ON FIXATION AND PRESERVATION OF GELATINOUS PLANKTERS

ABSTRACT

The technics adopted for fixation and preservation of gelatinous plankters, collected during the International Indian Ocean Expedition, could not explain for the bad state of their preservation as the data available in most cases were not comparable. Hence a series of experiments were set up at the IOBC to investigate into the old and new fixing and preserving procedures for gelatinous plankters. Results showed that one percent formaldehyde in sea water prepared from stock formaldehyde neutralised with excess calcium carbonate is the best among the test preservatives used.

The working group on plankton methodology, UNESCO/SCOR/WG-23 was established in 1968 to study the problems associated with the proper fixation and preservation of the zooplankton samples, as a result of the deterioration noted in the plankton samples collected from 1959 to 1965 by the International Indian Ocean Expedition. The experiments (UNESCO, 1968) designed by the working group for determining proper fixatives and preservatives were carried out in different centres. Studies on the fixation and preservation of gelatinous plankters conducted at the Indian Ocean Biological Centre as part of the above investigation is reported here.

The gelatinous plankters include those with higher water content like the coelenterates (medusae and siphonophores), ctenophores, sipunculoides, echinoderm larvae, tornaria and tunicates (salps, doliolids and larvaceae), and those with low water content such as the anthozoans, tomopterids, heteropods and chaetognaths. Studies on both the groups were conducted for finding out the best mode of preservation.

IIOE samples collected by different nations were not fixed in a standard manner. Both picric acid and formaldehyde in different concentrations were used. Later the plankton was sorted and preserved in 4 to 5% formaldehyde. At the time when this study was initiated a large percentage of the jelly plankters were seen in a deteriorated condition. About 70% of the medusae were unidentifiable. In no case morphological characters could be seen clearly. In most specimens the epidermis was stripped free from the mesogloea. It was often seen in the containers as a thin, collapsed structureless envelope, occasionally with the velum and rarely with the manubrium and tentacles. It appears therefore that both poor preservation and mechanical damage due to poor handling

were responsible for the poor condition of these organisms. Being fragile they are easily affected by both these factors.

Among the siphonophores few have retained their shape and characters. The physonectid siphonophores were seen in a contracted, flabby or fragmented condition and hence the counts of nectophores and well preserved bracts represented only parts of colonies. The nectophores in the majority lacked nectosac which possessed the radial canals. In the calycophorid siphonophores most of the species had lost the nectosac. The inferior nectophores were separated from superior nectophores in the polygastric stage and usually bracts separated from gonophores in the eudoxid stage. Cystonectid colonies were found highly contracted and many of them lacked tentacles.

Majority of anthozoan larvae also underwent violent contraction at the time of fixation. Their internal organs were often found to be projected outside the actinopharynx. In a number of specimens the ectoderm had separated almost completely from the rest of the body. A good number of zoanthideans were shrunken and adpressed out of shape.

Ctenophores such a *Pleurobrachia* sp. and *Beroe* sp. were broken up and partly dissolved leaving behind tentacle sheaths and comb rows as evidence of their presence. In fact many of the jelly taxa got deflated, became flabby and with agitation fragmented, superficially resembling decay. In chaetognaths and heteropods strong contractions were noticed with shrinkage in the region of the fins and tactile bodies. *Pterosagitta draco* had lost collarette completely. Muscular disintegration was noticed in the case of flaccid species such as *Sagitta enflata*.

Bipinnaria, ophiopluteus, echinopluteus, auricularia and brachiolaria larvae of hemichordates were also in a bad state of preservation. Ciliated bands of these larvae were highly damaged. In a number of preserved samples when recounted, their number varied from those made at the times of collection due to dissolution in the preservatives. Appendicularians were the worst affected by protein solubilization. They had decayed tails and extremely transparent trunks.

In the case of salps and doliolids the test was not affected but the stolen, the muscle structure and the pharynx have disintegrated almost completely.

Fresh plankton was collected from clear brackish and sea water and those in good condition were selected for the experiments. The plankton was kept in sea water in the ratio 1 : 9 and fixed (Balachandran, 1973a) by the addition of various test fixatives (Table I). In addition with a view to select a suitable narcotic for jelly plankters 18 narcotizing agents (Balachandran, 1973b) were tried. Fresh live jelly plankters properly fixed by the different fixatives after careful narcotization, were used in the experimental series on test preservatives (Table I and Table II). Initially the pH

TABLE - 1

Test fixatives and test preservatives used during investigations

Fixatives	Alcohol				Dowicil '100'	Formaldehyde									Osmic acid	Bouin (Hot)	Picric acid	Zenker's fluid	Smith's formol bichromate
Diluents used	Distil-led water	Tap water	Sea water		Sea water	Distil-led water			Tap water			Sea water			Distil-led water	Distil-led watet	Distil-led water	Distil-led water	Distilled water
Strength (%)	75	75	75	98	10 20 30	1	2	4	1	2	4	1	2	4	2				
Condition of organism*	B	B	B	B	A A S	A	A	B	A	A	B	S	S	B	S	S	B	B	B as fixative
	B	B	B	B	S S A	A	A	B	A	A	B	S	A	B	S	S	B	B	B as preser-vative

* S = Satisfactory; A = Average; B = Bad.

