

ABSTRACTS OF PAPERS

**Eighth Annual Albert L. Tester Memorial Symposium**

**7-8 April 1983<sup>1</sup>**

The Albert L. Tester Memorial Symposium is held in honor of Professor Albert L. Tester who, at the time of his death in 1974, was Senior Professor of Zoology at the University of Hawaii. The faculty and students of the Department of Zoology proposed an annual symposium of student research papers as a means of honoring, in a continuing and active way, Dr. Tester's lively encouragement of student research in a broad range of fields within marine biology. Papers reporting original research on any aspect of biology are solicited from students at the university and these papers are presented at the symposium, which takes place during the spring semester. Income from contributions to the Albert L. Tester Memorial Fund of the University of Hawaii Foundation is used to provide two prizes for the best papers by graduate students in the Department of Zoology. Papers are judged on quality, originality, and importance of research reported, as well as on the quality of the public presentation. Judges include several members of the faculty of the Department of Zoology as well as winners of the symposium from the preceding year, when possible. In addition, a distinguished scholar from another university is invited to participate in the symposium as a judge and to present the major symposium address. This year Dr. Robert Paine of the University of Washington, Seattle, participated in the symposium.

**A Role for Phagocytes in Endotoxin Protection from Oxygen Toxicity**

JOHN T. BERG<sup>2</sup> AND RICHARD M. SMITH<sup>2</sup>

Breathing pure oxygen causes pulmonary edema and death in rats and other mammals. Phagocytes may be responsible for this edema since they accumulate in the lungs during exposure to pure oxygen and are known to release tissue-damaging free radicals upon stimulation. The recent observation that endotoxin provides protection from oxygen toxicity by greatly reducing pulmonary edema is significant. The present study was designed to test the hypothesis that endotoxin provides protection from oxygen toxicity by reducing

exposure of lung tissue to phagocyte-derived free radicals. The protocol consisted of first lavaging phagocytes from the lungs of rats which had been injected with either endotoxin or saline prior to oxygen exposure and then assessing the ability of these cells to release free radicals by zymosan-luminol chemiluminescence (C1). The protective action of endotoxin was confirmed: three endotoxin injected rats (EIR) all survived 67 hours in pure oxygen while only one of six saline injected rats (SIR) survived a similar time period in pure oxygen. Endotoxin was also found to significantly reduce pulmonary edema. C1 data indicated that EIR phagocytes were capable of generating free radicals at an average rate 3 times greater than the rate measured in

<sup>1</sup> Manuscript accepted June 1983.

<sup>2</sup> University of Hawaii at Manoa, Department of Physiology, Honolulu, Hawaii 96822.

SIR phagocytes. Since this reduced ability of SIR cells to release free radicals may have resulted from substrate or cofactor depletion within a previously overactive cell, it may be that SIR phagocytes produced more free radicals in vivo than EIR phagocytes. An increased in vivo release of free radicals by phagocytes following oxygen exposure was also suggested from a comparison of resting

C1 in cells lavaged from air-breathing and oxygen-breathing rats. Phagocytes from oxygen-breathing rats produced more than three times as much C1 prior to zymosan stimulation (resting level) than cells collected from control rats breathing air. These data support the hypothesis that phagocytic cells are involved in endotoxin protection from oxygen toxicity.

## The Isolation and Preliminary Characterization of Canavanine Resistant Mutants of HeLa Cells

ROBERTA LYNN BRASHEAR<sup>3</sup>

HeLa cells were mutagenized with ultraviolet light and selected for survival at 39°C in the presence of L-canavanine, a deleterious analog of L-arginine. Cells were exposed to the analog for 24 hours, then washed and allowed to grow in normal media at 35°C for a second 24-hour period. This selection procedure was repeated two additional times and surviving cells and colonies were then removed from the culture dishes and cloned at 35°C.

