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Further Studies on the Pressure Tolerance of Deep-Sea Crustacea, with Observations Using a New High-Pressure Trap

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Abstract

A deep-sea benthic trap is described with which amphipods (*Tmetonyx cicada*) were both collected and observed at their ambient pressure of 134 atm. *T. cicada* collected with decompression from the same depth and locality were more sensitive to subsequent recompression than those amphipods brought to the surface at their ambient pressure. *T. cicada* from 2700 m experience irreversible injury during decompression to atmospheric pressure. The pressure tolerance of the deep-sea mysid *Gnathophausia zoea* was measured and compared with the tolerance of mid-water decapods and the shallow-water *Crangon crangon*. *G. zoea* became more sensitive to high pressure with prolonged exposure to atmospheric pressure. Deep sea animals exhibit a tolerance to high pressure related to their normal ambient pressure; sensitivity to decompression is also related to normal ambient pressure.

Introduction

The role of hydrostatic pressure in the physiology and ecology of marine animals has stimulated a number of investigations in recent years. Biochemical experiments have been initiated (see Hochachka, 1971, 1975) and behavioural observations on whole animals have been reported by Naroska (1968), Brauer (1972), Menzies and colleagues (Menzies, 1974), Macdonald and Teal (1975), and by Wilcock *et al.* (1978).

The behavioural experiments leave little doubt that pressure acts on the neuromuscular system of all grades of shallow-water animals and point to the existence of specific adaptations in deep-water animals.

The study of the ways in which deep-sea pressures affect experimentally convenient preparations is in its infancy; preliminary reports recently have been published on such systems as neurones from the supra-oesophageal ganglion of *Helix* sp., the squid giant axon under voltage clamp and the rat superior cervical ganglion (Harper *et al.*, 1975; Henderson and Gilbert, 1975; Kendig and Cohen, 1975). Most notably perhaps, Campenot (1975) has shown that neuromuscular transmission in shallow-water and deep-water decapods is differentially affected by pressure in the 50 to 200 atm range. These recent investigations should not overshadow the pioneering contributions of Regnard (1891), Ebbecke (1935) or more recently, of Spyropoulos (1957).

This paper reports experiments which use behavioural criteria to measure the pressure tolerance of freshly collected deep-sea animals. Since the application of pressure to ordinary shallow-water animals invariably elicits hyperexcitability and eventually convulsions, the pressure which triggers convulsions provides a single-number measurement of pressure tolerance, the pressure-convulsion threshold. At pressures higher than the convulsion threshold, motor activity is reversibly inhibited ("pressure-paralysis"), presenting a second criterion for measuring tolerance to pressure. These two responses have been designated Types I and II, respectively (Macdonald, 1972) and *R*, and *T* by Menzies (1974). Most ordinary shallow-water animals exhibit pressure convulsions at around 50 atm and pressure paralysis at about 150 atm. It is therefore of considerable interest to investigate the pressure-response of deep-sea animals which normally live at similar or much higher pressures. Preliminary experiments have shown that the deep-sea ostracod *Giganto-*

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cypris mülleri and the amphipod *Lanceola sayana* do not exhibit any hyperexcitability at increased pressure. We postulate a graded tolerance to pressure, manifest in the animal's motor activity, and related to the normal pressure at which the animal lives.

The hypothesis has been partially tested by using a new method for collecting benthic animals at their ambient high pressure, and by carrying out more detailed measurements on a pelagic animal than has hitherto been possible. Some of the results are consistent with the graded tolerance hypothesis, whilst others reveal complications. The work was carried out on R.R.S. "Challenger" in the vicinity of 55°N, 11°W in the Rockall Basin during cruises in September, 1974, June, 1975, and July, 1976, comprising a total of 27 working days at sea.

New methods and equipment used in the work with benthic amphipods are described, followed by experiments with the deep-water mysid *Gnathophausia zoea*; both sets of results are then discussed.

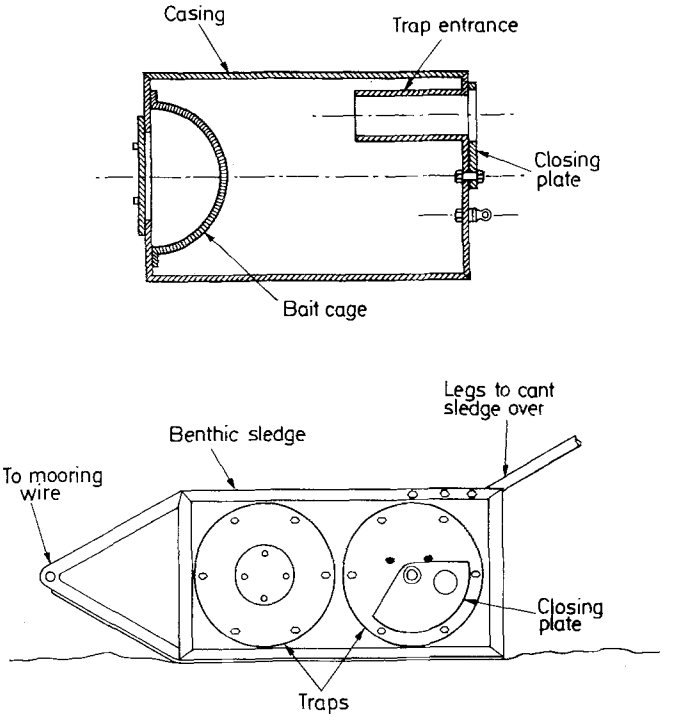


Fig. 1. Simple plastic traps (30 cm internal diameter) and benthic sledge used for initial trials. Samples recovered in these traps undergo decompression as they are brought to the surface

Benthic Amphipods

Materials and Methods

In order to collect animals with the minimum of damage it was decided to use baited bottom traps. A number of simple plastic traps were used, the walls of which were solid, and not an open mesh, in order to simulate the proposed pressure-retaining trap as closely as possible (Fig. 1). The traps were cylindrical, with an internal diameter of 30 cm and an overall length of 55 cm. The re-entrant tube fitted to one end of the trap allowed the animals to enter but prevented their escape. Re-entrant tubes of 2.5, 5, 6, and 8 cm diameter were used. Separate closing devices were also fitted: either (a) a weighted plate which seals the trap during ascent and descent, (b) a spring-loaded plate which seals the trap immediately the bait is taken or (c) a flap valve fitted across the trap entrance, allowing the animals to enter but preventing their escape.