TABLE - II

Test preservatives used in addition to those given in Table I

Preservatives	Ethylene glycol						Phenoxetol									
Diluents used	Distilled water		Tap water		Sea water		Distilled water			Tap water			Sea water			
Strength (%)	25	50	25	50	25	50	0.5	1.0	2.0	0.5	1.0	2.0	0.5	1.0	2.0	
Condition of organisms	B	S	B	S	B	S	S	S	S	S	S	S	B	B	B	as preservative

B = Bad ; S = Satisfactory.

(UNESCO 1968) of the different test series ranged from 3 to 8.5 at intervals of 0.5. The subsequent change in pH and in the morphological characters of taxonomic importance of the organisms at the time of fixation and at intervals of 1, 7, 15 and 30 days ; 2, 3, 6 and 12 months and 2, 3, 4 and 5 years after fixation were recorded.

Jelly plankters fixed and preserved after narcotization were found in excellent condition compared to those fixed without narcotization. MSS 222 and Benzamine hydrochloride were equally good narcotising agents when compared to those already in use such as magnesium chloride, magnesium sulphate, menthol, 2% chloral hydrate and Gray's mixture.

Of the various fixatives tried 1–2% formaldehyde proved advantageous over others. Of equal efficiency was 2% osmic acid solution. Though the penetrating capacity of osmic acid is extremely poor, it could do well with gelatinous plankters. However in view of its cost and poisonous nature its use may be restricted to ctenophores only. Fixing by the sudden addition of hot bouin also yielded good results. The disadvantages noted were that while bouin produced a yellowish colour, osmic acid produced a dark colour to the specimens.

Of the various preservatives tried, 1% formaldehyde in sea water gave better results. 10% Dowicil '100', 50% ethylene glycol and 0.5% phenoxetol were not as satisfactory as 1% formaldehyde.

Throughout the experimental series a tendency towards reduction of pH was noticed. After 5 years the lowest value noted was 5.5. The rate of fall in pH was found to be directly proportional to the ratio of specimens to the preservative, a ratio of one-part of specimens to nine parts of preservatives being appropriate. Specimen condition at low pH (acidic) was better than at high pH (basic). A pH varying from 5.5 to 7.0 was found appropriate for jelly plankters.

The various neutralising agents used were found to damage the tissues proportionate to their concentration. Concentration in excess of 1% of borax, hexamine, calcium carbonate, sodium bicarbonate, sodium ascorbate etc. interfered with sample condition. When hexamine, borax or sodium bicarbonate were used as buffer in excess of 1% concentration, pH tended to increase sharply even with slight evaporation. Sodium acetate, calcium carbonate or Rochelle salt at 1% strength did not increase the pH and hence may be preferred as neutralising agents for jelly plankters. Concentrated formaldehyde when neutralised with excess calcium carbonate powder and used in making 1% formaldehyde could maintain a pH of 6 for more than one year. One to 10 % sodium acetate could maintain a pH around 6 for 5 years. While borax bleached the specimens, sodium acetate helped to maintain their colour. It was found better to neutralise the stock solutions especially when calcium carbonate is used as

a neutralising agent. Use of Tris, Dowicil 'A', and sodium benzoate could not improve the preservative quality of the storage fluid, whereas propylene glycol and phenoxetol (Balachandran, 1974a) improved the quality of the preservative.

Of the three diluents used namely distilled, tap and sea water in the preparation of fixatives and preservatives filtered sea water was preferred as the buffering effect of sea water is well pronounced, because of its higher salt content which also helps in reducing the evaporation rate. However the initial pH of 7 of 1% sea water–formaldehyde was reduced to 6.0 in 2 years.

Shrinkage of the organisms after fixation largely depended on the water content of the jelly plankters. The displacement volume of these organisms was less in preserved condition and depends on the specific taxa and the time elapsed following preservation. In the case of salps a loss of about 85% of live volume was observed three years after preservation. Though chaetognaths showed a loss of 45% initially, no further reduction was noticed. The initial loss for crustacea was 8% which rose by 5% at the end of three years.

Based on the results it can be concluded that only an isotonic preservative which can minimise loss of weight and volume can keep the organisms in good shape. One per cent formaldehyde in sea water prepared from stock formaldehyde neutralised with excess calcium carbonate more or less satisfies this condition and is found to be the best among the test preservatives used. It is also found useful to change the preservative preferably once in 6 months in order to maintain the pH and to avoid formaldehyde layering. This is desirable especially if commercial formaldehyde is used as the iron impurities in it also tend to form precipitates. Storage in air-conditioned room was found better than storage at room temperature. Observation revealed that plankton samples in dark coloured containers retain their colour and pigmentation better than those kept in transparent jars. In addition to the use of proper fixatives and preservatives, careful handling of jelly plankters is also important. Particular care has to be taken in the methods of collection, handling and storage of jelly plankters (Balachandran, 1974b).

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