Six clonal lines were isolated and retested for L-canavanine resistance at 39°C. Three of these were also tested for canavanine resistance at 35°C. The cell lines all demonstrated resistance to the analog at 39°C and were then tested for uptake of radio-labelled L-arginine

and L-phenylalanine at an external concentration of 100  $\mu$ m. Uptake levels in the mutant lines were measured at both 35°C and 39°C and compared to the levels of the parental HeLa cell line. Three cell lines were also tested for defective tRNA synthetase activity by measuring the amount of L-arginine coupled to tRNA.

Results indicated that the mutant cell lines showed reduced uptake at 39°C. The mutants displayed varying degrees of L-canavanine resistance at both 35°C and 39°C. The mutant lines which demonstrate L-canavanine resistance only at 39°C appear to be temperature-sensitive mutants and will be the subject of further genetic, biochemical, and physiological characterization.

## *Nautilus belauensis* (Mollusca: Cephalopoda): A Test of the Vertical Migration Hypothesis Using Telemetry

BRUCE A. CARLSON,<sup>4</sup> JAMES MCKIBBEN,<sup>5</sup> AND MICHAEL V. DEGRUY<sup>6</sup>

Observations on the chambered nautilus made by Arthur Willey between 1894 and 1897 suggested that nautilus undergo extensive, daily, vertical migrations. His conclusions have been accepted as fact in all subsequent accounts of nautilus biology, but they have never been directly substantiated. Recently, some investigators have questioned this ac-

<sup>3</sup> University of Hawaii at Manoa, John A. Burns School of Medicine, Department of Genetics, Honolulu, Hawaii 96822.

<sup>4</sup> Waikiki Aquarium, 2777 Kalakaua Ave., Honolulu, Hawaii 96815.

<sup>5</sup> California State University, Physics Department, Long Beach, California 90840.

<sup>6</sup> 25/2 E. Manoa Road, Honolulu, Hawaii 96822.

count of nautilus behavior suggesting instead that nautilus have a wide vertical distribution and do not undergo regular migrations between deep and shallow water.

We monitored the movements of *Nautilus belauensis* in Palau from 19–26 June 1982 by attaching a neutrally buoyant sonic transmitter to the shell of an adult male nautilus. Use of a unidirectional hydrophone allowed us to pinpoint the horizontal position of the animal, and a depth sensitive strain gauge

built into the transmitter relayed depth information. The nautilus moved back and forth ca. 3 km along the reef face and moved vertically between depths of 85 and 467 m. The greatest change in depth during an observation period was 178 m upward in two hours. Water temperature between the maximum and minimum depths ranged from 8°C to 24°C. Generally, the animal was found in deep water in the daytime and at shallower depths at night as predicted by Willey.

## Some Aspects of the Early Life History of Mahimahi (*Coryphaena hippurus*) Reared in the Laboratory<sup>7</sup>

SHARON HENDRIK<sup>8</sup>

It is difficult to obtain information on the early life history of pelagic marine fishes because studies of larval stages of fish usually require expensive and time-consuming ship-board operations. However, information on growth rates, development, behavior, and ecology of larval fish can be determined in controlled laboratory studies if suitable culture techniques can be developed and a source of viable eggs can be found.

Mahimahi, *Coryphaena hippurus*, is a locally common and commercially valuable species. Wild caught adults can be maintained in captivity and will repeatedly spawn without hormone induction. In the present study, eggs were collected from captive adults and stocked into 400 liter cylindrical black fiberglass rearing tanks. Temperature, light cycle, food density, and food type were controlled. Growth rates were determined by daily measurements of standard length, head depth,

body depth, and eye diameter of 10–20 live larvae. Feeding and swimming behavior were documented daily. Dry weight and caloric content of larvae were also determined.

At hatching (day 0) larvae ranged in length from 4.3 to 5.4 mm and exhibited little growth through day 6. By day 4, all yolk was absorbed and larvae initiated feeding. Heaviest mortalities usually occurred on days 6 and 7. This was probably due to unsuccessful initiation of first feeding and supports the “critical period hypothesis” which states that larvae that do not initiate feeding soon after yolk absorption suffer a higher incidence of mortality. After day 6, larvae that successfully initiated first feeding began exponential growth and reached a mean length of 6 mm or more by day 8. Larvae completed metamorphosis by day 35, and by 6 months were sexually mature with a mean length of 53.5 cm.

