Rec. Zool. Surv. India, 90(1-4) : 265-303, 1992

ESTUARINE ANIMALS : A MANUAL ON COLLECTION, PRESERVATION AND MAINTENANCE

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I. INTRODUCTION

All estuarine biologists either working at research or academic institutes should be aware of atleast the simple collection procedures of estuarine animals and their accurate identification for environmental studies. After knowing such procedures an attempt should be made to build a reference collection centre covering the local flora and fauna which will later assist in various studies connected wtih ecology, commercial exploitation, conservation and environmental assessment studies. The present manual is a tool towards such a goal which deals with the general collection, preservation and maintenance procedures of estuarine animals.

II. COLLECTION

Collection of intertidal, planktonic, nektonic and benthic animals are to be made by using suitable gear. The collection equipment recommended are very simple in design and can be obtained from manufacturers within the country (Appendix a). The main criteria for estuarine gear should be of light weight as these are to be operated in shallow waters of estuaries from indigenous craft and fibre-glass boats operated with outboard motor engine.

a. Intertidal :

This area covers varied habitats having different fauna and flora inhabiting mud flats to salt marshes and mangroove swamps. Here the collections are mainly hand picked by using blunt forceps, chisels, hammers, showels, spades, crowbars, brushes, scoopnets, wide mouth pipettes, sieves of different aperture sizes (2.5mm - 0.5mm) and also by using formaldelyde, bleaching powder etc., in pools exposed at low tide

marks. One can visit this area frequently and make representative collections. Most of the animals (sessile or motile) are either buried in the mud, sand or associated with aquatic vegetation; attached to dead wood, rocks, within empty shells, oyster beds; under stones and other substrates. A tide table chart of the area is essential for collections as the animals will be exposed only during low tide and the area should be reached well before the low tide time. If one reached after the low tide, it is impossible to make representative collection as the area will be submerged during high tide water and one has to wait till the next low tide which may take few hours and that time may not be suitable for making collections as it may be dark at that time. It is better to stay at the spot overnight to observe the tidal exposure of the coast and to collect the animals during both high and low tides for better representative collections.

b. Plankton :

Planktonic animals range from few microns to few centimeters along with bigger forms viz., medusae, jellyfishes, salps. All these organisms are collected through plankton nets made of bolting silk cloth which are towed behind a boat at low speed. The most widely used plankton net (Fig. 1) consists of a circular metal ring of 30 cm. in diameter, with a canvas collar of 10 cm. height followed by a concial net of bolting silk approximately 100 cm. height ending in a canvas cloth of 10 cm. height at the narrow end (Cod end) to which a tubular metalic bucket is clamped. The bottom of the bucket is covered and clamped with the same bolting silk cloth used for the plankton net. Three bridles are tied to the eyes provided on the metal ring which in turn are connected to a small ring or shackle which is connected to a towing rope for towing the net behind the boat at a reasonable distance (5-10 mts) to avoid the disturbances caused by the Boat. The plankton collected in the bucket after towing for a period (15-30 min.) is transferred to the container after washing the sides of the bucket and the bloting silk cloth clamped to the bucket using 10% neutral formalin for fixing and preservation. Do not use water for washing the sample after collecting plankton into the container, which will dilute the preservative thus spoiling the plankton sample in due course.

Different types (numbers) of bloting silk cloth should be used to collect different types of plankton-macro (0-4) micro (5-15) and nanoplankton (15-25).

c. Nekton :

The nektonic animals are capable of swimming against currents and include mainly decapods, squids, fishes, reptiles and mammals. Most of these animals can be collected by different types of traditional nets used by fishermen - stake nets, scoopnets, cast nets, hand nets, siene nets, Midnapore shooting nets, bag nets, drift nets, inshore drag nets, hook & line and chinese dip nets, (Figs. 2 & 3). The smaller nektonic forms like fish larvae are collected with plankton nets as they are too small to be sampled through the fishing gear.

d. Benthos :

The benthos are those animals and plants which live on the floor/underneath

and they range from subtidal to the deeper parts of the estuary. The quipment used for the collection of benthic organisms are mostly a variety of grabs and corers which are designed to penetrate the bottom and grab the sediment sample with the associated fauna and folora. The retrived sediment sample is passed through sieves of different aperture sizes (2 mm - 0.05 mm). The macrobenthos are those which are retained in the sieves of 0.5 mm while meiobenthos and microbenthos are those which pass through 0.5 mm sieves and below.

The most common grab is the Peterson grab which consists of 2 jaws which are hinged together and are held open at the time of lowering. As soon as the grab touches the bottom, the clamp is released facilitating the grab to close its jaws and while doing so it takes a bite of the floor containing a segment of sediment sample with its associated organisms. Here we recommend use of a light weight grab of less than 8 Kg weight which can be operated in shallow waters from country crafts (Fig. 4). In addition to the above Peterson grab, a modified version of Van Veen hand grab (Fig. 5) is found to be highly suitable in estuaries of shallow depths. Detailed account of the various instruments used in the collection of benthos is given by Holme & McIntyre 1971. The benthic organisms are also collected by using dredges, beam trawl and Agassiz trawl (Figs. 6-8). They are used for collection of organisms crawling on the floor but not for burrowing and others living within the sediment. A light weight dredge with a square mouth of 30 cm which can be hand hauled from a small boat is suited for the collections of smaller epibenthic organisms from a limited area. For the collection of bigger epibenthic organisms over a wide area a beam trawl or Agassiz trawl are well suited for sampling, however these are difficult to operate from a country boat. A smaller version of these trawls with a mouth width of one meter can be operated from fibre glass boats with outboard motors. The dredges and trawls should not be operated for more than 15-20 minutes as there is a chance of clogging its mouth with large sediments and making it difficult to lift them.

