of different animals, there have been no experimental or field observations confirming or refuting the hypothesis. A single exception is the report (*ALPHA HELIX, New Guinea*, 1969; *Science 173*, 1971) that pony fish are stimulated to emit bioluminescence by exposure to light, but no quantitative data were obtained. The experiments described here provide such data and give strong support to the hypothesis.

Living pony fish (*Gazza minuta*) were obtained from a local fisherman in Ambon, Indonesia, and maintained aboard ship in a tank with running sea water. Experimental studies were carried out in a dark room with single individuals (40-45 mm standard length, 5-6 gm), placed in a beaker with aeration. A variable intensity blue light was placed above the animal, with a diffuser at about 15 cm; a photomultiplier to measure the ventral luminescence was beneath at about 25 cm. Measurements of bioluminescence were made with the light off; about 10 sec was required for this. Upon illumination, the ventral bioluminescence emission of the fish occurs, but the response is a slow one, increasing to a maximum at about 60 min. The plateau reached was proportional to the light intensity over the range studied. Upon

turning off the light the bioluminescence decreased, but again not rapidly; a half time for the decay of bioluminescence in the dark was about 2 min.

From visual observations, made with the light source coming from the side, we determined that ventral bioluminescence is actually occurring when the irradiating light is on, and that the appearance of luminescence is indeed slow. The important question is whether or not the bioluminescence is of the intensity required to obscure the silhouette. Visual observations suggest that it is; experiments will be carried out to test this.

Tapeta Lucida in Fishes

J. A. C. Nicol, University of Texas, Port Aransas

A reflecting layer of tapetum lucidum is found in the eyes of many fishes, especially those living under conditions of low illumination. It is located behind the retina, either in the pigment epithelium or in the chorioidal layer, and it can be either a diffuse or a specular reflector. A surprisingly wide variety of materials is employed as reflectors. In the form of thin crystals, ordered in regular layers, they reflect light by constructive interference of rays arising from superposed surfaces. Or, as minute spheres, of the order of a wavelength of visible light, they back-scatter light over wide angles. During the cruise of the R/V ALPHA HELIX, there were opportunities to study several such systems. Anchovies were obtained in Ambon from fisherman using lights. The anchovy eye has a complicated two-tier tapetum in the pigment epithelium. It includes a set of reflecting plates overlying the cones, and a scattering layer of tiny crystals, that serves the rods. Photomechanical movements allow the two tapeta to operate under diurnal and nocturnal conditions, respectively. The reflecting material is guanine. Bulleyes, Priacanthidae, which have a specular chorioidal taeptum, were collected in Banda. A white diffusing tapetum was found in the eye of *Howella*, Cheilodipteridae. It was restricted to part of the fundus, lay in the pigment epithelium, and was made of lipid material. Many lanternfishes, Myctophidae, were examined, most of which had blue or violet tapeta lucida of metallic appearance. They occupied the entire fundus, or part only, and they were formed of regularly arranged crystals in the pigment epithelium

cells. No tapetum was found in some species from the deep waters. Spectrum reflectivities were measured, and materials were collected for further studies by light and electron microscopy. Particularities of tapetal structure related to vertical distribution will be considered.

Luminescent Fishes

J. R. Paxton, Australian Museum, Sydney

Among the benthic fishes obtained by otter trawl in the Arafura Sea were four specimens of a luminous eel representing a new genus and species in the family Congridae, the first eel known to be luminescent. The luminescent system is internal with a light organ associated with the digestive system anteriorly where the intestine joins the stomach. An extension of the intestine posterior to the anus may also be involved in light production. Cultures of luminous bacteria were obtained from one specimen and sent from Ambon to a colleague at Monash University. The digestive and luminous system from one specimen will be sectioned to determine the fine structure and exact location of the bacterial colonies. Emission spectra were recorded on the ALPHA HELIX by Dr. Wampler.

Previous experience with pempherid fishes in Australia had indicated that one species for which a *Cypridina* luciferin-luciferase reaction was known also had luminous bacteria associated with their digestive system. The problems of obtaining uncontaminated bacterial cultures from the luminescent organs have prevented confirmation of a dual luminescent system. Apogonid fishes of the genus *Apogonichthys* are also known to have a *Cypridina* reaction and luminous bacterial cultures were obtained from ALPHA HELIX specimens. Light organs were fixed for electron microscopy to ascertain where the luminous bacteria are located.

Among the specimens obtained by midwater trawl were a series of evermannellid fish of the genus *Coccorella*. This species was found to have a luminescent

system associated with the pyloric caecae and intestine and is the first known luminous species in the family. Also taken were two specimens of an apparently new species of alepocephalid fish related to the genus *Rouleina*. The specimens had superficial photophores on the body and fins as well as a second set of deeper photophores on the ventral midline and head. These deeper organs flouresce red under ultraviolet, as do the photophores found in stomiatoid fishes. Remnants of a mucous sheath similar to those found in a few stomiatoids was present on the smaller specimen. This new species may have considerable significance in the study of relationships of the family Alepocephalidae.

Two nights of canoe fishing with the fisherman of the Banda Islands yielded more than 200 specimens of *Anomalops katoptron* and a few of *Photoblepharon palpebratus*, both of which are luminescent. Observations were made on the nocturnal habits of both species. The large series of *Anomalops* will allow studies on stomach contents, variation of light organ size with length and sex, and age and growth; otoliths were also taken for the latter study.

Studies on Midwater Fishes

Bruce H. Robison, University of California, Santa Barbara

The Indo-Pacific region has long been considered the center of origin for many of the most important families of midwater fishes, however, the midwater fauna of this region is still poorly known. The large collection of fishes made during this expedition will be of great significance in determining global distribution patterns, taxonomic relationships, and aspects of the evolution of midwater fishes.

Each of the midwater communities sampled is distinctive in terms of its faunal composition and organization despite the relative proximity of of the Seas which they inhabit. Initial comparisons of ecological structure suggest that similar species replace each other in adjacent Seas and that common ecological roles or niches can be defined by examining these repeating patterns. The terms of the similarity, and thus the definition of the niches, will be elucidated by the information we collected on vertical distribution patterns, chemical composition, age and growth (more than 600 pairs of otoliths were obtained), form and color (about 250 species were photographed in color), luminescent systems and visual systems.

For example, in the Banda and Halmahera Seas the dark, soft-bodied, *Lampanyctus niger* is the most abundant myctophid between 800 and 1000 m while the firmer-bodied, silver-blue *L. nobilis* is dominant between 600 and 800 m. Their counterparts in the Sulu Sea, *L. lineatus* and *Triphoturus turneri* are more slender fishes (probably reflecting the lower Sulu productivity) yet their musculature, color, depth range, otolith shapes, eye

structure and luminous tissue are comparable to the respective southern species.

Of equal interest are those situations where an abundant fish has no obvious replacement. *Anopolgaster* and *Idiacanthus* are common in collections from the Banda and Sulu Seas but not Halmahera. *Scopelengys* and *Sternoptyx* are very abundant in the Banda Sea, less so in the Halmahera Sea and uncommon in the Sulu Sea. *Bolinichthys blacki* is evenly distributed throughout the entire sampling area. These patterns undoubtedly reflect aspects of community structure that will not become apparent until the fish data can be correlated with stomach content analyses and hydrographic information.

The collection of fishes is remarkable for the number of forms which are rare in other oceanic regions or are as yet undescribed. Additionally, the collection contains unusually large and diverse representations from the families Searsidae, Astronesthidae, Chiasmodontidae, Chielodipteridae, Melanostomiatidae, Evermannellidae and the myctophid genus *Diaphus*.

Midwater Trawling: Summary of Operations and Gear Development

Bruce H. Robison and J. J. Childress,
University of California, Santa Barbara

One hundred and eighty midwater trawl hauls were made during the expedition, ninety-six of which were discrete-depth samplings. Three hauls were run in the Timor Sea, five in the Ceram Sea, ninety-nine in Banda Sea, thirty-seven in the Halmahera Sea, one in the Molucca Passage, four in the Celebes Sea and thirty-one in the Sulu Sea.

Extensive sampling in the Banda Sea provided two replicates of a day/night vertical series of discrete-depth hauls. Single day and night series were run in the Halmahera and Sulu Seas. Oblique hauls with the net continuously open were alternated with those in the vertical series. Neuston tows and night-light, dip-net stations were also conducted.

In each of the three major sampling areas the depth range covered was from 0 to 2000 m. The water column was sampled in 50 m increments above the thermocline, in 150 - 200 m increments between the thermocline and 1000 m, and in 500 m increments below 1000 m.

The basic trawl was an RMT-8, modified to operate from the ALPHA HELIX by the inclusion of vertical lifting cables. In operation, the standard cod-end was alternated with the Childress-Barnes thermally-protected cod-end. A messenger operated double trip mechanism was employed to open and close the net at depth.

The highly successful operation of this new and previously untried trawling system was due in large part to the cooperation and assistance we received from the crew of the ALPHA HELIX. We are particularly grateful to Captain W. Bristol, engineer Bill Collins, seaman John Smith and to the resident technician Tom Forhan for their efforts.

The Jumping Mechanism of a Large Tettigoniid

Peter J. Simmons, Australian National University

Jumping of orthopteran insects involves a rapid change in force exerted around the metathorasic femero-tibial joint from flexion to extension. The way in which this is achieved was examined in the "Banana Piper," a large Tettigoniid. Mechanical measurements indicated that there is a special point of balance at a femero-tibial angle of about 45°. At this part only a small increment in tension in either the flexor or the extensor tendon is required to cause a large movement of the tibia. Myograms indicated that it receives identical innervation to the main flexor muscle. This muscle may be important in causing changes in mechanical properties of the knee joint.

Visual Responses by a Species of Aeschnid Dragon Fly

Peter J. Simmons, Australian National University

Children on Banda Neia amuse themselves by catching dragon flies in an interesting and unusual manner. This is of interest because only one species, a large Aeschnid, is ever caught by their method. They employ flexible sticks, half a meter long, with a small white object, often a flower or ball of cotton wool, at the end. On seeing a dragon fly, they follow its flight path with the stick, vibrating it slightly. Sometimes the dragonfly is attracted to the object at the end of the stick and lands quite heavily on it.

Circadian Rhythms in Corals and Cowries

Beatrice M. Sweeney, University of California, Santa Barbara

It is commonly thought that most corals expand their polyps only at night. The truth of this generalization was borne out by observations on various reefs where only about 20% of all corals were expanded during the day. Experiments to test whether contraction of coral polyps is a direct response to light or is timed by a circadian clock were conducted as follows. Forty-three Fungids (at least 5 species), two specimens of *Tubipora musica*, and one of *Euphyllia rugosa* were collected from reefs in Banda and maintained in aquaria at the shore station. Individuals were observed at frequent intervals while they were exposed to natural light. After several days, specimens were moved during the night to a darkened aquarium where they remained in darkness for at least a week. Visual observations were continued, the specimens being briefly illuminated with weak light. Similar experiments were carried out with cowries (8 species of *Cypraea* and 1 *Ovula*), in which both the expansion of the mantle to cover the shell and the activity of the animals were scored.

In the aquarium in natural light, the polyps of most of the Fungids expanded only at night. In continuous darkness, this behavior continued for at least 5 circadian cycles ($T < 24$ hours). Specimens differed in the distinctness of this circadian rhythm. In continuous darkness, polyps spend more time expanded than in natural light and they contract when illuminated by a flash from a strobe. Thus contraction of polyps can be directly affected by bright light. The polyps of *Tubipora* are expanded only during the day in

natural light and continue one cycle of expansion in darkness. The polyps of one species of *Fungia*, *Podebrachia* and *Euphyllia* were always expanded.

All the cowries examined were active only at night in natural light and continued this behavior in continuous darkness. Some species also showed a circadian rhythm in mantle expansion, while in two (*C. isabella* and *C. erosa*) the mantle remained expanded most of the time.

This is apparently the first demonstration of circadian rhythmicity in corals and cowries.

Phytoplankton Studies in the Banda, Halmahera, Celebes and Sulu Seas

Beatrice M. Sweeney, University of California, Santa Barbara

A total of 131 plankton collections were made in the seas visited by this expedition. A 35μ mesh nylon phytoplankton net with an 18 cm opening was towed along the surface for various distances and the organisms were washed into a bottle at the cod end. The numbers of organisms in water samples concentrated by centrifugation were occasionally also determined. Interesting phytoplankton species were removed from the collections for further study, measurement and culture. Some organisms were isolated during the day and tested for bioluminescence the same evening, using a photomultiplier photometer.

All samples contained a wide variety of phyto- and zooplankton, but the species composition differed in different localities. Only in the mid-Sulu Sea were sparce plankton samples encountered. Dinoflagellates and most diatoms were represented by relatively few individuals. However, a large population of *Chaetoceros*, composed of at least 5 species and restricted to the inner harbor at Banda, was observed April 10-21, and again May 1 until we left the area on May 28. During this time, only a few *Chaetoceros* were found outside the inner harbor, suggesting that the water of this harbor is exchanged relatively slowly, in spite of strong tidal currents in the harbor entrances. Weda Bay in the Halmahera Sea supported a very large population of the blue-green alga, *Trichodesmium* (5 species) was 600,000 filaments per 1. water on May 19, 1975 at noon.

A remarkable feature of the collections generally was the presence of

very large species of both dinoflagellates and diatoms (*Pyrocystis noctiluca*, dia. 300 μ; *Pyrocystis* sp. 900 x 240 μ, and unidentified dinoflagellate 265 x 100 μ, *Coscinodiscus* sp. 800 x 700 x 700 μ; *Rhizosolenia* sp. 1000 x 300 x 300 μ. Cultures of these species have been initiated.

A few bioluminescence flashes could be evoked from every plankton sample tested. Isolated cells of *Pyrocystis* (2 sp.) *Gonyaulax polygramma* and several species of *Peridinium*, and one Ostracod were bioluminescent. All other organisms tested were not luminous, including 6 species of *Ceratium*, *Ornithocerus steinii*, *Trichodesmium*, (5 species), *Chaetoceros*, a number of different radiolarians, several medusae, copepods and tintinnids and a larvacean.

Three interesting examples of algal-algal symbiosis were encountered in the plankton collections. *Noctiluca* with motile green flagellate symbionts was collected just south of the Halmahera Sea and in the Basilan Straits. Three common species of *Rhizosolenia* were observed to contain short filaments (6-12 cells) of a red blue-green alga with a terminal yellowish heterocyst. *Ornithocercus steinii*, the cell body of which does not contain plastids, usually carries orange bodies outside the cell between the two girdle frills. These differ from zooxanthellae in lacking internal structure and resemble chloroplasts. Another rod-shapped organism was associated with the wing on the hypotheca. These three symbiont-containing cells were examined in detail while alive and were fixed for light and electron microscopy.

The small number of green *Noctiluca* collected (50 cells) restricted work with this species. However, it was possible to show that the symbiont stains blue with iodine and so contains starch. It is thus probably not *Protoeuglena*, as claimed by Subrahmanyan. Cultures of both *Noctiluca* and symbionts from crushed *Noctiluca* have been initiated on several media. Cultures, if successful, and electron microscopy should make possible the better characterization of the symbiont.

Studies of the Chemical Similarities of Earthworm Bioluminescence

John E. Wampler, University of Georgia

While luminous earthworms have been reported from many different parts of the world, no attempt has been made to investigate the chemical similarities of the luminescence reactions to different worms. This expedition offered an opportunity to investigate such similarities between the bioluminescence system of the North American earthworm, *Diplocardia longa*, and species from Australia and New Zealand. It also lead indirectly to the discovery of bioluminescence in at least three different Australian species (Jamieson, unpublished results). Since some non-luminous worms in North America appear to contain luciferin activity which can stimulate emission with purified *D. longa* system), this possibility was also investigated for several species of non-luminous earthworms from the Indonesian area.

With the help of Tony Whitaker of the D. S. I. R. of New Zealand, several specimens of *Octochaetus multiporus* were collected in Whitman's valley near Upper Hutt on the North Island of New Zealand. *O. multiporus* is a large (avg. 18" x 1/2") oligochaete found in this case in moist soil having a high clay content. The bioluminescence of this worm had previously been reported as orange in color. The luminescence is exuded in the coelomic fluid from the dorsal pores of the animal when it is subjected to violent tactile stimulation. With all of the worms examined the luminescence of the milky, whitish fluid was quite bright but of a yellowish-green color. The color was verified by viewing the luminescence through

through various colored filters. The luminescence material was easily solubilized and its luminescnece strongly stimulated by addition of dilute hydrogen peroxide. A frozen specimen of *O. multiporus* was transported to the ALPHA HELIX where an extract was made. This extract luminesed brightly upon addition of dilute hydrogen peroxide; the *in vitro* sprectrum having a maximum around 570 nm is very broad and still appears yellowish-green to the eye. Emission from the crude extract was greatly stimulated by addition of pure *D.longa* luciferin with little if any change in the emission spectrum. *D. longa* luciferase did not stimulate the luminescence.

With the help of Dr. Barrie Jamieson of the Department of Zoology, University of Queensland, several specimens of a recently discovered and, as yet, unidentified merinephric, parichaetin earthworm were collected from the vicinity of Mt. Warning in Queensland, Australia. The luminescence of this worm appeared blue-green and was again elicited as an exuded slime when the worms were violently stimulated. This worm is small (avg. 2" x 1/16") and inactive and was found in the moist soil of the forest floor very near the surface. Preliminary data suggest that the luminescent system of this worm is particulate and is definitely peroxide stimulated. Ten specimens were transported to the ALPHA HELIX but only two small ones survived. A crude extract was prepared from the exuded slime. This extract was stimulated to emit by addition of hydrogen peroxide and luciferase.

D. longa luciferin gave no stimulation and, in fact, appeared to inhibit the reaction slightly.

Of the ten species of non-luminous earthworms collected on the islands of Ceram and Banda, none appeared to contain stimulating components for the *D. longa* bioluminescence reaction.

Bioluminescence Emission Sprectra of Luminous Animals from the Southeast Asia Area

John E. Wampler and James M. Anderson, University of Georgia
Peter J. Herring, Institute of Oceanographic Sciences, Wormley, U.K.

Bioluminescence emission spectra can be extremely useful in investigations of the biochemistry of the light reactions. Observations of sprectral similarities have lead in the past to elucidation of chemical similarities and may in the future point the way to understanding the biological role of bioluminescence for some animals. In order to be able to collect, preserve, and catalog a wide variety of spectra from the many different animals to be collected on this expedition, a computerized instrument system was designed and built at the University of Georgia to allow rapid collection of luminescence spectra from transient and weak emission sources. This system is a specialized version of the absolute spectrofluorimeter previously described (Wampler and DeSa, *Applied Spectroscopy* 25, 263). During the course of the expedition over a hundred spectra were recorded from a wide variety of marine and terrestrial luminescent forms. The experience gained from this expedition has led to several alterations in the instrumentation and in the computer program which controls it and will be quite useful in the continuing effort to improve the utility of the instrument system.

Spectra were obtained from at least 12 different species of fish, some as yet unidentified; seven species of shrimp; five coelenterate species; four echinoderms (brittle stars); two polychaete worms; three species of squid; two species of firefly from Ceram; several different pyrosoma; several

species of fungi from Australia, Banda and Ceram; a sea cucumber; an as yet unidentified centipede from Banda; and the New Zealand earthworm *Octochaetus multiporus*. In many cases the luminescence spectra were structured and in several instances the structure was obviously due, in part, to more than one emission component. Perhaps the most interesting spectra are those of the searsid fish which show at least two emission components, a broad violet band with a maximum around 410 nm and a structured green component in the 470-500 nm region. The relative contribution of these two components was seen to vary with time with a slow but steady increase in the violet band. This band may represent the shortest wavelength bioluminescence yet reported.

Variations in the luminescence spectra between specimens, between different species, and even between light organs of the same animal were also observed. The widest variation seen between species were the four brittle star species. Each brittle star spectrum exhibited a structured band with a major peak between 510 and 530 and side peaks or shoulders. Another interesting variation was the difference between the esca and barbel light organs of a single angler fish specimen (linophryne), where the barbel light organ has a spectrum significantly greener (corrected maximum at 497 nm) and more symetrical than that of the esca (corrected maximum at 491 nm). This spectral difference could of course be explained in several ways, different chemistry, different enzymes, environmental effects, or most simply a filter effect of the light organ. In fact a filter effect was demonstrated for the effect of the body wall on the spectrum of the bacterial emission of a pony fish

(leiognathidae); in this fish the dissected light organ (corrected maximum at 496 nm) has a spectrum different both in position and shape from the spectrum as viewed through the body wall of the intact animal (corrected maximum at 504 nm).

