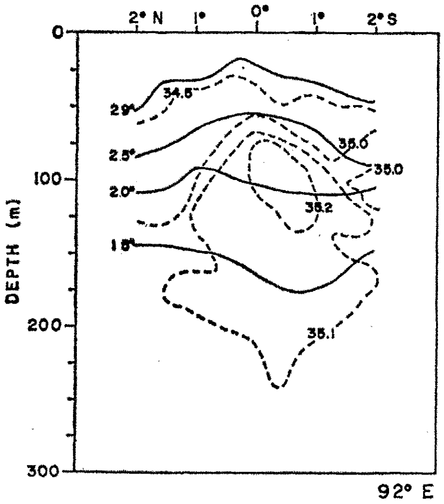

Fig. 3. Vertical section of temperature (solid lines, °C) and salinity (dotted lines, per mille) at 92°E (19–25 April 1963).

sharper than on any of the other meridional sections and showed no tendency to spread at the equator. Associated with the thermocline spreading at 92°E were low values of dissolved oxygen and high values of inorganic phosphate in the surface layer. The meridional distributions of temperature, dissolved oxygen, and inorganic phosphate on the 61°E section, where the presence of the undercurrent was suggested but not clearly established, all showed spreading of the isopleths at the equator. The weakening of the thermocline at the equator was not observed on the other sections where the undercurrent was not present. Thus, even in the Indian Ocean, where the undercurrent is not as well developed nor as steady as in the other oceans, these indications of upwelling of water from the thermocline may be found.

Within the thermocline along the equator a salinity maximum is found whose value at the maximum decreases from west to east. On our sections at 85°E, 89°E, and 92°E this maximum was found to be isolated in the meridional plane from water of a comparable salinity (Fig. 3). This salinity maximum is present throughout the year and would require eastward transport of water of high salinity for its maintenance. The eastward undercurrent measured on the sections at 79°E, 89°E, and 92°E provides the required eastward transport. Metcalf *et al.* have described an analogous relationship between the Atlantic Undercurrent and a salinity maximum at the equator (*3*).

The sign of the slope of the sea surface did not change with the phase of the monsoon. The dynamic topography of the sea surface relative to 1000 decibars on the July equatorial section clearly showed a slope up toward the east of 5×10^{-8} (*5*). The three separate determinations of the slope of the sea surface during the February to May cruise indicated on the eastern side of the ocean a slope of half the magnitude but of the same sign. However, on the equatorial sections of both cruises (July and April) the slope of the deeper isobaric surfaces on the eastern side of the ocean changes sign and becomes negative at 100 to 125 decibars. Thus the eastward velocity component in the thermocline is associated with an eastward pressure gradient roughly comparable to that found near the core of the undercurrent in the Pacific.

These studies show that an equatorial undercurrent does exist in the Indian Ocean with many of the properties associated with the undercurrents of the Pacific and Atlantic. There is no evidence that it is restricted to only one phase of the monsoon although its maximum development occurred during the end of the northeast monsoon. The tendency for the undercurrent to develop in the Indian Ocean is more marked on the eastern side of the ocean than on the western side. The undercurrent did not appear on either of our westernmost sections at 53°E. However, the observed current structure is certainly different from that typically observed at the equator in the other ocean. The speed of the eastward flow in the undercurrent of the Indian Ocean is only half that found in the Pacific. Although the eastward velocity component does appear to be steady over periods of weeks when the undercurrent is developed and can be traced over half the width of the ocean, there were times at which the undercurrent was either weakly developed or not present (*6*).

