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**Notes on the
Preservation of Marine Animals**

by

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Introduction

At sea, especially in the Antarctic, we often take enormous quantities of plankton and bottom living animals in our nets and trawls. There was a famous occasion in the "Scotia" (Wilton, Pirie and Brown, 1908) when, after a highly successful operation on the Burdwood Bank, half a ton of material was brought on board and the whole of the scientific staff, plus many other helpers, were kept busy for the next 24 hours sorting and bottling the catch. Kemp and Hardy (1929) report a similar incident and I can recall many others. In the fixation and preservation of such masses of material, there is little time for fine techniques and we have to resort as a rule to bulk tactics. The purpose of this paper, therefore, is to describe what we used to do in the Discovery Investigations and on the B.A.N.Z. (Mawson) Antarctic expedition.¹ I shall deal mainly with the bottom fauna, the bulk preservation of plankton being a matter with which everyone is familiar.

General procedure

The contents of trawls and dredges would be carefully emptied out on to the poop and all animals that it was supposed would contract if plunged straight away into fixative were placed immediately into bowls, basins, deep glass dishes or other suitable receptacles containing cold, fresh-drawn sea water liberally sprinkled with menthol crystals and left there to relax in the cold air on deck or on the swing table in the cool deck laboratory if the weather was rough. Some animals take longer to relax (i.e. become anaesthetised) than others. Holothurians, for instance, may take up to 12 hours. Sea anemones often take much less and indeed sometimes may turn themselves almost inside out if left under menthol too long. The best way to ascertain the depth of the anaesthesia is to keep prodding the specimens from time to time with forceps. When fully relaxed and expanded the animals were fixed for 24 hours in 10% neutralised seawater formalin, then transferred to weak alcohol and finally after a further 24 hours, to strong (75%) alcohol. We always had a ready-to-use supply of weak alcohol to hand because the original strong spirit into which the specimens were put was kept after being changed for fresh. Most other animals (and plants) were fixed, without any preliminary treatment, in 10% formalin for 24 hours, transferred to weak alcohol and, after a further 24 hours, to their final strong alcohol. Algae, however, apart from calcareous algae, were left as a rule in their formalin fixative which would be changed for fresh after a day or two.

Animals that react well to menthol include hydroids, alcyonaria, corals, sea anemones, holothurians, polychaetes, nemerteans (sometimes), Gephyreans, Polyzoa (perhaps) and nudibranchs. Most molluscs, however, show little tendency to expand.

In changing from the first (weak) to the second (strong) spirit, we always used to rock and wash the animals gently to and fro in their tubes or jars to rid them of the white precipitate that comes down when alcohol is mixed with sea water. This ensures a clean, clear and handsome preparation. In bottled material the fluids were changed by straining off through fine-meshed silk gauze (12 meshes to the linear inch) held, stretched taut, across half of the mouth of the jar. This ensured that small organisms that might be adhering to the larger specimens were not lost.

Bottom apparatus

On the high Antarctic continental platform we have used a variety of gear, notably the conical dredge, small rectangular dredges approximately 3 x 1 ft. in opening, the Monegasque trawl and the large 40-ft. otter trawl. The conical dredge is a sure and excellent sampler (or indicator) of the substratum, almost whatever its nature may be. It does not work on level rock completely free from stones, but even there we can generally tell from the 'shine' and markings on the metal rim that the bottom is in fact rocky. All these gears have been used with good effect on the right kind of bottom. But the bottom as a rule is seldom right. Far too often our dredges and trawls quickly become hitched up,

¹ For more elaborate treatment of marine animals the reader is referred to the excellent handbooks, Instructions for Collectors, especially No. 9A, issued by the British Museum (Natural History).

damaged and sometimes lost on the rocks, stones and erratic boulders with which it is so much encumbered. However, there is distinctly trawlable and dredgable ground off the great ice barriers such as exist at the head of the Ross Sea. There the bottom is covered with a deep ooze of exceedingly fine texture which (Marr, 1962) seems to be strangely free from major obstruction. We even used the large 40-ft. otter trawl (OTL) there without mishap and I believe that the still larger commercial otter trawl (OTC) could be used in this field with equal success. There are other places, too, round the continental platform where the OTL has been used with impunity, but they seem to be few and far between. Echo-ranging and submarine photography, however, may lead to the discovery of more and more of them.

Descriptions of bottom apparatus commonly used in Antarctica will be found in Kemp and Hardy (1929), Johnston (1937) and Mawson (1940). I must mention here, however, a contrivance we used with particularly rewarding results. This was the skids and frame of Russell's bottom townet (Russell, 1928) to which we attached an ordinary coarse-meshed (1 in.) dredge bag instead of the usual stramin net. We were especially successful with this on the deep ooze of the Ross Sea floor. The iron frame has a heavy chain footrope and this evidently stirs up the animals in the ooze (or crawling about on its surface) and they pass upwards into the bag clean and undamaged, the fine terrigenous material, which tends to swamp and injure the specimens, escaping through its coarse meshes. I have never in fact seen bottom animals brought to the surface in such perfect condition as those that we took on the Ross Sea shelf with this modified gear.¹ The DRR (dredge, rectangular, Russell), as we called it, has been used with good effect on rough stony ground (Marr, 1962), but I would say that its effectiveness on deep ooze is perhaps its most remarkable feature.