## Detection of in situ Hybrids by a Monoclonal Enzyme-linked Assay

LEA R. HIRAOKA<sup>9</sup>

In situ hybridization has proved to be a useful tool for the localization of gene sequences in chromosomes. Specific DNA or RNA representing a gene or gene product is hybridized to its complementary chromosomal sequence and the site of the hybrid is detected by immunological or autoradiographic techniques. This method was derived from mem-

<sup>7</sup> This work was supported by the National Marine Fisheries Service, Honolulu Laboratory, Kewalo Research Facility.

<sup>8</sup> University of Hawaii at Manoa, Department of Zoology, Honolulu, Hawaii 96822.

<sup>9</sup> University of Hawaii at Manoa, John A. Burns School of Medicine, Department of Genetics, Honolulu, Hawaii 96822.

brane filter hybridization techniques and is advantageous since hybridization is detected directly in a cytological chromosomal preparation permitting a high degree of spatial localization of specific nucleic acid sequences.

In developing an immunological detection method to map single-copy genes, we have utilized the ADH (alcohol dehydrogenase) gene of Hawaiian *Drosophila* as a model system. This immunological method eliminates the use of radioactive material while producing a permanent record of the hybridization. Copy-RNA probe, made in vitro to the cloned ADH gene, is hybridized to polytene chromosome preparations. Mouse monoclonal antibody directed against DNA-RNA hybrid duplexes and conjugated to alkaline phosphatase is then reacted to the hybridized chromosome. The hybrid-antibody-alkaline phosphatase complex is

then visualized by providing the enzyme with a substrate mixture containing lead ammonium citrate and phosphate. Free phosphates liberated by the phosphatase react with lead to produce a white insoluble precipitate at the site of hybridization. The lead phosphate is then converted to a visible black precipitate (lead sulfide) upon reaction with sodium sulfide.

This technique has been successful in localizing repeated and "single-copy" genes in polytene chromosomes and is currently being tested with human metaphase chromosome preparations. A modified version of this technique has been successful in identifying DNA-RNA hybrids in solution. The latter technique (ELISA) could prove to be useful in the detection of pathogenic agents in tissue extracts and serum samples.

## The Distribution and Abundance of Coral-Feeding Butterflyfishes at Puako, Hawaii

THOMAS F. HOURIGAN<sup>10</sup>

Butterflyfishes (Family Chaetodontidae) are conspicuous inhabitants of coral reefs around the world. As the major family of piscine corallivores, they may have important effects on the corals themselves and the associated community. This study compares the distribution of coral-feeding butterflyfishes on a coral reef to the distribution of corals and other habitat factors.

Visual surveys of fishes were conducted in six distinct habitat zones along a continuous fringing reef at Puako on the island of Hawaii. The number of butterflyfishes (as well as numbers of other corallivores), territorial damselfishes, and potential predators, were counted along a transect line over an area of 500 m<sup>2</sup>. These surveys were repeated over four seasons, beginning in winter 1979. Surveys of butterflyfishes alone were conducted during the summers of 1981 and 1982. Food preferences were determined by observing indi-

vidual butterflyfishes for a minimum of four, 5-minute intervals in each habitat, during which feeding bites on different substrata were recorded. Bottom cover in the survey areas was determined by a standard quadrat method.

Twelve species of chaetodontids were observed along the transects. The majority of butterflyfishes in most habitats were corallivores, with *Chaetodon multicinctus* being the most abundant. Corallivore populations remained relatively stable over the course of this study. Significant correlations were found between the abundance of coral-feeding butterflyfishes and the abundance of preferred corals in different habitats. Correlations with total coral cover were, for the most part, not significant. The numbers of competitors or potential predators could not be shown to affect the distribution of these butterflyfishes. The presence of large numbers of territorial damselfishes, however, may be an important factor. Possible consequences of these patterns of the distribution and abundance of corallivores are discussed.