JOHN A. KNAUSS

Graduate School of Oceanography, University of Rhode Island, Kingston

BRUCE A. TAFT

Scripps Institution of Oceanography, University of California, San Diego

References and Notes

1. J. A. Knauss, *Science* **134**, 1684 (1961).
2. Current measurements were made near the equator in the Indian Ocean on the 31st cruise of the *Vitiaz*. See I. M. Ovchinnikov, *Ann. Géophys. Intern., Oceanol. Res., Art., Acad. Sci. USSR*, No. 4, 18 (1961). A subsurface eastward component of current was observed beneath a westward surface current on sections across the equator 67°E and 76°E during February. A detailed discussion of these observations has not been published, so that the characteristics of the current structure are unknown.
3. For a summary of the observational evidence of the Equatorial Undercurrent see R. B. Montgomery, *J. Oceanogr. Soc. Japan, 20th Anniversary Vol.*, 487 (1962). The most detailed velocity observations are, for the Pacific, those of J. A. Knauss, *Deep-Sea Res.* **6**, 265 (1960), and for the Atlantic, those of W. G. Metcalf, A. D. Voorhis, and M. C. Stalcup, *J. Geophys. Res.* **67**, 2499 (1962).
4. A number of theoretical models of the Equatorial Undercurrent have been published in *Deep-Sea Res.* **6**, 287–327 (1960).
5. J. A. Knauss and B. A. Taft, *Nature* **198**, 376 (1963).
6. The work reported here was part of the *Lusiad* Expedition, a joint University of California–University of Rhode Island venture, and a United States contribution to the International Indian Ocean Expedition. Support for the work was provided by the Office of Naval Research and the National Science Foundation. This report is a contribution from the Graduate School of Oceanography of the University of Rhode Island and the Scripps Institution of Oceanography of the University of California.

6 November 1963

Marine Sediments: Effects of a Tube-Building Polychaete

Abstract. *The marine tube-building polychaete,* Owenia fusiformis, *selects sand grains of tablet form for its tube. It can concentrate the mineral hornblende at least 25-fold and these concentrations may persist after the death of the worm.* Owenia *and a small anemone,* Zaolutus actius, *can act together to stabilize the sand surface against movement by wave surge. The result is the formation of an area of stabilized substrate, with which characteristic animals and plants are associated, in the midst of a region of shifting granular substrate.*

The tube-building marine polychaete, *Owenia fusiformis* Delle Chiaje, is reported to have a worldwide distribution (*1*). It is usually found in areas where the sediment consists of fine sand or a mixture of fine sand and silt. On the intercanyon shelf in La Jolla Bay, *Owenia* is most abundant at depths of 8 to 12 meters; usually, few individuals are found deeper or shallower than this. Most of the observations reported here were made, and specimens were obtained, while diving with self-contained underwater breathing apparatus.

The newly settled young appeared in large numbers in early April, although a few were observed at other times throughout the year—in July, September, January. Starting in May 1960 and continuing through February 1961, sets of 12 sand-core samples, each sample 35 cm² in area, were taken monthly at three different depths, approximately 9, 10, and 11 m. The *Owenia* were counted and the diameter of the muscular "collar" directly below the branchial crown was measured. This structure was selected as an index of size because it did not appear to be appreciably affected by expansion or contraction either in the living worm or during preservation, and because it determines the minimum inside diameter of the tube.

In every month, 100 to 200 individuals were measured. The median diameter of the collars was 0.44 mm in May, 1.12 mm in June, 1.60 mm in July, and 1.68 mm in August; the median remained constant at 1.68 mm through February. The median diameter of the collars of what appeared to be adult worms was 2.3 mm, indicating that they must have been at least 2 years old. The period of rapid growth coincides with the time of the spring bloom of phytoplankton (*3*). Gut contents consisted mostly of finely-ground, unrecognizable organic material; the recognizable components were worn fragments of brown and red algae, mica flakes and, infrequently, diatom cases. In May, the population density was one worm per 5 cm^2. This dropped to about one worm per 7 cm^2 in June and stayed at this level until collecting was discontinued in February. The individual tubes were not scattered at random but occurred in clumps (all indices of dispersion were significant at $<$ 1 percent). Within the clumps, the density reached two worms per 3 cm^2.

When large numbers of young had settled, the tubes stood out from the background of light-grey sand like short pieces of black string (Fig. 1). Microscopic examination of the tubes showed that they were composed almost entirely (95 percent or more) of tabular-shaped, heavy mineral grains, mostly (80 to 85 percent) green hornblende, ranging in size from 62 to 80 μ (*4*). Green hornblende comprises only about 4 percent of the sand at this depth, so the worms must have done a rather rigorous job of sorting. Counts of particles on these tubes indicated that the worms must have handled about 25,000 sand grains per centimeter of tube (0.6 mm outside diameter) constructed.

