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* Present address: Department of Chemistry, University of Maryland, College Park 20742.

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Recovery and Maintenance of Live Amphipods at a Pressure of 580 Bars from an Ocean Depth of 5700 Meters

Abstract. *Amphipods were collected from an ocean depth of 5700 meters in a windowed pressure-retaining trap, kept alive in the trap for as long as 9 days aboard ship, and transported to a land laboratory. Observations suggest that the animals can easily tolerate decompressions of 29 percent and briefly of 70 percent of the value of 580 bars, the pressure of their natural habitat. The average pleopod beat frequency was 106 beats per minute. Evidence suggests that food (fish bait) can have at least a 4-day residence time in the gut of these animals.*

This is an account of the recovery of live amphipods four times in succession from a depth of about 5700 m in the central North Pacific Ocean. The pressure at this depth is about 578 bars, a pressure that disrupts the integrated functions of prokaryotic (*1*) and eukaryotic cells (*1, 2*) and of higher metazoa (*2*) found in shallow waters.

The unusual properties of the deep sea (*2, 3*) are a consequence of the considerable water column above it. The deep-sea habitat is characterized by a low temperature, a high hydrostatic pressure, the near absence of light, and a level of cosmic radiation substantially lower than that at the surface. The high pressure (*4*) renders this environment and its inhabitants refractory to study. Animals collected remotely with trawls, corers, and grabs are often torn or abraded, but even when intact they succumb as an apparent result of decompression.

Two approaches are used to circumvent these difficulties. One approach is to study animals and microbes in situ with submersibles [manned (*5, 6*) and unmanned (*7*)] and with free vehicle (a freely falling package) instruments (*8*). The other approach is to capture organisms in pressure-retaining devices and to maintain them in the laboratory for study within these devices or within high-pressure aquariums (*9*). The control over animals for experimentation should be greater in the laboratory than is possible in situ and should allow for otherwise infeasible studies. There have been only a few reports of the recovery of compressed samples from the ocean. Jannasch *et al.* (*10*) have described devices and used them (*6, 11*) to recover water samples from depths of about 3000 m. Macdonald and Gilchrist (*12*) designed a device for the study of animals and demonstrated its pressure-retaining feature by recovering a sample of water from a depth of 2000 m. Samples of plankton have been recovered with the device from depths of 600 m.

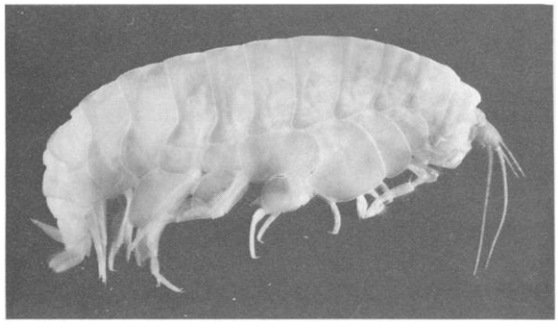

Fig. 1. Photograph of a preserved amphipod caught outside a PRAT in the central North Pacific Ocean and having an extended length of about 9 cm. Several genera occur in this locale (*14*).

The size and behavior of the animals sought in the deep sea determine the design of the capture gear. Hessler *et al.* (*13*) suggested on the basis of photographs that amphipods might be the animals to pursue in the deep sea because of their attraction to baited traps. The successes (*14, 15*) with which amphipods have been trapped in the deep ocean over the past few years fully support their contention. Amphipods (Fig. 1) may indeed become important experimental animals in deep-sea biology.

The pressure-retaining characteristics of the device employed have been described elsewhere (*16*). With this device it has been possible to retrieve amphipods (*15*) from the Philippine Trench with a retention in the trap of 75 to 85 percent of the pressure (about 960 bars) at the trench floor. It was not possible to determine the viability of these amphipods because of the limitations in ancillary equipment. Three equipment improvements made possible the study reported here. (i) A gas-filled hydraulic accumulator (*17*) was attached to the pressure-retaining trap to diminish the pressure loss arising from metal expansion and seal movement encountered during the ascent of the trap to the sea surface. (ii) Thermal insulation was provided to prevent the animals from being exposed to the warm surface waters of the ocean. (iii) A pumping system (*18*) was designed so that seawater at a high pressure could be circulated through the trap after its retrieval and thus could provide oxygen to and remove waste products from the water bathing the animals.