Ninety-one spectra were recorded in punched paper tape format. These will be added to the growing catalog of spectra being maintained at the University of Georgia. These spectra are readily available for study and comparison at any time and hard copies in graphical and/or tabular format are available on request.

An Examination of the Optical Mechanisms of Insect Ocelli

Martin Wilson, Australian National Museum

The function of the insect ocellus is mysterious. Our lack of understanding stems from a lack of basic information about the optical and physiological design of this organ. My time in Banda has been spent examining the ocelli of about twenty species of insects, mostly Orthoptera, and preparing them for electron microscopy.

Ocellar lenses from a species of large Acridid and a species of Cicada have been collected for examination of their optical performance. It is already apparent that in both these species the focal plane of the ocellar lens lies far behind the retina, moreover neither lens is capable of forming a good image. In contrast to the compound eye it seems that the ocellus is not concerned with spatial information. This is reasonable in view of the enormous convergence of the many receptor cells on to the very few second order neurons.

Of all the species examined Acridids were found to be peculiar in their posession of a vertebrate-like pupil. The radial movement of dense pigment to stop down the lens is mediated by the retina through illumination of only part of the retina does not provoke movement of only part of the pigment. The Cicada ocellus also exhibits an obvious light adaptation reaction. Light falling on the retina causes a pinkish blush to appear. The mechanism of the blush awaits electron microscopy though it is probably similar to the pigment movement in compound eyes. The ocelli of Tettigoniids, though often large and well developed, have neither pupil nor blush though only the electron microscop can reveal if there are ultrastructural responses to light adaptation.

ALPHA HELIX Southeast Asian Expedition

Philippines to Borneo Transit

June 18-21, 1975

Scientist, Marcia Rottman, University of Colorado

The objective of the scientific program enroute from Subic Bay, Philippines, to Cowie Harbor, Tawah, Sabah was to obtain plankton and sediment samples in the Palawan Passage in the South China Sea. Three one-meter oblique net tows were taken, two in relatively deep water with 300 m.w.o. and one in about 200 m of water with 200 m.w.o. Two neuston tows were also taken in association with the meter net tows. Nine grab sample stations were occupied in depths ranging from about 200 to 2000 m. Samples were recovered at eight of the stations.

Plankton samples will be used to extend knowledge of pteropod distribution in the South China Sea. The sediment samples will be used in a study of the distribution of pteropods and foraminifera with relation to living distributions, depth, and solution.

ALPHA HELIX SOUTHEAST ASIAN EXPEDITION

II Borneo (Comparative Physiology)

June 17 - August 16, 1975

Compiled By:
ALPHA HELIX Program
Scripps Institution of Oceanography
University of California, San Diego
La Jolla, California

ALPHA HELIX Southeast Asian Expedition
Phase II Borneo

June 17 - August 16, 1975

Chief Scientist, Brian K. McNab, University of Florida, Gainesville

There have been few studies of the comparative physiology of animals native to southeast Asia. The planned period of work for the R/V ALPHA HELIX in the southeastern Pacific in 1975 permitted our study of aspects of the comparative physiology and experimental ecology of selected terrestrial and aquatic animals of Borneo. Borneo was chosen because of its large land mass and diverse and distinctive fauna. The work of this expedition was accomplished in the Sultanate of Brunei.

The Borneo Expedition consisted of 12 biologists from 8 universities working for about 8 weeks in and around Bandar Seri Begawan, the capitol of Brunei, with most of the individual studies concerning the comparative physiology of indigenous aquatic and terrestrial animals.

For example, studies were made of the hematology of various mammals, especially the mouse deer and some fruit bats. The mouse deer has very small spherical blood cells and, consequently, a viscous blood. The mouse deer also has a high rate of heat production, but because of its small size, its weight of about 2 kg and its tropical distribution, it has some difficulty in regulating body temperature.

The rates of metabolism of some other Bornean animals vary with the diversity of food supplies. Thus, among Bornean bats, insectivorous species have low rates of metabolism and frugivorous species have high rates. An insectivorous bird was also shown to have a low rate of energy expenditure.

A study of the energetics of spiders showed that their metabolism varied

depending upon whether the spiders live in caves or not. This result is believed to occur at least in part because of the reduced food supplies available in caves.

A study of Bornean amphibians showed that terrestrial and aquatic species living in rain forests produced ammonia as their principal nitrogenous waste product, while arboreal species tended to use urea, especially when they were deprived of water. All amphibians studied from coastal and disturbed areas secreted urea.

A euryhaline teleost living in the intertidal waters of Borneo tolerated appreciable variations in salinity by means of modifying its permeability to water and salt.

Other studies were conducted on ant-mimicing spiders, on the fauna of pitcher plants and on the metabolism and water exchange of selected lizards and frogs.

Finally, a survey was made of the medical consequence of social change in indigenous peoples. It is suggested that the present intensive pressure to "modernize" will bring with it the anxiety of modern society and the consequent appearance of hypertension, heart attack and ulcers--problems which have been completely unknown in these peoples.

Our work could not have been accomplished except for the extensive aid given by many individuals. First of all, our stay in the Sultanate of Brunei was made possible by permission of the Sultanate itself. The British High Commissioner Mr. James Davidson and his First Secretary Mr. James Moffatt were instrumental in obtaining governmental permission. Mr. Jack Turner, Director of Marine, Mr. Sharifuddin, Curator of the Brunei Museum, Major M. Kaplan of the Royal Brunei Regiment and Mrs. Barbara Harrison were all of great assistance

during our stay. The biologists of this expedition greatly appreciate the aid freely given by the Captain and crew of the R/V ALPHA HELIX.

FIELD REPORTS

Relationship Between Phylogeny, Ecology, and Energy Expenditure in Spiders

J. F. Anderson, University of Florida, Gainesville

The major objective of my research was to test the hypothesis that the characteristically low rates of metabolism of arachnids are adaptations for survival when these predators face situations where prey are scarce. My previous work has demonstrated that field populations do face periods of complete or partial starvation, and species with low rates of metabolism survive starvation longer than those with higher rates. The latter observation was made by exploiting the variation in energy expenditure in very different species. I felt the hypothesis could be further strengthened by two additional lines of study. First, by studying energy expenditure in a group of spiders belonging to a single taxon but which live in different habitats, one could relate levels of energy expenditure to prey availability without differences in phylogeny complicating the interpretation of the results. Certain species of the family Liphistiidae are one of the few groups of arachnids suitable for this type of approach. I collected specimens of *Liphistius batuensis* in Batu Caves located 11 km NE of Kuala Lumpur, West Malaysia as an example of a species adapted to low prey density, a salient characteristic of cave environments. For comparison I obtained specimens of *L. malayanus*, a non-cave dwelling form at Templer Park a few km from Batu Caves. A total of 59 measurements of the rates of oxygen consumption were made at temperatures of 20, 25, and 30° C. These rates are 26, 38, and 61 µl O_2/g • hr for *L. batuensis* and 39, 56, and 71 µl O_2/g• hr for *L. malayanus* for the three temperatures, respectively. Comparison supports the original hypothesis since the species from the low energy cave habitat had lower rates of metabolism (average of 24% over the three temperatures) than the non-cave dwelling species. A second approach involved comparing rates of oxygen

consumption of ecologically equivalent but phylogenetically different species proved valuable. Hunting spiders belonging to the family Sparassidae were collected at Dato Gandi, Brunei. Oxygen consumption values from these specimens averaged 116 and 160 µl O_2/g • hr at 25 and 30° C, respectively, and are essentially identical to those obtained from North American hunting spiders belonging to different families but having the same niche. In conclusion, evidence from both approaches supports the hypothesis that the rates of energy expenditure are a function of prey availability associated with specific habitats and the various methods of prey capture of arachnids.

I wish to thank Drs. Jonathan Reiskind and Boo-Liat Lim for their aid in securing specimens of *Liphistius*.

Energetics of Temperature Regulation in the Lesser Mouse-Deer

B. K. McNab, University of Florida, Gainesville

Rates of oxygen consumption and body temperature in the lesser mouse-deer (*Tragulus javanicus*) were measured as a function of environmental temperature with a Beckman oxygen analyzer and a Schultheiss thermometer, respectively. A preliminary analysis of the data suggests that the adult mouse-deer has a high basal rate of metabolism and a high thermal conductance, compared to the values expected in mammals from body weight. The body temperature of adults is also high, about 38.5°C at the lower limit of thermoneutrality, but is rather variable at all ambient temperatures. At temperatures below 20°C body temperature falls to a mean of 37.5°C. In one immature mouse-deer weighing 67% of the adult, body temperature was much more variable and normally below that of the adult. At the lower limit of thermoneutrality, its body temperature was about 37.5°C and 36.5 at 16°C, some measurements falling to 34.4°C. The lower body temperature in the immature mouse-deer results from a small body size and a basal rate that is somewhat lower than that found in the adult (being equal to the value expected from wieght). Surprisingly, the immature mouse-deer had a lower thermal conductance than the larger adult. The adult and immature mouse-deer differ in another manner: the adult had a higher lower limit of thermoneutrality because at cool temperatures it did not attain the minimal conductance before raising its rate of metabolism. Thus, it almost appears as if the lower basal rate coupled with the small size in the immature mouse-deer requires the use of its minimal conductance to reduce energy expenditure without compromising thermoregulation.

These data have ecological implications. As in most, if not all, frugivores,

the mouse-deer has a basal rate of metabolism that is greater than expected from body weight. In its distribution the mouse-deer is limited to tropical environments; the northern limits are in southern Burma, Thailand and Indo-china. In fact, in Borneo the lesser mouse-deer is found in the lowlands, while the greater mouse-deer (*T. napoh*) is also found at high elevations. The apparent cold sensitivity of the lesser mouse-deer may well result from its small size.

I should like to thank Captain Wayne Bristol and the crew of the R/V ALPHA HELIX for their many kindnesses. Mr. C. R. Netto of Bandar Seri Begawan, the people of longhouse Amo B, Temburong, and Captain Anthony Hampton of the Royal Brunei Regiment all helped obtain palandok.

Energetics of Three Bornean Bats

B. K. McNab, University of Florida, Gainesville

In an extensive survey of the energetics of Brasilian bats McNab (CBP, 1969) showed that fruit-, nectar-, and meat-eating species had basal rates of metabolism equal to or greater than the values expected from weight, but insectivorous species had low basal rates. All species were characterized by high thermal conductances (as is to be expected in a tropical environment). The variations in basal rate were explained to be related to the seasonal availability of food in tropical environments; flying insects were thought to be the most seasonally variable of all food supplies, especially given the inflexibility of food habits among insectivorous bats.

The energetics of three bats were examined in Brunei: a small insectivore *Hipposideros*, a moderate-sized frugivore, *Cynopterus*, and a moderate-sized species that apparently is a nectavore, *Eonycteris*. All three species have high thermal conductances, in agreement with neotropical species, but there was appreciable differentiation among these bats in their energetics. *Hipposideros* had a basal rate only 70% of expectations, *Cynopterus* had a basal rate equal to 112% of expectations, and *Eonycteris* had a basal rate equal to about 96% of expectations. Thus these data are similar to the values found in neotropical species, and cut across taxonomic lines because *Hipposideros* belongs to the Rhinolophidae and the two "fruit" bats belong to the Megachiroptera.

There are, however, some noteworthy features in these bats. As in some South American species, *Cynopterus* has its lowest body temperature at intermediate temperatures that correspond to commonly occurring ambient temperatures. This behavior presumably permits a savings of up to 60% of the normal energy

expenditure. The nectivore *Eonycteris* is strictly a cave-dwelling species. It is an exceedingly sloppy thermoregulator. In terms of its gregariousness, body size, and to some extent its food habits, this species is similar to the neotropical phyllostomid, *Phyllostomus bicolor*. Both species are somewhat similar in the variability of body temperature. Nevertheless, I do not understand why *Eonycteris* should have such variable body temperature: is it related to its cave habits, or to its food, or both?

In any case, it can be concluded that the energetics of bats in the paleotropics are essentially similar to those in the neotropics, even though different taxonomic groups are normally present.

Reactions to Intermittent Visual Stimuli and Sea-Finding Behavior in Green Sea Turtles, *Chelonia mydas*

N. Mrosovsky, University of Toronto

Positive responses to light are important in the sea-finding behavior of sea turtles. These responses also occur in laboratory situations where stimuli can be standardized. It is also known that turtles base their sea-finding behavior on integration of cues from a wide field of view. The matter of integration of cues over spans of time has not been studied, though there are unconfirmed reports that some turtles are more attracted to flashing stimuli. To investigate these matters hatchling green sea turtles, *Chelonia mydas*, less than 1 week old, were presented with a choice between a plain light panel and one intermittently illuminated. The method was similar to that used by Mrosovsky and Shettleworth (*Behaviour 32:* 211-257, 1968) Eighty-eight animals were tested at each stimulus combination. Repeated presentations of one particular choice showed that the method was reliable and that there were no major changes with repeated testing. The main results were: 1) flashing light was less preferred than steady light, 2) the rate of flashes had little influence over the ranges studied, 1/sec to 14/sec, and 3) but the proportion of the time the light was off did affect the preference. Thus, if the off-period of the stimulus was 3 times as long as the on-period, it was less preferred than if the on-period was 3 times as long as the off-period.

These results suggest that a flashing stimulus is less preferred because it reduces the total amount of light reaching the turtle, and that turtles integrate information over time as well as space in their sea-finding behavior. This is a new observation, and it is hoped that future work will relate this to characteristics of the visual system and the optic tectum.

Metabolic Rates at Several Salinities in the Euryhaline Teleost, *Ambassus interrupta*

F. G. Nordlie, University of Florida, Gainesville

An extremely euryhaline form, *Ambassus interrupta*, very common in the tide channel between Muara and Bandar Seri Begawan, Brunei, was chosen for experimentation to determine whether or not the metabolic pattern with respect to salinity, previously determined in juvenile mullet is a general phenomenon for other species of euryhaline teleosts, and for individuals of all sizes. Two groups of individuals were selected for study--a group of small individuals with a mean weight of 1 g and a larger group with a mean weight of 4 g. Individuals of each size were acclimated to salinities of 10, 30, 50, 70 and 100% of full sea water. Rates of metabolism were determined for each group following an acclimation at 25°C. Results for the small fish were as follows: 10%-0.121 ccO_2/gm-hr; 30%-0.101; 50%-0.99; 70%-0.216; 100%-0.172. For the larger group the results were: 10%-0.087 ccO /gm-hr; 30%-0.084; 50%-0.070; 70%-0.078; 100%-0.085. It would appear from these data that the smaller *Ambassus* show a pattern of metabolism with respect to salinity very similar to that of the juvenile mullet. However, the larger *Ambassus* appear not to vary in metabolic rate from one salinity to another. Determinations of plasma Cl^- carried out for the larger group were as follows: 30%-126 meq/1; 50%-142; 70%-147; 100%-144. It thus appears that with the exception of the 30% sea water group, the plasma Cl^- is rather closely regulated so the lack of obvious alterations in the metabolic outputs of the larger group cannot be associated with a sloppiness of regulation.

I would like to thank the several people who helped with collecting including Dr. B. K. McNab, Dr. Walter Garey and Mr. Richard Beal and his associates in the Brunei Department of Fisheries.

Studies on the Pitcher Plant *Nepenthes gracillis* and the Fauna Associated with it, especially the spider *Misumenops nepenthicola*

J. Reiskind, University of Florida, Gainesville

The old-world pitcher plants, *Nepenthes*, were found to be abundant in the drier, exposed areas on the hills near Dato Gandi, Brunei. One species, probably *N. gracillis*, was studied. It is a ground form producing pitchers that rest near or on the ground. Ranging from yellow-green to dark maroon in exterior color and often speckled inside, they hold fluid (both of their own production and rainwater) in which captured insects are digested and in which dipteran larvae and other organisms live and develop. Also, the thomisid spider, *Misumenops nepenthicola*, was found in assocation with these plants.

M. nepenthicola was usually found sitting just below the lip of the pitcher, often not visible from above. When disturbed the spider would drop into the fluid at the bottom remaining inactive underwater for up to 3 minutes (but usually 30-60 seconds) and then would climb up just enough to pull its abdomen out of the water.

While laboratory experiments did not rule out the possibility that the spider could ambush insects as they enter the pitcher a more common (and the only observed) feeding technique was to capture struggling prey from the fluid. Hence the spider is truly commensal with respect to the plant.

The *Nepenthes* plant was studied with respect to certain aspects of its contained fluid as well as its live and dead animal contents.

The physical parameters measured were pH, viscosity and volume of the fluid. The unopened pitcher contained fluid with pH between 3.8 and 4.6 with

a viscosity of 1.5 to 1.7 that of water. Opened pitchers had pH between 2.0 and 5.2 and viscosities from 1.0 to 1.6 though most were close to 1.0.

Collections of the captured animals and those living in the pitchers were made. The captured organisms were mainly ants and termites while one found live mosquito and other dipteran larvae and some nematodes.

Correlations between the dead and living organisms and the various physical properties as well as between organisms will be sought.

Studies on the Mimicry of Ants by Spiders

J. Reiskind, University of Florida, Gainesville

Fifty-two species (or distant morphs) of myrmecomorphic spiders were collected and studied in West Malaysia and Brunei. All 14 species from Malaysia were in the family Salticidae. In Brunei 29 were salticids, seven clubionids and one each from the families Theridiidae and Thomisidae.

One objective of this study was to better understand the methods used by the living spider to give the illusion of an ant. To this end motion pictures were taken, some in slow motion, for careful analysis of mimetic behavior, especially modifications of the gait. Several new "techniques" used by the spiders were observed. They include 1) the lightening of the trochanter of the 4th leg to enhance the narrow pedicel of the spider (analogous to the petiole of an ant), 2) the light terminal segment of leg 1 that results in the antennae-like leg appearing raised off the ground when it is actually in contact, 3) the ability to raise the abdomen, and 4) the use of abdominal hair tufts to give the illusion of antennae on a posteriorly directed "head" and the spinnerets as "mouthparts."

In addition to these general observations many specific ones were made with respect to certain species:

(Species XLII)- This salticid has an insect-like "head" directed posteriorly. The knob-like posterior half of the abdomen includes tufts of hairs at the location of "antennae" and spinnerets that give the illusion of moving mouthparts. The posture of the spider tends to bring these spinnerets down periodically (attaching drag-line) and, when standing still, the visual illusion of an insect (not distinctly an ant) is very good. The spider does not move backwards,

however, and the function of this mimicry probably is to direct a predator's attack in the direction opposite the escape route.

(Species XXIII) - This thomisid has been reported from S. E. Asia as another spider in which its abdomen resembles the "head" of an insect; in this case an ant. Of the four specimens found one did have the abdominal eyespots and, while stationary, did give a fair illusion of an ant's head--vaguely *Oecophylla*-like. However, contrary to previous reports, this spider does not usually move backwards and has as good, if not better, an illusion of an ant's head when viewed from the anterior, with good eyespots and "face."

(Species XIX, XX, XXXVI, XLVI, XLIX) - All these salticid spiders are mimics of one dominant arboreal ant genus, *Crematogaster*. The ants are characterized by a heart-shaped, posteriorly pointed and shiny gasters. These mimics appear to be genus-specific with both the shape of the abdomen and its surface characteristics the major mimetic characteristics. Several of these species can also bob the abdomen, a behavioral trait of the model ants.

Few if any definitive species-specific mimic/model associations were found though several were likely due to the coincidence of the mimic and only one possible ant model. What is lacking in such cases is some sort of unique character possessed by the model and mimicked by the spider. Such an example is (Species XVIII) and the moderate-sized *Camponotus* ants found in Muara, Brunei.

The main collections were made at several locations in and around Kuala Lumpur, Malaysia and in the Islamic Sultanate of Brunei at Muara, near Bandar Seri Begawan, near Labi, in Temburong and at Dato Gandi along the Brunei River.

Nitrogen Excretion and Rates of Rehydration in Bornean Amphibians

V. H. Shoemaker, University of California, Riverside
L. L. McClanahan, California State University at Fullerton

Representatives of 13 species of anurans were collected for study from a variety of habitats and localities in northern Borneo. These included coastal areas (Tawau, Sahab; Muara, Brunei; Gadong, Brunei), a disturbed region of the interior (Bangar, E. Brunei), and primary forest (Sungai Rampayoh, near Labi, Brunei).