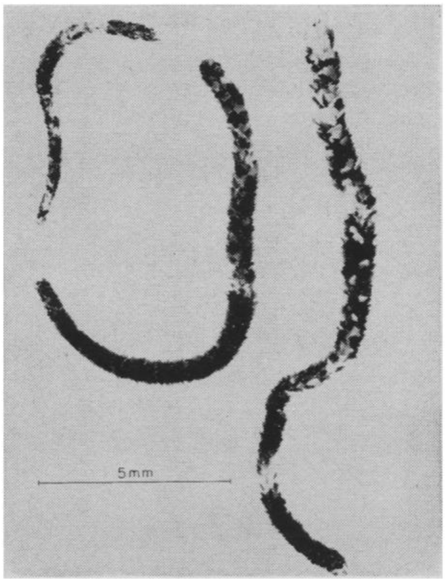

Fig. 1. Tubes of juvenile *Owenia fusiformis*. The dark particles are mostly green hornblende; the light particles are fragments of shell.

The possibility that the worms made use of surface texture, or specific gravity, or chemical differences when sorting particles was discounted by the occurrence of shell (calcite) as the second most abundant material in the tubes, by the absence of certain heavy minerals such as garnet, and by the fact that the heavy minerals of tablet form were present in the same proportions in the tube as they were in the sand. Size is apparently one criterion because all the particles were of about the same size. But size alone cannot be the basis for sorting because quartz, which constituted about 90 percent of the sand but was not used, provided a large supply of particles in this size range. However, a combination of size and shape would provide a basis for the observed discrimination because the quartz, and the heavy minerals such as garnet, that were not used are equant.

As the worms increased in size, larger particles were used to form the tube. Up to the size reached in August (collar diameter $\sim$ 1.7 mm) the tubes were made almost entirely of heavy minerals, though the proportion of shell fragments used had increased with increasing size. When the tubes reached an outside diameter of over 2 mm, the size of particles used had increased so that very little of the heavy mineral fraction of the sand could be used. Tubes of this size or larger contained a considerable amount of quartz, but they also showed that there had been strong positive selection for pieces of shell, entire shells of small gastropods, spines of the sand dollar (*Dendraster excentricus* Eschscholtz), and fragments of its test. All of these have at least one surface that is relatively flat. Tubes could, therefore, be found in all stages, from those of juveniles which were entirely "black" through those with dark "tails" to those that showed no evidence of the juvenile tube and were of coarser texture than most of the surrounding sand. Scholl (*5*) reported a similar preferential selection of platy materials by an intertidal tube-building polychaete, *Phragmatopoma californica* (Fewkes).

The process of tube-forming as described by Watson (*6*) involves a great deal of manipulation by a special "Lippen-organ." During this process the sand grains are either accepted or rejected. The worm increases the diameter and length of its tube by adding onto the upper rim. The particles are attached by their lower portion so the tube is imbricated upwards. As a result, when the worm twists the tube in the sand, the vertical movement is mostly downward and the worm can thus compensate for the increased tube length.

In La Jolla Bay, the worms obtained the particles in two ways: wave surge stirred up the sand and moved the suspension past and through the branchial crown; on days when there was little or no surge, the worms were seen to bend the tube over and pick up particles from the adjacent sand. Tubes reached lengths of 12 to 13 cm and outside diameters of 4 to 5 mm. As they grew larger there was a progressive decrease in the length of the "black" portion, suggesting that the bottom end was either constantly worn off or cut off by the worm (*6*).

The thin organic layer of the tubes to which the sand particles are attached is apparently a quick-setting proteinaceous material. It gave strong positive xanthoproteic, biuret, and sulfhydryl tests; after acid hydrolysis, it gave a positive ninhydrin test but negative tests for sulfate and reducing sugar (N, 7.7 percent). The living worm was able to protect the organic layer against bacterial attack, but empty tubes started to decompose within a week or less in aerated seawater in the laboratory. This means that the concentrations of heavy minerals formed by the worms would not persist long after their death if the tubes were in the sand layer that is subject to stirring by surge. On the other hand, the long tubes sometimes reached below this layer and either death of the mature worm or the gradual attrition of the juvenile tube could result in little pockets of heavy mineral concentration in the sand.