<sup>10</sup>University of Hawaii at Manoa, Department of Zoology, Honolulu, Hawaii 96822.

## Setagenesis as a Molt-Staging Technique in the Slipper Lobster, *Scyllarides squammosus* (Milne Edwards)

COLIN J. LAU<sup>11</sup>

A preliminary study was conducted to determine a method of molt-staging the slipper lobster, *Scyllarides squammosus* (Milne Edwards). In this species, pigmentation obscures those descriptive features conventionally used in determining molt stages. Setagenesis (setal formation) is difficult to observe because of cuticular pigments that develop during late postmolt. A continuous series of molt stages was described from daily clippings of pleopods and uropods from animals held in captivity preceding and following ecdysis. When feeding stopped, lobsters were classified in premolt condition (proecdysis) from macroscopic evidence (e.g., softness of the ventral branchial region,

compressibility of the ventral thorax) and subsequent molt. The setae of the excised appendage were chemically treated by various melanin removal methods in order to observe the degree of epidermal retraction and progressive eversion of the developing, underlying setae. Potassium permanganate treatment was most effective in melanin removal and caused no noticeable distortion of the epidermal tissues in intermolt and postmolt animals. Results indicate that pigment removal is a viable method for obtaining an objective record of molt stages for crustaceans when pigments mask the events of setagenesis during the molt cycle.

## Influence of Molt Frequency and Size Composition on Estimates of Populations of the Spiny Lobster, *Panulirus marginatus*<sup>12</sup>

LINDA M. B. PAUL<sup>13</sup>

Lobster fishery management recommendations are based largely on data from trap-caught animals. Comparisons of catch/effort ratios over time and analysis of the size structure of captured populations are two common methods of estimating field populations of spiny lobsters. These estimates can be distorted by lobster activity and behavior variables which may be related to size and sex composition of the population, molt stage, and water temperature.

In this study, molt intervals of individually marked spiny lobsters (*Panulirus marginatus*) were recorded for 15 months in the labo-

ratory. Carapace lengths (CL) for males ranged from 4.1 to 12.6 cm; female carapace lengths ranged from 4.1 to 9.1 cm. Results indicated that molt interval increased linearly with carapace length. A significant shift to a longer interval was detected for males larger than 6.6 cm CL during the winter months when tank temperatures fell below 24°C. Analysis of covariance indicated no significant difference between molt intervals for males and females although the mean number of molts per year for females larger than 5.1 cm CL was less than that for males for those size classes tested. There was no significant difference in molt intervals between lobsters missing one or more appendages and those missing none.

Lobsters were almost never found in a trap less than 6 days before or after a molt. Computation of the catchability coefficient ( $q$ ) using laboratory population and catch/effort

<sup>11</sup> University of Hawaii at Manoa, Department of Zoology, Honolulu, Hawaii 96822.

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<sup>13</sup> University of Hawaii at Manoa, Department of Zoology, Honolulu, Hawaii 96822.

data resulted in a 17–19% increase in the value of  $q$  when pre- and post-molt lobsters were excluded from the calculations. Catch/effort data, therefore, indicate catch of intermolt lobsters only, resulting in an underestimation of the total population.

Expectancy tests for equal probability of capture by size and sex indicated that both

male and female lobsters between 7.1 and 7.6 cm CL were slightly trap prone. The existence of trap prone lobsters in a fully recruited but sublegal size class will cause population estimates based on size class distribution frequencies to overestimate the number of juveniles not yet recruited to the fishery.