The traps were deployed at depths to 2700 m using a simple and inexpensive mooring (Fig. 2). A pair of traps were normally cast, mounted together in a protective benthic sledge which was attached to a 70 m ground line and anchor. A 6 mm diameter steel cable was used for the main mooring wire, which was suspended from inflatable buoys at the surface. To facilitate recovery of the mooring, the buoys were coupled by a slack

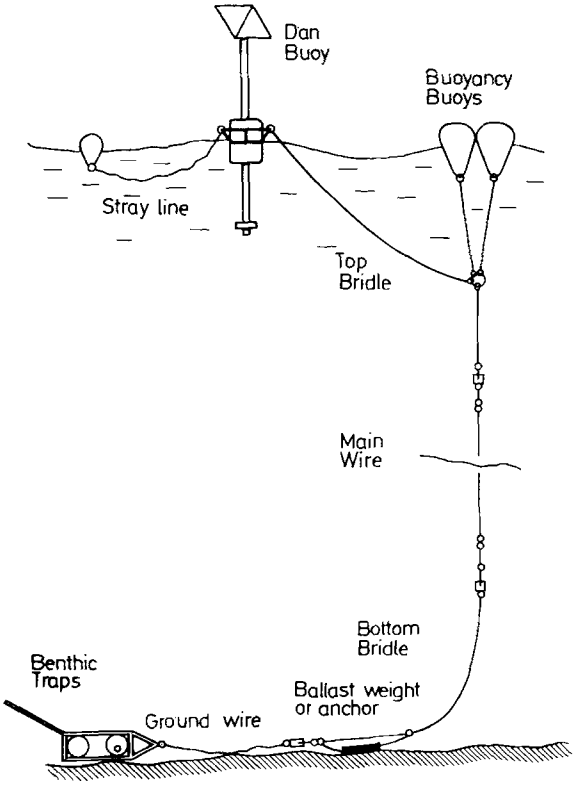


Fig. 2. Mooring used for deep-water traps. Main wire has a maximum length of 3000 m. (See text for further details.)

wire to a Dan buoy and stray line. The deck operation required to cast and recover the traps was fairly complex owing to the arrangement of the winches on the ship. At the start of each cruise the mooring wires and other lines were measured out and wound on to the appropriate winch drums. Normally one winch would be used to pay out and haul in the mooring wire while a second independent winch handled the buoy lines and the ground line. Normally a trap could be cast or recovered in 1 h 15 min.

Traps were left on the sea floor for periods of between 8 and 24 h. Initially only one of the traps was baited with fish in a standard manner, while the other trap was used to test closing devices and other variables. The traps generally remained where they were cast, except those in 2700 m depth which were dragged a few miles by the currents. No difficulty was encountered in locating the surface buoys with conventional navigation and radar, although accurate fixes from the satellite navigation system were helpful.

Normally 10 or more hagfish and many hundreds of amphipods were trapped at 1300 m depths, irrespective of the bait, entrance diameter or closing mechanisms used. Catches at greater depths were sparse, typically a dozen amphipods per trap from 2700 m. Amphipods thus recovered experienced both decompression and a slight increase in temperature. Their activity was observed in pressure vessels mounted in the ship's laboratory. The vessels were either of 6 or 11 cm internal diameter, with a maximum working pressure of 500 atm. Low constant temperature was maintained by an enclosing water jacket through which refrigerated water was circulated. Other practical details are described in Macdonald and Gilchrist (1972).

A special trap is required for the collection of benthic animals at constant temperature and pressure. The experiments with the plastic trap had shown that an entrance aperture of 2.5 cm diameter was adequate, and that elaborate methods of retaining the sample within the trap whilst it remained on the sea floor were not required. A pressure-retaining trap was therefore constructed using a globe valve which the authors had developed in earlier work to trap and recover mid-water animals at constant temperature and pressure (Fig. 3).

The valve contains a ball which is drilled through by a 2.5 cm diameter hole. The valve is opened and closed by rotating the ball through a 90° arc. The stop is fitted to limit the rotation of the valve spindle, and a trigger mechanism can be used to control the valve operation. When the valve is open, a 2.5 cm diameter clear passage leads to the sample and bait chamber. The sample chamber is separated from the outer bait