Nitrogen wastes were collected quantitatively from animals kept in water and from animals deprived of water for three days. Rates of excretion of ammonia, urea and urate were measured. None of the species excreted significant amounts of urate, and the proportions of urea and ammonia varied widely between species. Stream dwelling species from the Labi area were ammonotelic, both in and out of water. Terrestrial and arboreal species from Labi were also ammonotelic when kept in water, but increased the proportion of urea nitrogen when water deprived. All species from coastal and disturbed areas were ureotelic and most became even more so when kept out of water.

Rates of cutaneous water uptake following moderate dehydration also varied greatly between species. Aquatic species from Labi had maximal rates of water uptake below 7 ml/100 g/hr. Semi-terrestrial species from all areas exhibited elevated rates of water uptake (23-40 ml/100 g/hr), and two arboreal species had exceptionally high rates of rehydration (55-80 ml/100g/hr).

All of the species lost water by evaporation at the high rates typical of amphibians.

We thank R. Slater and K. Connor of the 22nd S.A.S. Regiment, and Mr. C. R. Netto for invaluable assistance in the collection of animals. We are also grateful to Captain W. Bristol and the crew of the ALPHA HELIX for logistical and moral support, and especially to Chief Engineer H. O'Grady for exceptional bravery in the field.

Blood Characteristics in the Lesser Mouse-Deer, *Tragulus javanicus*

G. K. Snyder, University of California, Riverside

The objective of this study was to determine the oxygen affinity, blood buffer capacity and selected hematological parameters of Chevrotain blood. Thirty ml of heparinized blood were obtained by cardiac puncture. Samples were simultaneously tonometered in an Astrup microtonometer, AMT (Radiometer, Copenhagen) with either nitrogen plus nitrogen or room air plus carbon dioxide at 37° C from Goddard gas mixing pumps (H. Wosthoff o H. G. Bochum, Germany). Blood oxygen affinity was determined by mixing selected ratios of the oxy- and deoxy-blood and recording the resultant mixed-blood PO_2 and pH in a blood micro system BMS3 (Radiometer, Copenhagen) in conjunction with a digital acid-base analyzer PHM72. Hemoglobin was assayed as cyanmethemoglobin (HYCEL) and red blood cells counted in an improved Neubauer hemacytometer.

At 36 mmHg P_{CO2}, P_{50} was 40.6 mmHg and pH 7.215. At 71.1 mmHg P_{CO2} P_{50} increased to 44.4 mmHg while pH fell to 7.162 and at P_{CO2} of 14.2 mmHg P_{50} fell to 36.1 mmHg and pH rose to 7.348. The Bohr effect, expressed at delta log P_{50}/delta pH, was -0.545, slightly low for a mammal of this body weight (1700 gms). The P_{50} at pH 7.4, 34.0 mmHg, compared closely with that predicted for this weight mammal, however, blood buffer capacity was considerably greater than that of human blood.

Blood hematocrit, 48.1% and hemoglobin concentration, 18.1 gm%, were somewhat higher than that of mammals in general, however, #RBC, 23.3 mill/micron^3 were extremely high. The calculated mean cell volume, 20.6 micron^3, was predictably low as also was the RBC dimensions however, the mean cell hemoglobin concentration, 37.6 gm%, is in the normal range for mammals. Both lung and heart

body weight ratios, 0.86 and 0.60 respectively, were also within the range predicted for a mammal of this size while RBC osmotic fragility, 50% hemolysis at 0.7% NaCl was slightly greater than for human cell suspensions. Blood viscosity increased exponentially as a function of blood hematocrit, the relative viscosity at the normal hct of this animal was 8.0. The calculated oxygen transport function of this blood increased with hct to approximately 45% and then declined. The observed hct, 48%, then, is consistent with the predicted optimum for oxygen transport.

I thank Captain W. Bristol and the crew of the ALPHA HELIX for their invaluable assistance during this study and special thanks to Mr. C. R. Netto and to Luta Pilang and other members of the Iban Longhouse at Amo B, Temburong and Captain T. Hampton of the Royal Brunei Armed Forces for their assistance in obtaining the mouse-deer.

Oxygen Affinity and Buffering Capacity of Fruit-Bat Blood

G. K. Snyder, University of California, Riverside

The objective of this study was to examine selected blood gas parameters, blood hematology and organ-body weight ratios in the common short-nosed fruit bat, *Cynopterus brahyotis*. Animals, obtained by mist netting, were anesthetized with pentothal, ip, and exsanguinated via cardiac puncture into heparinized syringes. Separate blood samples were simultaneously tonometered in an Astrup microtonometer, AMT (Radiometer, Copenhagen) at 37° C with either nitrogen plus carbon dioxide or room air plus carbon dioxide. In all cases selected gas mixtures were obtained with Goddard gas mixing pumps (H. Wosthoff o H.G., Bochum, Germany). Blood oxygen affinity was determined by mixing selected ratios of the oxy- and deoxy-blood and recording the mixed-blood PO_2 and pH with a Radiometer blood micro system, BMS 3 (Radiometer, Copenhagen) in conjunction with a digital acid-base analyzer, PHM72. Hemoglobin was assayed as cyanmethemoglobin (HYCEL) and red blood cell counts (#RBC) with an improved Neubauer hemacytometer. Blood phosphates were determined with a modified Fiske and Subbarrow technique.

At 36 mmHg P_{CO2} blood P_{50} was 47.2 mmHg and pH 7.224. At 71 mmHg P_{CO2} the P_{50} increased to 54.5 mmHg while pH dropped to 7.071 and at 10 mmHg 50_{CO2} the values were 27.2 mmHg and 7.685. The Bohr effect for the fruit bat blood, expressed as delta log P_{50}/delta pH, was -0.497, approximately 50% of the value predicted for a mammal of this BW (BW= 33.5 $\pm$ 1.1SE gms). Blood P_{50} at pH 7.4, 38.1 mmHg, was also low while blood buffer capacity was comparable to that observed in man.

Blood hematocrit (hct), 36.7 $\pm$ 1.3SE, and hemoglobin concentration, 11.9 $\pm$ 0.6SE were low compared to mammals in general, however, the red cell count,

10.5 $\pm$ 1.91SE was high. Thus, the calculated mean cell volume, 37.2 $\pm$ 2.4SE microns^3 was relatively low, while the mean cell hemoglobin concentration, 32.4 $\pm$ 0.7SE was normal. Blood 2, 3 DPG was 11.31 mg/100 ml blood. Lung and heart body weight ratios, 0.90 and 0.98 respectively, were very similar to that predicted for a mammal of this body weight.

I thank Capt. W. Bristol and the crew of the ALPHA HELIX for their support and assistance during the course of this study and Dr. B. McNab for the capture of the animals.

Respiratory Metabolism and Evaporative Water Loss In the Common House Gecko, *Hemidactylus frenatus*

G. K. Snyder, University of California, Riverside
W. W. Weathers, Rutgers University

Selected physiological parameters were determined as a function of body temperature. Oxygen consumption was determined in a closed chamber respirometer with built in thermobarometer. The system consisted of two jars connected with a U-tube manometer filled with a dilute Evan's blue solution which served as a marker. A syringe was coupled to the jar containing the animal making it possible to quantitatively replace the oxygen consumed by the animal. Carbon dioxide was absorbed with 10% NaOH in the animal chamber. A volume of water was placed in the thermobarometer to approximate the combined volume of the animal and NaOH in the animal chamber. Temperature was maintained $\pm$ 0.1° C by immersing the entire chamber in a water bath perfused by a Forma microtemp bath and circulator (Forma Scientific Inc., Marietta, Ohio). Evaporative water loss was determined by periodic weighing while the experimental animals were placed over drierite in a constant temperature and respiration counted visually with a stopwatch.

Oxygen consumption averaged 57 microliters oxygen $\cdot$ gm^{-1} $\cdot$ hr^{-1} at 20.5° C and increased to 330 microliters $\cdot$ gm^{-1} $\cdot$ hr^{-1} at 35°C. Over this temperature range Q_{10} = 3.3, extremely high when compared with temperatures which average from 1.6-2.5. Respiration was 20 cycles/min at 20.5°C and increased to 30 cycles/min at 27.5°C. Above this temperature, respiration remained essentially constant. Between 20 and 30°C the Q_{10} for respiration was 2.0. In other words, breathing rate accounted for the bulk of the increased respiratory gas exchange at the lower temperatures while at the higher ones gas exchange was augmented by some

combination of increased tidal volume and/or oxygen extraction. Evaporative water loss was 1.3 mg $\cdot$ gm^{-1} $\cdot$ hr^{-1} at 20.5°C and increased to 4.0 mg $\cdot$ gm^{-1} $\cdot$ hr^{-1} at 35°C. At 30°C, EWL averaged 3.4 mg $\cdot$ gm^{-1} $\cdot$ hr^{-1}.

We thank Capt. W. Bristol and the crew of the ALPHA HELIX for logistical and moral support, and Dr. B. McNab and Dr. F. Nordlie for assistance in capture of the animals.

Hemodynamics of the Mouse Deer, *Tragulus javanicus* (Osbeck)

W. W. Weathers, Rutgers University

The mouse deer (*Tragulus javanicus*) possesses the smallest known erythrocytes. In addition to their small size (1.5-2.1 microns in diameter) the red cells are spherical in shape. Spherical erythrocytes should result in both less efficient gas exchange and increased blood viscosity. That the shape of the erythrocytes may have some hemodynamic consequences is suggested by the fact that spherical red cells in the camel are associated with arterial pressures over 200 mmHg. To determine whether any hemodynamic effect could be ascribed to the oval shape of mouse deer erythrocytes several circulatory paramters were examined in one female deer, with additional measurements on a second female.

The procedure involved lightly anesthetizing the animals with sodium pentothal (25 mg/kg; I.P.) and cannulating the right jugular vein and carotid artery with PE 90 polyethylene tubing. Arterial pressure was determined with a mercury manometer, while heart rate was obtained by visually counting the pulsations in the carotid cannula. Cardiac output was determined by dye dilution with a Waters Corp. model X-250 densitometer. Hematocrit was determined by centrifuging blood in microhematocrit tubes until constant hematocrits were obtained (80 min). Plasma volume was determined by dye dilution with Evan's blue dye. Data for the two animals are summarized in table 1.

Table 1. Circulatory parameters of mouse deer

	Animal 1	Animal 2
Body weight, Kg	1.626	1.015
Body temperature, °C	38.0	37.5
Hematocrit ratio, %	31.4	31.0
Plasma volume, ml · $100g^{-1}$	4.10	---
Blood volume, ml · $100g^{-1}$	5.79	---
Mean arterial press., mmHg	121	87
Cardiac output, ml$(Kg \cdot min)^{-1}$	101	---
Heart rate, beats · min^{-1}	184	188
Stroke volume, ml · $beat^{-1}$	0.89	---
PRU_{100} mmHg · kg^{-1} · min^{-1}	11.98	---

With the exception of plasma and blood volume, none of the values is unusual for a mammal of this size, nor for ruminants as a group. Apparently, small spherical erythrocytes pose no special hemodynamic problems for the mouse deer.

Thanks are due to Dr. P. D. Sturkie for the loan of the densitometer, and to Mr. R. Netto for assistance in obtaining the animals.

Thermoregulation in the Dusky Munia (*Lonchura fuscans*)

W. W. Weathers, Rutgers University

Resting oxygen consumption, evaporative water loss and body temperature were determined as functions of ambient temperature in 8 dusky munias (mean mass 9.6g). Rates of oxygen consumption were determined using an open flow system with the aid of a Beckman paramagnetic oxygen analyzer (Model F 3; 1% scale). Rates of oxygen consumption of O_2 in air entering and leaving the metabolism chamber and from the rate of air flow through the chamber (corrected to STPD). The metabolism chamber consisted of a 600 ml can equipped with ports for air entry and exit. All determinations were made at night on birds which were resting quietly in the dark.

The zone of thermal neutrality extended from approximately 32 - 37°C. Within this range O_2 consumption averaged 2.25 ml O_2 $(g \cdot hr)^{-1}$ or 2.44 Kcal day^{-1}. Below the lower critical temperature oxygen consumption increased at a rate of 0.23 ml O_2 $(g \cdot hr \cdot °C)^{-1}$.

Below 30°C body temperature (measured with a Schulthesis quick-reading Hg thermometer) remained constant as air temperature varied, and averaged 37.2°C, which is low compared with the body temperature of most species of birds. Above 30°C body temperature varied directly with ambient temperature, and increased at a rate of 0.54°C per degree increase in air temperature.

The rate of evaporative water loss (determined gravimetrically by collecting the water vapor given off by the bird with Drierite) remained fairly constant below 35°C and averaged about 8.0 mg $(g \cdot hr)^{-1}$ or 19.2% of body mass per day. This rate is low when compared with that of similar sized birds. Above 35°C the rate of evaporative water loss increased dramatically, reaching a maximum

of 41.3 mg $(g \cdot hr)^{-1}$ at an ambient temperature of 43.8°C. With one exception, dusky munias did not sufficiently increase evaporation to maintain body temperature below ambient temperature when subjected to heat stress.

I thank Dr. B. McNab for use of his oxygen analyzer and associated equipment.

Oxygen Consumption of Three Species of Tropical Ranid Frogs

W. W. Weathers, Rutgers University
G. K. Snyder, University of California, Riverside

The relation of the resting rate of oxygen consumption ($\dot{V}O_2$) to ambient temperature was determined in three species of frogs of the family Ranidae from Northern Borneo. Oxygen consumption was determined at temperatures between 19.5-35.5°C in closed circuit respirometers in conjunction with a thermobarometer, on animals which had been resting in the dark at thermoequilibrium for at least 1 hr. Oxygen consumption was determined at 5-15 min intervals for 1-1 1/2 hrs, and VO_2 taken as the mean of the three lowest values. Values for $\dot{V}O_2$ were as follows:

Species	N	Wb g	Ta °C	$\dot{V}O_2$ 10^{-3} ml $(g \cdot hr)^{-1}$
Rana macrodon	3	88.0	20	30
			25	51
			30	87
			35	150
Rana signata	6	3.5	20	50
			25	85
			30	145
			35	255
Rana sp.	3	2.6	20	62
			25	105
			30	175
			35	300

The rate of change in $\dot{V}O_2$ with temperature thus corresponds to a Q_{10} of between 2.82-2.94, which is high when compared with the Q_{10} of many temperate zone amphibians.

In an additional series of experiments of the relation of $\dot{V}O_2$ to time of day was determined in *Rana signata*. $\dot{V}O_2$ was determined at 30°C at 15 min intervals for 26 consecutive hrs, on five frogs. Mean hourly rates of $\dot{V}O_2$ (microliters g^{-1} hr^{-1}) ranged from 143 $\pm$ 8.6 (mean $\pm$ se) to 180 $\pm$ 13.1. Plotted as a function of time of day $\dot{V}O_2$ followed a sine curve with peaks at 0800 and 1900 hrs, roughly the times of sunrise and sunset.

We thank Drs. V. Shoemaker and L. McClannahan for the animals.

Social Changes in Rural Brunei and Their Medically Related Consequences

S. G. Wolf and T. D. Wolf, Marine Biomedical Institute

Data were gathered fron nine jungle dwelling communities, including six Iban long houses, two Dusun villages and one Punan long house as well as from anthropologically sophisticated informants who had lived for extended periods with the tribes and from reports available in the library of the Brunei Museum.

The rapid introduction since 1962 of modern communications, road, radios and helicopters; modern medicine with the virtual abolition of vector borne infectious diseases; the sharp reduction of infant mortality and the almost universally available schooling, together with governmental pressures to embrace the Moslem religion, have posed important and difficult challenges for the tribes whose stable pattern of life has been established over many centruies. In the process of a remarkably successful adaptation to these changes the tribes have maintained an extremely cohesive and mutually supportive family structure and have clung tenaciously to their traditional animistic beliefs in spirits, omens and dreams, and their reliance on their Medicine Men, despite acceptance by many of the religion of Islam and widespread reliance on modern medical services. There appears to be at present an effective equilibrium and, associated with this, an essentially healthy state among the inhabitants of the villages. We found no evidence of systemic hypertention, myocardial infarction, rheumatoid arthritis, peptic ulcer or ulcerative colitis.

Tentative conclusions from this brief study are suggested by previous experience with Roseto, Pa., an exclusively Italian community in the United States, whose salubrious state of health lasted only as long as the Old World attitudes and traditions were maintained while adapting to American economic

and political patterns. A prediction is therefore ventured, that as the newly educated youth of rural Brunei grow up in a world alien to their unschooled parents, and as the anxiety-relieving powers of old traditions and practices are eroded, the chronic diseases of western society may make their appearance.

ALPHA HELIX SOUTHEAST ASIAN EXPEDITION

III Visayan Sea (Corals and Sea Snakes)

August 21 - October 2, 1975

R/V ALPHA HELIX

VISAYAN SEA EXPEDITION

August 21 - October 2, 1975

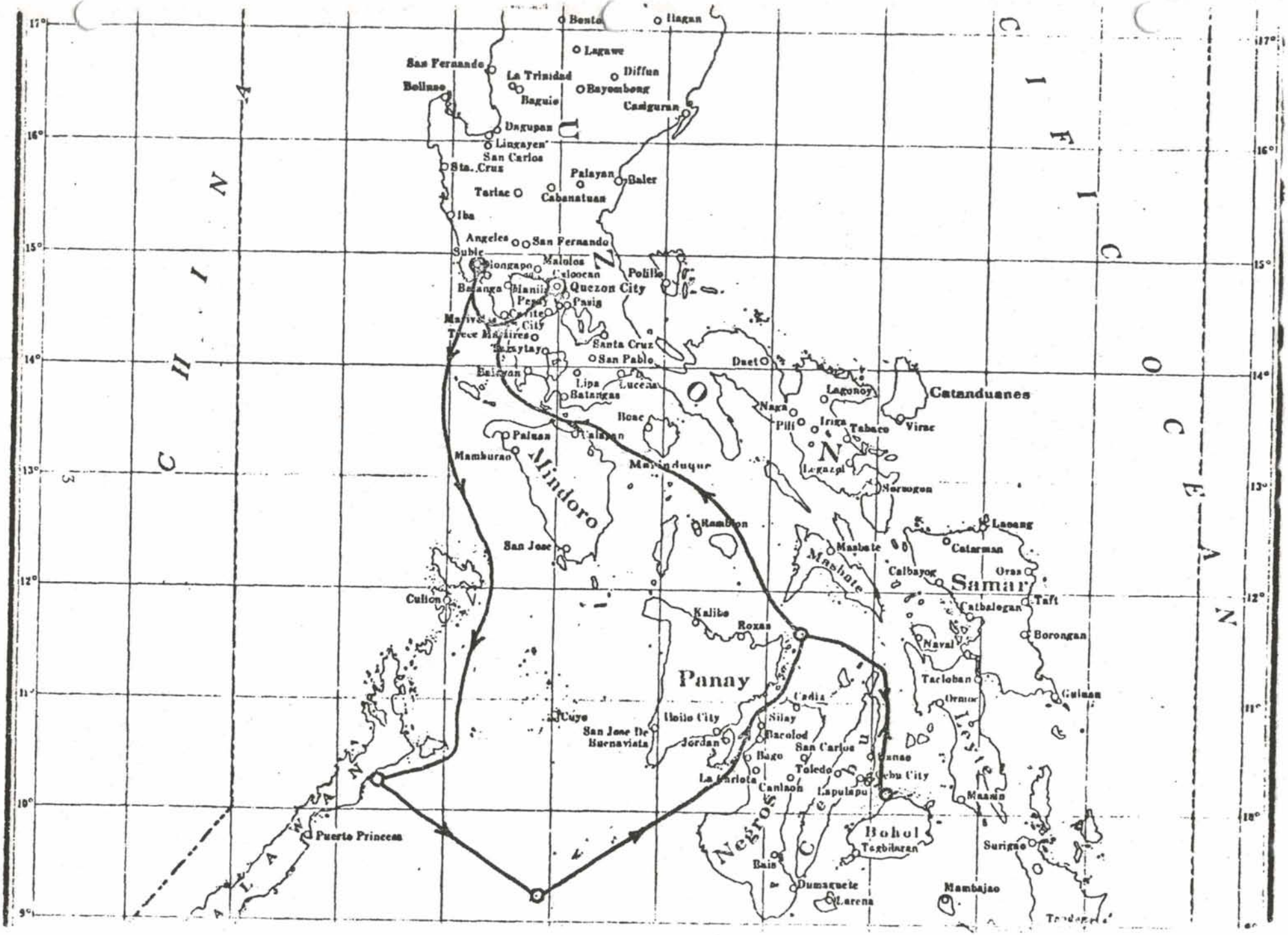
William A. Dunson, Chief Scientist
Pennsylvania State University

Our operations were carried out in the central Philippines, primarily in the Visayan and Sulu Seas, with the cruise track taking us from Manila to Subic Bay, to Green Island Bay of Palawan Island, across the Sulu Sea to the isolated and seldom visited Tubbataha Reefs, up the east coast of Panay to the Gigantes Islands, and across the Visayan Sea and down the east coast of Cebu to the extraordinary double barrier reefs along the north coast of Bohol. The major portion of our work was conducted at Green Island, at the Gigantes Islands and at Bohol. The high population density of the Philippines coupled with a thriving export trade in coral and shells and a past propensity for dynamite fishing had led us to expect rather poor conditions on the reefs. We were pleased to discover that many of the reefs were apparently healthy despite the intensive exploitative pressures on them. However, there is a desperate need to document the present state of reef development to determine if degenerative changes are occurring.