## **An Experimental Study of the Effects of Piscivorous Predators on Reef Fish Recruitment, Survival, and Community Structure**

ROBERT E. SCHROEDER<sup>14</sup>

For both theoretical and practical reasons it is important to understand the extent to which predation by piscivorous fishes controls the structure of reef fish communities. This problem is being investigated at Midway lagoon in the Northwestern Hawaiian Islands. A quantitative index of recruitment rate is obtained by making sets of daily collections each season of new postlarval fishes that recruit to standardized attraction objects. Recruitment rate appears to be strongly seasonal. Visual census of pristine fish communities on eight patch reefs within the lagoon began in May 1981 and is ongoing. Following a year of baseline monitoring, four of these patch reefs are being experimentally manipulated by repeated spearing of piscivorous predators. Subsequent censuses of

these eight reefs have shown that noncryptic piscivorous predators are less abundant on experimental reefs subjected to spearfishing than on the control reefs. Preliminary results of the initial treatment period have demonstrated a response in recent postrecruit populations of several prey fish species. A trend of increasing population abundance has been shown in replicated experimental reefs (accompanying predator removals and occurrence of natural recruitment), at a time when a trend of decreasing population abundance was seen in replicated control reefs. This trend continued through the first year of treatment. These early results suggest that predators may be important in structuring reef fish communities.

## **The Possible Effects of Intraspecific Competition upon the Population Structure of a Solitary Vermetid Mollusk<sup>15</sup>**

THOMAS L. SMALLEY<sup>16</sup>

Intraspecific competition is known to have a major impact on the population structure and dynamics of sessile marine benthic organisms. Among sessile, suspension-feeding invertebrates, space has consistently been demonstrated to be a limiting resource, whereas the role of food limitation has been considered unimportant. However, access to food

<sup>14</sup> University of Hawaii at Manoa, Hawaii Cooperative Fishery Research Unit, 2538 The Mall, Honolulu, Hawaii 96822.

<sup>15</sup> This work was completed while author was a graduate student at the University of Guam Marine Laboratory.

<sup>16</sup> University of Hawaii at Manoa, Department of Zoology, Honolulu, Hawaii 96822.

implies access to space (and vice versa), thus the observation of competition for space does not justify the assumption that competition for food is unimportant.

Populations of a suspension-feeding vermetid mollusk, *Dendropoma maxima*, were studied in the field, and experimental manipulations were performed to determine if intra-specific competition for food occurs, and if so, if the interaction influences the size structure of the population. *Dendropoma maxima* is found in a wide variety of substrata including live coral. This study examined *D. maxima* populations found in living heads of the massive coral *Porites lutea*.

Vermetid density varied widely between coral heads, and, within any one coral head, the distribution of *Dendropoma maxima* was found to be strongly affected by the orientation of the coral head to the current. On average, 78% of vermetid populations were found on the exposed sides of the coral heads (i.e., facing the current). Calculation of dispersion patterns within coral heads, however, showed vermetids to be randomly dispersed

on both the high density exposed and low density protected surfaces. Size-frequency distributions between vermetid populations of different densities and within coral heads (i.e., exposed vs. protected) emphasize the absence of larger individuals in high density situations. Individuals at high density were significantly smaller than those at low density between coral heads and within coral heads. These results suggest a relationship between density and aperture size in *D. maxima* populations. Experimental manipulations (i.e., reduction of population densities) to measure the effect of population density on the size of the communal mucous nets revealed no significant difference in pre- and postmanipulation size of mucous nets.

Assuming that the relative size of communal mucous nets tells us something about the availability of food to individuals within the population, the results presented here suggest that intraspecific competition for food occurs and this interaction plays an important role in structuring these sessile vermetid populations in *Porites lutea*.

