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Maintenance of quality in fish eggs and larvae collected during plankton hauls

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The quality of preserved fish eggs and larvae in marine zooplankton collections is discussed in this section, as well as all the steps in the chain of events between collection, preservation and handling at sea, and sorting, identification, handling and storage ashore which could influence the quality of fish eggs and larvae, either adversely or favourably.

Careful examination has been made of fish eggs and larvae from a variety of sources, including material from all the oceans. Well-preserved collections have been in the minority. In many collections the condition of specimens ranged from fair to almost unrecognizable.

In some collections damage to specimens was due to the preservative which was either too strong (4 per cent formaldehyde or even stronger), or which had additives for buffering or colour retention which caused deterioration of quality (as for example, hexamine). In most instances, however, the poor quality of the specimens was not primarily due to the preservative but to collection techniques, delay in fixation, and incorrect handling of the material in the laboratory. There is ample evidence, however, which shows clearly that first-rate quality can be maintained in preserved fish eggs and larvae over an extended period of time.

Some stages of fish larvae are more fragile and subject to greater damage during collection and handling than other stages. As a rule, younger stages of fish larvae, i.e. yolk-sac larvae and small larvae prior to fin formation are more fragile and subject to greater damage during collection and handling than older stages of larvae. Some kinds of fish larvae are fragile at all stages. Thus, thin elongate larvae such as clupeids, engraulids, or stomiatoids are usually more easily damaged (gut torn, eyes lost, etc.) than compact stubby larvae such as carangids, scorpaenids and serranids. Early stage eggs (between fertilization and blastopore closure) are more subject to damage during collection than middle and late stage eggs.

Fish eggs and larvae in collections from tropical regions usually are in poorer condition, on the average, than fish eggs and larvae collected and handled in comparable fashion from temperate or subarctic regions.

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The poorer quality of fish larvae collected from tropical regions could be due, in part, to the temperature related phenomenon of more rapid deterioration of specimens that die during the course of a haul in tropical waters. This is at present only a supposition, but related to better substantiated observations of the adverse effects of delay, even of minutes, in preservation of plankton collections after the net has been retrieved aboard the vessel. The influence of duration of tow on quality of specimens in tropical regions should be critically evaluated. Perhaps in tropical waters, the time duration of a haul should be decreased and the number of hauls increased.

Collection at sea

Some aspects of quantitative sampling of fish eggs and larvae have little influence on quality of the specimens obtained; these would include such items as the need to cover the entire depth range of the eggs and larvae of the fishes being investigated, the need for uniformity in gear deployment and for freedom from clogging during a haul. The critical problem area in sampling as regards quality is that of net avoidance by larvae. To decrease this, there has been a trend to increase speed of hauling. However, the value of the increased catch of larger larvae has to be weighed against two adverse effects of increased speed: increased loss of small eggs and larvae through the mesh apertures of the net due to increased filtration pressure at higher speeds (the extrusion problem) and poorer condition of the specimens retained.

Detailed personal observations on the quality of fish eggs and larvae as related to vessel speed were made on collections from the standard CalCOFI net (Ahlstrom, 1952), paired Brown-McGowan BONGO nets (McGowan and Brown, 1966), Gulf III sampler (Gehringer, 1952), Gulf V sampler (Arnold, 1959), Clarke jet-net (Clarke, 1964), Bary high-speed plankton sampler (Bary *et al.*, 1958). The unencased nets (CalCOFI, BONGO, and Gulf V) were hauled at speeds between about 1.5 to 5 knots, while the encased nets (Gulf III, jet, and Bary) were hauled at speeds between about 2 to 8.5 knots. For all gears, fish eggs and larvae were in the best condition from hauls made at slow vessel speeds, and damage to specimens became increasingly greater with increase in speed of hauling. Filtration pressure on eggs and larvae resulting from increased towing speeds may be the most critical factor influencing quality of fish eggs and larvae at the time of collection. Such observations of damage resulting from increased towing speeds are not unique to fish eggs and larvae. Tranter and associates, for example, recorded progressive damage to appendicularia between hauling speeds of 1.5 and 2.4 m/sec (Tranter and Smith, 1968).

Where hauls have been made at slow vessel speeds, or taken vertically with such gear as the Hensen egg net or the standard IIOE net (Currie, 1963) it may be assumed that fish eggs and larvae would be brought aboard within the retrieved net in good condition, and the following comments are made for fish eggs and larvae in that state.

Preservation

After the net is brought aboard it is washed down to dislodge organisms clinging to the meshes of the net. Water pressure should be sufficient to wash down the net, but not so strong as to damage specimens. This is another danger point. The cod-end bucket or sac containing the plankton sample is detached and the sample is preserved at once. Delay in preservation can be a critical factor with regard to quality of the material.

The following details illustrate the adverse effects of delay in preserving plankton collections.