The abundance of experimental material, the fine weather, and the lack of previous work in Philippine waters were all factors leading to a most productive scientific effort. We are especially indebted to Tom Forhan, our marine technician, to Renato Garcia, a scientist from the Philippine National Museum, and to Capt. Coleman for invaluable assistance.

On occasion, previously planned studies were put aside temporarily when an unusual opportunity presented itself. For example, Dr. Karen Porter studied a most interesting series of pools at various levels in and above

the intertidal zone of Turnina Islet. Dr. Heatwole devoted a day to surveying the fauna of an isolated coral cay with thousands of nesting boobies on it. Drs. Minton and Tamiya worked on the little known venom of the mangrove snake which we found in large numbers in a Palawan swamp. Dr. Dunson performed some temperature and salinity tolerance tests on tadpoles found living in extremely hot coastal pools. The "virgin" state of studies on Philippine coral reefs is well illustrated by the discovery of Dr. Pichon of 10 genera of corals previously unrecorded from the Philippines. Dr. Hartman's sponge collections also contain numerous new records for the Philippines and many new species as well.


Benta
Ilagan
Lagawe
San Fernando
La Trinidad
Diffun
Bolinao
Bayombong
Baguio
Casiguran
Dagupan
Lingayen
San Carlos
Sta. Cruz
Palayan
Baler
Tarlac
Cabanatuan
Iba
Angeles
San Fernando
Subic
Olongapo
Malolos
Caloocan
Polillo
Balanga
Manila
Quezon City
Pasay
Parañaque
Cavite
City
Santa Cruz
San Pablo
Daet
Batangas
Lipa
Lucena
Lagonoy
Catanduanes
Naga
Boac
Pili
Iriga
Tabaco
Virac
Palauan
Mamburao
Mindoro
Mansanduque
Legazpi
Norsogon
Romblon
Laoang
Masbate
Catarman
Calbayog
Oras
Samar
Catbalogan
Taft
Borongan
Culion
Kalibo
Roxas
Naval
Panay
Tacloban
Ormoc
Guiuan
Cuyo
Iloilo City
Silay
San Jose De Buenavista
Jordan
Bacolod
San Carlos
Bago
Toledo
Cebu City
La Carlota
Canlaon
Lapulapu
Maasin
Puerto Princesa
Negros
Cebu
Bohol
Tagbilaran
Bais
Dumaguete
Larena
Surigao
Mambajao

SCIENTIFIC PARTICIPANTS

1. Dr. William A. Dunson, Department of Biology, Pennsylvania State University, University Park, PA 16802.
2. Mr. Renato Garcia, National Museum, Manila, Philippines.
3. Dr. Willard Hartman, Peabody Museum of Natural History, Yale University, New Haven, Conn. 06520.
4. Dr. Harold Heatwole, Department of Zoology, University of New England, Armidale, NSW 2351 Australia.
5. Dr. Sherman Minton, MD, Department of Microbiology, Indiana University Medical Center, Indianapolis, Indiana 46207.
6. Dr. Michel Pichon, Department of Marine Biology, James Cook University of N. Queensland, Post Office James Cook University, Qld. 4811 Australia.
7. Dr. James Porter, School of Natural Resources, University of Michigan, Ann Arbor, Mich. 48104.
8. Dr. Karen Porter, Department of Biology, University of Michigan, Ann Arbor, Mich. 48104.
9. Dr. Roger Seymour, Department of Zoology, Monash University, Clayton, Victoria 3168 Australia.
10. Dr. Nobuo Tamiya, Department of Chemistry, Tohoku University, Aobayama, Sendai, Japan.
11. Dr. Robert Trench, Department of Biology, Yale University, New Haven, Conn. 06520.

Extraordinary tolerance of some Philippine tadpoles to high temperature and salinity

William A. Dunson

In searching for snakes in a mangrove swamp I inadvertently waded through a shallow tidal pool on Luzon which contained large numbers of anuran tadpoles. In an estuarine area which was about 20% sea water this was in itself interesting. However, the temperature of the water was quite high as I noticed with my own dermal sensors. On returning at noontime with a thermometer I was amazed to find water temperatures of 41.8-42.0°C. Nighttime temperatures were about 30C. Since the highest previous reported field temperature for tadpole pools was about 39°C (in Australia), I felt that this situation definitely deserved further study. Two additional pools were briefly studied on Luzon and Bohol Islands. In both cases pool temperatures around noon were near 42°C. Thus it seems that tadpoles in shallow ponds are regularly exposed to these high temperatures for a period of perhaps 4 to 6 hours on cloudless days. Frogs caught in and near the ponds were tentatively keyed out to Rana moodiei. Tadpoles were preserved for later identification. A series of temperature and salinity tolerance tests were carried out on one batch of tadpoles from Subic Bay mangroves (Luzon). From their natural habitat of 20% sea water, tadpoles were transferred every six days to 40%, 60%, 80% and 100% sea water (at 22-25°C). Almost all survived through the 60% sea water, and one third remained healthy (feeding on spinach) after six days in 80% sea water. All died within three days after transfer to 100% sea water. All temperature tolerance tests were subsequently carried out in 20% sea water. Tadpoles were acclimated to room temperature (25-25°C) and to 35°C. Survival times at test temperatures of 40-45°C were considerably longer for the 35°C acclimated group. The mean survival time at 42°C

for this group was 42 hours, indicating that the tadpoles in the pools at 42 C were not moribund. Moreover survival at 40C was excellent even at 10 days. It seems likely that the upper incipient lethal temperature lies slightly above 40°C. It would be most interesting to acclimate tadpoles to 40°C and then test their tolerance, but the metamorphosis of my specimens precluded this. Although comparative data are limited, it appears that these tadpoles are among the most resistant anurans, if not the most resistant, to high temperatures and salinities.

Water balance in sea snakes

William A. Dunson

Recent studies have shown that the true sea snakes (family Hydrophiidae) and the marine file snake (family Acrochordidae) have posterior sublingual salt glands that secrete highly concentrated NaCl solutions into the tongue sheath. However in some sea snakes these glands are, for some unknown reason, very poorly developed. Apparently the skin and/or the kidney-gut complex assume a more important role in ion and water balance in these species. It is also possible that there is a relation between the extent of dermal respiration and the relative permeability of the skin to water and salts. The only sea snake previously studied, Pelamis, is essentially impermeable to Na and has a low permeability to water in comparison with fresh water snakes (Table 1). In Pelamis water efflux and influx are almost balanced, with the slight net loss being made up by feeding, not by drinking. In the survey of whole body water permeabilities conducted during the Visayan Sea Expedition a totally unexpected finding was that some sea snakes have very high water effluxes and influxes (Table 1). In fact in some cases effluxes are comparable with those of fresh water species which rapidly dehydrate and die in sea water. The sea snake H. ornatus however maintains a high rate of influx as well as efflux without drinking. Influxes of snakes with their heads held out of water or with the cloacal opening sutured shut, remained high, demonstrating that the skin is the main organ of exchange. It now appears that the skin of marine snakes is the major route for water exchange with the environment (the respiratory tract is an additional minor source of water loss). The remarkably high water efflux and influx in some hydrophids is exceptional. Most species have rates of exchange of 0.5 ml/100 g h and less. However the unknown mechanism by which the high rates of dermal influx are maintained in Hydrophis ornatus and H. inornatus is especially interesting and deserves further studv.

Table 1. Water exchange between reptiles and seawater.

Species/normal habitat	Mean efflux, ml/100 g h	Mean influx, ml/100 g h
A. Fresh water		
Queen snake, Regina	4.9	---
Water snake, Natrix sipedon	1.8	---
B. Estuarine		
Salt marsh snake, Natrix	0.5	---
File snake, Acrochordus	0.5	0.5
Dog-faced water snake, Cerberus	0.3	0.2
C. Marine-all sea snakes		
Laticauda (3 species)	0.2	0.2
[1] Pelamis	0.2	---
Lapemis	0.5	---
Hydrophis spiralis	0.6	0.5
Hydrophis cyanocinctus	0.7	0.7
Hydrophis ornatus	1.3	0.9
Hydrophis inornatus	1.8	1.2

[1] Dunson and Robinson (in press)

ASSOCIATIONS BETWEEN ALGAE AND SPONGES

W. D. Hartman

Perhaps as many as 10% of the sponges inhabiting shallow Philippine waters (to a depth of 2 to 3 meters) grow in association with algae. These associations appear to vary from facultative to obligatory and include the following general categories. (1) The surface of the sponge may be covered with a growth of filamentous algae usually attached to protruding spicules (e.g., Cinachyra, one of the most widely distributed genera of sponges in shallow tropical waters). (2) Filamentous green algae may be bound together by diaphanous films of sponge tissue to make up discrete and recognizable growth forms in which the oscules of the sponge are apparent at the surface (e.g., Sigmadocia is a common sponge genus entering into such relationships in which the algal component forms the predominant mass of the association). (3) Zooxanthellae or zoocyanellae (comprising unicellular or filamentous bluegreens) may occur intracellularly or extracellularly in sponges. It is the purpose of this work to examine the last mentioned associations in greater detail.

Zooxanthellae were found in association with only one sponge species, namely, Spirastrella vagabunda, in the areas visited. One of the three populations of this sponge studied lacked zooxanthellae. An attempt was made to find out if labeled photosynthetically fixed carbon is released by the algae to the sponge cells. Zooxanthellae were isolated from the sponge by homogenizing, filtering out large animal particles with gauze and centrifuging and washing the resulting suspension. Sedimented zooxanthellae (about 0.1 ml wet-packed volume) were suspended in 2 ml of sea water (without and with the addition of DCMU to provide a dark control) or in host tissue homogenate. To each sample 100 μ Ci ml^{-1} $NaH^{14}CO_3$ was added, and the samples

were incubated for one hour in sunlight or under fluorescent lights in the laboratory. Processed samples were read in a Beckman liquid scintillation system or were studied with two-dimensional paper chromatography to identify the photosynthetic products released. The latter studies will be completed at a later date. Results of the first method of analysis are given in Table 1 which reveals a low but significant percentage of photsynthetic products released to the medium in thc presence of host homogenate. These values are to be compared, however, with 20 to 50% release of soluble ^{14}C compounds by various marine cnidarians studied by authors. Previous work indicates that only some of the zooxanthellae in sponges are intracellular whereas all are intracellular in cnidarians. Perhaps this is a factor in explaining the different values in the two phyla. Material for light microscopic autoradiography is available for future studies of the incorporation of ^{14}C compounds into animal cells. Studies of the ultrastructure of the algae within their host sponge will be undertaken at a later date.

Unicellular bluegreens have been found in at least six species of Philippine shallow water sponges but only one, a species of Dysidea, proved to contain both unicellular and filamentous bluegreens. This species was studied by methods identical to those described above. Results are shown in Table 1. Experiment 1 differs from the remaining three for unknown reasons. Varying amounts of lysis of the algal cells were apparent in experiments 2 through 4, but time was not available to analyze the causes of the phenomenon. If experiment 1 is valid, then bluegreens release more fixed carbon to the host Dysidea than is true of the spongicolous zooxanthellae studied here. Material for autoradiographic study of the incorporation of ^{14}C compounds into animal cells as well as fpr ultrastructural studies of the symbiosis are available.

Table 1. Relative amounts of soluble ^{14}C compounds released by symbiotic algae from sponges.

	SEA WATER ONLY		WITH HOST HOMOGENATE		DARK FIXATION (DCMU)
Expt. No.	Total ^{14}C fixed (CPM)	% released to medium	Total ^{14}C fixed (CPM)	% released to medium	% of photo-synthesis
Spirastrella vagabunda					
1	7.6×10^5	1.8	5.7×10^5	7.0	8
2	8.0×10^5	1.6	4.7×10^5	6.4	10
3	7.1×10^5	2.4	6.1×10^5	7.0	6
Dysidea sp.					
1	4.4×10^5	2.1	1.1×10^5	12.3	2
2	0.7×10^5	7.8	0.3×10^5	4.2	5
3	2.2×10^5	5.1	1.0×10^5	4.3	4
4	4.5×10^5	4.1	2.9×10^5	4.3	3

OBSERVATIONS ON THE PALAWAN MANGROVE SNAKE

Boiga dendrophila multicincta

S. A. Minton

Twenty-three specimens of *Boiga dendrophila multicincta* were collected in a mangrove swamp near the mouth of the Barbicon River, northeast Palawan. All were basking on vegetation at heights of 0.5 to 5 m and usually remained motionless until touched. Temperatures of 6 individuals were close to that of the ambient air. Diurnal basking presumably alternates with nocturnal activity; this was partially confirmed by observation of captive snakes. All but one individual were about 1.2-1.4 m in length and presumably adult. Three females were gravid and deposited clutches of 5, 5, and 6 eggs 12, 16, and 23 days after capture. Two snakes' stomachs contained rodent remains indicating prey about the size of subadult *Rattus rattus*. The presence of cloudy eyes indicated more than half the snakes were preparing to shed their skin. The snakes bit readily as a defensive reaction; no evidence of envenomation accompanied bites inflicted on members of the expedition.

TERRESTRIAL BIOTA OF NORTH ISLET, TUBBATAHA REEF

Harold Heatwole

North Islet is a small (300 x 100 m) sand cay located nearly in the center of the Sulu Sea. Despite its small size and its remoteness it has a rather large terrestrial biota. One species of vascular plant is present, a low, succulent species. Herbivores consist of two species of bugs. The majority of species depend on the carrion and guano of the numerous nesting gannets and upon marine-derived carrion (dermestid beetles, earwigs, three species of flies). Four species (collembola, silverfish, terrestrial isopods, and earthworms) are feeders on organic soil, detritus, or the fungi growing on detritus. There were two species of predators, a spider and a centipede. By far the most abundant species was an omnivorous ant. Terrestrial vertebrates were represented by one species of finch (2 individuals only) and a swallow (one individual).

CARDIAC RESPONSES TO TEMPERATURE IN SEA KRAITS

Harold Heatwole

It was previously reported that the sea krait, Laticauda colubrina in an aquatic situation undergoes marked increases in the heart rate during periods of breathing. Since this species is amphibious, resting much of the day in rock crevices above high tide, it was of interest to measure its heart rate out of water at a series of temperatures. In contrast to the aquatic situation, heart rate at a given constant temperature remained rather uniform and showed only a slight breathing tachycardia.

Q_{10}'s were high, being slightly over 3 in the temperature ranges below 25° and slightly under 3 in those above 25°.

When temperature changed steadily, there were marked adjustments in heart rate. Snakes being warmed from cold narcosis elevated their heart rate precipitously when they reached about 24°C and it remained high until body temperature reached about 28°C whereupon it decreased sharply and then rose gradually with increasing temperature. The accelerated heart rates in the 24-28°C range would facilitate heart transport in a cold snake in a warming environment. Field temperature data indicate this species normally operates at a temperature of 27-30°C. Similar results were obtained on Laticauda semifasciata. The effect was very slight in the tree snake, Boiga dendrophila multicincta.

In L. colubrina, mean heart rate at 20°C was 14 beats per minute; in an aquatic situation this species has a breathing heart rate of 29 bpm and an apneic one of about 10. Thus the terrestrial heart rate is closer to apneic heart rate than to the breathing heart rate and supports a previous contention that this species in water does not undergo true diving bradycardia but rather displays breathing tachycardia.

BREATHING RHYTHM OF THE HOMALOPSINE SNAKE, CERBERUS RHYNCHOPS AND THE SEA KRAIT, LATICAUDA COLUBRINA

Harold Heatwole

In contrast to true sea snakes and file snakes which have prolonged periods of apnea between breaths regardless of whether they are submerged or at the surface, Cerberus and Laticauda alter breathing rhythm in these two situations. When diving they have the typical sea snake pattern mentioned above but when floating at the surface or on land, they adopt the more regularly spaced breathing pattern typical of land snakes. In the sea krait, breathing rate is rather insensitive to temperature within the range normally encountered by the snakes in nature. At 25-30° C voluntary submergence times of inactive Cerberus average 25-35 minutes (depending on the individual) and are independent of ambient levels of oxygen and CO_2 as indicated by experiments in which nitrogen and CO_2 were bubbled through the water in which the snakes were kept. Submergence time is greatly decreased by activity.

HEAT TOLERANCE OF MARINE SNAKES

Harold Heatwole

Nine species of marine snakes were heated in sea water at a rate of about 1°C per 15 minutes until they died. The upper lethal limit was remarkably uniform not only within species, but among species. Three species of sea kraits had mean values of 39.7° (Laticauda colubrina), 39.7° (Laticauda semifasciata) and 39.3° (Laticauda laticaudata); sea snakes were 39.0° (Astrotia stokesii), 39.8° (Pelamis platurus) 39.5 (Hydrophis ornatus) and 37.8° (Lapemis hardwickii). The homalopsine snake, Cerberus rhynchops, had a mean value of 39.9°C. With the exception of the low value for Lapemis, none of the above species-differences were statistically significant By contrast the file snake, Acrochordus granulatus was significantly more heat tolerant (41.7°C).

RESISTANCE OF SOME AMPHIBIANS AND REPTILES TO SEA SNAKE VENOMS

Sherman A. Minton

Tamiya (abstract, this expedition) has shown that sea snakes (Laticauda colubrina and Hydrophis ornatus) and some colubrid snakes (Boiga dendrophila and Cerberus rhynchops) have extremely high resistance to sea snake venoms. In an effort to elucidate the mechanism of this immunity, mixtures of recently collected snake serum and muscle were incubated with dilutions of fresh venom and injected intramuscularly into mice. Results of one such experiment are shown in Table 1.

Venom and Dose	Incubation with 30 m, 37°	Result Deaths/Animals injected
Laticauda colubrina	Laticauda colubrina	0/3
2.3 mouse LD_{50}	Serum	
same	Fresh Human Serum	1/3
same	Normal Saline	3/3
none	Laticauda colubrina	0/3
	Serum	

Experiments with higher doses of L. colubrina venom and with Hydrophis ornatus venom resulted in death of all venom injected animals, although survival time was prolonged in animals receiving mixtures of snake serum and venom. Incubati of snake venom solutions with minced snake muscle (Laticauda semifasciata and Cerberus rhynchops) for 60 min. at 35° resulted in no neutralization of the venom. Evidently factors in serum and crude muscle preparations play little role in the resistance of snakes to sea snake venoms.

Frogs (Rana sp.) weighing approximately 20 gm survived 5 mouse LD_{50} of Laticauda colubrina and Hydrophis ornatus venoms injected into the dorsal

lymph sac but succumbed to 10 LD_{50}. Geckos (Cosymbotus platyurus) weighing 2.1 to 4.3 gm succumbed to these sea snake venoms at doses of approximately 0.1 mouse LD_{50} for H. ornatus and 0.05 for L. colubrina by intramuscular injection. Young Gekko gekko of approximately the same weight of Cosymbotus adults survived these doses, but too few were available to determine the limits of their tolerance. Two G. gekko weighing 16 and 18 gm died within an hour following intramuscular injection of 5 mouse LD_{50} of H. ornatus venom. In both lizards and frogs, generalized flaccid paralysis is seen prior to death, and respiration ceases well before heartbeat. In frogs the paralysis may be preceded by a period of hyperactivity. The susceptibility of small geckos to sea snake venom is as great or greater than that of mice, hence high resistance to these toxins is evidently not a universal characteristic of squamate reptiles.