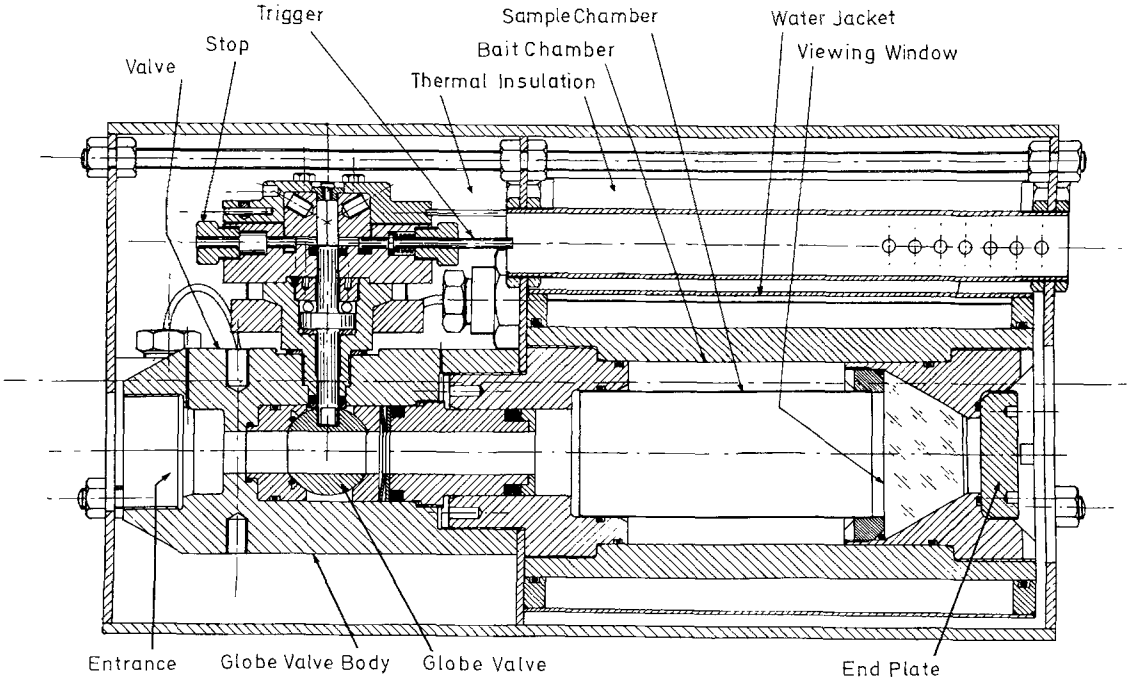


Fig. 3. Benthic trap for recovery of deep-sea animals at constant temperature and pressure. Internal diameter of sample chamber is 7.5 cm

chamber by a 7.5 cm diameter, cylindrical stainless-steel gauze mesh. A conical plastic window is fitted to one end of the pressure vessel and allows the catch to be viewed. The window is in the form of a truncated cone 5 cm thick with a 60° included angle which affords a viewing aperture 5 cm in diameter. When the trap is lowered to depth, an end plate is fitted to the window mount. This keeps the low pressure face of the window at atmospheric pressure and prevents any leakage from the window during recovery. A maximum operating pressure of 400 atm was chosen to simplify machining of the trap body, although the globe valve is designed to operate at 1000 atm.

When the trap is prepared for a cast, an extensible drawbar is coupled to the end of the trap and attached to the groundwire of the mooring (Fig. 2). The globe valve is connected by a cable to the drawbar and during descent from the surface the valve is kept closed by the drawbar tension transmitted through the cable. Additionally, the trap is internally pressurized to match the proposed trapping depth. When the trap reaches the sea floor the tension on the drawbar is reduced, the external pressure matches the pressure within the trap and a spring opens the globe valve. Conversely, on being hauled up off the sea floor, the drawbar tension closes the valve which holds the water at its ambient pressure.

The retention of pressure in this type of equipment has been discussed by Macdonald and Gilchrist (1969, 1972). When a thick-walled steel vessel such as the pressure trap is raised from depth to the surface, it can be shown that the pressure of water trapped inside will decrease by 5% because of the elasticity of the steel. The total pressure fall off is much greater, due to local distortion and movement of the seals. However a 160 ml hydraulic accumulator charged with nitrogen at 80 atm is coupled to the interior of the pressure trap to increase the compressibility of the system and thus decrease these pressure losses to a negligible amount. The accumulator has a maximum safe working pressure of 330 atm and was therefore isolated during those laboratory experiments which involved pressures higher than 330 atm.

On board ship after recovery, the protective steel housing round the trap, the drawbar, and the window end plate are removed. The trap is then transported to the laboratory and coupled to a pressure pump and gauge. The trapped pressure and the animals are then checked and ice packs placed on the outside of the trap to keep the contents cold. The responses of the animals to pressure changes are then studied in the same way as in animals recovered with decompression and subsequently recompressed.

During the 1976 Challenger cruise, a total of 6 casts were made with the pressure trap and on three occasions deep-sea amphipods were collected at their ambient pressure of 134 atm. Using the plastic traps, amphipods were collected from three depths on the continental slope – 1200 to 1300, 2000 and 2,700 m – and were subsequently subjected to various pressure tests in groups at a temperature between 5° and 8°C, which corresponds to those prevailing at depth in the Rockall Basin (Ellett and Martin, 1973). The motor activity which was used to score the amphipods' tolerance is termed a spasm, which is a well defined stage in the amphipod's response to increased pressure. The initial effect of compression is to stimulate normal locomotion, but at higher pressures the amphipods often flex dorsoventrally. This is probably comparable to the convulsions seen in decapods, but unfortunately in amphipods it is not always clearly discernible from normal movements. Somewhat higher pressures elicit a strong dorsal flexion or spasm which is sustained for 30 sec in the first instance. The onset pressure for a spasm is the main criterion used in the amphipod experiments, but it cannot be equated with the convulsion used in the work with *Gnathophausia zoea* (see below). At pressures higher than the onset pressure for spasms the amphipods become immobilised and remain dorsally flexed, in marked contrast to their normal, ventrally curled posture.

Results

Most experiments used stepwise compression, with 50 atm increments at 5 min intervals. This is referred to as rapid compression, in contrast to a slower rate of compression which will be mentioned later.

Experimental animals were fixed after use and subsequently identified.