The traps (Fig. 2) were deployed as free vehicles (*19*) that sink to the sea floor, rest there for a predetermined time, discard ballast, and rise to the sea surface where they are recovered onto the ship. The entrance to the pressure-retaining chamber of a trap is shut within a few seconds after the release of the ballast used to sink the free vehicle. The free vehicles were equipped with a radio transmitter and a flashing light to assist in locating them on the sea surface and were usually recovered within 1.6 km of the deployment site.

Seawater was circulated through the pressure-retaining amphipod traps (PRAT's) after recovery. The heart of the pumping system consisted of an air-driven high-pressure pump (*20*) that was compatible with seawater. A piece of 316 stainless steel tubing having an inside di-

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Table 1. Data on live retrievals.

Date (1977)	Position	Depth (m)	Pressure (bars)		Time on sea floor (minutes)	Time in surface water (minutes)	PRAT number	Station number
			At depth	In PRAT				
8 June	28°37.4N, 155°24.03W	5782	586	559	608	73	2	654
9 June	28°23.19N, 155°24.95W	5549	562	565	751	21	1	669
16 June	28°36.82N, 155°30.60W	6049	613	407	829	20	2	740
20 June	28°35.65N, 155°20.39W	5741	582	414	545	15	2	781

ameter of 0.76 mm and an outside diameter of 1.59 mm was connected to the output of the high-pressure pump and to one of the high-pressure valves on the trap. The tubing (6.1 m long) was wound into a coil and immersed in the constant-temperature bath so that seawater flowing though the coil was cooled before entering the trap. This tubing also served to dampen the pressure fluctuations of the pumping cycle and to partly meter the flow of seawater. A 6.7-m length of the 316 stainless steel tubing was connected to the output of the trap; its inside diameter (0.15 mm) served as the primary mechanism for metering the flow of seawater. With an air pressure of 2.14 bars, seawater entered the trap at a rate of 3.4 cm^3/min and a pressure of 565 ± 12 bars in a continuous and unattended mode of operation. The supply of seawater for the traps was collected from the open ocean and was passed through filters having a pore diameter of 0.22 μm. Surface seawater has an O_2 concentration of about 4 cm^3 per liter of seawater. Thus, this flow rate should have allowed for the utilization of O_2 in the trap at about 0.8 cm^3/hour without any reduction in the concentration of O_2.

Aspects of four sequential live retrievals are summarized in Table 1. Two pressure-retaining amphipod traps were used in the same general locale of the central North Pacific Ocean. The traps rested on the sea floor for about 12 hours, since over this period a sizable quantity of animals would be attracted and so there would be a high probability that some of them would enter the pressure-retaining portion of the trap. The time spent in the deep ocean was also sufficient to chill a trap to the ambient temperature of about 1.2°C. Insulation was provided around the trap to protect the animals from the warm surface waters (about 24°C). The insulation maintained the temperature of a PRAT at less than 6°C for 1 hour while surrounded by warm surface waters and at even lower temperatures as the length of time spent in the surface waters diminished. Three of the trap recoveries were rapid (Table 1), and the traps were placed in a 2°C bath within 10 minutes.

The principal objective of this study was to retrieve live animals and maintain them at their deep-sea environmental conditions for at least 1 week. Another objective was to evaluate the logistic problems encountered in transporting a PRAT back to our laboratory at Scripps Institution. Successful transport to and maintenance in the laboratory are important since it then becomes possible to carry out experimentation on events such as molting, limb regeneration, and genetic processes that will require a long period of observation. The animals recovered at station 781 (Table 1) were kept alive for 9 days aboard ship, for another day in transit to our land laboratory, and for still another day at the laboratory.