SALINITY TOLERANCES OF INSULAR LIZARDS

Sherman Minton, Harold Heatwole and William Dunson

Several species of widespread lizards, commonly found on rather isolated islands and which have been suspected of being flotsam-transported were tested for their tolerance to salinity conditions likely to be experienced during such voyages. Lizards were individually placed on seawater soaked absorbent cotton pads and periodically checked for survival. Survival time for a given species was longer for larger animals than for small ones and longer for intact animals than for those with skin injuries or broken or regenerating tails. Intact adults of the geckos Hemidactylus frenatus and Lepidodactylus cf lugubris survive 30-50 hrs in comparison to 6 1/2 hrs for a rainforest species (Cyrtodactylus phillipinicus). A skink (Emoia atrocostatum) inhabiting mangroves, beaches and other saline habitats survived 115-380 hrs under the conditions described above and 39-99 hrs when submerged in sea water except for the head. Although the putative flotsam dispersed species are unusually salt tolerant, the noteworthy result of the experiment was that the geckos would be able to tolerate only a short trip if exposed to salt water. Transport via canoes or other human agency may have been more important than flotsam in distributing these species to remote islands. One H. frenatus was observed in the prow of a small local boat returning from an offshore island to the mainland.

Distribution, diversity, and food habits of Philippine sea snakes

Sherman Minton and William Dunson

During the Visayan Sea Expedition approximately five weeks were spent in the Sulu, Visayan and Camotes Seas. More than 1000 sea snakes were purchased from fishermen or collected by night-lighting or diving. About 200 specimens were preserved for shipment to the Field Museum of Natural History at Chicago for identification by Dr. H. Voris. Five hundred snakes were used in various experiments or shipped back to laboratories and the remainder were released. There are reliable reports of approximately a dozen species of true sea snakes (Hydrophiids) in the Philippines. Of these we caught examples of ten species. With the addition of a new record for the area, Astrotia stokesii, the total observed diversity was 11 species. There were also two marine snakes of different families, Acrochordus granulatus (Acrochordidae) and Cerberus rhynchops (Colubridae) that were abundant in certain areas. There are certainly a few additional species present which were not collected, especially from mud bottom habitats which were not sampled extensively. Although overall sea snake diversity is lower in the Philippines than in Indonesia or certain parts of Northern Australia, certain species are locally abundant. Hydrophis ornatus is the commonest snake off NE Panay. For example, one night's sample of 198 snakes from dip netting under lights at the Gigantes Islands was 63% H. ornatus, with the remainder divided among seven other species. Two other species were caught at other times in this area; thus 10 of the 11 total species were found in the immediate vicinity of the Gigantes Islands. This is quite a respectable diversity even in comparison with Ashmore Reef (Australia) where 11 species are coexisting in one localized area. In fact it is doubtful whether a greater diversity than this is ever found within a circular area of a diameter

approaching 5 kilometers. Some large catches of snakes caught by divers in 6 to 10 fathoms were almost entirely Laticauda semifasciata. The Visayan Sea is known to be a favorite haunt of this species which is found in enormous numbers breeding at Gato Island north of Cebu. No true sea snakes were caught in the Sulu Sea and few were present along the north coast of Bohol (Camotes Sea). The shallow, productive Visayan Sea is an exceptionally good habitat for sea snakes. The 6-10 fathom shoal areas are especially productive. Stomach contents were collected from each species for subsequent identification. All species fed only on fish and considerable specialization was evident. Laticauda colubrina feeds only on several species of eels, whereas L. semi-fasciata disgorged eels and other types of fish. An astounding discovery was the specialized feeding of H. ornatus on a single species of poisonous plotosid catfish, and nothing else. A surprising number of healthy specimens of this species had catfish spines protruding from their bodies.

A NOTE ON THE VENOM OF THE PALAWAN MANGROVE SNAKE, Boiga dendrophila multicincta

S. A. Minton and N. Tamiya

Venom was obtained from several specimens of Boiga dendrophila multicincta by collecting directly from the fangs in capillary tubes. Quantities were very small, averaging 0.006 ml per snake. The venom was colorless and viscid. It was immediately diluted 1-1000 with saline and stored in the refrigerator. Two mice injected intramuscularly with 0.2 ml of this dilution died; one of 4 mice injected with 0.1 ml died. Hemorrhages remote from the site of injection were noted in these animals, and their blood did not clot after death. There was no hemorrhage or necrosis at the site of injection.

Venom in a dilution of $1\text{-}10^{-4}$ increased coagulation time of freshly drawn human blood from 5 min. to 12 min. (Lee-White Method); in a dilution of $1\text{-}10^{-5}$ there was an increase to 8 min. Clots were abnormally soft and friable. One of three mice injected with 0.1 ml of dilute venom solution showed prolongation of clotting time from 2 min. to 7 min. 5 hr. after injection, this returned to normal 3.5 hr. later. One animal died 4 1/4 hr. after injection; coagulation time was normal 20 min. before death. The third mouse survived and showed no prolongation of clotting time.

A survey of the reef coral fauna, and observations on coral reef morphology and ecology in the Philippine islands.

Michel Pichon

During the Alpha Helix "Visayan Sea" expedition, the following coral reefs of the Philippine islands have been investigated: Capones island (Luzon), Green island (Palawan), Tubbataha (Sulu sea), Gigante island (Panay), Danajon Bank (Bohol). Observations and data have been gathered on these reefs from the following standpoints: hermatypic coral fauna, reef morphology and reef ecology.

I. Reef coral fauna

In each of the above mentioned localities, a sample of the hermatypic coral fauna was collected, but due to the special circumstances of the expedition, collections were reduced to the necessary minimum. Specimens of the species collected have been presented to the National Museum of the Philippines, Manila.

From a generic standpoint, all genera mentioned by Nemenzo (1955-1961), but one (Physophyllia) were found, and in addition, ten hermatypic genera, representing new records for the Philippine fauna were collected. These include: Montastrea, Physogyra, Oulophyllia, Cynarina, Acanthastrea, Diaseris, Scapophyllia, Pseudosiderastrea, Madracis, Parascolymia. When taking into account these new additions, the Philippine scleractinian hermatypic fauna now comprises 66 genera. Biogeographical comparisons with other areas of the western pacific region remain difficult for the various localities have been inequally sampled. As it stands at present, it appears that generic diversity of hermatypic scleractinians in the Philippines remains slightly lower than that of the indonesian archipelago and that of the Great Barrier Reef Province.

II. Reef morphology

The reefs investigated during the cruise represent different morphological reef types:

-Gigante islands: From reef coral communities without reef construction, to small fringing reef without boat channel.

-Capones island: small fringing reef on the lee of a volcanic island.

-Green island: platform reef with vegetated and intertidal sand cays - The reef is differentiated into a windward (S.E.) and leeward (NW) side.

-North Tubbataha: atoll shaped reef isolated in the Sulu sea, with a relatively shallow (20 m) central lagoon, and several sand cays, intertidal or permanently emerged on the reef flat. The outer slope is characterized by a coral reef platform sloping gently to 10-12m, and by a vertical drop-off, down to at least 60 m.

-Danajon Bank, North Bohol: Reef system composed of a double barrier, an extremely rare feature - Between the shore and the inner barrier depths of 27m are found, and the depth of the elongated lagoon or channel between the inner barrier and the outer barrier is about 25 m on an average - Both reefs are characterized by the width of the reef flat, which varies between 1 and 2 nautical miles.

Coral communities are mostly developed on the slopes, as well as on the outer part of the outer barrier. In other parts of the reef flat, sediment deposits are dominant.

III. Reef ecology

Most of the reefs investigated are relatively or well protected from intense hydrodynamic actions (no heavy swell of oceanic origin), and the gradient of zonation related to this particular factor is weak or not apparent. Similarly, the conditions of sea water transparency were poor, on an average, low illumination intensities being reached at moderate depths. As a consequence, the vertical zonation is condensed in a comparatively small depth range. The sedimentation of fine particles appears to be a dominant environmental factor: Sand or muddy sand deposit (mostly of skeletal origin) are present in most biotopes and often prevent a lush growth of scleractinian corals. On the contrary, seagrass communiti are well developed (particularly on the reef flats), the dominant species, being Enhalus acoroides and Thalassia hemprichi.

-The fringing reefs do not display, as it is often the case with this type of reef, a clearly marked zonation, and community structur and composition can be demonstrated by quantitative methods (quadrats or linear transects).

-On Green Island Reef, the main features of benthic reef zonation are in accordance with the general model suggested by Pichon (1973) for Indo Pacific coral reefs, and can be summarized as follows:

A. Frontorecifal (fore-reef) ensemble

1. Outer slope coral communities
2. Outer reef flat, without sediment deposit

B. Epirecifal (epi-reef) ensemble

1. Boulder tract
2. Secondary coral communities of the inner reef flat (with an abundance of the brown alga Turbinaria.

3. Sedimentary deposits of the inner reef flat, including:

a. Sea-grass beds

b. Sargassum zone (on dead coral blocks)

c. Sediment accumulations, including sand cays.

C. Postrecifal (backreef) ensemble

1. Coral communities of the inner slope

2. Lagoon floor and lagoon reef formations.

-The situation in Danajon bank is more complex, due to the existence of a double barrier, and probably to the geological history of the area. (Marine limestone outcrops are found on both the inner and the outer barrier). It appears that at present, the processes of cementation and reef construction are extremely slow, if not nil. This can be explained by the local environmental conditions above-mentioned: low intensity of hydrodynamic factors, rate of sedimentation. The reef flat of the inner barrier, as a whole, can be considered as an enclave of the "Epirecifal Ensemble" into the Postrecifal ensemble. It can tentatively be suggested that the reef flat of the inner barrier corresponds to the series of biotopes 3-c (see above) of the Epirecifal Ensemble.

BIBLIOGRAPHY

Faustino, L. A. (1927) Recent Madreporaria of the Philippine islands. Monogr. 22. Bur. of Science, Manila

Nemenzo, F. (1955-1967) Systematic studies on Philippine shallow water Scleractinians Nat. & applied. Science Bull. Univ. Philippines.

Pichon, M. (1973) Recherches sur les peuplements à dominance d'Anthozoaires dans les recifs coralliens de Iulear (Madagascar) Arch. originales CNRS n° 9250.

Abundance and Diurnal Movement of Demersal Zooplankton on Philippine Coral Reefs

K. Porter, University of Michigan

Previous studies of tropical marine zooplankton sampled open water populations using nets towed from research vessels. Such studies in near shore waters detected zooplankton associated with coral reefs and lagoons. Recent studies in the Caribbean Sea (Porter, 1973) sampled the plankton directly adjacent to the reef face using diver-pushed tow nets. An association of zooplankton which spent the day living in the interstices of the reef and migrating into the water column during the night (demersal plankton) was identified. This reef-generated plankton constitutes a major food source for reef invertebrates and planktivorous fish. In this study the demersal plankton of three Philippine reefs were quantitatively sampled using vertical migration traps designed to continuously sample the plankton rising from the reef surface.

Each trap consisted of a square, PVC frame tethered to the reef. Each frame was divided into 0.37 m^2 quarters covered by 1.5 m high cones of 6 mil polyethylene sheeting. Cones were topped with 2 l polyethylene bottles filled with just enough air to keep the cones and bottles suspended vertically above the reef face. The bottles were entered via 3 cm D plastic tubes which ended 3/4 of the way into the bottles. Vertically migrating zooplankton entered the bottles, dispersed and were trapped. Plankton remaining in the cone were also collected by squeezing the water in the cone up into the collecting bottle. A hole in the bottle covered with 64 μ Nitex netting allowed the excess water to flow through the bottle while retaining the zooplankton. Traps were placed in 30 feet of water over various substrate types. Bottles were collected at 10:00 and 16:00 and more frequently on several occasions. Samples were preserved in 10% buffered

formaldehyde and a crude but nondestructive quantification of the samples was made by measuring the volume of settled plankton. Samples were taken to the University of Michigan for species identification and enumeration. The trap samples will be compared on the basis of substrate type and their composition will be related to nonquantitative net hauls and light trap samples also taken during the course of this study.

Table 1 shows the diurnal pattern of emergence with 3-6 times more plankton rising from the reef at night than during the midday hours. Daytime samples contain primarily open water copepods and true plankton that entered the trap. Night samples contain a mixture of large invertebrates commonly associated with the benthos during the day. Broad expanses of reef generate more demersal plankton than do patch reefs or areas with low coral cover. Large differences exist between reefs.

Table 1. Emergence Rates of Demersal Plankton From Reef Areas Covered by Mixed Species of Encrusting Corals. Zooplankton Production is Expressed as the Settling Volume of Zooplankton Rising from a Square Meter of Reef Surface in an Hour (cc m^{-2} hr^{-1})

Location	Midday Period	Dusk-Night-Dawn Period
Green Island, Palawan		
patch reef	0.12 ± 0.02 (8)*	0.39 ± 0.03 (15)
reef face	0.09 ± 0.01 (14)	0.54 ± 0.14 (15)
North Gigantes Island, Panay		
western reef face	0.09 ± 0.01 (16)	0.60 ± 0.07 (15)
Bohol Island		
outer barrier reef face	0.09 ± 0.01 (4)	0.34 ± 0.07 (3)

*$\bar{x} \pm$ S.E. (n)

Community Structure in Tropical Island Splash Pools

K. Porter, University of Michigan

A series of pools are formed during the rainy season on basalt steps on the eastern shore of Turnina Island, western Visayan Sea. Some pools are temporary, being innundated at high tide or drying if daily rains do not occur. Salinities of permanent pools vary from brackish near the sea to fresh on the higher steps. Pools containing rafted debris such as coconut husks, seed pods, leaves and twigs have brown water and a detritus covered bottom. Those without debris are clear with a green algal floc on the bottom. In all pools, temperatures vary diurnally from 26 to 35° C. Oxygen is saturated at midday and near depletion (1.0 ppm) before daybreak, pH ranges from 7.6 to 8.9 in the pools.

Faunae consist of ostracods, mosquito larvae and pupae, and dragonfly nymphs. This depauperate fauna is probably the result of the seasonal nature of the ponds and the distance from the mainland. Distributions are summarized in Table 1.

Table 1. Physical, Chemical and Biological Aspects of Tropical Splash Pools on the Eastern Shore of Turnina Island*

Pool	Salinity	Permanence	Debris	Color	Fauna	Benthos
1	F	P	C	C	O,Dr	A
2	F	P	C	C	O,M	A
3	S	P	D	B	M	D
4	F	P	D	B	M	D
5	F	P	C	C	O,Dr	A
6	S	T	D	C	--	-
7	F	T	C	C	O	A
8	F	T	C	C	O,M**	A

*S = salinity above 10%; F = salinity below 10%; T = temporary; P= permanent on a seasonal basis; C = clear; D = detritus; B = brown water; O = ostracods; Dr = dragonfly nymphs; M = mosquito larvae and pupae; A = green algae.

** Mosquito larvae do not survive a drying up of the pool. Ostracods can survive dessication.

To determine the factors causing the distribution of faunae in the pools a series of survivorship studies were conducted using animals and water from pools 1, 2, 3, and 4. Food webs were outlined using gut analyses and *in situ* and laboratory experiments.

Temperature of 5, 10, 20, 25, 30, 35, 40 and 45 and artificial salinities of distilled water, 1, 5, 10, 20 and 35% were varied in combination. Survivorship and fecundity was also determined in combinations of water and benthic food from each pond. Ostracods and mosquitoes were tested. Ostracod mean survival times were less than 24 hours in ponds and artificial salinities of 10% or more. Distributions are therefore strongly determined by salinity. Food quality is also important in that although ostracods could survive in all nonbrackish waters, green algae are required as a food source for reproduction. Permanent ostracod populations are therefore restricted from nonbrackish pools.

Factors regulating green algal distribution were determined by measuring rates of ^{14}C - carbon fixation of the whole benthic community in the various pool waters and in artificial salinities. Photosynthesis was reduced by brakish water and by brown water. This indicates that brown water inhibits photosynthesis of green algae and that the inhibitory effect is not pH dependent. Janzen (1974)

suggests that brown waters are toxic due to the leaching of secondary plant compounds from plant materials in the waters.

Mosquito larvae survived to pupation and emergence in all salinities, pool waters, and food types. Dragonfly nymphs were not tolerant of brackish water. The absence of mosquitoes from some fresh pools was due to the heavy predation of nymphs on the larvae. This was confirmed by the introduction of nymphs from mainland pools into pool 2 with a subsequent decline in numbers of mosquito larvae.

Salinity and water quality therefore play a role in green algal distributions. Salinity and food quality (the presence of green algae) in turn determines the distribution of ostracods. Predatory dragonfly nymphs exclude mosquito larvae from the Turnina pools that are otherwise suitable for their survival. The presence of nymphs in only some of the fresh pools is probably due to the chance visit of egg laying females. The exclusion of dragonfly nymphs from brackish water provides a refuge for the mosquito larvae in those brackish pools. Temporary pools which dry up on occasion during the rainy season are subject to chance colonization by mosquito larvae. Only the ostracods, however, can survive the drying up period.

This study illustrates the importance of physical, chemical and biological and stochastic factors in determining the community structure of aquatic communities.

Social Facilitation of Feeding in Three Size Classes of the Striped Catfish *Plotosus anguilaris* (Bloch)

K. Porter, University of Michigan

Five schools of 30-50 uniform sized juvenile Striped Catfish, *Plotosus anguilaris* (Bloch) were observed feeding at Green Island, western Sulu Sea; Bohol outer barrier reef, Southern Camotes Sea; and Turnina Island, western Visayan Sea. Estimated average individual body length in the respective schools was 1.5, 1.5, 3.0 and 5.0 cm. Feeding sites included sand, coral rubble and reef with 30-50% living coral cover. Fish fed in a tail-up position, browsing on the substrate. Browsing schools moved across the substrate with a leading group of individuals which directed school movements into areas where intensive feeding activity occurred. Leading individuals were periodically replaced, in a leap-frog fashion, by groups of individuals from the rear. Individuals maintained no permanent positions in the school. Similar feeding behavior has been described for ground feeding bird flocks and represents a social facilitation of feeding in which the costs and benefits of leading the group in a food search are shared by all members of the goup. The uniform size of individuals in each plotosid school and the breeding habits of the species suggest that members of each school are siblings. Schooling behavior is maintained throughout adulthood.

Schools do not react to the presence of a diver by changing course, dispersing or clumping. Schools pass around and regroup behind a hand that is passed through them. Movements of the school, therefore, do not seem to be primarily related to escape from predators. However, schooling itself may have antipredatory significance. Individual *P. anguilaris* possess venemous pectoral spines reported to inflict serious wounds (Marshall 1964). They also have distinctive black and white

stripes typical of warning coloration. Schools may be easily recognized and avoided by a predator that has experienced ill effects from ingesting an individual or individuals from a school.

The Effect of Dynamite Fishing on Demersal Zooplankton Production of Philippine Coral Reefs

K. Porter and J. Porter, University of Michigan

Dynamite fishing is practiced extensively in the Visayan and Camotes Seas. Its effect on the zooplanktonic component of the reef ecosystem was examined by sampling demersal plankton abundance in blasted and undisturbed areas at the Bohol Island outer barrier reef.

Plankton migration traps were placed in 30 feet of water over 1) a dense 1.5 m high stand of branching *Acropora cuneata*, 2) mixed encrusting coral species, 3) dynamite blasted rubble of *Anacropora* and 4) natural coral sand adjacent to the reef. Traps were collected at 10:00 and 16:00 and the settling volumes of plankton collected were determined.

Plankton emerged from the reef primarily during the period including the dark hours (Table 1). Sand patches and blasted rubble generated less demersal plankton that did living coral areas. Branching, three-dimensional coral generated more demersal plankton than did encrusting coral.

Previous studies (Porter 1973) showed that the bottom dwelling plankton that migrates into the water column at dusk and returns to the reef at dawn (demersal plankton) constitutes the major source of food for corals and planktivorous reef fish. This study shows that dynamite fishing as used in the Philippines, or any activity that reduces the living coral cover and the three-dimensionality of the reef, reduces the demersal plankton available for the growth and regeneration of the reef ecosystem.