Amphipods from 1200 to 1300 m Depth

Table 1 summarizes the results from experiments on *Tmetonyx cicada* brought to the surface with decompression. Rapid recompression to 300 atm caused spasms in 50% of the total. The 1976 amphipods were slightly more sensitive, with 50%

Table 1. *Tmetonyx cicada*. Onset pressure for spasms in amphipods collected with decompression from 1200 to 1300 m. Compressed in 50 atm steps at 5 min intervals. Temperature, 5° to 8°C. Amphipods removed from trap for no more than 19 h, with 90% for no more than 14 h, before the experiment

Year	Total no. of traps cast	Total no. of amphipods	No. starting spasms at 300 atm	No. showing spasms at lower pressure
1974	2	52, in groups of 5-19	41	0
1975	4	57, in groups of 3-10	17	0
1976	10	18, in groups of 3	5	9 at 250 atm
Total		127	63 (49%)	

Table 2. *Tmetonyx cicada*. Onset pressure for spasms in amphipods collected with decompression from 1300 m depth at 56° 26' N; 9° 31' W. Compressed as in Table 1. Temperature, 6°C

Trap no.	Experiment no.	No. of amphipods and length (mm); all female unless otherwise stated	No. starting spasms	Onset pressure for spasms (atm)
1976				
A(i)	B1b	3; 20, 16, 12.5	0	(experiment stopped at 300 atm)
A(i)	A1a	3; 20, 16, 14.5	3	250
A(i)	A2b	3; 17, 12.5♂, 12.5	2	300
			1	350
A(i)	A3a	3; 18, 16.5, 11.5	3	300
A(vi)	A9a	3; 12, 14, 19	3	250
A(vi)	A11	3; 12, 13.5♂, 19	3	250
Total		18	9 (50%)	250
			15 (100%)	Mean = 273±31.9*

*Ignoring first experiment which stopped short of convulsions.

undergoing spasms at 250 atm (Table 2). Discounting the first experiment in Table 2, the mean onset pressure for spasms is 273 ± 31.9 (standard deviation) atm. In three other experiments the amphipods were rapidly recompressed to 130 atm, i.e., to their normal ambient pressure, held for 3 h and then compressed to 150 atm and thereafter in steps of 50 atm. Of 10 individuals thus treated, 6 underwent spasms at 350 atm. The mean onset pressure for spasms was 335 ± 24 atm for the group (Table 3).

Three lots of amphipods were collected in the high-pressure trap at 134 atm and pressure tests were carried out on them. Once the trap was secured in the ship's laboratory, the activity of the amphipods was observed for 30 min, and in all three cases it appeared vigorous and normal. No obvious response to a change in light intensity was seen. In Trap 1, about 20 individuals could be seen crowded at the window; indeed they may have been attracted to the light. In Trap 2, only about 7 amphipods were visible at a time, but Trap 3 contained 15 or more amphipods close to the window.

After the initial period of observation, the amphipods were pressurised to 150 atm and thereafter in 50 atm steps at 5 min intervals to a maximum of 400 atm. Trap 1 was compressed to only 330 atm because the accumulator isolating valve was absent from the circuit. In all three experiments typical dorsal-ventral flexing movements were seen at 350 atm (Traps 2 and 3), and at 330 atm (Trap 1). No spasms were seen at pressures of less than 400 atm, at which pressure the majority of amphipods in Traps 2 and 3 underwent typical, well defined spasms (Table 4). There is no doubt that the onset pressure for spasms in these animals is close to 400 atm.

Following compression to 400 atm, the amphipods began to become immobilised. After 5 min exposure they were smoothly decompressed to 130 atm over approximately 1 min, held at that pressure for 40

Table 3. *Tmetonyx cicada*. Onset pressure for spasms in amphipods collected with decompression from 1300 m at same locality as those in Table 2. Amphipods were rapidly compressed to 130 atm, held at that pressure for 3 h, then compressed to 150 atm and thereafter as in Table 1. Temperature, 6°C

Trap no.	Experiment no.	No. of amphipods and length (mm); all female unless otherwise stated	No. showing spasms	Onset pressure for spasms (atm)
1976				
A(iii)	A10	3; 17.5, 14.5♂, 9	3	300
A(vii)	A13	4; 18.6, 17, 14.5♂, 8.5?	4	350
A(ix)	A16	3; 19, 17, 14	2 (+ 1 doubtful)	350
Total		10	3 (33%)	300
			10 (100%)	Mean = 335±24[a]

[a]Significantly different from mean in Table 2.

Table 4. *Tmetonyx cicada*. Onset pressure for amphipods collected at ambient pressure from 1300 m depth at same locality as those in Table 2. Compressed to 150 atm, and thereafter as in Table 1. Temperature, ca. 5°C

Trap no.	Experiment no.	Trapped pressure ± 5.5 atm / ambient pressure (calculated)	No. of amphipods normally visible	No. of amphipods recovered from trap after experiment. Length 6.5 - 20.5 mm	Onset pressure for spasms
1976					
A(i)	A6	134.9 / 129.2	20	37	No spasms seen at 330 atm, the maximum applied[a]
A(ii)	A15	134.9 / 132.7	7	28	400 atm (majority)
A(iii)	A18	133.6 / 132.2	15	23	400 atm (majority)

[a]The data show that the majority undergo spasms at 400 atm and that none convulse at 330 atm.

Table 5. *Tmetonyx cicada*. Activity at 200 atm. Amphipods were collected from 1200 to 1250 m depth, with decompression. Temperature, 5°C

Trap no.	Experiment no.	Duration of exposure to 200 atm	No. of amphipods in experiment (and length)	Activity at end of exposure: No. crawling or swimming	Activity at end of exposure: No. in normal curled position with pleopods moving
1974					
I(i)	26	4¾ h	19 (20-12 mm)	2	15 (+ 2 obscured from sight)
1975					
II(ii)	A11	4 h	5 (18-14 mm)	3	2
II(ii)	A12	4 h	5 (17-14 mm)	0	5
			Total 29	Total 27 (93%)	

min and then decompressed to 1 atm in 50 atm steps at 5 min intervals. In the case of Trap 1, air bubbles appeared at 1 atm because air was accidentally trapped during the initial compression before lowering (see "Materials and Methods"). Traps 2 and 3 showed no bubbles on decompression and in both cases the amphipods exhibited increased activity, including some mild spasms, during the final decompression step to 1 atm.

In summary, Tables 1-4 show a series of onset pressures for spasms in *Tmetonyx cicada*, ranging from 400 atm for the majority of amphipods recovered at their ambient pressure to a mean onset pressure of 273 atm for the amphipods recovered with decompression in 1976.