III. COLLECTION TREATMENT IN THE FIELD

1. Sorting :

The intertidal, planktonic, nektonic and benthic animals are to be sorted immediately after collection. The planktonic samples are to be fixed in 5-10% neutral formalin. The nekton samples are sorted out group wise (appendix - B & C), fixed and preserved in 10% neutral formalin. Bigger organisms need a slit in the belly through anal opening. Benthic organisms retrieved after sieving through a set of sieves of 2 mm down to 0.5 mm are weighed, fixed and preserved in 10% neutral formalin. For groups like sponges, coelenterates, annelids, mollusca and echinoderms special treatment of anaesthetization and fixing is needed and the details of such procedure are givne in Table I.

b. Anaesthetization :

Wherever anaesthetization is necessary, crystals of menthol, megnesium sulphate, magnesium chloride, chloral hydrate are sprinkled over the clean estuarine waters containing the animals allowing them to expand gradually (Table I).

c. Fixation :

Formalin is the best field fixative which is available commercially as 38% aqueous solution of formaldehyde gas. For dilution purposes the 38% solution is treated as 100% formalin and animals are fixed in 5-10% neutral formalin sollution generally. The 10% solution is prepared by adding 9 parts of water to 1 part of commercial formalin. (Before dilution, 1 litre of commercial formalin should be neutralized either with 20 gms of Borax or 200 gms of Hexamine).

d. Preservation :

After fixation (Killing in formalin) the liquid fixative generally becomes diluted and dirty. Further some groups are not to be preserved in formalin even for short time. Therefore, suitable preservative should be used before packing them in containers as it may not be possible to unpack the collections immediately at the laboratory (Table I).

e. Containers :

It is always preferable to use screw capped plastic tubes and wide mouth plastic bottles, jars and drums with screw caps for keeping the collected material in the field. The bigger animals — crabs, squids and certain fishes etc., should be wrapped in cheese cloth (gauze cloth) and packed in bigger screw capped, wide mouth plastic drums. The collections contained in the smaller tubes are to be packed in 1-5 litre screw capped wide mouth plastic jars with cotton padding all around.

f. Labelling :

Every sample either kept in the tubes or wrapped in cheese cloth should contain invariably an internal label printed on a tracing paper. Avoid paper labels as they become soft and disintegrate. Labels should be written with water proof black Indian Ink or with soft lead pencil (Fig. 9).

g. Documentation :

As it is not possible to write all the details on the field label, it is important to keep collection data in a field note book. The sample number given on the label should be same as mentioned in the field note book/station book so that the detailed field data can be transferred on to the permanent label in the laboratory. Data sheets are to be filled while collecting the plankton, nekton and benthos and proforma data sheets are given for guidance (Appendix - d-f).

h. Packing :

The final field packing of the collections kept in different small plastic containers are packed in steel trunks with sufficient soft padding material all around. The big plastic drums can be carried after covering with gunny bags. All these packages should contain a destination label on the outside as well as inside.

i. Photographs :

Photographic presentation of the collection spots as well as the specimens in live condition, preferably in situ and also fixed is necessary for better record of the collections and also for future publication purposes.

IV. COLLECTION TREATMENT IN THE LABORATORY

The collections brought from the field will be unpacked and transferred to suitable containers with final preservatives (Table I).

a. Sorting :

Eventhough the samples were well cleaned and preserved in the field, it needs further attention as it is not possible to have thorough cleaning of the sample in the field. As there is every chance of some specimens being damaged during transit, only the specimens in good condition should be finally preserved. After that, the collections are sorted out finally groupwise (Appendix - C). For subsorting the collection samples of lower groups a good binocular stereo microscope with wide field eyepieces and good illumination is necessary.

b. Preservation :

After subsorting, either the collections were transfered to 5-10% neutral formalin or to 75% ethyl alcohol (i.e. 4 parts of rectified spirit and one part of water). Soft bodied animals are to be transfered to 75% alcohol by passing them through 30% and 50% alcohol for few hours to avoid strinkage. The bigger animals like fishes, crustaceans are to be washed before transfering them to 75% alcohol (Table I). It is advisable to preserve bigger animals in formalin as they require large quantity of ethyal alcohol which is expensive as well as difficult to procure and maintain, while the formalin preservative is cheaper and need less attention, though it is unpleasent to work.

c. Containers :

Wide mouth glass jars and bottles of different sizes provided with plastic screw caps or glass stoppers are ideally suited for long term preservation of animal collections. While preserving the smaller specimens, it is better to use small specimen tubes (glass/plastic) which will be plugged with cotton and kept immersed in the preservative using the above mentioned glass jars/bottles with cotton padding at the bottom and top. The large specimens which cannot be kept in glass jars should be stored in plastic tanks fitted with trolly frames and tight lids.

d. Labelling (unnamed) :

After the transfer of the materials to the suitable containers, a suitable label filled with all field data written in water proof Indian Ink should be kept inside (Figs. 10-13). These labels are very important for future study of the collections as it may take even a long time to look into them.

e. Registration (unnamed register) :

The unnamed material thus collected and preserved must be registered serially in a good bound ruled ledger register using water proof Indian Ink only. The format of the unnamed register is given in the (appendix-g).

f. Documentation :

It is useful to prepare a list of collections, group wise present in the unnamed collection for a better inventory as the unnamed register will not give a ready information of all the unnamed collections of a particular group.

g. Holding of the unnamed collections : (unnamed collection room) :

A seperate collection room with shelves capacity is necessary for keeping and arranging the unsorted, sorted and registered collections group-wise. The plan of arrangement of collections should be displayed at the entrance of the collection rooms.

h. Upkeep :

The future of the collection depends upon the upkeep and maintenance i.e. routine check up, including the filling of preservative whenever necessary and dusting of the containers as it may take even years to work out the unnamed collections. It is better to evolve a routine procedure for checking the collections as the collections increase through years.

i. Photographs :

As it is not possible to photograph all the specimens in the field, efforts should be made to photograph them in the laboratory with a view to prepare line drawings at a later stage.