Table 1. Emergence Rates of Demersal Zooplankton Expressed as Settling Volumes of Zooplankton (cc m^{-2} hr^{-1})

Substrate	Midday Period	Dusk-Night-Dawn Period
(1) *Acropora cuneata*	0.14 ± 0.01 (4)	0.68 ± 0.06 (4)
(2) plating coral	0.09 ± 0.01 (4)	0.34 ± 0.07 (3)
(3) dynamited rubble	0.08 ± 0.02 (8)	0.24 ± 0.01 (8)
(4) sand	0.05 ± 0.01 (8)	0.20 ± 0.05 (8)

* $\bar{x} \pm$ S.E. (n)

INTERSPECIFIC SPACE COMPETITION AMONG INDO-PACIFIC REEF CORALS

James W. Porter

Of sixty-six scleractinian coral genera observed in the Philippines, species from sixteen of these genera scattered in nine families were tested for extra-coelenteric destruction of each other's tissue to determine a competitive hierarchy. In striking contrast to Caribbean species, all of which are arranged in a linear, fixed, and uni-directional "pecking order," interactions between touching Pacific corals are much reduced, with only five or possibly six of the 120 possible non-repetitive combinations of the above sixteen species showing strong interaction within forty-eight hours of being placed in contact in flowing sea tanks. Strongest aggressors are members of the mussid and favid families. Rapid overgrowth of one species by another, however, is very common on the reef in water less than seven meters. Branching species in the following genera may tentatively be arranged in order of diminishing overgrowth ability: Acropora over Montipora over Pocillopora over Seratiopora over Porites over Pectinia over Echinopora over Merulina over Cyphastrea over Turbinaria over Favia. Hemispherical species are uniformly slower growing than these branching forms. Space was never under any circumstances monopolized by one species or genus indicating a relative balance of space-competitive abilities.

PRODUCTION AND RESPIRATION VALUES FOR INDO-PACIFIC REEF CORALS

James W. Porter

In situ investigations on the production and consumption of oxygen were conducted on four morphologically distinct species of Indo-Pacific reef corals to test the hypothesis that the Surface/Volume Ratio and other gross morphological characteristics could indicate the relative dependence of the coral on autotrophy or heterotrophy in meeting its daily metabolic needs and the coral's niche separation from other coral species. The respirometer recorded photosynthetically active radiation (PAR), oxygen levels in three closed chambers with the test corals, and automatically fully exchanged the water in the chambers once every hour with new reef water.

Results are summarized in the table below and indicate that although the $P_{net\ day}/R_{total\ night}$ values are all in excess of unity at 7m, they are achieved by differing production and respiration values. In general, branching species do have the higher production values except for certain unusual Indo-Pacific species such as Fungia actiniformis and Plerogyra lamelosa which have greatly enlarged tentacles filled with zooxanthellae.

Species	P_{net} in $mgO_2\ gChl_a^{-1}$ per microeinstein	$R_{total\ night}$ in $mgO_2\ gChl_a^{-1}\ hr^{-1}$	$P_{net}/R^*_{total\ night}$
ɛropora cf. cuneata	6.0×10^{-3}	1.17	2.1
erogyra lamelosa	10.6×10^{-3}	1.03	4.1
ɔrites lutea	3.9×10^{-3}	1.46	1.0
·agia actiniformis	28.0×10^{-3}	5.25	2.1

Assuming 400 microeinsteins average light over a 12 hour light period (a common average value at 7m in the Philippines during rainy season) and a 12 hour dark period.

Observations of *Acanthaster planci* feeding on Philippine Coral Reefs

James Porter, Michel Pichon and Karen G. Porter

Acanthaster planci were observed feeding on the coral reefs of the Capones Islands, Luzon; Green Island, Palawan and Bohol Island outer barrier reef. No sightings were made at North Gigantes Island, Panay. All localities, except Capones Islands, were research stations at which at least 50 diver hours were spent under water. No evidence of extensive population growth or reef destruction by the Crown-of-Thorns was found.

POTENTIALS FOR NUTRIENT PROCUREMENT IN XENIA.

Robert K. Trench

The alcyonarian octocoral Xenia contains unicellular dinophyceae (zooxanthellae) within the endodermal cells. Because these coelenterates appeared to lack the morphological attributes associated with a carnivorous habit, the concept that they depended entirely on their zooxanthellae for nutrients was generated.

In this study, a reinvestigation of the nutritional potentials of Xenia was conducted. Emphasis was placed on (a) the amounts and identity of photosynthetic products moving from symbionts to hosts, (b) the ability of Xenia to ingest, digest and assimilate exogenously supplied animal protein and (c) the kinetics of uptake of dissolved glycine from the ambient environment.

The evidence so far obtained shows that the zooxanthellae release in vitro 40-60% of the total carbon fixed following one hour incubations in $NaH^{14}CO_3$. Analysis of media and cell extracts by paper partition radiochromatography demonstrated selective release of metabolites by the algae. The major substance released was glucose, accompanied by minor traces of alanine and glycolic acid. These algae appear different from those of corals and sea anemones which release large quantities of glycerol.

When colonies of Xenia were offered suspensions of india ink mixed with a homogenate of lobster muscle, it was possible to observe them ingest such particles which could be traced into the coelenteric cavity. Experiments were also conducted using ^{35}S-labelled bacterial proteins mixed with lobster homogenate. After feeding, these specimens were fixed for subsequent analysis by autoradiography, which should provide information on digestion of protein and assimilation of amino acids, assuming the animals do possess these capabilities.

Studies on the kinetics of uptake of glycine showed that Xenia could remove this amino acid from solution with high efficiency ($K_s = 3 \times 10^{-6}$M). Apparently Xenia cannot effect uptake from concentrations lower than 1.5×10^{-7}M glycine. In all instances, when external concentration of glycine was 10^{-5}M or less, net influx of the metabolite was clearly demonstrable. At glycine concentrations of 10^{-4}M or greater, the evidence suggests zero flux or net efflux. Since it is unlikely that the ambient concentrations of free amino acids in the natural habitat occupied by Xenia would ever attain concentrations of 10^{-5}M, it is likely that free amino acids represent a resource potentially exploitable by these organisms. Specimens have been prepared for ultrastructural analysis to determine the existance of a microvillated epidermal epithelium, which is usually positively correlated with the capacity for transport.

The preliminary conclusion of this study is that Xenia utilizes primary photosynthate from its algae while simultaneously retaining the capacity for heterotrophic nutritional input. The organisms are very likely particulate suspension feeders.

Effect of Pressure on Blood Gases in Diving Sea Snakes

Roger S. Seymour

If all of the cardiac output in sea snakes equilibrated with lung gas, increased gas tension in the lung during diving would be reflected closely by arterial blood gas tension. However, intracardiac and pulmonary shunting has been demonstrated in sea snakes. The proportion of venous blood effectively bypassing the lung should increase as the lung collapses during a dive. This hypothesis was tested at atmospheric pressure by controlling the amount of gas in the lung and examining the oxygen tension difference (ΔP_{O_2}) between the lung and pulmonary veins. ΔP_{O_2} increased with progressive deflation of the lung suggesting that the collapsed portions of the elongate lung continued to be perfused but did not exchange much gas.

In another set of experiments, the lung was flushed with air and the snakes were compressed to as much as 5 ATA. Arterial P_{O_2} remained less than 50 % of the lung P_{O_2} which reflects considerable pulmonary bypass. The presumed advantages of this shunting are that O_2 may be metered from the lung reserve, thus minimizing the dangers of O_2 toxicity and loss of O_2 through the gas permeable skin and that similarly blood P_{N_2} may be kept relatively low which reduces the potential for bubble formation upon decompression. However, snakes occasionally formed bubbles in the blood following repeated dives to 4 or 5 ATA probably because the P_{N_2} in the water of the compression chamber increased and prevented adequate cutaneous N_2 loss. This was indicated by the formation of bubbles on the skin during decompression. No deaths were correlated with N_2 bubble formation in the blood under the experimental conditions.

CUTANEOUS OXYGEN UPTAKE IN AQUATIC SNAKES

Harold Heatwole and Roger Seymour

Cutaneous oxygen uptake was measured in eight species of aquatic snakes. The sea snakes had values similar to those previously reported except that Hydrophis ornatus had unusually high values which correlates with its known high skin permeability to other substances (e.g. water). Cerberus rhynchops, a colubrid snake inhabiting mangrove swamps had very low cutaneous O_2 uptake as did the semi-aquatic sea kraits.

Effect of CO_2 on Ventilation in a Sea Snake and an Arboreal Snake

Roger S. Seymour

Sea snakes can eliminate much of their CO_2 production cutaneously. This capability is correlated with a relatively high blood P_{CO_2} and low ventilation rate.

Ventilation was compared in the arboreal snake, Boiga dendrophila, and the sea snake, Laticauda colubrina, breathing various mixtures of air and CO_2. In the pure air breathing state, minute volume was about 8 times higher in Boiga than in Laticauda (97.7 vs 10.8 ml $min^{-1}kg^{-1}$). The difference was mainly because of a higher tidal volume (78.8 vs 8.0 ml kg^{-1}), the frequencies being similar (1.3 vs 2.0 breaths min^{-1}). Minute volume increased 4.2 times at 3% CO_2 in Boiga but 7.2% CO_2 was required to elicit the same factorial increment in Laticauda. The blood P_{CO_2} in the air breathing state averaged 13.4 torr in Boiga and 31.8 torr in Laticauda. These results indicate that the arboreal snake is more dependent on pulmonary ventilation to eliminate CO_2 and is more sensitive to elevated blood P_{CO_2} than is the sea snake.

FUNCTION OF THE SACCULAR LUNG IN *LATICAUDA COLUBRINA*

Roger Seymour and Harold Heatwole

The posterior saccular portion of the lung of the sea krait was ligated under cold narcosis and the effect of this treatment on buoyancy control and ventilation studied. Deflation of the saccular lung caused little impairment of swimming or diving in large tanks. However, inflation of the tied off section caused problems but did not prevent the snake from diving or swimming underwater.

Tying off of the saccular lung did cause a significant decrease in submergence time and when the ligature was removed, submergence time increased. Thus, although the saccular lung lacks an extensive vascular bed for gaseous exchange it apparently facilitates ventilation of the anterior vascular segments of the lung.

Anaerobiosis in sea and land snakes

Roger S. Seymour and John Baldwin

Enzyme preparations from skeletal muscle of snakes representing four families (Hydrophiidae, Elapidae, Colubridae, Acrochordidae) will be compared after the expedition. Preliminary work on Laticauda colubrina showed low activity of muscle lactate dehydrogenase and phosphorylase and high activity of hexokinase relative to the terrestrial elapids, Demansia textilis and Australaps superba. The respective phosphorylase:hexokinase ratios were 11.4, 43, and 242. This suggests a lower reliance on anaerobic pathways in the sea snake.

During the Ashmore Reefs Expedition, gas tensions and lactate concentrations were analyzed in sea snake blood, the results indicating that voluntary dives were accomplished aerobically. To supplement this respiratory information, blood samples from naturally diving and swimming sea snakes were taken within 1.5 minutes of capture. In 16 individuals of three species, lactate averaged only 15.4 mg%, indicating that activity and diving usually occurs aerobically. However, two snakes had high blood lactate (ca. 110 mg%) which suggests that anaerobic pathways are occasionally employed, possibly during feeding.

Cardiovascular Responses in Aquatic and Terrestrial Snakes

Roger S. Seymour

Because of their shape, snakes should show relatively great variations in blood pressure depending on the posture of the body. This study examines the role of peripheral resistance and heart rate in the regulation of central blood pressure in snakes from totally aquatic, semiaquatic, and terrestrial-arboreal habitats.

On first analysis, three trends appeared. Mean blood pressure increased with the degree of terrestriality. In general, the heart was proportionately farther from the head in aquatic snakes. During head-up tilting experiments, arboreal and some of the semi-aquatic snakes regulated blood pressure rather precisely through a combination of changes in heart rate and peripheral resistance whereas the hydrophiids appeared to have lost this ability, the head blood pressure becoming negative at relatively low tilting angles.

Species	Habitat	Mean Blood Pressure
Boiga dendrophila	Arboreal	74
Laticauda colubrina	Semiaquatic	38
Cerberus rhynchops	Semiaquatic	34
Laticauda semifasciata	Semiaquatic	31
Acrochordus granulatus	Aquatic	31
Hydrophis ornatus	Aquatic	23
Hydrophis sp.	Aquatic	27

SEA SNAKE VENOM STUDIES

Nobuo Tamiya

Milking of venom from sea snakes was performed on five species during the expedition as described previously (Tamiya and Puffer, 1974). The yield and lethal dose are given in Table 1. The values for Hydrophis inornatus have not previously been reported.

The results on Laticauda colubrina and L. semifasciata are in good agreement with those described by Reid (1956) and Arai et al (1964), respectively. The toxicity of the venom of the Hydrophis ornatus reported here is 1.5 times greater than values reported for this species by Tamiya and Puffer (1974), while the toxicity of Hydrophis spiralis venom is half of that described by Carey and Wright (1960).

The high lethal activity of these snake venoms (0.081 - 0.67 µg/g body weight of mice, i.m.) suggests their high content of neurotoxins, the LD_{50} values of the purified preparations of which are 0.075-0.015 µg/g.

The effects of the venoms of two species of sea snakes (Hydrophis ornatus and Laticauda semifasciata) were tested on sea snakes (Hydrophiidae), on Colubrid (mangrove snake of the genus Boiga and water snakes of the genus Cerberus) and on file snakes (Acrochordidae) in collaboration with Drs. S. Minton and H. F. Heatwole. It was found that Hydrophid and Colubrid snakes are not susceptible to sea snake venoms, whereas Acrochordid snakes are about 150 times and 20 times more tolerant than mice (on a µg venom/g body weight basis) to H. ornatus and L. semifasciata venoms, respectively. The results are shown in Table 2.

A sea snake Laticauda colubrina (female, 329g) was injected with 3750 µg (11.4µg/g body weight)and her electrocardiogram recorded. After a rise in heart

to 60/min for 7 min (probably due to handling as similar results were obtained on a snake handled and a needle inserted but no venom injection), the heart rate went down to the normal value of 38 and stayed constant until the experiment was terminated.

It is of interest that snakes, especially Hydrophiidae and Colubridae, are tolerant to sea snake toxins, which are shown to block the neuromuscular junction binding to acetylcholine receptors (Tamiya and Arai, 1966, Sato et al, 1970). The venom of a sea snake Lapemis hardwickii is equally lethal (on a μg/g body weight basis) for both mice and snappers, the latter being among the prey fishes of the snake (N. Tamiya, H. Puffer and J. McCosker, observation during the Ashmore Reef Expedition on R/V Alpha Helix, 1972-1973). Laticauda colubrina and L. semifasciata sera gave only marginal protection to mice injected with sea snake venoms.

Further experiments are planned with the dried venom preparations.

References

Arai, H., Tamiya, N., Toshioka, S., Shinonaga, S., and Kano, R. (1964) J. Biochem. 56 568-571.

Carey, J. E. and Wright, E. A. (1960) Nature 185 103-104.

Reid, H. A. (1956) Trans. Roy. Soc. Trop. Med. Hyg. 50 517-542.

Sato, S., Abe, T. & Tamiya, N. (1970) Toxicon 8 313-314.

Tamiya, N. & Arai, H. (1966) Biochem. J. 99 624-630.

Tamiya, N. & Puffer, H. (1974) Toxicon 12 85-87.

Table 1. Yield and lethal dose of sea snake venoms.

	Yield/head		Protein concentration 1cm**	LD_{50} 20g mouse i.m.	
	Maximum ml	Average ml	A_{280}	µg/g	10^{-6}ml/g
Hydrophis inornatus	0.035	0.014	150	0.13	0.87
H. ornatus	0.107		105	0.084	0.80
			184	0.081	0.44
				0.075	
H. spiralis	0.010		250	0.67	2.6
Laticauda colubrina	0.270	0.16	403	0.22	0.56
			382	0.25	0.65
				0.265	
L. semifasciata	0.160	0.021	130	0.30	2.3
				0.27	

*wet fresh venom volume/g
**approximately equal to mg/ml

Table 2. Lethal activity of *Hydrophis ornatus* and *Laticauda semifasciata* venoms on snakes.

Venom	Test Animal*	Dose (i.m.) µg/individual	Dose (i.m.) µg/g	Dose (i.m.) Relative amount/g	Outcome***
LD_{50} *Hydrophis ornatus*	Mouse Av. 20g	1.5-1.7	0.075-0.084	1**	50% death
	Hydrophis ornatus (3) 21-36g	283	7.8-13.5	104-180	survival
	Laticauda colubrina (6) 66-329g	560-7500	8.6-36.4	114-485	survival
	Laticauda laticaudata (1) 81g	1130	14	186	survival
	Bioga dendrophila (1) 134g	1130	8.4	112	survival
	Cerberus rhynchops (3) 14.4-28.8g	750-3000	52-101	694-1340	survival
	Acrochordus granulatus (3)				
	12.0g	62	5.22	70	survival
	12.0g	130	10.8	145	survival****
	11.5g	261	22.7	302	death
Laticauda semifasciata	Mouse Av. 20g	5.3-5.9	0.265-0.300	1**	50% death
	Cerberus rhynchops (3)	175-1040	5.3-47.8	20-180	alive
	Acrochordus granulatus (7)				
	9.25-13.0g (5)	130-520	11.4-40.0	43-151	death
	11.3g	52	4.51	17	survival
	21.3g	52	2.44	9.2	suvival

*Numbers in parentheses show the number of animals used.
**Lethal dose values for mice are taken as unity.
***Outcome after 48 hours.
****Very ill for 24h.

ALPHA HELIX SOUTHEAST ASIAN EXPEDITION

IV Bindoy (Nautilus)

October 8 - November 13, 1975

Compiled By:
ALPHA HELIX Program
Scripps Institution of Oceanography
University of California, San Diego
La Jolla, California

ALPHA HELIX Southeast Asian Expedition

IV Bindoy (Nautilus)

October 8 - November 13, 1975

Chief Scientist, James R. Redmond, Iowa State Univeristy

Biologists have long recognized the key position among the cephalopods occupied by the pearly nautilus. It is the sole survivor of the once extensive fauna of shelled cephalopods and shows many apparently primitive features presumably characteristic of these extinct forms. Their modern coleoid descendants, the squids, octopuses, and other dibranchiate cephalopods, are the most complex of all the invertebrates and are different from the nautilus in many ways. Some of the major differences include the absence of an external shell, a very different tentacular apparatus, a reduced blood volume and loss of the hemocoel, the presence of branchial hearts, and more highly developed sense organs and central nervous system. Thus, there are three compelling reasons for studying the nautilus. First, in itself it is a unique and fascinating animal; second, it is the only living animal that can provide direct and detailed information concerning the physiology of an otherwise extinct major group of animals and; third, it can yield clues to the selective pressures and evolutionary steps which lead to the most complex of the invertebrates, the modern cephalopods.

There have been a number of studies on Nautilus. Most of these have been anatomical, but there are also scattered studies on living specimens. However, the remote location of the nautilus, the absence of laboratory facilities, and the tendency of the nautilus to live at moderate depths have made it difficult to study. The combination of the laboratory facilities of the ALPHA HELIX and a large population of *Nautilus pompilius* found in the southern Philippines by

Dr. Norine Haven have provided an excellent opportunity for an intensive study of living nautilus.

A group of 11 scientists from 4 countries met the ALPHA HELIX in Manila and sailed south about 400 miles to the southern Tañon straits between Negros and Cebu islands. Most of our work took place just off shore from the town of Bindoy on Negros. This particular site was chosen because Dr. Haven's previous experience here indicated the presence of large numbers of nautilus. With the exception of Dr. Pedro Gonzales, who collected specimens for the National Museum of the Philippines, all the scientists aboard were concerned with research on *Nautilus pompilius*.

We were fortunate in many ways. Weather was good, the water was calm, and we did obtain an adequate supply of nautilus. Some of the fisherman on Bindoy take nautilus in traps placed on the bottom in approximately 300 m of water. We are greatly indebted to Mr. Wilson Vailoces, a friend of Dr. Haven, who arranged with the fishermen to bring their daily catch of nautilus directly to the ALPHA HELIX. We had originally planned to trap our own specimens, however, the abundant supply provided by Mr. Vailoces saved us untold hours of labor and contributed greatly to the success of our research. In addition, the generous hospitality extended by the officials and citizens of Bindoy, especially Mr. Vailoces' family, made our stay at Negros very pleasurable.

We obtained a considerable body of information concerning respiration, blood pressure and flow, properties of the hemocyanin, biochemistry of blood and muscle, physiology of the eye, renal physiology, growth, buoyancy, ovarian development and general biology of *Nautilus pompilius*. Samples of blood and other tissues were also prepared for ultrastructural examination and additional

biochemical studies at home institutions.

As is always the case in such expeditions our success depended in large part upon the activities of many other individuals. We deeply appreciate the unstinting support provided by Captain Garrett Coleman, the officers and excellent crew of the ALPHA HELIX, and our technician, Mr. Walter Schneider. Their help and friendship made our stay aboard the ALPHA HELIX most enjoyable. We are also indebted to Dr. Walter Garey and Mrs. Joan Murry for their help in the planning and logistics of our trip. We thank the government of the Republic of the Philippines for allowing us to conduct these studies in their territorial waters. The research was supported by grant BMS 75-00149 from the National Science Foundation.