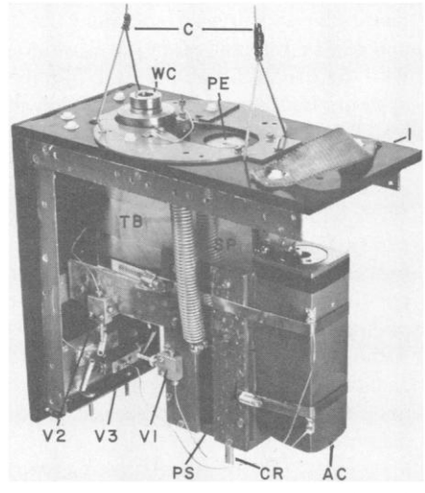

Fig. 2. Photograph of a PRAT taken with only two sides of the insulating box (*I*) shown. The PRAT sinks to the sea floor with the ballast connected to the rod (*CR*). On the sea floor, the floats (not shown) fastened to the cable (*C*) lift the trap body (*TB*) and stretch the two springs (*SP*) (only one visible) until the piston comes to rest on the stop (*PS*). The entrance (*PE*) into the chamber in the *TB* is then open. Animals are attracted to bait in half of a minnow bucket (not shown), which is secured to the circular plate at the top of the apparatus. When the ballast is released, the piston is pulled into the trap body as the springs contract and the piston seals the chamber of the trap [see (*16*)] when the piston end (*PE*) is at the position shown. The release of ballast also allows the floats to return the PRAT to the sea surface. The gas-containing accumulator (*AC*) is disconnected after retrieval with valve *V3* and its charge of N_2 is vented with valve *V1*. Valves *V2* and *V3* are used to connect the trap to a high-pressure seawater circulating system (*18*). One of the two windowed closures (*WC*) is visible. The *PE* diameter is about 5 cm.

What is the range of hydrostatic pressures and what are the rates of change in pressure that deep-sea organisms will tolerate? These questions are important to understanding the role of hydrostatic pressure as an environmental variable contributing to the zonation of species in the water column and the evolution of species in the ocean. Moreover, answers to these questions could lead to methods for handling deep-sea organisms outside of pressure vessels even if for only short periods of time. I made some observations that bear on these questions. The first recovery of live animals (Table 1, station 654) was achieved without a significant loss in pressure. An animal was seen in my earliest observations (approximately 45 minutes after the recovery of the free vehicle) and it exhibited swimming activity, beating of pleopods, and resting states. About 1.5 hours after the recovery, an inadvertent decompression occurred over 30 minutes and resulted in the pressure of the trap dropping to 172 bars—a 70 percent reduction from the natural environmental pressure and recovery pressure. The decompression was then detected, and the pressure in the trap was increased to 565 bars within 3 seconds. Observations for 2 hours after the recompression did not reveal any swimming animals. However, two swimming animals were seen 8 hours after the recompression. Thus at least two animals survived a decompression of nearly 400 bars for about 30 minutes and a rapid (3-second) recompression.

Evidence that the animals may be affected by even smaller decompressions was obtained at station 781. The trap was on board the ship 15 minutes after the free vehicle arrived at the sea surface. Within an additional 10 minutes the trap was in a water bath at 2°C and the pressure in the trap, initially 414 bars, was immediately increased to 572 bars. Observations revealed that there were five small animals lying in the chamber of the

trap and displaying very little motion. During the first hour after the recompression, the activity of the animals increased dramatically. By 4 hours after the recompression, the animals were very active and were swimming—darting from one part of the trap to another. This greatly increased activity could have been due to the introduction of fresh seawater into the trap begun concurrently with the recompression or to the recovery of the animals from the adverse effects of decompression. The first trap (station 654) contained many more animals (24) than the last one (station 781) and had actively swimming animals in it prior to the introduction of any seawater. All of these observations support the hypothesis that the amphipods were adversely, albeit not irreversibly, affected by a decompression of about 28 percent rather than by a lack of fresh seawater.