V IDENTIFICATION

a. Labelling (named) :

All identified material should be properly labelled on a good quality drawing paper using water proof Indian Ink. The size of the label should be proportionate to the size of the container (Figs. 14-16).

b. Registration (named) :

All the identified collections are to be entered in a bound ruled register made of good quality ledger paper. The entries of the named collections should be made with water proof Indian Ink. The format of the named registar is shown in the appendix - h.

c. Containers :

This being the final arrangement of the collection, containers of suitable size should be selected keeping in view the size of the specimen to be kept inside. There should be at least 25% extra space around the specimens in the container so that they will be kept always immersed in the preservative. The containers should be of clear glass with plastic screw caps preferably with a liner inside to reduce the evaporation of the preservative. For tube collections (smaller specimens) and tank collections (very big specimens) procedure mentioned under (IV. c) can be followed. The named collections pertaining to small/big specimens of the same species from different localities are to be kept either in big glass jar/tank without mixing their localities. The label as written in (V. a) should be inserted in the container.

d. Documentation (catalogue cards) :

For ready information of the named collections, data belonging to each species available in the collections are to be entered in the species catalogue cards (Fig. 17) and are to be kept in the index cabinets as per the suggested classified list (appendix-c).

e. Preservation :

Proper upkeep of the named collections is necessary. Regular checking of the collections and filling of the preservative should be done at intervals without fail.

f. Holding of named collections (named collection room) :

A seperate room for keeping the named collection is necessary and the room should be provided with shelves capacity so that the collections can be arranged as per the classified list (appendix - c). Special thought should be given in arranging and making them exhibited clearly as these collections itself should act as an aid to the identification for the future study of unnamed material.

The collection room should be protected from sun light and better lighting arrangement should be made so that the specimens can be seen clearly. All the identified collections should be handled (removed or replaced) by the qualified staff only.

VI. LOANS

a. Preparation of list :

All the specimens (named/unnamed) should be sent out only after filling in the proforma in quadruplicate with all details and duly checked and signed by the concerned. The proforma for sending the loan collection is given of (appendix-i).

b. Packing and Forwarding :

Proper care should be taken in selecting the packing material. They should be

either light weight and unbreakable corrugated boxes, screw caped tins or thermocole boxes so as to withstand the transit through rail/road/air. Good padding around the specimens with light cotton wool is very essential, otherwise the specimens will be pressed from all sides. Before closure of the packing, 3rd copy of the loan form duly filled should be kept inside the package. After closing the packet with a brown paper and sealing with cellotape, a despatch lable containing from and to addresses should be pasted (fig. 18). It is better to insure/register the collection parcel while sending it with in the country/abroad.

c. Despatch & Receipt :

Proper record of the specimens sent out and received should be maintained in a register for further correspondence (appendix - J & K).

VII. LITERATURE

a. References group-wise :

Literature references cards (Fig. 19) are to be maintained group-wise/species-wise/area-wise for preparation of reports, faunal lists and for other purposes. These cards should be kept in the index cabinets of 5" × 3" size for early reference. It is necessary to keep species cards and photography cards (Fig. 17, 20 & 21) also in the index cabinets of sizes 6" × 4" and 8" × 5" respectively.

b. Reference library :

A reference library covering with books, journals and reprints dealing mainly with taxonomic work (group-wise) is essential for the study of the animals so collected, preserved and maintained in the reference collection centre.

VIII. PARTIAL BIBLIOGRAPHY (PERTAINING TO COLLECTION PROCEDURES)

Ghosh, A.K. and Sengupta, T 1982. *Handbook on Insect collection, Preservation and study*. Zool. Surv. of India, Calcutta, 64 pp.

Holme, N.A. 1964. Methods of sampling the benthos. *Adv. mar. Biol.*, **2** : 171-260.

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Hulings C. Neil and Gray S. John. 1971. *A manual for the study of Meiofauna*. Smithsonian Contributions to Zoology, **78** : 84 pp.

Hureau, J.C. and Rice, A.L. 1983. *Guidelines for marine biological reference collections*. Unesco reports in marine science, **22** : 48 pp.

Kapur, A.P. (Ed.), 1968. *A Handbook for Zoological collections*. Zool. Surv. of India, Calcutta, 152 pp.

Lincoln, J. Roger and Gordon Sheals, J. 1979. *Invertebrate animals, collection & preservation.* British museum (Nat. Hist.), London, 149 pp.

Newell, G.C., and Newell, R.C. 1973. *Marine plankton, a practical guide.* Hutchinson Educational Ltd., London : 244 pp.

Michael, P. 1984. *Ecological methods for field and Laboratory investigations.* Tata McGraw-Hill Publishing Company Limited. New Delhi, 404 pp.

Unesco, 1968. *Zooplankton sampling.* Unesco monographs on oceanographic methodology, **2** : 174 pp.

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Zoological Survey of India, 1980. *Proceedings of the workshop on techniques in Parasitology,* 148 pp.

TABLE 1. Narcotization, Fixation and Preservation of Estuarine Animals

Narcotization/ Anaesthetization	Fixative or Kill	Preservative first change	Preservative Final	REMARKS
1	2	3	4	5
BOTTOM DEPOSITS (MUDS, SANDS & OOZES ETC.,)				
None	5% neutral formalin (1 part of commercial formalin and 19 parts of sea water).	70-90% alcohol (made from rectified spirit of 90% alcohol).	90% alcohol	Use of formalin ensures the best fixation of soft parts.
MEIOFAUNA				
6% Magnesium Chloride solution for 10-15 minutes.	Bouin's fluid for soft fauna. 5% neutral formalin containing 2% glycerine for hard fauna.	70% alcohol for soft fauna 5% neutral formalin for hard fauna.	First change is final	
PLANKTON				
None	5-10% neutral formalin for 48 hours.	5-10% neutral formalin	First change is final	Large animals are to be separated from the plankton before fixation.
PROTOZOA				
None	50% alcohol or 5% neutral formalin for foraminifera and radiolarians.	70-90% alcohol 5% neutral formalin.	First change is final	
PORIFERA				
None	50% alcohol for 12 hours.	50% alcohol after 12 hours.	70% alcohol	Do not use formalin Marine sponges are kept for two hours in fresh water before drying.