While checking over the fish eggs and larvae from the southern pattern of a CalCOFI cruise in the late 1950s it was noted that about half of the collections were showing serious deterioration with many larvae too disintegrated to identify or to measure. On investigation, all the poor collections were found to have been taken on one of the two scientific watches. The scientist-in-charge, when questioned, admitted that there had been a time delay of about 20 min in preserving samples. The effect of this delay on the condition of fish eggs and larvae was disastrous.

The following methods are used at the National Marine Fisheries Service, La Jolla.

Samples are preserved in quart (946 ml) jars. The plankton is emptied into the jar and the residue of plankton in the cod-end bucket is washed down into the jar. The jar should be at least three quarters full before formaldehyde is added. Then add 50 ml of concentrated commercial formaldehyde (40 per cent), followed by 20 ml of a saturated solution of borax in sea-water, and the jar is then filled to the top with sea-water before capping, after which it is gently inverted a few times to ensure thorough mixing of the fixative with the specimens. The final concentration of the formaldehyde is about 2.2 per cent. This concentration is not necessarily put forward as the optimal amount for preservation of fish eggs and larvae but it produces good quality preserved specimens, and has proved to be satisfactory for many years.

Jars containing plankton should always be completely filled (Parts I and V), otherwise the concentration of formaldehyde may be altered when using the above method of fixation. The contents will also slosh about when the ship rolls, thus damaging fragile fish larvae.

In order to preserve natural colour in biological specimens, investigators have added antioxidants such as emulsified concentrate of Butylated Hydroxytoluene (BHT) (Waller and Eschmeyer, 1965), Butylated Hydroxyanisole (BHA), (Toyama and Miyoshi, 1963) and Sodium Ascorbate (Yoshida, 1962). Red pigments have been shown to be important in identification of tuna larvae (Ueyanagi, 1966; Matsumoto *et al.*, 1972). Dr W. J. Richards (NMFS, Southeast Fisheries Center) (personal communication), reports variable success with BHT in preserving red pigments in fish larvae, particularly tunas, after extensive tests, and finds that the best procedure for studying evanescent pigment in fish larvae is to examine specimens immediately after collection and preservation.

Storage of collections aboard the research vessel

To minimize damage to delicate organisms during storage on the vessel, samples should be stored in the most stable part of the vessel. Temperature of storage may be important, particularly in tropical regions. This, again, has been one of the uncontrolled variables that might effect specimen quality. Experiments are needed to test the effect of storage temperature on the condition of plankton organisms, especially during cruises of rather long duration.

Handling the collections ashore

PLANKTON VOLUME DETERMINATIONS

Not all laboratories make a plankton volume determination as the first shore-side step in processing a plankton collection, but many do, including our laboratory. This measurement is of value as a rough measure of zooplanktonic biomass, but the determination also has a practical value in subsequent handling of the collection. Large samples may have to be aliquoted and the fraction to be sorted will often depend on sample size.

Damage to specimens during handling for volume determination should be minimal. The plankton is separated from the preserving liquid by pouring the sample through a fine-meshed draining cone. The plankton is retained in the cone until drainage of liquid from the cone diminishes to an occasional drop. The volume of drained plankton is determined by measuring the change in volume of a partly filled graduated cylinder on its addition. The sample is again filtered to concentrate it, and returned to a preserving liquid. At this time, it is advisable to place the sample in new 2 per cent formaldehyde solution rather than return the sample to its original preserving liquid (Parts I and V). The practice at La Jolla has been variable in this respect.

SORTING

At our laboratory, separation of fish eggs and larvae from the other plankton constituents has top priority, and this is done before any other organisms are removed. Usually the total sample is sorted for eggs and larvae. Because of the danger of formaldehyde poisoning, the plankton is separated from its preservative and placed in fresh water for sorting. Deterioration of quality can occur if plankton is kept too long in fresh water. Fortunately, most samples of fish eggs and larvae can be completely sorted during 1 day. The fish eggs and larvae are placed in vials, preferably 2-dram, and preservative added. We use 1.05 per cent buffered formaldehyde, prepared with tap or distilled water. The plankton is returned to its preserving liquid.

When sorting of a sample is not completed during a working day, the sorted portion is kept separate from the unsorted, but preservative is added to each. The

sample should not be allowed to remain in fresh water overnight although, unfortunately, this sometimes happens. Occasionally samples require several days to sort. If allowed to remain in fresh water without preservative for this length of time, marked deterioration could result. The problem is compounded by holidays or week-ends. Unless carefully monitored, more damage to specimens can occur during sorting in fresh water than during any other shoreside operation (Parts V and VI).

IDENTIFICATION

During identification of eggs and larvae, the preservative is retained in the sample (1.05 per cent buffered formaldehyde); hence the problems outlined for sorting in fresh water do not exist. If an investigator is ultra-sensitive to formalin the larvae can be transferred to ethanol or similar preservative. Damage to specimens can result from handling with forceps or while measuring, but such damage is minimized by experienced workers. Unless damage to specimens results from the preservative itself, which can act beyond this step during subsequent storage of specimens, the quality of the preserved material will have been determined by preceding events (already discussed) by the time the collection reaches the ichthyoplanktologist for identification and study.