The following observations relate to the feeding habits of these animals. The bait attracting the animals caught at station 669 was available to them for ingestion. One of the live animals caught had a greatly distended abdomen; this condition is observed only in animals satiated with bait. The distended condition persisted for 4 days. The death of the animal on the fourth day was due to an inadequate amount of circulating seawater. A long residence time for this kind of food (fish) in the gut of an amphipod is implied by the long duration of the distended condition. This animal did not appear to produce fecal material. In contrast, the amphipods caught at station 781 produced fecal pellets during the early portion of their captivity. The animals from station 781 had probably not eaten the bait used to attract them to the trap since the bait was wrapped in a nylon net. Although fecal material was not eaten immediately, it may have been eaten later since it eventually abruptly disappeared. Curiously, an animal in the trap that was incapacitated for about 6 days and died on the seventh day of captivity was not bothered (eaten) by the other healthy animals in the trap for 11 days. Amphipods from shallow water are known to be cannibalistic and to eat their own molts (*21*)—adaptations that would seem to be desirable in a food-scarce environment such as the deep sea. Cannibalism varies among species (*21*) and between sexes (*22*). If deep-sea amphipods prove not to be excessively cannibalistic, then it may be possible to maintain breeding cultures (*23*).

Amphipods respire by moving water over gills located near the ventral aspect of the thoracic coxae. The ventilation is accomplished by the beating of pleopods; the beating frequency is affected in shallow-water organisms by a number of factors (*24*), including the partial pressure of CO_2 and O_2 in the water and the size of the animal. Values for the ventilation frequency are tabulated (*24*) for amphipods living at 1 atm and 14°C and have a range of 12 to 184 beats per minute. Observations were made of the ventilation frequency with about eight animals in the traps from stations 654, 669, and 781, at a pressure of 572 bars and at a temperature of about 2°C. For 63 observations, the average value was 106 beats per minute, the standard deviation was 33 beats per minute, and the range was 41 to 181 beats per minute. Thus the ventilation frequency for deep-sea amphipods is not much different, if at all, from that for shallow-water ones. The 63 values observed include what I believe, based on the activity of the animals, are values for healthy and unhealthy animals. Values in the range of 90 to 150 beats per minute are probably indicative of healthy animals (*25*).

This study demonstrates that deep-sea amphipods can be trapped for physiological studies in a pressure-retaining device and can be maintained in the laboratory with the use of a high-pressure pumping system circulating seawater through the trap. The components of the systems are small enough that they can be transported by air to land-based laboratories. Work with these animals will provide the means for measuring rates of biological processes in highly controlled laboratory conditions, for determining the effect of pollutants on deep-sea life, and possibly, if the deep-sea animals can be bred as shallow-water amphipods have been, for understanding genetic processes in the deep ocean.

A. ARISTIDES YAYANOS
Scripps Institution of Oceanography, University of California, San Diego, La Jolla 92093

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17. This is a cylinder whose interior is divided into two parts by a freely moving piston. One compartment is filled with N_2 at an initial pressure somewhat less than that at the depth at which the trapping will be conducted. The other compartment is filled with seawater and is connected to the chamber of the pressure-retaining trap. The N_2 is about ten times more compressible than seawater at a depth of 10,000 m.

    The following calculation shows how the accumulator diminishes the effects of seal movement and of metal expansion encountered during the ascent of a trap from the sea floor. The compressibility of a substance (in this case, seawater) is defined by

    $$\beta_{sw} = -(1/V_{sw})\,(\partial V_{sw}/\partial P)_T$$

    where β_{sw} is the compressibility of seawater, V_{sw} is the volume under consideration, P is the pressure, and T is the temperature. The pressure dependence of β_{sw} is insignificant for the purpose at hand. At maximum ocean depths, $\beta_{sw} \cong 35 \times 10^{-6}$ bar^{-1}. The volume of seawater under consideration is that in the pressure-retaining chamber of the trap having a volume designated by V_{ch} and approximately equal to 450 cm^3. Therefore, substituting V_{sw} with V_{ch} gives