## Single Domain Magnetite Crystals and Their Possible Arrangement in Magnetoreceptor Organelles in the Yellowfin Tuna, *Thunnus albacares*

MICHAEL M. WALKER<sup>17</sup>

Behavioral conditioning experiments have previously demonstrated repeatable responses to earth-strength magnetic fields in the yellowfin tuna, *Thunnus albacares*. I conducted experiments to determine whether the organization of magnetite particles located in tissue contained within the ethmoid bones of the skull was suitable to provide the physical basis for responses to magnetic fields in this species. Magnetometry experiments tested for natural remanent magnetization (NRM) of the ethmoid tissue and for the rate at which the tissue acquired or lost magnetization in progressively increasing inducing or alternating

fields. Further experiments monitored the change in moment with time on warming from  $-196^{\circ}\text{C}$  to  $20^{\circ}\text{C}$ . Extraction, recovery, and examination of the crystals in the transmission electron microscope (TEM) permitted both an independent test of the findings of the magnetometry experiments and unique chemical identification of the magnetic material.

The frozen ethmoid tissues of seven yellowfin tuna exhibited no NRM in a superconducting magnetometer. All acquired significant induced moments (saturation isothermal remanent magnetization, sIRM) when exposed to an inducing magnetic field pulse. On warming from liquid nitrogen to room temperature, the moments retained by the samples decayed exponentially, suggesting that the orientation

<sup>17</sup> University of Hawaii at Manoa, Department of Zoology, Honolulu, Hawaii 96822.

of the magnetite particles became randomized by thermal agitation as the tissues thawed. The progressive IRM acquisition and alternating field demagnetization studies permitted the following conclusions: (1) the ethmoid tissues contained no detectable multidomain particles or high coercivity contaminants such as hematite; (2) the magnetite particles interact significantly; and (3) the particles exhibit a narrow size frequency distribution with average lengths and diameters of 50 and 40 nm, respectively. Crystals observed in TEM showed a narrow size range, averaging  $45 \times 38$  nm, and a nonoctahedral crystal form. They are thus unlike any known geologic

or synthetic magnetite. The crystals are too small for individual use in magnetoreception. However, the detection of interactions between particles suggests that the crystals are organized into chains. The interaction energies of the chains with the geomagnetic field could therefore be large enough for use in magnetoreception. The loss of acquired moment by the ethmoid tissue implies that the chains are at least partly free to rotate. This observation suggests that a mechanoreceptor which monitors position or movement of the chains is a suitable means to link the crystals to the nervous system.

## The Effects of Reef Isolation and Habitat Structure on the Fish Communities of Small Artificial Reefs

WILLIAM J. WALSH<sup>18</sup>

The development and dynamics of fish communities on six small concrete block reefs in Kona, Hawaii, were investigated by means of visual censuses over a 27 month period. To examine the effect of reef isolation on community parameters, pairs of reefs were constructed in three locations relative to a 370 m<sup>2</sup> sand patch. One pair was isolated within the sand patch, one was on its edge abutting the natural reef, and one pair was on the natural reef itself. The effect of habitat structure was studied in two of the locations where different types of blocks were used to construct the reefs within each pair.

All reefs were rapidly occupied, particularly by adults moving from adjacent areas. Eleven species were recorded on the reefs 10 days after establishment. A cumulative total of 50 resident species were recorded in all daytime censuses. Between-census turnover in species composition was high (37.4%) and maximum time of persistence for most species was < 6 months. Movement of adult fishes between

natural and artificial reefs appeared responsible for high species variability.

Juvenile recruitment to the reefs was scant, averaging < 1.5 individuals per reef per census. Settlement was highly patchy with a strong element of stochasticity. Social interactions did not appear to influence the pattern of juvenile settlement. Overall recruitment failed to show distinct seasonality due to the scarcity of juveniles and the presence of winter-recruiting wrasses. Between-year variability in recruit strength was evident for a number of species.

Habitat structure had little effect on the parameters investigated for both diurnal and nocturnal censuses. This was attributed, in part, to the small numbers of species and individuals which occupied the reefs and the presence of unanticipated openings between blocks.

Isolation substantially increased the numbers of species and individuals occupying a reef both during the day and at night. Species turnover was also significantly lower on one of the isolated reefs.

The results of this study suggest that the factors influencing reef fish community structure may depend on the location of the reef.

<sup>18</sup>University of Hawaii at Manoa, Department of Zoology, Honolulu, Hawaii 96822.