The resistance of *Tmetonyx cicada* to a prolonged exposure at 200 atm following rapid compression is shown in Table 5. Periodic observations showed that the am-

Table 6. *Eurythenes* sp. Revival and onset pressure for spasms in amphipods at 6°C, collected with decompression from 2000 m depth

Trap no.	Experiment no.	No. of amphipods recovered (all female) + length	No. moving after recovery at 1 atm	No. moving shortly after application of 200 atm	No. moving after 30 min at 200 atm	Onset pressure for spasms after holding at 200 atm
1976						
C(i)	A24	10; 34-17 mm	0	10 (7 vigorous)	10	5 at 450 atm
C(ii)	A28	5; 30-14 mm	2	5	5	4 at 450 atm
Totals		15	2	15	15	9 (60%) at 450 atm

Table 7. Revival of different species of amphipods brought to surface with decompression from 2700 m depth. All amphipods were experimented on within 30 min of removal from the trap at ca. 5°C

Year and trap no.	Experiment no.	Temperature (°C)	No. of amphipods recovered, species and length	No. showing movement after recovery at 1 atm	No. showing movement on compression to 250 atm	No. showing feeble movement after exposure to 250 atm
1975						
II(ii)	C14	4	11 *Eurythenes* sp., 25-16 mm	1	4	1, after 2 h
II(iii)	C17	5	11 Not identified	2	9	4, after 2 h
II(i)	B2	5	15 *Tmetonyx cicada*, 16-11 mm	0	3 (300 atm)	3, after 1 h 30 min
1976						
B(i)	A12	6	6 *Paralicella tenuipses chevreux*, 9-12 mm	0	6	4, after 1 h 30 min
Totals			43	3 (7%)	22 (51%)	12 (28%) after 1 h 30 min - 2 h

phipods retained a normal level of activity compared to controls kept at atmospheric pressure.

Amphipods from 2000 m Depth

Two lots of *Eurythenes* sp. were collected with decompression from 2000 m depth. Table 6 summarises the extent to which rapid compression to 200 atm revived them. The onset pressure for spasms in 60% was 450 atm when compression steps were resumed after holding the amphipods for 30 min at 200 atm.

Amphipods from 2700 m Depth

In contrast to the individuals from 2000 m depth, those from 2700 m showed only feeble signs of revival after rapid recompression to 250 atm (or 300 atm in one case) which was not sustained. The species included *Tmetonyx cicada* and *Eurythenes* sp. (Table 7). Signs of revival usually appeared at pressures of less than 250 atm.

The Pelagic Mysid *Gnathophausia zoea*

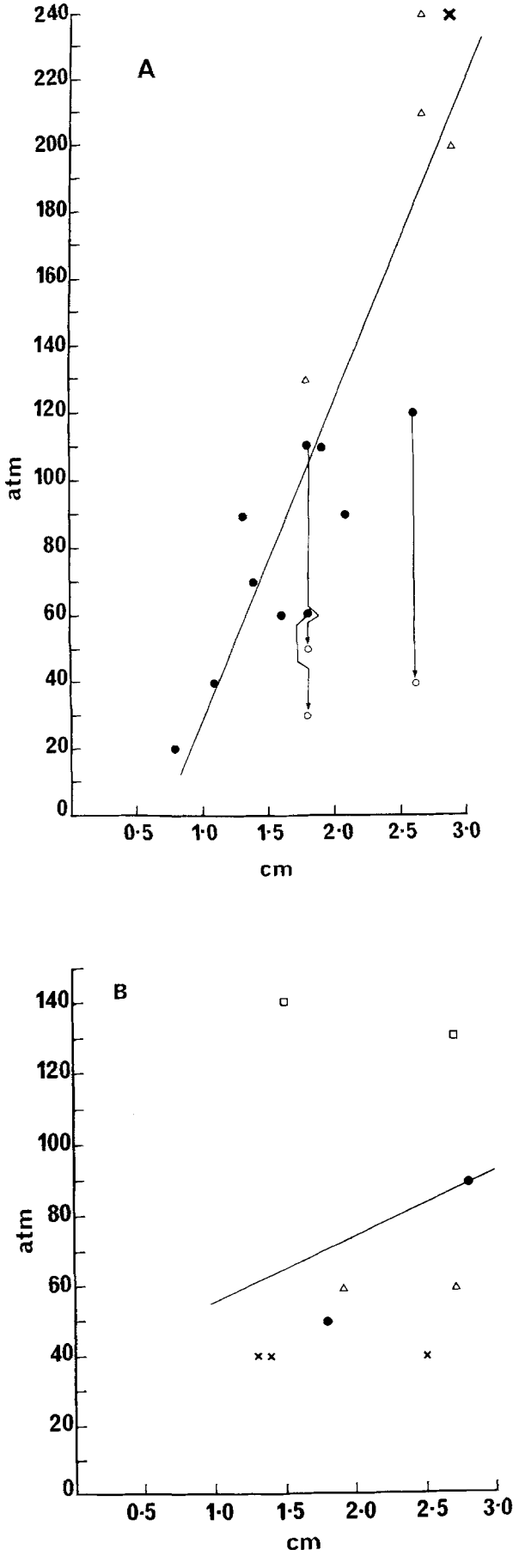
Materials and Methods

Gnathophausia zoea was collected in a 2 m diameter ring trawl fitted with a cod-end bucket 1 m long x 20 cm in diameter. On most occasions the trawl fished at depths of less than 1000 m for approximately 6 h, and in about half the trawls 1 to 4 healthy individuals would be collected.

Gnathophausia zoea remains active for several days when kept at 5°C at atmospheric pressure. Pressure experiments were usually carried out with the 11 cm diameter pressure vessel, but smaller individuals were occasionally accommodated in the 6 cm diameter vessel. Compression was either rapid, as with the amphipods, or slow – 10 atm increments at 6 min intervals, i.e., 100 atm h^{-1}.