Although *Owenia* was usually found

at depths around 10 m, the densest population observed was inshore of this at a depth of about 6 m. This formed an approximately elliptical bed, 60 m by 150 m, with its long axis oriented parallel to the beach. When first observed in June 1956, it consisted of worms that were at least 15 months old. It was examined on frequent dives until it disappeared in July–August 1957. Although numerous young *Owenia* settled elsewhere in the region during April 1957, none settled in the bed. Since 1957, only very widely scattered individuals of *Owenia* have been seen at this depth.

As one swam seaward across the bed, a gradual increase in the density of worms was noted until the maximum of three worms per 2 cm^2 was observed about 1 m from the seaward edge. At the seaward edge the density was still about one worm per 1 cm^2. The shoreward edge was rather poorly defined but the seaward edge was sharply marked and there were no worms for about 100 m seaward of it. From samples taken throughout the area covered by the bed, the density of the bed as a whole was estimated to be 500 to 1000 worms per square meter.

The unusual location of the bed, the absence of other beds at this depth, the shape of the bed, and the density and distribution of the *Owenia* population within the bed can all be explained if it is assumed that the presence in the surf zone of large numbers of the pelagic larvae in a state ready to settle happened to coincide with the presence of a rip current at this location. The head of most rip currents in this region occurs at a depth of about 6 to 7 m. Underwater observations of sediment in suspension indicate that their seaward edge is often very sharp, so that while larvae would progressively settle out as the current got weaker, most of them might be dropped just as the head was reached. This, in conjunction with the net inshore movement of water near the bottom, would produce the sharp seaward edge and the skewed distribution of the population. The lateral currents feeding into the inshore end of the rip could concentrate larvae from a large volume of water; the lateral motion at the head of the rip would tend to distribute them in a bed elongated parallel to the beach. Provided that there was a period of relative calm after settlement, and that the number of worms settled caused some stabilization of the substrate in spite of their small size, the bed could be established. Once established, it would be able to withstand normal surge and should persist for a considerable time.

Associated with the *Owenia* was a small anemone (*Zaolutus actius* Hand) (*7*). There was an average of 0.95 (759/795) anemone per worm tube. The foot of the anemone was attached to the worm tube 2 to 5 cm below the sand surface and the anemone stretched outward and upward to just above the surface. The combination of worm tubes and anemones had a profound stabilizing effect on the bottom sediment. In contrast to the surrounding sand, there were no ripples in the sand where the animals were situated and little or no sand was put into suspension by the wave surge. Further evidence of the stabilization was provided by the rather frequent occurrence of breeding individuals of three invertebrates that were not found elsewhere on the sand and that, on the basis of their structure and behavior, would appear to require a stable substrate. There were: a long-legged isopod, *Munna ubiquita* Menzies; a pycnogonid, *Oropallene heterodentata* Hilton; and a small hermit crab, *Pagurus* sp. (*7*). In addition, a golden-brown diatom film grew on the sand surface of the bed at various times of the year. In La Jolla Bay, outside of the *Owenia* bed, this film is commonly seen on sand only at depths greater than 10 m. Other biological and geological (*8*) evidence indicates that disturbance of the bottom sediment by surge action decreases very markedly at about this depth.

When first seen the bed was relatively continuous. By October 1956, some relief had developed in the form of depressions 10 to 15 cm deep, 30 to 40 cm wide, and 50 to 100 cm long, with the long axis oriented in the direction of surge movement. The network of worm tubes and anemones, however, still prevented ripple formation and sand movement on most of the bed area. The depressions were apparently started by the activities of various rays: the shovelnose guitarfish, *Rhinobatos productus* (Ayres); the thornback, *Platyrhinoidis triseriata* (Jordan and Gilbert); the round stingray, *Urolophus halleri* Cooper; and the bat ray, *Myliobatis californicus* Gill; all of these dug holes in the sand to bury themselves and, perhaps, fed on the worms (*9*). From this time until the bed disappeared, many more rays were noted in the area than we would have expected from our extensive diving experience at this depth. There was also a marked concentration of the swimming crab, *Portunus xantusii* (Stimpson), one individual of which was seen eating an *Owenia*. By February 1957, a considerable part of the bed was broken up into separate hummocks that were 5 to 10 cm above the level of the surrounding sand, but their surfaces were still sufficiently stabilized to allow the growth of the diatom film. In mid-July a slight reduction in the density of *Owenia* was noted; by 6 August the density had dropped precipitously to 2.2 worms/m^2, and the *Zaolutus* had declined to 0.3/m^2. Even the sand tubes were gone. Their absence suggests that the bed was destroyed in late July—the organic matrix would have required about a week to decompose. Thus a feature that had provided an area of over 7000 m^2 of stable substrate for more than two years, totally disappeared within a matter of weeks.

