

A Review of the Nature and Causes of Deterioration in Zooplankton Samples

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Introduction

During 1960-1965 the Indian Ocean Biological Centre received about 2,000 zooplankton samples collected by different ships, from different areas in the Indian Ocean, showing different states of preservation. This necessitated a study on a standard procedure for fixation and preservation of zooplankton. An understanding of the nature and causes of deterioration noted in different taxa by the specialists concerned is a prerequisite for an insight into the problem of fixation and preservation. It is attempted, therefore, to review some of the works carried out so far on these lines along with certain original observations.

Nature of Deterioration in IIOE Material

Comments on zooplankton curation have been made by Fleminger (1967 and 1968), Glover (1967), Hansen (1967), McGowan (1967), Tranter (1967 and 1969), Brinton (1967), Ahlstrom (1967 and 1968), McKenzie (1967), Fenaux (1967), Buckman (1969), Nair, R. V. (1967), Thomson (1967), Gruner (1968), Greze (1967), Rao (1967), Be' (1967), Rass (1968), Williamson (1968) and Vannucci (1969). Deterioration in mate-

rials other than I. I. O. E. is noticed by Fauvel (1953) and others.

Shelled organisms showed varying stages of deterioration—whitened, chalky and softened nature of the calcite tests of Foraminifera and the shells of Pteropoda; sometimes the tests of the Foraminifera are completely dissolved leaving only body whorls. Robust shells of Pteropoda survive the decalcification process. The hard resilient, translucent pteropod shells in general show better preservation. McGowan (1967) observed the following sequence of dissolution of shells: (a) Shells turn white, (b) become chalky, (c) become flakey, (d) fall apart and (e) the flakes dissolve at pH 7. McKenzie (1967) found ostracods with all appendages intact and the shells not affected.

Gelatinous organisms such as medusae, salps and appendicularians decompose, making identification difficult. The musculature and mesogloea of the medusae decompose leaving only a thin, collapsed, structureless epidermal envelope, occasionally the velum remains, the manubrium and tentacles are rarely seen. In the case of salps and doliolids the test does not suffer much, but the stolon decomposes. The muscle structure and pharynx completely disintegrate.

Medusae, salps and doliolids deflate, become flabby, and with agitation tend to fragment. This process superficially resemble decay. Sometimes nectosac and somatocyst of siphonophores decompose. Appendicularians are the best indicators of protein solubilization since they are without cuticle; the larvacean body decomposes and dissolves away and the tail becomes softened, transparent and unrecognizable. Among chaetognaths almost all *Pterosagitta draco* lose their characteristic epidermal collarette and the less spectacular collarettes of other species are usually lacking (Thomson 1967). In the flaccid species, *Sagitta lyra* and *S. enflata*, the muscle walls collapse inwards. In some cases muscular torsion is evident resulting in an abnormal shortening and thickening of bodies. Chaetognath ovaries are found in a decomposed state. According to Fauvel 1953, Polychaetes become soft, sticky and transparent due to deterioration of internal tissues, but due to a tough cuticle, the general shape and consistency is retained. Glandular pigments also disappear. Pinnules of Tomopteridae are missing.

The internal tissues of a number of organisms in a sample may be in a state of moderate to total disintegration making them fragile and inferior for taxonomic studies due to loss of structures. Earlier stages of deterioration include frothy aspect and opacity of cephalothorax of Eucalanidae, loss of resiliency of the cuticle to slight pressure applied by needles and one or more ruptures of the dorsal cuticle, of the cephalothorax accompanied by extrusion of internal tissues through the rupture (Fleminger 1967 and 1968). Partial to

complete loss of definition of internal organs and muscle tissues together constitute advanced signs of deterioration. Egg sacs of Copepods decompose. In many cases within the tougher exoskeleton or test, tissue dissolved into a cloudy fluid is available. Where this fluid has disappeared into the preservatives the specimens are transparent. An identifiable remnant of exoskeleton, test or cuticle record the presence of organisms in the sample and where such resistant remnant is not present, no identifiable record of the organism's presence is left as in LARVACEA. The exoskeleton of crustacea is not much affected. The loss of eyes and limbs is commonly noticed. According to Williamson (1968) well over half the decapods show some degree of decay of the soft protoplasm and cases in which only the exoskeleton, remains are by no means rare. Guener (1968) noted many of the Amphipod specimens rather being transparent indicating a decay of tissues. Also he noted variations in the condition of Amphipods from cruise to cruise.

Ahlstrom (1968) claims that while half the fish larvae material sorted at IOBC is rather good to excellent condition, the other half ranges from passable to very poor. Loss of eyes, torn, ripped intestine and gut are common damages. Specimens have either lost their pigment entirely or it is badly faded. Deterioration is noticed in the form of separate, swollen myomeres and separation of flesh from skeletal parts, occasionally only the skeleton parts remaining. Rass (1968) noticed in the case of some fish larvae melanin pigment faded to brown or pale yellow. Also he found most samples in more or less satisfactory state

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inspite of the larvae being partly macerated with consequent fading of the melanin pigments and in having white spots sedimented on the surface of their bodies.