Results

Gnathophausia zoea normally shows bursts of pleopod activity as pressure is in-



creased. Although the pleopods remain vigorous, they are often temporarily disorganised after a compression step. At a characteristic pressure, individuals undergo a sudden, violent dorsal-ventral flexion (a convulsion), a movement which is clearly based on the normal escape response but which appears to be induced by pressure. The mysids were never seen to convulse at elevated pressure in response to noise, light or other obvious external stimuli. *G. zoea*'s pressure convulsions and an associated fanning of the telson are similar to those described in *Crangon crangon* and other decapods by Wilcock *et al.* (1978).

The rhythmic activity of the heart and pleopods is also used to score the well being or otherwise, of the mysids. The heart can be seen with a stereobinocular microscope through the transparent dorsal region of the body, and provided the mysid is securely strapped to a transparent plate with elastic bands, the heart rate may be monitored at pressures in excess of the convulsion threshold pressure.

Convulsion Threshold Pressures

Slow compression is appropriate to determine convulsion threshold pressures and early experiments yielded high but rather variable values. At least three factors were discovered which contribute to this variability, namely size (presumably age), the length of time the mysid was kept at atmospheric pressure before an experiment (time out of trawl) and the number of individuals confined within the pressure vessel.

Fig. 4A shows the relationship between size and convulsion threshold for mysids kept at atmospheric pressure for

◀ Fig. 4. *Gnathophausia zoea*. (A) Convulsion threshold pressures of different-sized mysids; abscissa, size (cm), using distance from eye to posterior edge of carapace as a convenient index; ordinate, threshold pressure (atm); each point refers to an individual mysid which was subjected to slow compression on its own within 15 h of removal from the trawl; cross: 1974, triangles: 1975, circles: 1976 cruises; the points connected by arrows refer to a second threshold measurement 24 h after the first; the regression of threshold on size is plotted, and correlation coefficient is 0.89; mean convulsion threshold is 151 ± SD 65 atm. (B) Mysids similarly subjected to slow compression in 4 groups indicated by the symbols; the mean convulsion threshold is 72 ± SD 38 atm, and there is no correlation between size and threshold

no more than 15 h after removal from the trawl. Three points (arrowed in Fig. 4A) refer to individuals which were experimented on within 3 h of removal from the trawl and subsequently tested 24 h later. All three showed a substantially lower threshold pressure. Fig. 4B shows the effect of grouping the mysids in the pressure vessel.

Ten standard individuals, i.e., with an eye to posterior carapace measurement of 1.8 cm or more and which had been removed from the trawl for less than 15 h, were slowly compressed at 5° to 6°C and were found to have a mean convulsion threshold of 151 ± 65 atm. Similar individuals treated in groups had a mean convulsion threshold pressure of 72 ± 38 atm. The difference between these is significant at the P = 0.01 level.

During slow compression, the mysids continued to convulse intermittently, but above a maximum pressure the convulsions faded. This is shown in Fig. 5, which summarises pooled results from 8 experiments using standard individuals. The figure includes data from *Crangon crangon* for comparison.

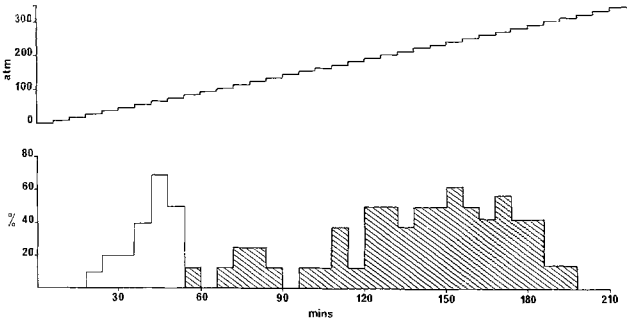


Fig. 5. *Gnathophausia zoea*. Successive convulsions in mysids subjected to slow compression to 350 atm at 5° to 8°C. Upper graph: pressure increase with time. Lower graph: pooled results of 8 experiments with standard individuals (measurement of 1 mysid was omitted, but it was almost certainly of standard size); abscissa: time (min), ordinate: percent of 8 individuals exhibiting convulsions in each 6 min interval between compression steps; histogram on left shows comparable responses in *Crangon crangon* (Wilcock *et al.*, 1978)

Heart Rate and Pleopod Activity

In two experiments, a pair of mysids were slowly compressed to 200 atm and observed at that pressure for 22 to 24 h. All 4 individuals showed typical discontinuous peopod activity (Fig. 6). The occurrence of beating and the frequency of the pleopod rhythm showed little decline during the pressure treatment. In other experiments in which the mysids were slowly compressed to 350 atm, the frequency and amplitude of the pleopods became so reduced after 45 min at 350 atm that counts were only possible in 5 out of 9 individuals.

The pressure tolerance of the heart and the pleopods were compared in rapid compression experiments. Rapid compression to 200 atm affected the pleopods slightly more severely than slow compression at 200 atm. After 1 h at 200 atm individuals were further rapidly pressurised to 350 atm. In 5 out of 6 cases the pleopods ceased movement before the heart stopped. Fig. 7 shows typical results, including the stimulatory effect on the heart of the two separate phases of compression.