At this step, many kinds of fish eggs and larvae are separately bottled, and, of course, put in fresh preservative. Usually the eggs or larvae occupy only a small percentage of the container volume, hence the proportion of preserving liquid to organisms is large. Unquestionably, this is advantageous with regard to maintenance of quality during storage. While preparing this section, I have examined collections of larvae at our laboratory stored for 15 to 20 years. The larvae remain in excellent condition, with melanistic pigment still intense. There has been no observable deterioration of quality, and it follows from this that 1.05 per cent buffered formaldehyde in fresh water has proved itself to be a quality preservative for fish eggs and larvae.

STORAGE

A chief concern in curating identified collections of fish eggs and larvae is to prevent loss of specimens due to drying up. For 'dead' storage of specimens, such loss can be minimized if groups of vials containing specimens are stored in a bath of preservative within air-tight jars. Most of the Dana material at Charlottenlund (Denmark) is so curated. For collections that are consulted frequently, as in our eggs and larvae collection at La Jolla, this method of storage is impractical, due to the difficulty of retrieval. Our collection is curated for ready retrieval of any desired collection. Specimens are mostly stored in 2 dram (7.39 ml) vials sealed with a plastic cap that has an inner vinyl liner that permits firm sealing. We store the vials in cardboard boxes designed to snugly accommodate 60. Collections must be examined periodically and preservative added. Some years ago we used corks

for stoppers in vials. Although corks permit a good seal, they have the disadvantage of discolouring the larvae in some collections.

We have not investigated one aspect of storage—change in concentration of the preservative due to evaporation. When the preserving liquid is reduced by half due to evaporation, has the effect been to double the strength of formalin in the vial, or is formaldehyde lost along with water vapour during evaporation? This is an important point to settle. Perhaps in curating for evaporation, the old preserving liquid should be removed and new preservative added.

Specimens should be stored away from light. They lose even melanistic pigment if allowed to remain in the light for a period of time.

References

AHLSTROM, E. H. 1952. Pilchard eggs and larvae and other fish larvae, Pacific coast—1950. *U.S. Fish Wildl. Serv., Spec. Sci. Rep. Fish.*, no. 80, 58 p.

ARNOLD, E. L., Jr. 1959. The Gulf V plankton sampler. *Galveston Biological Laboratory fishery research for the year ending June 30, 1959* (U.S. Fish Wildl. Serv., Circ. 62, p. 111–13.)

BARY, B. M.; STEFANO, J. G. de; FORSYTH, M.; VAN DEN KERKHOF, J. 1958. A closing, high-speed plankton catcher for use in vertical and horizontal towing. *Pac. Sci.*, vol. 12, no. 1, p. 46–59.

CLARKE, W. D. 1964. The Jet-Net, a new high-speed plankton sampler. *J. Mar. Res.*, vol. 22, no. 3, p. 284–7.

CURRIE, R. I. 1963. The Indian Ocean standard net. *Deep-Sea Res.*, vol. 10, nos. 1–2. p. 27–32.

GEHRINGER, J. W. 1952. An all metal plankton sampler (model Gulf III). *U.S. Fish Wildl. Serv., Spec. Sci. Rep. Fish*, no. 88, p. 7–12.

MCGOWAN, J. A.; BROWN, D. M. 1966. *A new opening-closing paired zooplankton net.* (Univ. Calif. Scripps Inst. Oceanogr.-Ref. 66–23.)

MATSUMOTO, W. M.; AHLSTROM, E. H.; JONES, S.; KLAWE, W. L.; RICHARDS, W. J.; UEYANAGI, S. 1972. On the clarification of larval tuna identification particularly in the genus *Thunnus*. *Fish. Bull.* (United States), vol. 70, no. 1, p. 1–12.

TOYAMA, K.; MIYOSHI, G. 1963. Prevention from color fading of aquatic animals under preservation. I. Test on preservatives to retain red color in fish and crustacean specimens. *J. Tokyo Univ. Fish.*, vol. 50, no. 1, p. 43–8.

TRANTER, T. J.; SMITH, P. E. 1968. Filtration performance, p. 27–56. *Zooplankton Sampling*, Paris, Unesco, 2nd impr. 1974. (Unesco Monographs on oceanographic methodology, no. 2.)

UEYANAGI, S. 1966. On the red pigmentation of larval tuna and its usefulness in species identification. *Rep. Nankai Reg. Fish. Lab.*, vol. 24, p. 41–8. (In Japanese, English summary.)

WALLER, R. A.; ESCHMEYER, W. N. 1965. A method for preserving color in biological specimens. *Bioscience*, May 1965, p. 361.

YOSHIDA, Y. 1962. A way of making fish specimens with their original body colours kept. *Bull. Misaki Mar. Biol. Inst., Kyoto Univ.*, no. 3, p. 67–8.