In preserving the Ross collection we used the better part of 400 gallons of 94% commercial alcohol, and since the bulk of this had to be broken down to 75% it gives some idea of the wealth of life we encountered in these very high latitudes and of the size of the collections made.

Special techniques

Bottom deposits: muds, sands, etc. Fixed 10% formalin for 24 hours, then transferred to very strong (94%) alcohol. The prior use of formalin ensures that the soft parts of the small organisms contained in the sediments get the best possible bulk treatment.

Foraminifera and other very small animals. These would occur in the bottom deposits and in the residues, seivings, washings, etc. from trawls and dredges. They did not as a rule receive special treatment. Representative samples of such deposits and residues were placed in 10% formalin and after 24 hours, in very strong (94%) spirit.

Sponges. Very watery animals. Place at once into the strongest possible (94%) spirit. Change after 24 hours to alcohol equally strong.

Coelenterates: Hydroids, Alcyonaria, corals, sea anemones. Anaesthetise in menthol for 4-12 hours (or less if they appear to be relaxing too much), fix in 10% formalin for 24 hours, transfer to weak spirit and finally after another 24 hours, to strong (75%) spirit. Hydroids can occasionally be killed with the polyps expanded by shaking vigorously in formalin.

Holothurians. Anaesthetise for up to 12 hours in menthol, fix formalin² followed

¹ In a letter to Dr. Deacon, the late Dr. Stanley Kemp, a master at handling animals at sea, wrote, "Everyone is staggered at the superb condition of the Ross Sea collections." I do not write this vaingloriously, but simply to show what can be done with the right apparatus on the right kind of bottom, provided due care of the specimens follows once they are safely on board.

² Remember that holothurians are rather watery animals and that some have tough and rather thick skins. It is important, therefore, to inject the thicker-skinned ones with a dose of strong formalin (40% formaldehyde) through the anal opening in order to ensure proper fixation of the viscera. Do the same with the weak and strong alcohols.

as described above by weak and strong alcohols.

Starfish and Sea Urchins. Fix formalin followed by weak and strong alcohols.

Ophiuroids. Kill flat in fresh water. Fix formalin followed by weak and strong alcohols.

Grinoids. Insert with the brachials folded about them into long tubes containing 75% spirit. Change spirit after 24 hours.

Nemerteans. Try to get them to relax and expand under chloral hydrate. Menthol is sometimes very effective but it is a matter of trial and error. Fix formalin followed by weak and strong alcohols.

Polychaetes. Anaesthetise for as long as may be required in menthol, to which they react very well. Fix 10% formalin in tubes that just fit them. Leave tubes (corked) lying flat overnight. This allows the animals in the confined space of their narrow tubes to fix in their natural shape and ensures that they do not collapse upon themselves, as they might do in vertical tubes, or if the tubes were too wide for them. Transfer to weak, followed by strong, alcohol. It is bad practice to fix polychaetes, even if relaxed, on blotting paper soaked in formalin. If one does, they collapse dorso-ventrally and become unnaturally flattened.

Gephyreans. Anaesthetise for as long as necessary in menthol. Fix 10% formalin, followed by weak and strong alcohols.

Parasitic worms. Fix hot corrosive sublimate. Preserve in 75% alcohol.

Pycnogons. Fix 10% formalin, the large and very large ones with the legs extended fore and aft in long $\frac{1}{2}$ in. to 1 in. tubes. Transfer as before to weak, and later, strong alcohols.

Crustacea of all kinds. Fix 10% formalin (for the soft parts), following with weak and strong alcohols.

Nudibranchs, Tectibranchs, etc. Anaesthetise menthol for as long as necessary, fix 10% formalin, following with weak and strong alcohols.

Polyzoa. Anaesthetise menthol for up to 10 hours (or, say, overnight), fix 10% formalin, following with weak and strong alcohols.

Ascidians. Fix 10% formalin, following with weak and strong alcohols.

Cephalodiscus. Anaesthetise menthol, fix 10% formalin, following with weak and strong alcohols.

Fishes. Fix 10% formalin making a slit in the belly (except in very small specimens) to allow penetration to the viscera. After 24 hours, transfer to weak and finally to strong alcohol. Fishes are best preserved head down in tubes or jars that just fit them. Large fishes, with the label folded (print inside) and tucked under the gill-cover, should be sewn up in baft (or other cheap cloth) and preserved flat in tanks, preferably provided with trays, with a numbered bone label outside tied on to the wrapping.

Plankton animals

Large animals picked out from the plankton samples were treated in much the same way as above.