COELENTERATA – HYDROZOA				
Menthol, Magnesium Sulphate, Magnesium chloride or very dilute formalin.	10-20% neutral formalin	10% neutral formalin/ 70% alcohol.	First change is final.	20% neutral formalin is used for bigger forms.
COELENTERATA – SCYPHOZOA				
Menthol, Magnesium Sulphate, Magneisum Chloride or very dilute formalin.	10-20% neutral formalin.	10% neutral formalin/ 70% alcohol	First change is final.	Larger specimens do do not need narocotization.
COELENTERATA – ANTHOZOA				
Menthol, Magnesium Sulphate or Magnesium chloride.	10-20% neutral formalin.	10% neutral formalin/ 70% alcohol.	First change is final.	Forms having calcareous skeleton should be preserved in 70% alcohol.
CTENOPHORA				
Chloral Hydrate	Chromic acid (1% 100 ml)/ Osmic acid (1% - 2 ml) mixture for 15 for 15 minutes.	Graded through 30, 40, 50, 60 & 70% alcohol.	70% alcohol.	Never use formalin solution.
PLATYHELMINTHES – TURBELLARIA				
Allow them to extend in clean water, remove excess water and Add 10% alcohol or 1% Hydroxylamine.	70-90% alcohol/5% neutral formalin for 12 hours.	70-90% alcohol	First change is final.	
PLATYHELMINTHES – TREMATODA				
Allow them to extend in 1% salt solution and transfer to a small quantity of clean solution.	10% neutral formalin/70-90% alcohol 70-90% alcohol for 12 hours.	5% neutral formalin/ 70-90% alcohol.	First change is final.	Forantomicalstudies use either Bouin's or Susa's fixatives. Bigger flukes are to be fixed on a slide under the pressure

				of cover slip. Bouin's fluid (Picric acid 75 ml, formalin 25 ml, acetic acid 5 ml) Susa'sfluid(saturated mercuric chloride. solution 80 ml, Sodium Chloride 0.5g, Trichloroacetic acid 2 g, Acetic acid 4 ml, and formalin 20 ml.
PLATYHELMINTHES – CESTODA				
Same as above	Same as above	Same as above	Same as above	The Specimens are to be stretched on a glass sheet and use small quantity of fixative over them.
NEMERTINEA				
Chloral Hydrate or Magnesium sulphate for 6-12 hours.	10% neutral formalin/ 30-50% alcohol for over night.	5% neutral formalin/ 70-90% alcohol.	First change is final.	Susa's or Bouin's fluid are good fixatives for anatomical studies.
ASCHELMINTHES – ROTIFERA				
Add drop wise 5% neutral formalin.	10% neutral formalin.	5% neutral formalin.	First change is final	
ASCHELMINTHES – GASTROTRICHA				
6%.solution of Magnesium chloride.	10% neutral formalin.	5% neutral formalin/ 70% alcohol.	First change is final	
ASCHELMINTHES – KINORHYNCHA				
Distilled water	10% neutral formalin	5% reutral formalin.	First change is final.	

ASCHELMINTHES — NEMATODA				
None	5% neutal formalin/70% hot alcohol for over night.	5% neutral formalin.	First change is final.	Parasitic nematodes are to be washed in saline water for few minutes and they shouldnotbepressed.
ASCHELMINTHES — NEMATOMORPHA				
None	5% neutral formalin for 30 minutes.	70% alcohol/5% neutral formalin.	70% alcohol.	
ACANTHOCEPHALA				
1% salt solution for few minutes.	5% neutral hot formalin/70% hot alcohol for 30 minutes.	70% alcohol.	First change is final.	
ENTOPROCTA				
Menthol or Magnesium Sulphate	Commercial formalin (undiluted)/ Bouin's fluid.	70% alcohol.	First change is final.	
BRYOZOA (POLYZOA OR ECTOPROCTA)				
Same as above	Same as above	Same as above	Same as above.	Calcified Bryozoa are to be washed in fresh water and are to be preserved in 70% alcohol only.
PHORONIDAE				
Menthol/Magnesium sulphate/ slow addition of alcohol.	10% neutral formalin for 48 hours.	5% Neutral formalin/70% alcohol.	First change is final.	
BRACHIOPODA				
Slow addition of alcohol.	90% alcohol.	70-90% alcohol.	First change is final.	A piece of stick should be kept between the valves

				before fixing for the penetration of fixative inside.
SIPUNCULA				
Slow addition of alcohol, Magnesium Chloride, Menthol.	90% alcohol or 5% neutral formalin for 12 hours.	70-90% alcohol.	First change is final.	
ECHIURA				
As above.	5% neutral formalin for 12 hours	70% alcohol.	First change is final.	
ANNELIDA/POLYCHAETA – ARCHIANNELIDA				
Slow addition of alcohol, menthol or or magnesium chloride.	10% neutral formalin 90% alcohol for 48 hours.	70% alcohol/5% neutral formalin	First change is final.	Non pelagic forms are to be preserved in alcohol only.
ANNELIDA / HIRUDINEA				
Slow addition of alcohol, chloroform or magnesium sulphate	5% neutral formalin/50% alcohol for 12 hours.	5% neutral formalin/ 70% alcohol.	First change is final.	Small forms should be straightened by pressing them between two glass slides held together by rubber bands at the time of fixation.
ARTHROPODA – CRUSTACEA (SMALLER FORMS)				
None	10% neutral formalin for 24 hours.	70-90% alcohol.	70% alcohol.	
ARTHROPODA – CRUSTACEA (LARGER FORMS)				
Chloral hydrate or 1-2% formalin to avoid limb shedding.	10% neutral formalin for 3-4 days.	70-90% alcohol.	70% alcohol.	
ARTHROPODA – XIPHOSURA				
	Inject undiluted formalin ventrally	70-90% alcohol.	70% alcohol.	The specimens can

	into the tissues and kept in 10% neutral formalin for 48 hours.			be preserved in dry condition after fixation.
ARTHROPODA – PYCNOGONIDA				
	70-90% alcohol.	70% alcohol.	First change is final.	
TARDIGRADA	5% neutral formalin/70-90% alcohol.	70% alcohol.	First Change is final.	
MOLLUSCA				
Small amounts of alcohol, magnesium sulphate, magnesium chloride, menthol chloral hydrate for 24 hours.	10% neutral formalin/70-90% alcohol. alcohol.	70-905 alcohol/ 5% neutral formalin.	First change is final.	Shelled mollusca can can also be preserved in dry condition after removing the soft parts
CHAETOGNATHA				
	5% neutral formalin/70-90% alcohol.	5% neutral formalin/ 70% alcohol	First change is final.	
ECHINODERMATA				
Magnesium sulphate or Menthol for holothurians ; immersion in fresh water for few hours for brittle and feather stars.	10% neutral formalin/70-90% alcohol.	70-90% alcohol.	First change is final.	These are uncommon in estuaries. One or two small holes are to be made in the skin around the mouth of echinoids for better penetration. of the fixative and preservative. Hard bodied echinoderms can be preserved in dry condition after fixation in formalin.