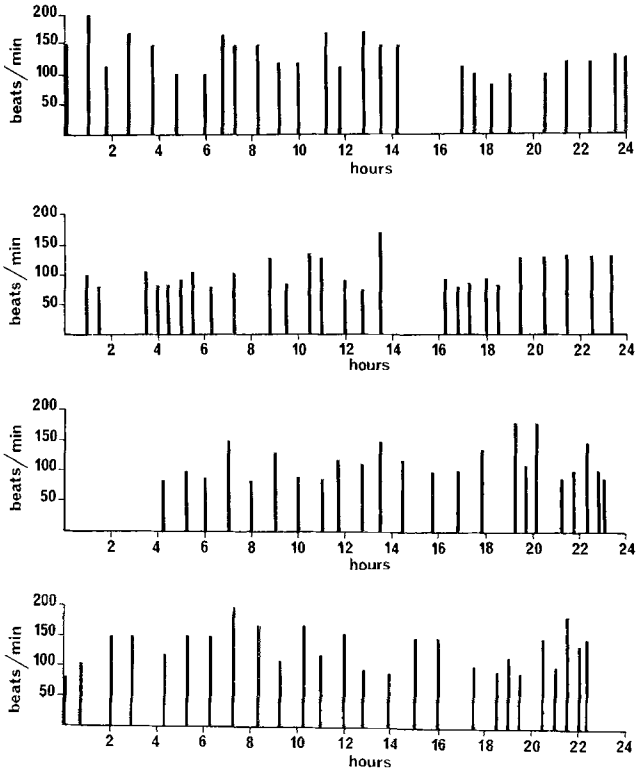


Fig. 6. *Gnathophausia zoea*. Sustained pleopod activity in mysids subjected to slow compression to 200 atm and held at constant pressure for 24 h. Abscissa: time, starting when 200 atm was reached; ordinate: pleopod beats min^{-1}. Results of two experiments each using 2 mysids are shown. Each vertical line refers to pleopod rate at time of observation. Activity was discontinuous, and often the pleopods were stationary

Tolerance to Anoxia at Atmospheric Pressure

In view of the above findings, and the known sensitivity of some pelagic ani-

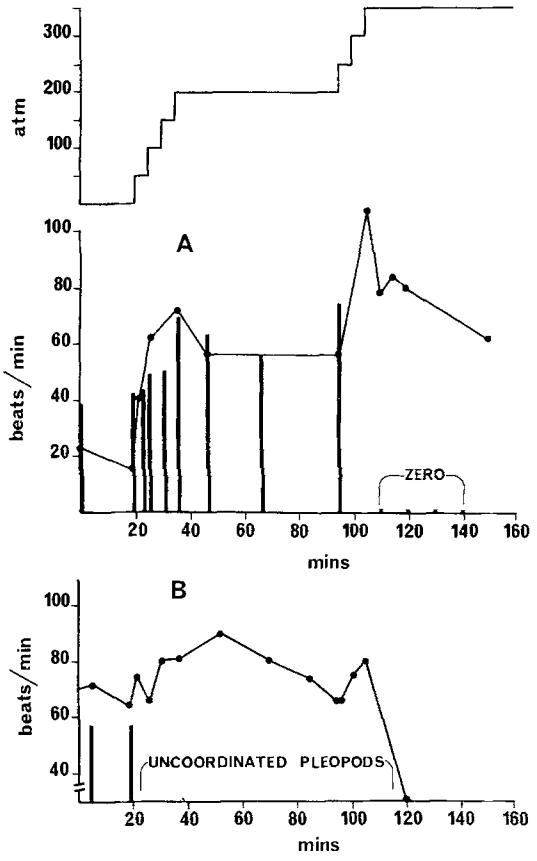


Fig. 7. *Gnathophausia zoea*. Heart rate and pleopod rate in mysids following rapid compression to 200 atm and then to 350 atm at 5°C. Top graph shows pressure increase with time. Lower graphs show typical results from Experiments A and B. Abscissae: time; ordinates: rate of beating; dots: heart; vertical bars: pleopods. Note in both cases the heart continues to beat while the pleopods are severely affected. Individual A had been removed from the trawl for more than 15 h. It was not seen to convulse, probably because it was too tightly strapped down. Individual B convulsed at 150 atm and was a standard animal

mals to anoxia, *Gnathophausia zoea* was tested for its ability to survive without oxygen. This was carried out at atmospheric pressure by purging a column of sea water with nitrogen and monitoring the pO_2 at the base where the test animal was confined, with an oxygen electrode. Mysids thus deprived of oxygen rapidly became immobilized. Of three mysids subjected to anoxia for 1 h, two recovered normal activity on being returned to normal sea water, and all four mysids which were deprived of oxygen for 4 h subsequently recovered.

Discussion and Conclusions

Amphipods collected with decompression from 2700 m showed only partial and temporary revival when restored to 250 atm pressure in contrast to those from 2000 m which recovered fully on compression to 200 atm. The amphipods collected with decompression from 1300 m depth exhibited a level and repertoire of activity which would be considered normal in a shallow-water benthic species. The results suggest that the decompression experienced by amphipods from 2700 m (270 atm) caused largely irreversible effects whilst decompression from 2000 m (200 atm) impaired activity in a way which was readily reversed by recompression.

Tmetonyx cicada was obtained from both 2700 m and 1300 m depths, some 90 miles apart, and *Eurythenes* sp. was likewise brought up from depths of 2700 and 2000 m. We do not know whether the difference in the response to decompression between the two groups of the same species is acquired in their lifetime, perhaps during a migration down the continental slope, or is genetically determined.

Decompression of *Tmetonyx cicada* from 1300 m (130 atm) caused slight effects which were only apparent when the onset pressure for spasms was used as a criterion. Those amphipods which were brought to the surface at their ambient pressure had a higher onset pressure for spasms than those retrieved with decompression. Of the former group, none underwent spasms at 330 atm and the majority showed spasms at 400 atm. Those which were collected with decompression but held at 130 atm for 3 h prior to the pressure test showed a mean onset pressure for spasms of 335 atm (Table 3). Without the 3 h adaptation period the mean onset pressure for spasms was 273 atm. The differences between these values are significant both in those cases where a statistical test can be applied (compare Table 2 with Table 3), and also, in our judgement, where it cannot (compare Table 4 with Tables 2 and 3). We conclude that retrieval with decompression reversibly sensitizes the amphipods to subsequent compression.

A comparison of the pressure tolerance of these benthic amphipods with the tolerance of a shallow-water benthic species reveals large differences (Table 8). The littoral *Marinogammarus marinus* has an onset pressure for spasms of around 50 atm in comparable experimental conditions. At 200 atm, only 20% of *M. marinus* exhibit any activity and this is the feeblest sign of movement, quite unlike the deep sea *Tmetonyx cicada* or *Eurythenes* sp. (see Tables 5 and 6 of present paper, and Macdonald, 1976).