FIELD REPORTS

Preliminary Report on Studies on Reproductive Biology of *Nautilus*

J. M. Arnold, University of Hawaii

There are many open questions concerning the reproductive biology of *Nautilus pompilius* that have yet to be answered. Although of obvious significance, *nautilus* embryos have never been studied and little is known about either the male of female reproductive morphology or physiology. Unfortunately, embryos were not obtained during this cruise but many reproductive tissues were collected for later histochemical and ultrastructural examination. No definitive answers can be provided until these tissues have been analyzed in detail, so this report consists of a list of tissues collected and some tentative results based on preliminary observations.

From the male reproductive tract the following tissues were fixed for electron microscopy and histochemistry: testis, spermatophoric organ, spermatophoric gland, vas deferens, Needham's sac, penis, spadix, spadix glands, and spermatophores from several places along the reproductive tract. From the female, several stages of oogenesis were fixed and embedded as well as immature and mature nidamental glands and vulva. It appears that there is an accessory organ associated with the developing oocytes which functions in vitellogenesis. Chromosomal squashes were made of the testis and tentatively it appears that *Nautilus pompilius* has 21 pairs of chromosomes. Tissues of interest to several other investigators were also prepared for electron microscopy.

Hemodynamics in the Chambered Nautilus, *Nautilus pompilius*

G. B. Bourne, University of Calgary, K. Johansen, University of Aarhus and J. R. Redmond, Iowa State University

The nautilus cardiovascular system follows the molluscan plan of a linear sequence of vessels. There is a single ventricle pumping blood into a major aorta and some other vessels. This blood passes by way of various arterial branches to the organs. Blood is collected from organs in large sinuses which eventually lead to the vena cava. After the vena cava enters the pericardial cavity it divides into four afferent branchial veins which conduct blood to the ctenidia. After oxygenation in the ctenidia blood returns to the ventricle by way of four efferent branchial veins.

We have accumulated evidence for three pumping systems propelling the blood through the animal's body. The ventricle forms the systemic pump. Blood pressure in the sinuses, vena cava and efferent branchial veins is boosted by a venous pump formed by rhythmic contractions of the respiratory muscles. Finally there is a branchial pumping system formed by the pair of renal appendages and the pericardial gland which are found on the root of each afferent branchial vein. Visual examination of the pericardial structures shows that the systemic heart and the branchial pumps beat at the same frequency of about 10-12 beats/min. The venous pump is driven at the much higher frequency of 40-50 beats/min. The ctenidia and aorta also show contractile properties which could serve to propel the blood.

Blood pressure was measured by miniature strain guage pressure transducers in the following areas of the vascular system: ventricle, major aorta, hemocoel, vena cava, afferent branchial veins, efferent branchial veins. The pressure

in the aorta showed a systolic range of 32-50 cm water and a diastolic range of 6-35 cm water. Occasionally negative pressures were recorded from the vena cava and efferent branchial veins.

Blood flow to the head was measured by an electromagnetic flow transducer. The flow pulse has a similar appearance to that of mammals with a small back flow at the end of each cycle.

Respiratory Physiology

G. B. Bourne, University of Calgary, K. Johansen, Univeristy of Aarhus, and J. R. Redmond, Iowa State University

Two basic approaches were used to investigate respiratory physiology. Direct measurements of oxygen uptake and percent oxygen extraction from inspired water were made using a flow through respirometer. Water samples for P_{O_2} measurements were taken from the inflow and outflow of the chamber, and, by means of catheters, from the inhalent and exhalent water passed directly through the branchial chamber of the experimental animal. The second approach involved the implantation of small catheters in the vena cava, efferent and afferent branchial vessels. Blood samples taken from these vessels were analyzed for P_{O_2} and pH. These latter measurements will be discussed in a separate section of this report.

Pending complete analysis of the data, the following trends appear. At normal temperatures of 17-18°C, the "resting" oxygen uptake appears to be 0.5-0.8 O_2/min/kg wet weight. Oxygen extraction is low, usually between 5-15%, with extraction dropping as breathing rate and amplitude increase. *Nautilus* can withstand hypoxic conditions very well. While oxygen uptake drops somewhat as the P_{O_2} of inhaled water falls, it is not until the latter drops below about 40 mm Hg that the respiratory system fails. Even then *Nautilus* can survive very low P_{O_2}'s for several hours (see Hochachka's report on metabolism). Bubbling oxygen through the inhaled water appears to depress the frequency and especially the amplitude of breathing motions suggesting that the respiratory motions are keyed to oxygen levels in the water and/or blood.

Respiratory Properties of the Blood of *Nautilus pompilius*

G. B. Bourne, University of Calgary, K. Johansen, University of Aarhus, and J. R. Redmond, Iowa State University

Oxygen equilibrium curves of Nautilus blood were determined using a modified mixing method whereby the P_{O_2} is measured after mixing set volumes of fully oxygenated and deoxygenated blood. The Bohr effect and buffering properties of the blood were obtained by varying the P_{CO_2} of the equilibruim gases using a Wosthoff gas mixing pump. Eschweiler tonometers were used for tonometry and a Radiometer BMS3 and PHM72 were used for P_{O_2} and pH measurements. Equilibrium curves were determined at 18 and 24°C. Oxygen capacity was measured according to the method of Laver 1967 (*J. Applied Physiol. 22:* 1017).

The equilibrium curves are distinctly sigmoid and showed a P_{50} value at 18° C and pH 7.45 of 17 torr increasing to 18.5 torr when the blood was acidified to pH 7.25. The Bohr values were low ($\emptyset$ = -0.17). The temperature effect on the O_2 binding was moderate, increasing the P_{50} from 17 torr at 18°C to 25 torr at 24°C at pH 7.40. Oxygen capacity of Nautilus blood varied from 1.5 to 3.0 vol %.

Further analysis of the oxygen equilibrium curves will be done later as well as further work on the kinetics of the O_2 binding to hemocyanin.

Collections from the Tañon Strait, Cebu and Negros Islands, Philippines

P. C. Gonzales, National Museum of the Philippines

I was fortunate to be included in the scientific party of this ALPHA HELIX expedition, which carried out physiological and behavioral studies in vicinities of Bindoy, Negros Oriental and of Alegria, Cebu in the Tañon Strait. Neither a physiologist nor a behaviorist, I came into the program as an observer and a collector, both of which proved profitable to me, expecially the latter.

Given the free use of the excellent facilities of the ALPHA HELIX, I was able to collect specimens from the littoral, the sublittoral, even the abyssal zones of the sea. The littoral and upper sublittoral work, at least down to 200 feet, was carried out with the help of SCUBA; the abyssal by means of traps and otter trawl.

The various methods used in the collections yielded an excellent variety of biological materials. These specimens, including fishes, crustaceans, cnidarians, sponges, and echinoderms are now being identified and studied by different workers. Thus, all identifications in this present report are tentative.

Collections:

Sponges:

1. Spongiidae	8
2. Callyspongiidae	2
3. Haliconidae	4
4. Adociidae	2
5. Clathriidae	1
	17

Cnidaria:

All corals and gorgonians are not yet identified even to families due to the absence of the researcher on corals. Total corals 51

Echinoderms:

1. Echinasteridae
   Echinaster sp. 1

2. Pentacerotidae
   Culcita novoguinea 2
   Choriaster granulatus
   Pentaster obtusatus

3. Oreasteridae
   Protoreaster nodosus

4. Linckiidae
   Linckia laevigata
   Nardoa tuberculata

5. Archasteridae
   Archaster typicus

6. Ophiocomidae
   Ophiocoma scolopendrina

7. Temnopleuridae
   Salmacis sphaeroides

8. Diadematidae
   Echinotrix calamaris
   Echinotrix diadema

9. Cidariidae
   Prionocidaris faculosa

10. Echinometridae
    Echinometra mathei oblonga

11. Others are still unidentified

Total echinoderms 75

Crustaceans:

1. Portunidae
   Portunus
   Thalmaita
   Charybdis

2. Penaeidae
   Penaeus

3. Ocypodidae
   Uca

4. Majidae
5. Xanthidae
6. Calappidae
7. Dorippidae
8. Paguridae
9. Grapsidae
10. Thalassinidae
Thalassina anomla
11. Dromiidae
12. Gecarcinidae
13. Unidentified families 9

Total crustaceans 339

Shells:

1. Conidae
2. Cypraeidae
3. Ovulidae
4. Haliotidae
5. Volutidae
6. Muricidae
7. Ostraeidae
8. Strombidae
9. Nautilidae
10. Loliginidae
11. Phyllidae
12. Littorinidae
13. Terebridae
14. Unidentified

Total shells 117

Fishes:

1. Atherinidae
2. Brotulidae
3. Hemiramphidae
4. Serranidae
5. Labridae
6. Coridae
7. Lethrinidae
8. Apogonidae
9. Pomacentridae
10. Congridae
11. Uranoscopidae
12. Tetraodontidae
13. Lutjanidae
14. Mullidae
15. Plotosidae

Total fishes 187

Observations on the Biology of *Nautilus pompilius*

N. H. Haven, Hopkins Marine Station

Function of mantle cavity organs

Both a male and female *Nautilus* were transplanted into shells where the living chamber was partially replaced by plexiglass. With the mantle reflected it was possible to make photographic records of ala infundibulae as water was pumped into the mantle cavity, as well as the use of the funnel for exhalent pumping and maneuvering as the animal swam.

Visual pigments

Animals were dark adapted and the eyes excised and frozen for later analysis of visual pigments. The pigments are of particular interest because *Nautilus* not only lives at considerable depths, but also lacks the lens characteristic of other cephalopods.

Artificial fertilization

Several sets of eggs were artificially fertilized and kept for 16 and 36 hrs respectively. They were preserved in Bouin's fixitive and will be examined for possible cleavage after having undergone proper staining.

Diet

The gizzards of several animals were examined for analysis of their natural diet. Preliminary examination revealed decapod crustacean, shrimp, and one sea urchin spine.

Juveniles

Several attempts were made to trap juveniles. These traps at 100 fathoms and two set at 50 fathoms off the island of Cebu produced no *Nautilus*.

Metabolic Features of *Nautilus* Muscle

P. W. Hochachka, University of British Columbia

Within the molluscs can be found representatives spanning a range from facultative anaerobic to obligatorily aerobic metabolic organization. Because of its interesting phylogenetic position, *Nautilus* is an organism of importance in any attempt to understand the evolution of metabolism in the molluscs in general and in cephalopids in particular. One indication of the metabolic capacities of a tissue can be obtained from studies of enzyme profiles. Which enzymes are present? In what quantities?

Accordingly, I assayed several key enzymes functioning at important control points or branchpoints in energy metabolism of the *Nautilus*. Most of the work focussed on the retractor, the funnel, the heart, and the spadix muscles. The funnel and heart show the highest aerobic potential as judged by enzyme activities (high citrate synthase, malate dehydrogenase and GOT levels; lower pyruvate kinase and octopine dehydrogenase levels). The retractor and spadix on the other hand have relatively high levels of enzymes of anaerobic metabolism. These studies will be completed at my home laboratory, but even at their preliminary state they clearly indicate that *Nautilus* metabolism is a complex mix of anaerobic and aerobic capacities, a long way from the obligatorily aerobic nature of the fast squid, *Symplectoteuthis*, as well as from the facultatively anaerobic bivalve molluscs.

Nautilus Blood Metabolite Studies

P. W. Hochachka, Univeristy of British Columbia

As a result of my tissue metabolite studies and the blood pH studies of Johansen, Bourne, and Redmond, it became particularly important to get some indication of changes in blood metabolites under different physiological states of the animal. The outstanding feature of the *Nautilus* metabolism, of course, is that anoxic stress (i.e. anaerobic metabolism) leads to one extremely basic end product (arginine) plus one relatively mild acid (octopine). The usual vertebrate situation is a potential drop in pH with anaerobic activation. In this context, therefore, I turned some attention to measuring arginine and octopine levels in *Nautilus* blood. The most important outcome of these attempts thus far is the discovery that the standard perchlorate extraction used so successfully on tissues does not work well on *Nautilus* blood, because it releases into solution potent inhibitors of enzymes used for metabolite assays. The inhibitors are assumed to be heavy metal ions (although I should mention that adding EGTA or EDTA to my extracts did not alleviate the problem). Hence, it was with some difficulty that I found a technique (salting out) for deproteinizing *Nautilus* blood that seems to work well and not interfere with enzyme assays for metabolites. The estimates of ocotopine and arginine that I obtained were as follows:

	Octopine, mM	Arginine, mM
Arterial Blood pH about 7.4	0.15	0.9
Venous Blood pH about 5.4	0.27	0.6

If these values are correct, they suggest that these 2 metabolites alone

could account for a pH gradient between arterial and venous blood of up to 0.7 pH units, and these would of course change with different physiological states. Also, if these values are correct, they are important for they indicate extremely large concentration gradients may occur for these metabolites between blood and tissues (muscle at least). For this reason, I assayed ocotpine and arginine levels in other tissues to supplement my value for muscle. Except for muscle, all other tissues I examined had quite low concentrations of both these metabolites. Hence, if there is a gradient problem between blood and tissues for these metabolites, it is acute only when the blood passes muscle.

The blood metabolite measurements, moreover, bear upon the question of the fate of octopine. Unlike lactate, which readily floods from the tissue into blood, octopine appears to be more strongly retained in muscle, where presumably at the end of a work burst, when the oxidation potential is favorable, it is reconverted to arginine and pyruvate. Certainly, the kinetic properties of the enzyme (a relatively high affinity for octopine) are consistent with this model. However, one has yet to account for the arterial-venous differences in these metabolites.

Apparently, the kidneys and/or the gills are the only tissue candidates that could monitor the blood in such a way as to account for our results. Accordingly, I measured the amount of ODH in these tissues. Of these, the gill had about 2 times higher amounts. Its affinity for octopine is about about 2.5 times higher than is that of the muscle enzyme (the gill enzyme being measured using a crude supernatant fraction as an enzyme source). It is therefore possible that we are dealing here with an isozyme system for ODH comparable to that of LDH in the vertebrates. This possibility will be further investigated.

Nautilus Muscle Anaerobic Metabolism:
Octopine and Arginine as End Products of the Process

P. W. Hochachka, University of British Columbia

When *Nautilus* muscle performs "burst" work (e.g. a sudden retraction into its shell) or hypoxic work, lactate is not accumulated. We have always assumed that octopine does in its stead; so have some other workers. But no one has ever demonstrated its production previously in any simple way relating to muscle energy requirements. Some workers have looked for its production in *Sepia* under what I think now must have been relatively mild working conditions; but they reported that the product was not formed. Since my preparations of ODH were free of contaminating dehydrogenases (such as, for example, LDH, MDH, alpha-glycerophosphate dehydrogenase) I was able to quantitatively measure octopine and arginine production in *Nautilus* muscle under various conditions. In two chronically hypoxic animals, for example, octopine levels in retractor muscle were as high as 36 mM while arginine levels reached 60 mM. Typically, taking *Nautilus* from its shell appears to strongly activate the retractor, and resting values of these metabolites were at first difficult to obtain. Octopine concentrations would always be in the 20 mM range, no matter how quickly we tried to remove the animal from its shell. On John Arnold's suggestion, I turned to the spadix, a sturdy male reproductive organ with potent anaerobic capabilities. The spadix does not seem to be activated by removal of the organism from its shell. Moreover, it consists of 4 cirri, numbers 2 and 3 being similar in size. Hence number 2 could serve as a control while we studied number 3, or vice versa. Hartline rigged a pH "special" stimulator. We typically chilled the animal prior to experimentation. The spadix was

excised and kept chilled (in iced sea water) while we dissected out the appropriate cirri. The cirrus was then mounted and while forced to work against a 50 g weight was given a single stimulus of 15 second duration. Tissue samples were taken at 15 sec intervals for the 1st min, then at various time periods during recovery. Samples were frozen within 2-3 sec in -80°C ethanol. Known weights of muscle were homogenized in 15% perchlorate to inactivate enzymes and solubilize metabolites. The perchlorate was precipitated with 1.4 M K_2CO_3 (about equal volumes) and the extract was brought to about pH 8. I then assayed for octopine and arginine. Two important results emerged. Firstly, during such spadix activation, arginine production momentarily exceeds the cells capacity to handle it; hence, it accumulates to values over 70 mM. (I shudder to think of the pH consequences.) When the arginine peak begins to decline it is only about 50% accountable for by simultaneous octopine production by ODH. The rest presumably is recycled to arginine phosphate. The second conclusion that can be made unequivocally is that formally both arginine and octopine are unquestionable end products of anaerobic metabolism in the *Nautilus* spadix. The spadix experiments settled once and for all a rather fuzzy area in cephalopod metabolism.

Subsequently, we found that if the entire Nautilus is chilled in iced sea water, the retractor muscle can be dissected out without activating it. Hartline, in one case, completely removed the retractor without witnessing a single contraction. In such preparations, we were able to repeat the spadix type of experiment, in this case however also varying the length of the stimulus. Essentially the same results were obtained at 15 sec stimulus as with the spadix. However, when the stimulus time went to 240 sec, the arginine peak

was much lower, while octopine levels were much higher, indicating that in these more extreme conditions, the arginine pool was being depleted more drastically.

The percholate extracts were frozen after use for further metabolite studies.

Nautilus ODH: A cephalopod analog of vertebrate LDH

P. W. Hochachka, University of British Columbia

Muscle metabolism in *Nautilus* (as in other cephalopods) differs from vertebrate muscle in at least 2 fundamental ways: 1) arginine phosphate replaces creatine phosphate as the muscle phosphagen, and 2) ODH, octopine dehydrogenase, replaces LDH, lactate dehydrogenase. During anoxic or hypoxic stress, or in "burst" muscle work, lactate therefore does not accumulate but octopine does. The octopine is formed from pyruvate and arginine with NAD for mainline glycoysis being regenerated from NADH in the process. My colleagues, Jeremy Fields and John Baldwin, and I had earlier this year examined the catalytic and regulatory properties of ODH in squid and octopus muscle. I, therefore, was particularly interested in studying the *Nautilus* homologue. In *Nautilus*, ODH activity occurs at highest levels in the retractor and spadix muscles, in lowest activities in the kidneys and heart, and at intermediate levels in the liver. I partially purified the enzyme from retractor muscle and briefly examined its catalytic properties. The enzyme follows hyperbolic saturation with respect to either substrate. The Michaelis constant (K_m) for pyruvate varies from about 0.1 to 0.5 mM depending upon arginine concentrations, while the K_m for arginine varies between 2-5 mM, depending in this case upon pyruvate levels. Each substrate increases the enzyme affinity for the cosubstrate. As a result, when the availability of both increases simultaneously (as presumably must occur *in vivo*) and in constant ratio, the enzyme displays a "flare up" in activity (sigmoidal saturation curves). This appears to be the only metabolite regulation of the enzyme. However, arginine may play other unexpected regulatory

functions: firstly, through substrate inhibition at high levels, and secondly, through a pH effect. Arginine, it must be emphasized, is an extremely strong base; an aqueous solution for example will absorb CO_2 from the air. By titrating Sigma grade arginine, I found that in the physiological pH range, for each 1 mM arginine formed at least 0.8 mM H^+ must be simultaneously generated in order to prevent a dramatic pH rise. During "burst" work or anoxia, one function of ODH might be to "bleed off" some of the arginine formed from arginine-P phosphorolysis. However, it would be to the muscle cell's distinct disadvantage to completely deplete its arginine-arginine-P pool. Hence, it is probable that the cell must sustain a rising pH under extreme conditions of anoxia or emergency "burst" work. In this context, drastic ODH inhibition by high pH (and by high arginine) can be viewed as an arginine conservation mechanism. Moreover, it implies that arginine functions at an important metabolic branchpoint: one branch represented by arginine kinase, leads back to arginine-P, while the other represented by ODH leads to octopine. The details of this competitive branching point situation will be further considered upon my return to Vancouver.

The Pyruvate Branchpoint in *Nautilus*

P. W. Hochachka, University of British Columbia

One observation arose that I'm not certain how to handle: in fresh extracts of *Nautilus* muscle I could always find ample activity of lactate dehydrogenase, measured either in the forward or backward directions. Yet, in the tissue experiments, I could not detect lactate accumulations (this will be checked with a more sensitive instrument at my home laboratory). In any event, I can unequivocally conclude that lactate does not accumulate in the tissues in high quantities.) That presents us with a paradox of the pyruvate branchpoint; namely, the requirement for distributing pyruvate between octopine and lactate.