HEMICHORDATA				
Small quantity of alcohol/magnesium chloride.	10% neutral formalin for 24 hours.	5% neutral formalin/ 70-90% alcohol.	5% neutral formalin/ 70% alcohol.	
UROCHORDATA				
Magnesium sulphate or Menthol.	Commercial formalin.	5% neutral formalin/ 70-90% alcohol.	5% neutral formalin/ 70% alcohol.	
CEPHALOCHORDATA				
None	10% neutral formalin.	5% neutral formalin.	First change is final.	
FISHES				
None	10% neutral formalin for 2-3 days.	10% neutral formalin/ 70% alcohol.	10% neutral formalin/ 70% alcohol.	Make a slit in the belly for large specimens for better fixation.
AMPHIBIANS/HEPTILES/BIRDS/MAMMALS				
Use chloroform for large specimens.	10% neutral formalin for 2-3 days.	10% neutral formalin/ 70% alcohol.	10% neutral formalin/70% alcohol.	Make an incision in large specimens for better penetration of the fixative and preservative. Crocodiles, Turtles, Birds and Mammals can be skinned and preserved following taxidermic procedures.

Appendix - a

ADDRESSES OF SUPPLIERS

OCEANOGRAPHY INSTRUMENTS

The General Engineering & Scientific Works
C-4, Industrial Estate
BERHAMPUR (Gm) - 760 008 Orissa

Hydro - Bios
C/o K.L.B. Sales & Service
1E/17 Jhandewalan extension
NEW DELHI - 110 055

Krishna Plastics
Dewan Road, Ernakulam
Cochin - 682 011

SIEVES AND SOIL TESTING INSTRUMENTS

Geologist's Syndicate (Pvt) Ltd.,
137, Biplabi Rashbehari Basu Road
Calcutta - 700 001

NYLON THREAD/NETS

Garware Marine Industries Ltd.,
Raheja Centre
12th Floor
Nariman point, BOMBAY - 400 021

Biseswarlal Jagadish Prasad
Jaunlipatty
Cuttack - 753 009

BOLTING SILK

Dadia Textiles
Cotton exchange Building
Room No. 514, Kalbadevi Road
Bombay - 400 002

Shiva Scientific Company
Plot No. 329
Venkatram Nagar
Chitlapakkam
Madras - 600 014

PLASTIC CONTAINERS

Tarson Products
818, Marshall House
331, Netaji Subhas Road
Post Box No. 560
Calcutta - 700 001

Polylefins Industries Ltd.,
Post Bag No. 23
H.P.O. Akola - 444 001

Polyene General Industries Pvt. Ltd.,
11-A, Industrial Estate
Guindy
Madras - 32

Sintex Plast Containers
Plastic Division
The Bharat Vijay Mills Ltd.,
Kalol (NG) - 382 721 Gujarat

GLASS BOTTLES

Alembic Glass Industries Ltd.,
Baroda.

Appendix - b

Taxonomic list suggested for preliminary sorting

Porifera
Coelenterata
Ctenophora
Platyhelminthes
  Turbellaria
Nemertinea
Entoprocta
Ectoprocta (Bryozoa)
Phoronidae
Brachiopoda
Sipuncula
Echiura
Annelida
Crustacea
  Decapoda
  Other crustacea
Pycnogonida

Mollusca
Echinodermata
Protochordata
Fishes

Appendix - C

Taxonomic list suggested for final sorting

PROTOZOA
- Foraminifera
- Radiolaria

PORIFERA
- Calcarea
- Hexactinellida

Demospongiae

COELENTERATA
- Hydrozoa
- Scyphozoa
- Anthozoa

CTENOPHORA

PLATYHELMINTHES
- Turbellaria
- Trematoda
- Cestoda

NEMERTINEA

ASCHELMINTHES
- Rotifera
- Priapulida
- Nematoda

ENTOPROCTA

ECTOPROCTA

PHORONIDAE

BRACHIOPODA

SIPUNCULA

ECHIURA

ANNELIDA
- Polychaeta
- Oligochaeta
- Hirudiinae

CRUSTACEA
- Branchiopoda
- Ostracoda
- Copepoda
- Mystacocarida
- Branchiura
- Cirripedia
- Malacostraca
  - Leptostraca
  - Syncarida
  - Peracarida
    - Mysidacea
    - Cumacea
    - Tanaidacea
    - Isopoda
    - Amphipoda
  - Stomatopoda
  - Euphausiacea
  - Decapoda

PYCNOGONIDA

CHAETOGNATHA

MOLLUSCA
- Polyplacophora
- Aplacophora
- Gastropoda
- Scaphopoda
- Pelecypoda
- Cephalopoda

ECHINODERMATA
- Asteroidea
- Echinoidea
- Echinoidea
- Holothuroidea
- Ophiuroidea
- Crinoidea

HEMICHORDATA

UROCHORDATA

CEPHALOCHORDATA

ELASMOBRANCHII

ACTINOPTERYGII

Appendix - d

Estuarine Biological Station
Zoological Survey of India
Hillpatna, Berhampur (Gm)
Orissa, 760 005 India

Net/Bucket Zooplankton Sorting Sheet for Estuarine/Brackish and Lagoonal waters.