    $$\partial P/\partial V_{ch} = -(V_{ch}\beta_{sw})^{-1}$$

    This relationship to a first approximation gives the change in pressure in a sealed chamber as a result of changes in the chamber volume. Substituting the above values gives $\partial P/\partial V_{ch} = 63.5$ bars per cubic centimeter change in V_{ch}. The attachment of a gas-containing accumulator to the trap results in a V_{ch} of about 650 cm^3, and that is part seawater and part gas. The compressibility of the gas-liquid interior of the chamber is greater than the compressibility of seawater alone (β_{sw}) and has a value of about 160×10^{-6} bar^{-1} at maximum ocean depths. Therefore, $\partial P/\partial V_{ch} = 9.6$ bars per cubic centimeter change in V_{ch} and shows by how much an accumulator can reduce the pressure loss encountered as a result of seal movement and metal expansion. The actual value of $\partial P/\partial V_{ch}$ is a function of the volume of the accumulator, the depth at which the sampling will occur, and the amount of N_2 initially in the accumulator.
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20. An air-driven pump (model DSHW-225, Haskel Engineering and Supply Company. Burbank, Calif.) was used. The pump body is made of a stainless steel compatible with brines. Ideally, the pump body should be made of the titanium alloy (6A14V) which was used to make the pressure-retaining trap. The pumping cycle causes a ± 14-bar fluctuation in the hydrostatic pressure inside the trap. This could be reduced by at least an order of magnitude by a feedback signal from sensors on the pressure gauge that could regulate the air pressure regulator and the air flow to the pump. No effect of these pressure fluctuations could be detected on the behavior of the animals.

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25. The taxonomic description of the amphipods caught has not been completed but will be a part of the studies in progress by other participants on the expedition.

26. I am greatly indebted to R. V. Boxtel, B. Minor, and R. Wilson for untiring assistance and helpful discussions; to Dr. K. L. Smith, Jr., for his help as chief scientist, for helpful discussions aboard the R.V. *Thomas Washington*, and for his criticisms of the manuscript; and to M. E. Horn, the ship's first officer, for the speedy recovery of the free vehicles. Supported under National Science Foundation OCE 76-12017 and OCE 76-80874 and under Energy Research and Development Administration contract EY-76-03-0034-241-142.

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Mineralization Kinetics: A Constant Composition Approach

Abstract. *A new method is described for studying, reproducibly, the kinetics of crystallization of minerals under conditions of constant solution composition even at very low supersaturations. For calcium phosphates the method provides direct evidence for octacalcium phosphate as the precursor to hydroxyapatite precipitation at physiological* pH.

The precipitation of sparingly soluble salts from their supersaturated solutions finds application in a wide variety of scientific fields. The carbonates of calcium and magnesium are of great interest in limnology (*1*), oceanography (*2*), and sedimentology (*3*). Important manifestations of nucleation and crystallization of calcium phosphates are the transformations in soil following phosphate fertilization, removal of phosphate from wastewater by the addition of lime, formation of teeth and bones, and pathological stone formation in the urinary tract. Precipitation of the sulfates of calcium and barium creates serious problems in desalination and in oil-field scale formation. In this report we describe a new and highly reproducible method for studying rates of mineralization, even at very low supersaturation, with a precision hitherto unobtainable.

Although numerous spontaneous precipitation studies of minerals have been made, they suffer from the disadvantage that the size and size distribution of the solid particles change during the course of the reaction. Also, the usual assumption of homogeneous nucleation (*4*) is open to question, since it is doubtful if any medium is sufficiently free from foreign nucleating sites to preclude heterogeneous nucleation (*5*). The problems associated with the irreproducibility of the results of such studies were overcome with the development of seeded growth techniques (*6, 7*), which enabled the effects of factors such as temperature, supersaturation, and ionic strength to be studied quantitatively (*8–10*).