The few remaining isolated clumps of *Owenia* plus *Zaolutus* appeared to be acting normally. A group of *Owenia* and *Zaolutus* from the bed brought into the laboratory on 6 July experienced high temperatures (23° to 25°C) and scarcity of food, yet they persisted until September under what must have been relatively unfavorable conditions. From outward appearances, they were representative of the majority of the individuals in the bed.

Continuous records of temperature and wave heights taken at the end of the Scripps Pier (at the same depth and about 150 m north of the bed) indicated no unusual values for this period. There had been a net lowering of the sand level by 10 cm during the period 26 June to 2 August but this was about usual for the season (*8*). The continued presence of normal numbers of *Dendraster excentricus* Eschscholtz and *Renilla köllikeri* Pfeffer, both of which would be more susceptible to removal, also discounts the possibility that there had been a period of unusually violent scour by wave surge action. The available information suggests that neither physical conditions nor disease nor age of the *Owenia* caused the destruction of the bed. The most likely explanation seems to lie in the activities of the rays. A relatively large school of bat rays could have accelerated and completed the process that had led to dissection of the bed into hummocks by February 1957.

E. W. Fager

Department of Oceanography,
Scripps Institution of Oceanography,
University of California, San Diego

References and Notes

1. P. Fauvel, *Polychètes Sedèntaires*, vol. 16 in *Faune de France* (Lechevalier, Paris, 1927), pp. 202–204.
2. I thank A. O. Flechsig and R. J. Ghelardi for their excellent assistance.
3. W. E. Allen, *Bull. Scripps Inst. Oceanog. Univ. Calif.* **5**, 293 (1945).
4. Tube analysed by G. A. Rusnak.
5. D. W. Scholl, *Compass* **35**, 276 (1958).
6. A. T. Watson, *J. Linnean Soc. London, Zool.* **28**, 230 (1901).
7. *Zaolutus actius* was identified by Cadet Hand; *Oropallene heterodentata* Hilton was identfied by J. W. Hedgpeth; all other organisms were identified by the author on the basis of published descriptions.
8. D. L. Inman and G. A. Rusnak, *Beach Erosion Board, Tech Mem. No. 82* (U.S. Corps of Engineers, Washington, D.C., 1956); D. L. Inman, *ibid., No. 100* (1957).
9. R. Ridge, unpublished work indicates polychaetes may be a major item in bat ray food in some locations.
10. Supported in part by National Science Foundation grant G-7141, and by the Marine Life Research Program, the Scripps Institution's component of the California Cooperative Oceanic Fisheries Investigations, sponsored by the Marine Research Committee of the State of California.

26 September 1963

Diglyceride Release from Insect Fat Body: A Possible Means of Lipid Transport

Abstract. *Lipid is released in the form of diglyceride from the fat body of the cecropia silkmoth* (Hyalophora cecropia), *in both adults and pupae. This diglyceride, in the form of a complex with hemolymph protein, is the most probable means by which lipid is transported in this insect.*

Although lipids are of vital importance to many insects as metabolic fuel, little is known about the transport of lipids from the organ of storage (fat body) to sites of utilization. This report indicates that in pupae of the American silkworm (*Hyalophora cecropia*), long-chain fatty acids are transported in the form of diglycerides which are probably conjugated to hemolymph (blood) proteins.

When palmitic acid-1-C^{14} was injected into cecropia pupae and the lipid extracted from the pupa a few hours later, almost all the recovered radioactivity was found in the neutral fat fraction. The pupae used in these experiments were held at 6°C for about 6 months. Morphological examination revealed that they had not begun adult development at the time of the experiments, which took place on the day the animals were returned to room temperature. Judging by previous experience, these pupae were ready to begin development. Palmitic acid was injected as the potassium salt, albumin complex (*1*), and radioassays were performed with a liquid scintillation counter (*2*); internal standards were used to correct for quenching. When lipid was extracted separately from the fat body and hemolymph (blood), it was noted that the specific activity of the neutral fat in the hemolymph was more than 120 times greater than that in the fat body. This suggested that either the hemolymph is capable of synthesizing neutral fat from unesterified fatty acid or that the lipid is synthesized in the fat body and released into the hemolymph. Our subsequent finding, that hemolymph alone has only a negligible capacity to incorporate labeled palmitate into neutral fat, lends credence to the latter hypothesis.