Code No : Sample No : Field data Sheet No :
Date of Collection : Time of coll : (Day/Night)
Collector : Analyst :
Date of Sorting : Duration of Sorting : (in hours)
Name of water body :
Location : Country : State :
District : Taluq : Village :
Sampling station Name and No :
Section (if estuary, state-Head/middle/mouth) : (if lagoon/back water, state-direction-NE/SE etc.,) :
Latitude : Longitude :
Depth (Sounding, m) : Depth of haul (m) :
Type of haul (vertical/surface) :
Duration of the haul :
Gear : Diameter : Mesh :
Volume of water filtered (m^3) :
Volume collected in ml : (displacement Vol.) Biomass mg/L (wet/dry weight) :

Abundance indicated by + against the group.

Sl. no.	Group	Total no. in sample	Total volume ml	%
1.				
2.				
3.				
4.				
5.				

Appendix - e

Estuarine Biological Station,
Zoological Survey of India,
Hillpatna, Berhampur (Gm)
Orissa 760 005 India.

Nekton Sorting Sheet for Estuarine/Brackish and lagoonal waters.

Code No : Sample No : Field data sheet No :
Date of Collection : Time of Collection (Day/Night) :
Collector : Analyst :
Date of Sorting : Duration of Sorting : (in hours)
Name of water body :
Location : Country : State :
District : Taluq : Village :

Sampling Station Name and No :
Section (if estuary, state-Head/middle/mouth) :
(if lagoon/backwater, state-direction-NE/Se etc.,) :
Latitude : Longitude :
Depth (Sounding m) : Depth of haul (m) :
Gear : Mesh :
Duration of the haul :
Biomass g/m² (wet/dry weight) :

Abundance indicated by + against the group			
Sl. No.	Group	Total no. in sample	Percent (%)
1.			
2.			
3.			
4.			
5.			

Appendix - f

Estuarine Biological Station,
Zoological Survey of India,
Hillpatna, Berhampur (Gm)
Orissa, 760 005 India.

Benthos Sorting Sheet for Estuarine/Brackish and lagoonal waters.

Code No : Sample No : Field data sheet No :
Date of Collection : Time of Collection : (Day/Night) :
Collector : Analyst :
Date of Sorting : Duration of Sorting : (in hours)
Name of water body :
Location : Country : State :
District : Taluq : Village :
Sampling station name and No :
Section (if estuary, state-Head/middle/mouth) :
(if lagoon/backwater, state-direction-NE/Se etc.,) :
Latitude : Longitude :
Depth (m) : Gear :
Area : Wet Sieve No :
Biomass g/m² (wet/dry weight) :

Abundance indicated by + against the group			
Sl. No.	Group	Total no. in sample	Percent (%)
1.			
2.			
3.			
4.			
5			

Appendix-g

ZOOLOGICAL SURVEY OF INDIA, ESTUARINE BIOLOGICAL STATION, BERHAMPUR
REGISTER OF UNNAMED COLLECTIONS

Regn No.	Date of Regn.	Name of Survey	Particulars of specimens	No. of Exs	Locality including Habitat in detail in detail	Date of collec-tions	Host and Loca-tion if if any	Collec-tor or Donor	Field coll. No.	Date of des-patch	Date of rece-ipt	REMARKS Wet/Dry
1	2	3	4	5	6	7	8	9	10	11	12	13
127	20-3-84	Extensive Survey of Brahma-giri and near by areas, Puri Dist.	Mollusca/ Bivalvia	30 exs	Arakakuda (Village) Chilka mouth	23- 2 -84		C.A.N. Rao & Party.				

Appendix - h

ZOOLOGICAL SURVEY OF INDIA, ESTUARINE BIOLOGICAL STATION, BERHAMPUR
REGISTER OF NAMED COLLECTIONS

Regn No.	Zoological name	Locality	Name of Collector	Date of Collection	No. of examples/ Nature of Collection	Date of entry	Order & family	Det. by	REMARKS Wet/Dry
1	2	3	4	5	6	7	8	9	10
1.	*Nephthys Polybranchia* Southern	Arkakuda village, Chilka mouth lagoon	C.A.N. Rao	23.2.1984	5 examples	9.1.1986	Polychaeta Nephthy-didae	C.A.N. Rao	In pirit

Appendix - i

Forwarded to :

_____

_____

_____

_____

REGISTER FOR SENDING SPECIMENS
ZOOLOGICAL SURVEY OF INDIA
ESTUARINE BIOLOGICAL STATION
HILLPATNA BERHAMPUR (GM)

760 005 ORISSA INDIA

Sent by
Dated
No. of Packages
Mode of despatch

LOAN GIFT EXCHANGE IDENTIFICATION AND RETURN

Sl no.	Regn. No.	NAME	LOCALITY	COLLECTOR	No. of specimens	REMARKS

☐ Received in good condition ☐ Date of Receipt ☐ Name

☐ Signature

Appendix - j

ZOOLOGICAL SURVEY OF INDIA, ESTUARINE BIOLOGICAL STATION, BERHAMPUR
CENTRAL REGISTER FOR DESPATCH OF ZOOLOGICAL MATERIAL

Serial lot No.	Date of entry in register	REFERENCE		Name and address of Sender	Particulars of material	Purpose	Disposal to sections	Initials of Person making entry.	REMARKS
		Diary No. Date	File No.						
1	2	3	4	5	6	7	8	9	10
1 to 27	18.12.84	1390/ 11.12.84	17-1-82 Tech.	Dr. Kaza V. Rama Rao Officer-in-Charge Zoological Survey of India, Hillpatna, Berhampur (Gm).	Unidentified Mollusca as per the list.	For Identification	To The Mollusca section, Z.S.I. 8, Lindsay Cal - 16.		Collections have been sent through Mr. S.S. Khora, Junior Research fellow of this station.