Gnathophausia zoea's pressure tolerance can best be compared with that of *Crangon crangon* and the mesopelagic decapods *Sy-*

Table 8. Pressure-tolerance of benthic and pelagic amphipods from different depths

Depth (m)	Species	No. of collections	Total no. of amphipods observed	Temperature (°C)	Onset pressure for spasms during rapid compression	Source
Benthic						
10	*Marinogammarus marinus*	Many	Many	3, 13	Majority at 50 atm	Macdonald (1972, 1976)
1300	*Tmetonyx cicada*	16	127 (18 see Tables 1 and 2	5-8 5-8	49% at 300 atm 50% at 250 atm)	This paper
1300	*Tmetonyx cicada*, recovered at ambient pressure	3	42	5-8	Majority at 400 atm None at 330 atm	This paper
2000	*Eurythenes* sp.	2	15	6	60% at 450 atm	This paper
Pelagic						
Shallow oceanic	*Parathemisto* sp.	5	24	5-8	58% at 150 atm	This paper
Deep sea	*Lanceola sayana*	4	6	5-8	No spasms	Macdonald and Teal (1975)

Table 9. Mean convulsion threshold pressures of *Gnathophausia zoea* (Mysidacea) and various decapods individually compressed at 5° to 8°C. Values ± standard deviations. Number of individuals used is given in parentheses. nd: no data

Species	Slow compression (increments of 10 atm, 6 min intervals)	Rapid compression (increments of 50 atm, 5 min intervals)	Source
Crangon crangon	50 ± 3 atm (22)	71 ± 26 atm (7)	Wilcock *et al.* (1978)
Systellaspis debilis	nd	125 ± 41 atm (6)	Macdonald and Teal (1975)
Acanthephyra purpurea	nd	141 ± 58 atm (6)	Macdonald and Teal (1975)
Gnathophausia zoea	151 ± 65 atm (10)	91 ± 58 atm (6)	This paper

stellaspis debilis and *Acanthephyra purpurea*, all of which show convulsions based on their escape reflex. The values in Table 9 must be qualified by the differences between the animals. *Crangon crangon* is a shallow-water, bottom-dwelling decapod. Taxonomically it differs considerably from *G. zoea* and it beats its pleopods only infrequently. The mesopelagic decapods are more similar to *G. zoea* in maintaining a pleopod rhythm. Two points of comparison are interesting. First, the convulsion threshold pressures of *C. crangon* and *G. zoea* are different, and the distribution of successive convulsions during slow compression is also very different (Fig. 5). Unfortunately, only rapid compression convulsion thresholds for free-swimming *S. debilis* and *A. purpurea* have been measured (Macdonald and Teal, 1975). Table 9 shows these values, 125 and 141 atm, respectively, and the lower convulsion threshold measured with rapid compression of 6 *G. zoea*, all of which were full size but two of which were removed from the trawl 18 h prior to the experiment.

Autonomic activity provides a second point of comparison. The beating of the pleopods and the heart in *Systellaspis debilis* and the pleopods in *Acanthephyra purpurea* is stopped by rapid compression to 200 atm (Macdonald and Teal, 1975), whereas *Gnathophausia zoea* continues autonomic activity for 1 h after rapid compression to 200 atm. It appears that *G. zoea* is slightly more pressure-resistant in this respect than either of the two mid-water decapods. Its pleopods also remain active for 24 h after slow compression to 200 atm, a markedly more pressure-tolerant performance than *A. purpurea* is capable of (Macdonald and Teal, 1975). In both *S. debilis* and *G. zoea* the pleopods are immobilised before the heart. *S. debilis* is highly susceptible to anoxia, which would account for its lesser tolerance to paralytic pressures. *G. zoea* (also *Crangon crangon* and *Lanceola sayana*) survived periods of anoxia of more than 1 h, and periods of pressure-paralysis (see also Childress, 1975).

The increase in convulsion threshold pressure with increased size of *Gnathophausia zoea* suggests that age-dependent factors influence pressure sensitivity (Fig. 4A). According to Clarke (1962), adult *G. zoea* in the Pacific are only found below 500 m depth, and probably do not extend much deeper than 1000 m. Other species, *G. gigas* and *G. ingens*, have well documented vertical distributions,

with the larger individuals being more common at greater depths.

The results of both the amphipod and the *Gnathophausia zoea* experiments may be interpreted to mean that decompression to, and holding the animals at, 1 atm, sensitizes them to subsequent compression. The three cases in which *G. zoea* was pressure tested soon after being removed from the trawl and then tested again 24 h later show that the prolonged exposure to atmospheric pressure can sensitize these mysids. Obviously other explanations are possible. It may be that the first convulsions modify the animals' responses to subsequent pressure treatment, but this is certainly not the case in *Crangon crangon* which shows highly reproducible individual threshold pressures. We conclude that our pressure tolerance measurements made on deep-sea animals which are recovered with decompression give low values. Differences in tolerance between such animals and shallow-water forms therefore appear minimal.

In general, the results are consistent with the graded tolerance hypothesis, but we are now confronted with the task of measuring not only tolerance to high pressure but tolerance to decompression from the animals' normal ambient pressure. When *Tmetonyx cicada*, which had been retrieved at 134 atm were decompressed, some motor disturbances at around 50 atm were seen. Observations of decompression from much greater ambient pressures would be most interesting. Generally the evidence encourages the view that as an animal's normal ambient pressure increases, so it acquires a sensitivity to decompression. It is now technically possible to measure the relationship between ambient pressure and sensitivity to decompression in benthic animals and the 1000 to 3000 m depth range seems to be the critical one for the present. Bathypelagic animals may well show marked differences from benthic animals, especially in those species which move rapidly over a wide vertical range. It is our experience that recovering bathypelagic animals at their ambient pressure is very difficult and our pressure retrieval system is more suited to benthic animals.

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