Causes of Deterioration in Zooplankton Samples

Shortage of shelf life of plankton owing to deterioration as noted above is the outcome of a multitude of factors from a variety of sources which can be studied in four phases :

1. From field to the laboratory,
2. At the laboratory,
3. On the shelves and
4. Physiological.

1. *From field to the laboratory* : includes a chain of events from collection, fixation and handling at sea to transport to the laboratory.

(i) Poor ship board techniques at the time of collection : Hosing down the sides of the net with a strong jet of water is primarily responsible for loss of appendages and rupture of plankton. Mesh size and the type of bucket used also cause damage to plankton since the rate and direction of flow of water is related to the mesh size and type of bucket used. In a larger mesh size, the rate of flow water is greater and the zooplankton get crushed more, whereas, if the mesh is of a smaller size the net gets clogged soon thus preventing the faster flow of waters thereby increase the pressure and subsequent damage of the plankton. Similarly a completely closed plankton bucket protects plankton from frictional damage while the one

with a side window or a bottom filter leads to crushing of the plankton. The undivided long haul may cause damage to the catch. When the plankton collected in the bucket is filtered and transferred to the fixative, due to exposure to air, evaporation takes place leading to formation of artefacts which cause damage to organisms. Delayed fixation helps histolysis and promotes bacterial growth especially in tropics where organic material is more. The amount of fixation added should be proportional to the amount of organic matters present.

According to Tranter (1969) the interaction between net and water which constitute filtration performance influence the condition of the catch. The organisms filtered from the water accumulate upon the filtering surface. Damage to the organisms caught would appear to be caused by the pressure drop across the meshes (filtration pressure) which varies as the square of the approach velocity. Consequently the condition of the catch could probably be improved by lowering the speed of tow, by reducing the mouth area of the net or by increasing the area of gauze.

(ii) Sample size, ratio of sample volume to jar volume and length of cruise together constitute a second factor. Generally, the jars containing plankton are not filled completely with preservative. Ahlstrom claims that while the vessel rolls or pitches, the sloshing of organisms against the bottle as well as assemblage of fragile ones with crustacea or pteropod leads to mechanical damage, the extent of which is proportional to the length of cruise. This is particularly true for specimens which were damaged

previously, while the net was hauled and washed, due to mutilation. According to Hansen (1967) deterioration in some of the 'Dana' samples was caused by insufficient original preservation on board the ship. Inadequate preservative can cause decomposition.

(iii) The transport of samples across continents and oceans to the sorting laboratory and their detention at improperly ventilated, warm and humid custom warehouses for lengthy periods could constitute a third factor.

2. *At the laboratory*: Apart from handling defects the following factors might affect the conditions of stored plankton samples. (i) It was noted that the most probable cause of the deterioration is the mechanical and perhaps osmotic damage inflicted on the sample by successive biomass determinations. (ii) The use of tap water for subsampling and measuring the displacement volume; contaminated water could initiate bacterial activity, particularly in the absence of sufficient preservative in solution. (iii) The plankton fractionator might cause mechanical damage particularly as the plankton passes through the small exit holes. (iv) Damage due to pressure applied in between the organisms while water is being removed by blotting paper during displacement volume determination. (v) The effect of formalin concentration while plankton taxa are left for long periods in petri dishes, and exposure to air during sorting and (vi) Mechanical damage caused by brush in handling the specimens and by the label in the specimen tube and the cotton plug.

3. *On the Shelves*: The concentration and type of preservatives and buffers;

the effect of light and temperature; and the type of containers use (Currie 1967) would influence the condition of the sample. Additives such as Hexamine, Borax, Sodium hydroxide, Calcium carbonate, Sodium bicarbonate, Rochelle salt, Methanol and Tris HCl etc. have histochemical reactions on the tissues.

According to McGowan (1967) excessive acidity of zooplankton samples affects the organisms adversely. Crustacea have high content of unsaturated fat and because of their oxidation into fatty acids pH changes soon in samples with large numbers of crustaceans.

4. *Physiological*: Ahlstrom and Thraillkill (1963) have observed in the case of gelatinous organisms preserved in formalin, an 80% loss of weight and diminution of volume and in the case of nongelatinous forms a 20 - 30 % loss of weight. During the first few hours after formalinization, the diminution is very great and varies from one to another sample, and stabilises after a month.

Gelatinous organisms have a water content greater than 80% of the estimated biomass and the interstitial water component of organism was assumed to be approx. 24%. Hence, taken as a whole the average sample would have a water content of 886 parts per thousand. With these observations in mind perhaps the deterioration of organisms can be explained (McGowan, 1967).

Gelatinous organisms have a hydrostatic exoskeleton and an internal osmotic pressure maintained perhaps by Donnan's equilibrium, that gives them turgor. When the properties of their outer

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membranes are changed by denaturing and precipitation of their proteins by fixation, this equilibrium also changes and loss in weight and volume is observed. The inability of formaldehyde to keep the volume and weight of specimens unaltered could be responsible for subsequent deterioration.

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