To investigate the premise that the fat body releases newly synthesized lipid into the hemolymph, the following experiments were conducted. The total amount of labeled lipid extracted from the fat body and hemolymph was subjected to column chromatography (*3*). Results in Table 1 reveal that the radioactivity of the diglyceride fraction in the hemolymph was extremely high, both in total and specific activity, but that the triglyceride fraction had a very low specific activity. That the palmitate was converted to neutral fat in the fat body was demonstrated in the following manner. When isolated pupal fat body was incubated in saline (0.86 percent KCl in phosphate buffer, *p*H 6.7) containing labeled palmitate, there was a rapid incorporation of the fatty acid into neutral fat within 60 minutes. About 80 percent of the labeled neutral fat was in the form of diglyceride, while the remainder was triglyceride. However, of the total neutral fat found in this organ, almost 98 percent was triglyceride and less than 2 percent diglyceride. The specific activity of the diglyceride was almost 200 times greater than that of the triglyceride. These experiments in vitro suggest that palmitate is first incorporated into diglyceride by the fat body, and that a major fraction of the diglyceride is then released into the hemolymph. The remaining diglyceride in the fat body could conceivably be used for triglyceride synthesis by conventional biosynthetic means (*4*). Little, if any, of this triglyceride is released into the hemolymph. According to this view, one would expect the observed higher specific activity of neutral fat to be in the hemolymph rather than in the fat body.

To examine this hypothesis further, 1.5 g of fat body carefully dissected from a pupa was rinsed several times with saline solution and then incubated in 3 ml of saline solution containing 1 million count/min of palmitate (0.04 μmole), at 25°C. After 60 minutes the fat body was rinsed several times to remove excess fatty acid, and this "prelabeled" fat body was then incubated in cell-free hemolymph. The incubation media used in control experiments included saline or various albumin solutions. At the end of the incubation period, the lipid was extracted from the incubation medium with a mixture of isopropanol, heptane, and 1*N* H_2SO_4 (40 : 10 : 1 vol/vol) (*5*), and chromatographed on florisil as before. As shown in Table 2 and Fig. 1, the labeled diglyceride was rapidly released from the fat body into the hemolymph while little triglyceride was released. An appreciable quantity of free fatty acid was also released from the fat body into the hemolymph. While the release of labeled diglyceride appeared to be specific for the insect's own hemolymph, the triglyceride and unesterified fatty acids entered the albumin-containing media with the same facility as they entered the hemolymph. From these experiments, there is little doubt that the diglyceride fraction of the neutral lipid is important as a means of transporting lipid from the fat body to other metabolic sites.

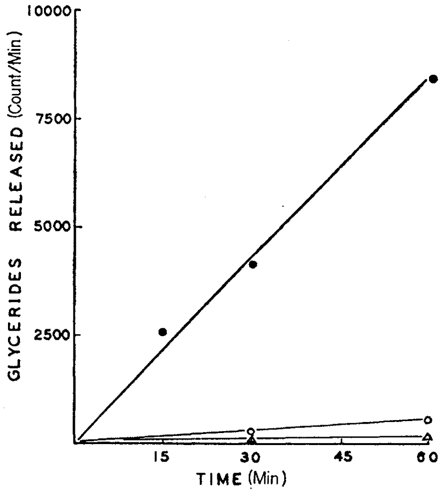

Fig. 1. The release of glycerides from pupal fat body. Samples (300 mg) of labeled fat body containing 19,900 count/min of triglyceride and 97,300 count/min of diglyceride were incubated with 1 ml of saline and 0.2 ml of cell-free hemolymph containing 1 μmole of glutathione (to inhibit blood tyrosinase) or with 0.8 ml of saline and 1 μmole of glutathione without hemolymph. Solid circles, diglyceride released into hemolymph; open circles, diglyceride released into saline; and triangles, triglyceride released into hemolymph.