For reasons that are not entirely clear, ODH essentially totally outcompetes LDH for the common substrate pyruvate. One reason might be relative enzyme affinities for pyruvate (about 0.2 mM for ODH versus a pyruvate K_m of about 0.5 mM for LDH), but this is not fully satisfactory, for at the high arginine levels we observed, arginine substrate inhibition of ODH sets in and should damper the competitive ability of this branch of the system. Only further work can clarify this problem.

If we accept the preliminary data at face value (and assume that no lactate in fact accumulates under any physiological conditions), then we are faced with yet another conundrum; why is LDH there in the first place?

Ultrastructural Features of *Nautilus* Muscle

P. W. Hochachka, University of British Columbia

While studying the metabolic properties of *Nautilus* muscle, the important question of muscle cell ultrastructure naturally arose. Dr. John Arnold imbedded samples of heart funnel, retractor, and spadix muscle. He will pursue the electron microscopy of the spadix in detail at the University of Hawaii, while I will be responsible for the electron microscopy of the retractor, funnel, and heart muscle, studies to be performed at the University of British Columbia.

An Unusual Swimbladder or Swimbladder Function

K. Johansen, Aarhus University and P. C. Gonzales, National Museum of the Philippines

During deep water (ca 450 m) bottom trawling along the coast of Cebu Island close to the town of Alegria in the Philippines a teleost specimen identified to the genus *Peristedion* was caught. The specimen could not be classified as any of the listed species of the genus in the Indo-Australian archipelago, (Weber and De Beaufort: *The Fishes of the Indo-Australian Archipelago*) and will later be identified after consultation with specialists.

When the fish surfaced it showed the typical signs of gas evolution in tissues caused by rapid decompression. A striking feature was two gas filled chambers located bilaterally immediately posterior to the last fill arch and following the contour of the pectoral bone. The surface of the chambers facing to the water side were thin and transparent while the posterior internal walls of the chambers were densely red from a rich vascularization. Gas was immediately sampled from the chambers and analysis revealed a partial pressure of oxygen of about 740 torr. Quite obviously the bladder gas composition would have been altered during decompression since gas was separated from the water by the thin and presumably gas permeable membranes. Dissection revealed that the fish also possessed a typically placed physoclist swimbladder dorsal to the gastrointestinal tract. The dissection unfortunately caused rupture of this bladder and its gas composition could not be analyzed.

Until more specimens of this remarkable fish are collected and further work possible some intriguing speculations into a largely unknown field of swimbladder function are invited. Whereas the so called "gas secretion" or deposition of gas into the physoclist swimbladder is a relatively rapid

process allowing the descent to greater depths to occur at reasonable speed, the reverse process of ascent must occur very slowly due to a very slow resorption of gas from the physoclist swimbladder since the resorption and resulting decompression of the bladder gas must occur via the blood stream. In the *Peristedion* matters may be different due to the unusual gas bladders located externally. If these bladders communicate with the main swimbladder through ducts having a sphincteric control of gas passage, gas could be directly vented to the external bladders upon ascent allowing rapid decompression of the main swimbladder. The compressed gas would presumeably rapidly diffuse through the thin walls of the external chambers making swift vertical ascent possible. Careful dissection will prove if this possibility shall go on record as pure fantasy.

A second possibility is that the two gas bladders are fully pledged swimbladders and possess an intrinsic mechanism for gas deposition. The strikingly vascular posterior walls of the chambers are clearly suggestive of a specialized function. The placement of the bladders bilaterally on the proportionately large head could presumably buoy the fish to an inclination of head higher than tail and thus favor ascending movement at low expenditure of energy.

The fact remains that the external gas chambers could not have been an artificial product of the rapid decompression in the trawl.

Tissues from the external chambers as well as from the typical internal swimbladder have been fixated for later light and electron microscopical studies.

Oxygen Uptake and Hemoglobin Function in the Leech, *Hirudo medicinalis*

K. Johansen, Aarhus Univeristy

The leech, *Hirudo medicinalis*, grows to extraordinary size (18-22 cm) in Southeast Asia. Specimens were collected in ponds near ricefields on Negros Island in the Philippines. Water temperature at the collecting sites exceeded 40°C at midday declining to about 20°C at night. Water oxygen tensions varied from supersaturation during daytime to hypoxic levels (P_{O_2} < 30 torr) at night. Oxygen uptake was measured on 10 leeches using closed respirometry. Water P_{O_2} and pH were measured using a Radiometer PHM 72 acid-base analyzer.

Oxygen uptakes averaged 32.6 µl/g/hr at high oxygen tensions (P_{O_2} 70 torr). At reduced oxygen tensions animals collected the same day showed a regulated uptake down to water oxygen tensions of about 30 torr. Specimens kept in captivity without feeding for 3 to 4 days showed reduced oxygen uptakes below 70-80 torr. At reduced oxygen uptakes the water in the respirometer became rapidly acidified suggesting that the animal has a high capacity for anaerobiosis.

Leeches have no specialized respiratory surfaces and gas exchange occurs through the skin. They allegedly possess a high hemoglobin concentration, but the respiratory properties of leech hemoglobin and its significance in gas exchange have hitherto not been evaluated. Blood was collected from 60 specimens and frozen for later determination of oxygen equilibrium curves and their relationship to pH, temperature, salts and organic molecules possibly having an influence on the binding of oxygen to hemoglobin. Fifty-five live specimens of the giant leeches will be brought to my home laboratory for direct studies on the role of hemoglobin in gas transport and acid-base balance.

Ventilation and its Control in the Sea Snake, *Laticauda laticauda*

K. Johansen, Aarhus University

A method was developed to directly measure tidal volume, breathing frequency and total pulmonary ventilation in unrestrained sea snakes. The method was used to study the normal ventilatory pattern in *Laticauda* as well as the ventilatory response to breathing hypoxic and hypercapnic gas mixtures.

The apparatus consisted of a chamber equipped with a vertical cylinder of a diameter slightly larger than the head of the snake. The system was filled with water to a level in the vertical cylinder. In order to breathe the snake must extend its head into the cylinder and break the water surface. Breathing will displace the water in accordance with the volume of gas inhaled or exhaled. The water displacement will cause a variable hydrostatic pressure in the chamber which was continuously recorded using a Statham strain gage transducer and an Offner-Beckman dynograph. The system was calibrated by adding known volumes of water to the cylinder when the snake was in the head-up breathing position while recording the resulting pressure changes. The respirometer was kept at 25°C and experimental runs lasted 6-12 hrs. Four specimens were used in the study.

Normal breathing in *Laticauda* is intermittent with apneic periods lasting from 60-120 sec between breaths. Only a single breath cycle occurs at each surfacing. Apneic periods are passed in the inspiratory position. A breath cycle starts with an active expiration lasting one second followed by a longer lasting (2 sec) inspiration. Voluntary dives could last as long as 8-12 min, and these were always followed by a much reduced interval between breaths and an increase in tidal volume. Tidal volumes during normal breathing ranged between 8-18 ml depending upon the size of the animal.

Ventilation response curves to graded hypoxia were obtained when breathing 10%, 5%, 3% and 1% oxygen in nitrogen. Only a slight ventilatory response to 10% oxygen was apparent, while breathing 5% oxygen caused a marked reduction in the apneic intervals between breaths. Tidal volumes were commonly doubled and ventilation could increase 6 times. Further reduction in inhaled oxygen concentration increased the frequency of breathing and tidal volume still further. Typically the lung residual volume increased during hypoxic breathing. Breathing hyperoxic gas caused a reduction in tidal volume and frequency compared to normal air breathing suggesting that normal ventilation is driven by an oxygen linked stimulus. Hypercapnic breathing (1%, 3%, 5%, CO_2 in air) had either no effect or a depressant effect on ventilation.

The data suggest that ventilation control in *Laticauda* is governed by a hypoxic drive. Aquatic snakes have a large component of CO_2 elimination occuring by cutaneous exchange. This may be the reason for the apparent insensitivity to CO_2 which affords an advantage to a diving species by permitting longer dives and reduced acid-base disturbance.

Electroretinogram of *Nautilus*

G. D. Lange and A. C. Hurley, Scripps Institution of Oceanography,
and P. H. Hartline, University of Illinois

Our previous studies of squid and octopus have led us to conclude that a series of cascaded low-pass filters approximate the light to electroretinogram generating process. Physical models such as chains of chemical reactions and synaptic events could account for such behavior. *Nautilus* has what appears to be a similar fundamental structure, so it is of interest to determine whether it falls into the above cephalopod pattern. Especially of interest are differences between *Nautilus* and other cephalopods which could be correlated with differences in the role which vision plays in their lives. Dynamic properties of *Nautilus* ERG generator were assessed by stimulating the eye with flashes and square wave or sine wave intensity modulated lights. An excised retina preparation was used (retina remains responsive for 24 hrs or more if cut open and maintained in the dark in oxygenated sea water). Computer analysis will be performed later.

Inspection of the results suggests that low-pass filter characteristics obtain in *Nautilus*. The *Nautilus* retina, however, is much slower than that of squid, octopus or cuttlefish. Examination of the frequency-response curves (Bode plots) of excised *Nautilus* retina at 22-24°C suggests that the attenuation of ERG at 0.2 Hz approximates the attenuation of squid or octopus ERG at 1 to 2 Hz. Similarly, examination of flash or square wave responses show about 5 times longer latency to onset and latency to peak in *Nautilus* as in octopus, squid and cuttlefish. Responses measured at different temperatures show a Q_{10} of about 2 for characteristic time constants. If it is assumed that *Nautilus*

normally lives at about 17°C (as is inferred from bathythermographs taken in trapping areas) the ERG of *Nautilus* seems to be one-tenth as fast as other cephalopods we have studied.

A striking aspect of *Nautilus* ERG is its rapid adaptation to light stimuli. It has proven difficult to study small signal response characteristics since this requires exposure of the eye to some mean light level and measurement of responses to small deviations from that level. Exposure of isolated retina or intact eye to moderate light intensities such as will allow 50% modulated sinusoidal stimuli to produce measurable amplitude responses causes distinct adaptation and thus a vanishing response. The retina must be dark adapted prior to each measurement. To obtain measurements of loss of sensitivity with exposure to light and gain of sensitivity with exposure to dark, we exposed retinas to trains of bright flashes after dark adaptation, or exposed them to dim, infrequent flashes after exposure to a bright adapting light. From the studies of light adaptation we can say that flashes which evoke near maximal responses cause a 20-30% decrement in response to succeeding flashes. From the studies of dark adaptation, we can say that recovery of sensitivity is neither exponential nor linear. Over an hour is required for complete dark adaptation at 22°C. Later analyses of flash response shape during light and dark adaptation will allow us to state how response kinetics change under differing states of adaptation.

Miscellaneous information of interest to comparative neurophysiologists includes the findings that: a) two flash facilitation occurs in *Nautilus* during recovery from an adapting flash b) background enhancement (increment in flash response size if a dim adapting light is simultaneously presented) occurs in *Nautilus* as in squid and ocotpus.

Retinal Responses of Cuttlefish

G. D. Lange and A. C. Hurley, Scripps Institution of Oceanography,
and P. H. Hartline, University of Illinois

A single specimen of cuttlefish was examined using flashes of light. Electroretinogram and optic nerve multi-unit recordings were made. The flash response shows less than 20 msec latency at 29° C. The latency is 50-70 msec at 14° C, thus showing a Q_{10} in the vicinity of 2. The eye is clearly responsive to very rapid changes in the visual world. Optic nerve responses can be recorded and exhibit short latency with transient and tonic components. No unit isolation was achieved, but this was very likely due to the short survival time of our experimental preparation.

This species would be favorable for study in the future, because we were able to obtain optic nerve responses several hours after the eye had been removed.

Ocular Gravity Reflex in *Nautilus*

G. D. Lange and A. C. Hurley, Scripps Institution of Oceanography,
and P. H. Hartline, University of Illinois

As in octopus, *Nautilus* tends to keep its eye fixed with respect to gravity. The eye is turned through an angle of 20° in order to accomplish this as the rest of the body rocks. This is particularly true during vigorous swimming when the animal rocks considerably due to the off-center thrust of the siphon jet.

The dynamics of the response to changes in body orientation imposed from outside the animal were studied. Animals were restrained in a device which could make sudden or sinusoidal changes in body position around an axis through the animal and a little behind and parallel to the eye stalks. Position of the eye was monitored with a television camera. Preliminary data analysis indicates a very fast (within the shortest time measurable by the techniques we could use aboard ship) initial compensatory movement followed by a slower correction.

Ablation of the statocyst organs associated with each eye fully abolished the response in the corresponding eye. Since *Nautilus* has a purely passive mechanism for floating upright in the water due to the buoyancy properties of the chambered shell the statocysts are not necessary for control of the attitude of the animal as a whole. It is possible, therefore, that their main function is the positioning of the eye. Further measurements should allow us to determine the precision of this mechanism (our best estimate now is that the eye is returned to its resting position after a perturbation to within 1 or 2 degrees). One can speculate that the precision of this mechanism might also be related to the precision with which the animal sees the world (its visual acuity).

Dynamics of Pupillary Response in *Nautilus*

G. D. Lange and A. C. Hurley, Scripps Institution of Oceanography
and P. H. Hartline, University of Illinois

Nautilus, like many other molluscs, but unlike other living cephalopods, has a lensless eye. It has been described as a pin hole camera eye. We have found, however, that there is a pupillary closing response to light much like that present in the lensed eyes of vertebrates. We have studied some of the properties of the pupillary light reflex considering it a simple form of behavior which might give us some knowledge of the input physiology of the central nervous system.

The data were obtained by televising the eye using a red light and close-up lenses. The red light had a twofold function 1) minimal stimulus to the retina and 2) enhancement of the pupil on the black and white TV. Careful data analysis will be made later but preliminary results indicate: 1) The response to a step of light has a fast component taking 30 sec or less which nearly completes the change in size. 2) There may be slower later components either in the initial direction or opposite. 3) A pupil will contract due to light in the contralateral eye. This response only requires that the contralateral retina be stimulated, as it occurs when the contralateral iris has been excised or even when the contralateral eye has been cut open leaving only a retinal cup. 4) Some direct effects of light on the iris or other non-retinal elements of the eye have not been totally ruled out. 5) The pupil opens when the animal struggles and also with urethane anaesthetic. 6) Electrical stimulation can open an excised iris which seems to close passively. The anatomy of the iris will be investigated later.

Growth and Buoyancy in *Nautilus pompilius*

A. W. Martin, University of Washington

The growth rate, length of life, and period of sexual maturity have not been defined for nautiloids. Working with *Nautilus macromphalus* the author has suggested four or five years as the probable time. It had been hoped that juvenile specimens of *N. pompilius* might be taken in the prolific Philippine fisheris so that catch distributions would show the presence of year classes. This hope was not realized. Enough specimens were obtained so that counts of shell rings can be made, which may reveal the life span.

Bound intimately to the growth rate is the problem of maintaining the correct buoyancy so that the animal will be neither too heavy as the shell grows, nor too light so that it floats to the surface. When only slightly positively buoyant the animal can swim down, but this proves to be much more difficult than swimming up.

Denton has suggested that, like cuttlefishes, *Nautilus macromphalus* can readmit water to chambers from which most of the water has been pumped by the sodium pump of the siphuncle. The energy needed can come from the hydrostatic pressure at depth. The sodium pump might be decreased in activity or even shut off. This idea is particularly attractive to paleontologists who would like the ammonites to have had a similarly effective physiology. Experimental evidence on this point has been lacking.

During the present cruise all water was drained from the recent chambers of a number of specimens, and the holes sealed with resin reinforced with a piece of nautilus shell chosen to have the correct curvature. When animals

were lowered to a depth of 180 m, and left for 36 hrs, water was readmitted to several chambers, not only into those drained previously which might have leaked water through the plugs, but also into higher chambers of the shell in which leakage was not a factor. The animals had been rendered positively buoyant by fastering corks to the shell so that if signals were generated by the animal to correct its buoyancy such signals would be present. The problems of detection and mechanism are obviously fascinating ones and go far beyond the intentions of the present cruise.

Studies on Renal Physiology of *Nautilus pompilius*

A. W. Martin, University of Washington

Preliminary experiments on *Nautilus macromphalus* in New Caledonia had defined some of the functions of the kidneys of this genus. With the expert surgical assistance available on the ALPHA HELIX it had been anticipated that conclusive experiments on the kidneys could be completed on this cruise. Unfortunately, although the surgery proved easy enough, the animals did not withstand the opening of the shell as had the specimens of *N. macromphalus* and no satisfactory *in vivo* experiments were completed. It was possible to catheterize the large pericardial coelomic nephropores without opening the shell but I was unable to tie a catheter in place, or to tie shut the other nephropore which also drains all of the pericardial coelomic space.

Many animals were available each day, so some *in vitro* experiments were performed. Minces of pericardial gland, renal appendage and liver were exposed to benzoic acid, with and without glycine to test for the formation of hippuric acid. The tissues were extracted with ether and the samples dried, but the hippuric acid, if any was formed, must be assayed in Seattle.

Fish tubules actively take up phenol red *in vitro*. It was thought that *Nautilus* kidneys might accumulate para amino hippuric acid *in vitro*. Minces were exposed to low and high concentrations of PAH but the medium failed to show any disappearance of the compound.

The renal appendages of modern cephalopods are bathed in a filtrate from the pericardial glands, which makes up the fluid of the urine and into which the renal appendages secrete various materials. The renal appendages of Nautiloids are isolated from any filtrate, which flows by 2 separate ducts to the mantle

cavity. Each appendage lies in its own renal coelomic space and has its own duct to the exterior. No fluid appears to leave this space which is usually occupied by a mass of calcium phosphate concrements. It has been assumed in the past that these concrements were waste products, voided only at long intervals. About 4 years ago the author suspected that the calcium phosphate is in reality a store of calcium for the rapid calcification of the new septa. It would require an urgent function indeed to secure the taking over of 4 of the 8 kidneys with consequent major shifts in function of the other kidneys. The urgency is suggested to lie in the necessity of calcifying each new septum before water can be withdrawn from the new chamber by the siphuncle. If air were present before some rigidity had been established in the thin organic membrane a change in depth would rupture the membrane. This explanation also accounts for the fact that concrements are absent in a considerable proportion of the animals examined. There may be up to 10 g of calcium carbonate in a septum of an adult animal, but there may be up to 5 g of calcium phosphate stored in the renal coeloms, so the fraction available very quickly is an appreciable one.

If this hypothesis is correct the calcium must be mobilized very quickly by the animal. In similar cases in the gastropods (although the kidneys are not involved) there is carbonic anhydrase on the concrements. Assays for carbonic anhydrase in pericardial glands and renal appendages, as well as concrements, proved negative. Since the material is calcium phosphate this may not be surprising, but the mantle areas tested were also negative. Frozen tissues have been prepared for the assay of phosphatase in Seattle, but this feature of kidney physiology is worthy of a good deal more study.

Viscosity of Blood

J. R. Redmond, Iowa State University

One of the curious features of the modern cephalopods, and especially the squids, is their high metabolic rate but low oxygen carrying capacity of the blood. The blood of the coleoid cephalopods is very sensitive to pH, the hemocyanin exhibiting a very large Bohr effect. The possibility exists that the oxygen carrying capacity of the blood is limited by the amount of hemocyanin that can be dissolved without unduely increasing the viscosity of the blood, and that the enormous Bohr effect (which is not present in *Nautilus*) may be a means of maximizing oxygen delivery at high partial pressures in a blood of low oxygen capacity. To examine the viscosity effect, samples of whole blood and ammonium sulfate precipitated hemocyanin are being returned to Iowa State University where the relationship between hemocyanin concentration and viscosity will be determined.

Honolulu to San Diego Transit

December 11 - 22, 1975

Scientist, Gary Lopez, Scripps Institution of Oceanography

The purposes of this work were: 1) The collection of water striders, Haliobates spp., for heavy metal analysis. The position of capture was recorded. It is hoped this study will reflect the extent of atmospheric pollutant fallout from the Honolulu-San Diego transect (for results of the analysis contact Dr. Lana Cheng, Scripps Institution of Oceanography. 2) The collection of Lepas spp. (barnacle) for distributional analysis.

Collection was done with a one meter neuston net at sundown, towing at two knots. Ten 30-minute tows were carried out. More than 60 Haliobates were taken in the ten tows, but no Lepas were captured.