The precipitation of calcium phosphate phases is of particular interest. Under solution conditions normally encountered biologically, hydroxyapatite [$Ca_5(PO_4)_3OH$, hereafter HAP] is the thermodynamically stable phase. However, most calcium phosphate solutions in crystal growth experiments are initially supersaturated with respect to four additional phases; in order of increasing solubility at physiological *p*H these are tricalcium phosphate [$Ca_3(PO_4)_2$, hereafter TCP], octacalcium phosphate [$Ca_4H(PO_4)_3 \cdot 2½H_2O$, hereafter OCP], anhydrous dicalcium phosphate ($CaHPO_4$, hereafter DCPA), and dicalcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$, hereafter DCPD). Hydroxyapatite does not normally precipitate directly from solution, although it has been shown to grow on HAP seed crystals at very low supersaturations (*10*). From experiments involving the mineralization and x-ray analysis of epiphyseal rat cartilage, Posner (*11*) identified an x-ray amorphous material of approximately TCP composition. This phase has also been proposed as a precursor to HAP in the formation of teeth and bone (*12*). In addition, both OCP (*13*) and DCPD (*14*) have been proposed as possible precursor phases.

The development of a *p*H-stat method (*15*) enabled studies to be made of calcium phosphate crystal growth on well-characterized synthetic and natural seed material under conditions of constant hydrogen ion activity. These methods, however, still suffered from the disadvantage that the calcium and phosphate ionic concentrations varied appreciably during the reactions. At each stage, therefore, the supersaturated solutions were metastable with respect to different calcium phosphate phases, which could form and subsequently dissolve as the concentrations in the supersaturated solutions decreased. Since the concentration changes became very small as the reaction proceeded, a relative analytical error of only a few percent in the total calcium or total phosphate concentration could preclude differentiation between the possible crystalline phases.

To overcome the problems associated with the changing solution composition during precipitation, a new method is reported here in which the chemical potentials of the solution species are maintained constant during the reaction. After the addition of well-characterized seed material to a stirred, stable supersaturated solution of calcium phosphate at the required *p*H, the concentrations of lattice ions were maintained constant by the simultaneous addition of reagent solutions containing calcium and phosphate ions, controlled by a glass electrode probe. The compositions of the reagent solutions were calculated from the results of exploratory measurements. The method is illustrated by three experiments at 37°C and the physiological *p*H of 7.40, in which the solutions were supersaturated with respect to all phases other than DCPD and DCPA. These are summarized in Table 1. In experiment 1377, the solution Ca/P molar ratio, *R*, initially 1.39, decreased by 4 percent

Table 1. Constant composition seeded growth of HAP crystals at *p*H 7.40, 37°C. Symbols: T_{Ca}, total calcium concentration; *R*, molar Ca/P ratio in solution at each time.

Experiment 1377*			Experiment 1777†			Experiment 2577‡		
Time (min)	T_{Ca} (m*M*)	*R*	Time (min)	T_{Ca} (m*M*)	*R*	Time (min)	T_{Ca} (m*M*)	*R*
0	0.800	1.39	0	0.800	1.450	0	1.200	1.33
5	0.788	1.39	20	0.797	1.457	1	1.197	1.32
13	0.770	1.38	34	0.796	1.457	3	1.208	1.32
30	0.755	1.35	48	0.797	1.450	5	1.214	1.32
40	0.752	1.35	60	0.794	1.457	7	1.234	1.31
			75	0.800	1.462	10	1.248	1.30
			90	0.797	1.457			
Mean					1.456			1.32
Standard deviation					0.004			0.01

*Initial solution: 150 ml of 0.800 m*M* $CaCl_2$, 0.575 m*M* KH_2PO_4, 0.854 m*M* KOH, and 5.0 mg of HAP seed. Titrant solutions: 10.00 m*M* $CaCl_2$ and 7.19 m*M* KH_2PO_4 with 12.81 m*M* KOH. †Initial solution: 150 ml of 0.800 m*M* $CaCl_2$, 0.552 m*M* KH_2PO_4, 0.457 m*M* KOH, 8.40 m*M* KCl, and 5.0 mg of HAP seed. Titrant solutions: 10.00 m*M* $CaCl_2$ and 6.90 m*M* with KH_2PO_4 with 11.91 m*M* KOH. ‡Initial solution: 300.0 ml of 1.200 m*M* $CaCl_2$, 0.900 m*M* KH_2PO_4, 0.715 m*M* KOH, 24.27 m*M* KCl, and 5.0 mg of HAP seed. Titrant solutions: 26.67 m*M* $CaCl_2$ and 20.00 m*M* KH_2PO_4 with 34.10 m*M* KOH.