Medusae and Siphonophores. Fixed and preserved in 10% sea-water formalin which as a rule was changed after a day or two.

Polychaetes. As for bottom polychaetes.

Pelagic Nemerteans. I have often found it useful to put them in a spoon, already dipped in formalin, and to brush and straighten them out with a good quality paint brush charged with formalin. Preserve in 10% formalin or in weak, followed by strong, alcohol as desired.

Crustacea of all kinds. As for bottom crustacea.

Leptocephali. Fix and straighten on formalin-soaked blotting paper by brushing gently with a formalin-charged brush. Preserve in 10% formalin.

Squid. Fix 10% formalin, following with weak and strong alcohols. With slender elongate specimens it is a good plan to fix in tubes that will just accommodate their widest parts, tail down, with the long tentacular arms, at full stretch, led well forward. As with Polychaetes, leave tubes flat overnight.

Fishes. As for bottom fishes, except that very small specimens need not be opened.

General notes on preserving

Bottling. The commonest type of bottle in use at sea is the ordinary housewife's preserving jar. Always remember to wipe the seating which takes the rubber ring clean of grit and dirt. Grit can cause a space to be left between ring and seating and eventually lead to evaporation and spilling. Remember, too, to hold the glass top of a screw-top jar firmly down on ring and seating with the left hand while screwing up with the right. If the glass top is allowed to revolve during the screwing process the ring invariably becomes unseated. We tried as a rule to use jars (and tubes) that fitted the animals we were preserving so that they would not wash about in the rolling ship. As a safeguard against maceration, we never filled our jars more than about half full of specimens. I have often opened a jar crammed to the top with soft-bodied watery animals which stank when I removed the lid.

Labelling. Always use a 2B, high quality pencil, the point well sharpened but with the keen edge slightly blunted by rubbing on the trousers. Attend to the point frequently. Dig deeply into the paper, but not so hard as to break the point, leaving as black an impression as possible. The correct amount of pressure is only acquired after considerable practice. Colour notes should go on the back of the label with a P.T.O. on front. Labels done in this way on good paper¹ are as legible today as they were when originally written, some of them 35 years ago. In many instances they are in fact more legible, the black lettering on a carefully executed label tending to become blacker on immersion in formalin and blacker still in alcohol. I have no doubt these early labels will continue to maintain this lasting quality for many years to come.

Use labels that fit conveniently into the tubes and jars into which the specimens go. Coil the tube labels horizontally inside near the top out of contact with the specimen, pressing them hard against the glass so that they take the circular shape of the tube. Let the station number and group or name of the specimen show clearly to the sorter. It is tiresome and very time-wasting for him to have to search about in a tube (or jar) for a too small label carelessly inserted. If the right size of label is used with the right tube or jar the whole of a horizontally coiled label should be visible to those who subsequently handle the specimens. Use the same procedure for jars, large labels for large jars, smaller ones for small jars, coiling horizontally as above but at the bottom of the jar.

With tubes it is bad practice to place the label vertically because this entails pushing it downwards the whole length of the tube with possible damage perhaps to a delicate specimen at the bottom.

Very small specimens were preserved in tiny vials, ca. $\frac{1}{8}$ in. diameter by ca. 1 in. long, plugged with cotton wool wrapped in tissue paper. In practice we found these were too small to take a legible label so we used to put them, plug up, vertically into somewhat larger tubes provided with a larger horizontally coiled label and with a soft bedding of cotton wool at the bottom.

As a safeguard against chafing of the print and written matter when preserving sand, or stones encrusted with sponges, Polyzoa, etc., it is advisable to put a second label protected by an envelope into the jar or other container that might be used.

¹ The paper used in pre-war days was known as "Antique parchment, cream wove" and was supplied by Waterlow and Sons Limited.

Plugging and storage of tubes. Plug all tubes¹ with cotton wool wrapped in tissue paper, pushing the plugs well home into the fluid so as to leave no air space or bubble. Immerse plugged tubes immediately in basins containing formalin or alcohol to prevent evaporation while they are accumulating to be stored away. In the final storage use 5- or 7-lb. jars with a soft bedding of cotton wool at the bottom. Pack tubes, plugs up, vertically into the jars adding cotton wool as necessary (but not too tightly) to prevent rattling about. Fill up with 75% alcohol or 10% formalin as required, adding a final padding of cotton wool at the neck liberally saturated with the fluid. Pack smaller-sized tubes in tiers separated from each other by a bedding (or beddings) of cotton wool. As you approach the narrowing neck of the jar it is permissible to lay very small tubes on their sides, on a slant, or however they will stow best. On no account, however, stow them upside-down because this allows the specimens to come in contact with the label, or even with the cotton wool should the paper wrapping happen to have got split, as it occasionally does, in the process of plugging. Such contact may lead to the damaging or loss of a delicate specimen, especially if there should be careless removal of the plug or label to which it may be adhering.

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¹ i.e., tubes containing specimens in their final alcohol or formalin.