Appendix k

ZOOLOGICAL SURVEY OF INDIA, ESTUARINE BIOLOGICAL STATION, BERHAMPUR
CENTRAL REGISTER FOR RECEIPT OF ZOOLOGICAL MATEIAL

Serial lot No.	Date of entry in register	REFERENCE		Name and address of sender	Particulars of material	Purpose	Disposal to sections making	Intials of person entry	REMARKS
		Diary No. Date	File No.						
1	2	3	4	5	6	7	8	9	10
1 to 24	4.1.85	Moll. 534	29/84	Dr. K.V. Surya Rao Officer-in-Charge Mollusca Section Zoological Survey of India. 8. Lindsay Street, Calcutta - 700 016.	Identified mollusca Collection as per the list except lot No. 8, 14, 15.	Received after Identification.	To The O/C EBS, ZSI Berhampur.		Lot Nos. 8, 14, 15 were kept in mollusca section Z.S.I Calcutta for further study.

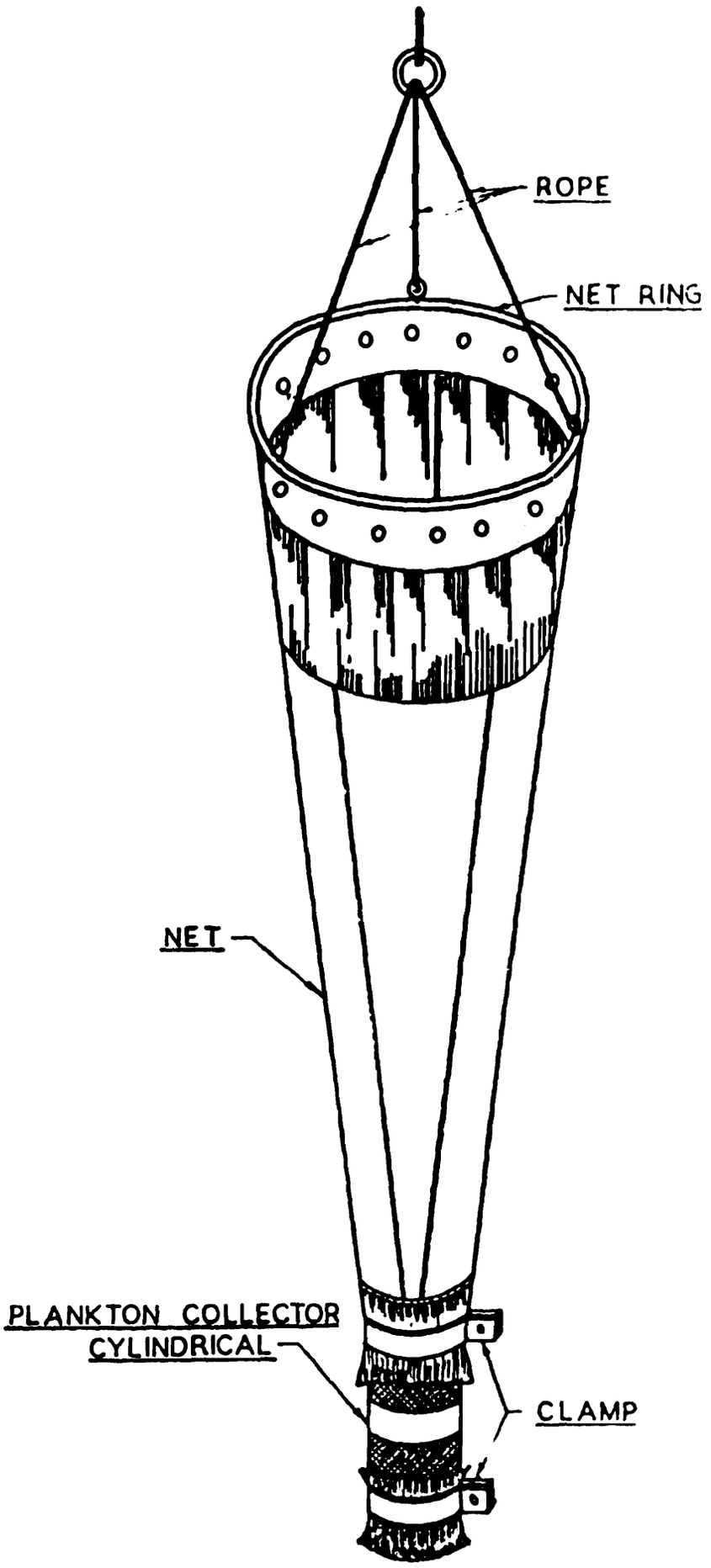

Fig. 1 Standard Plankton net

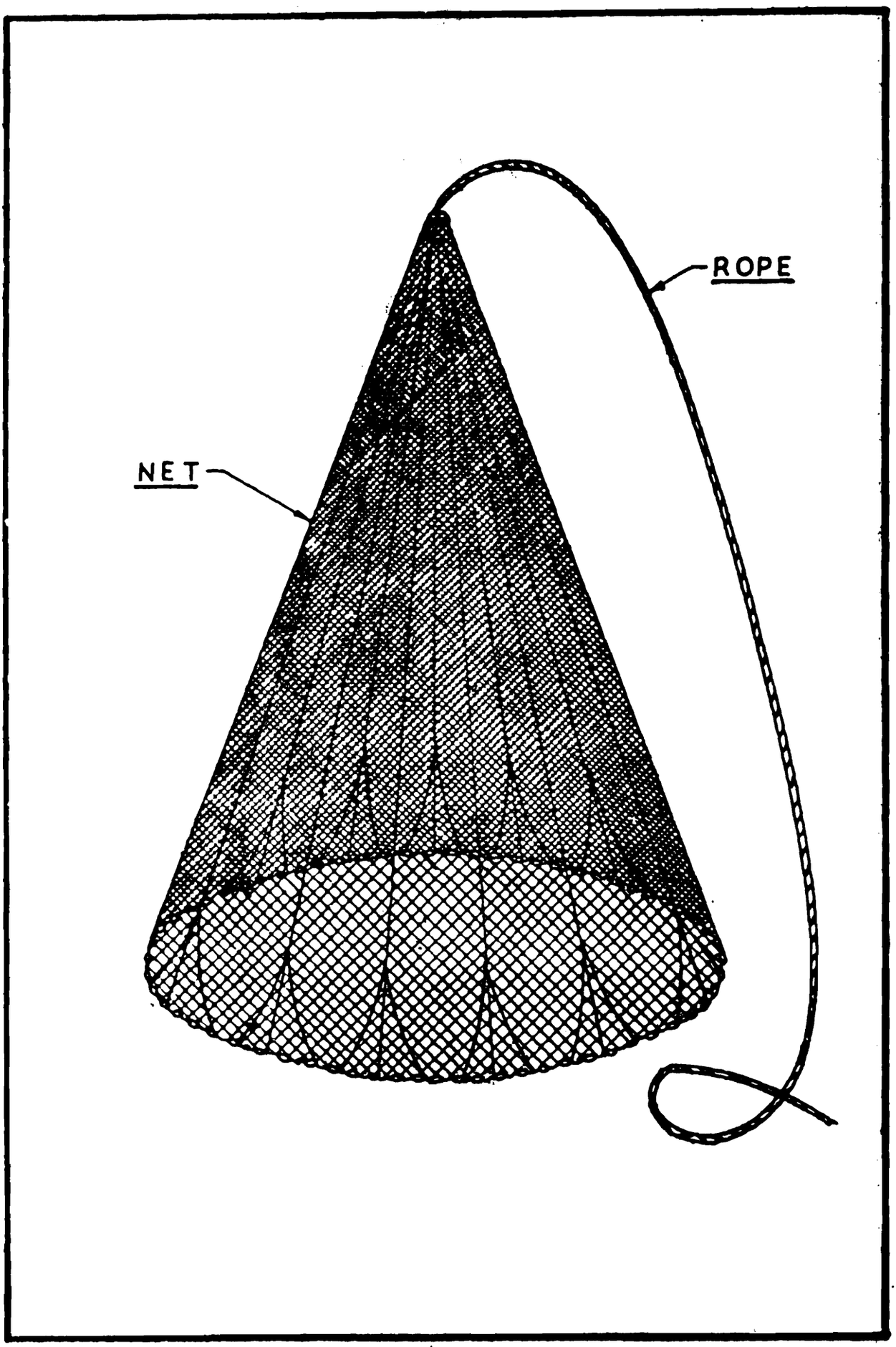

Fig. 2 Cast Net

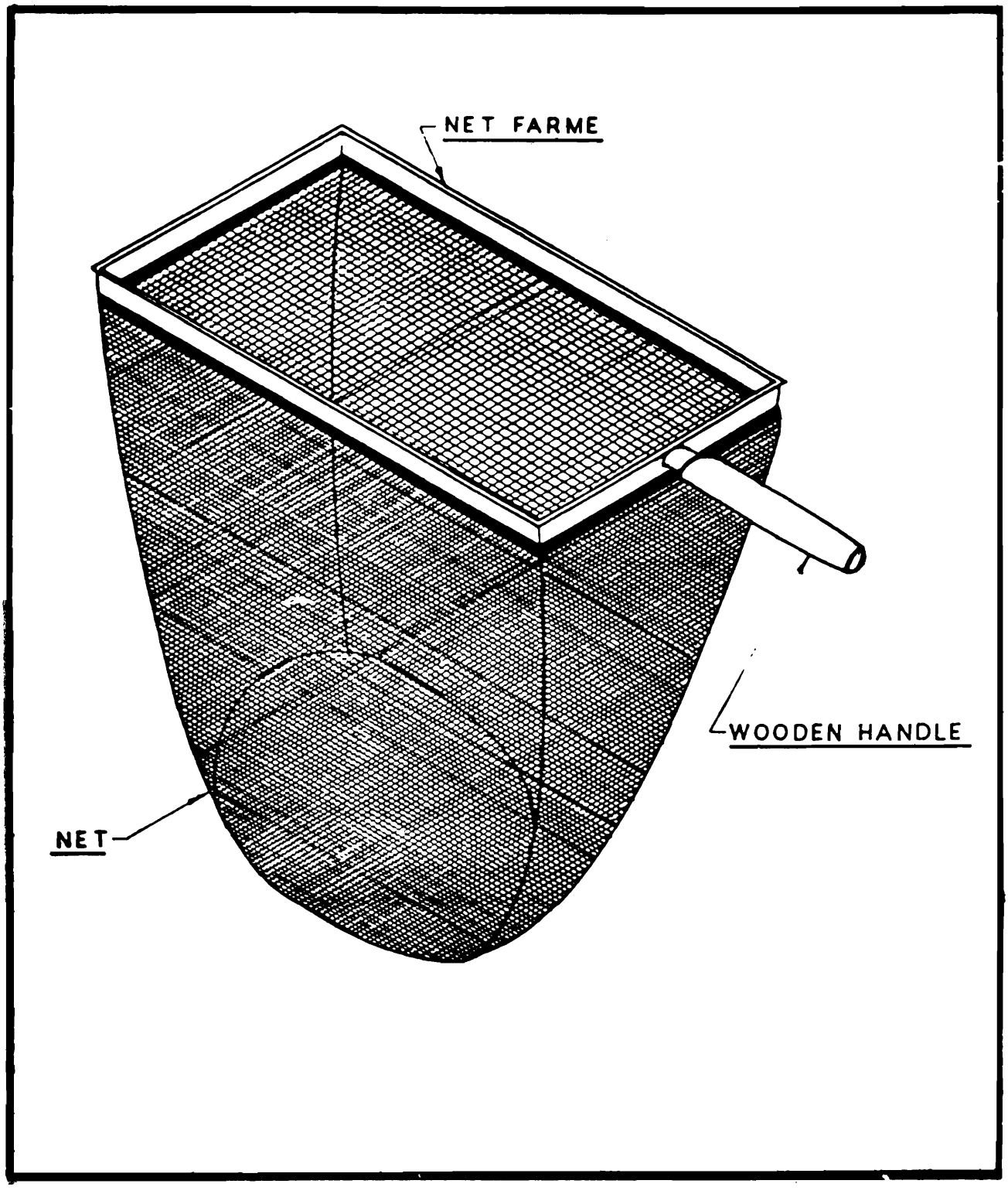

Fig. 3 Scoop net

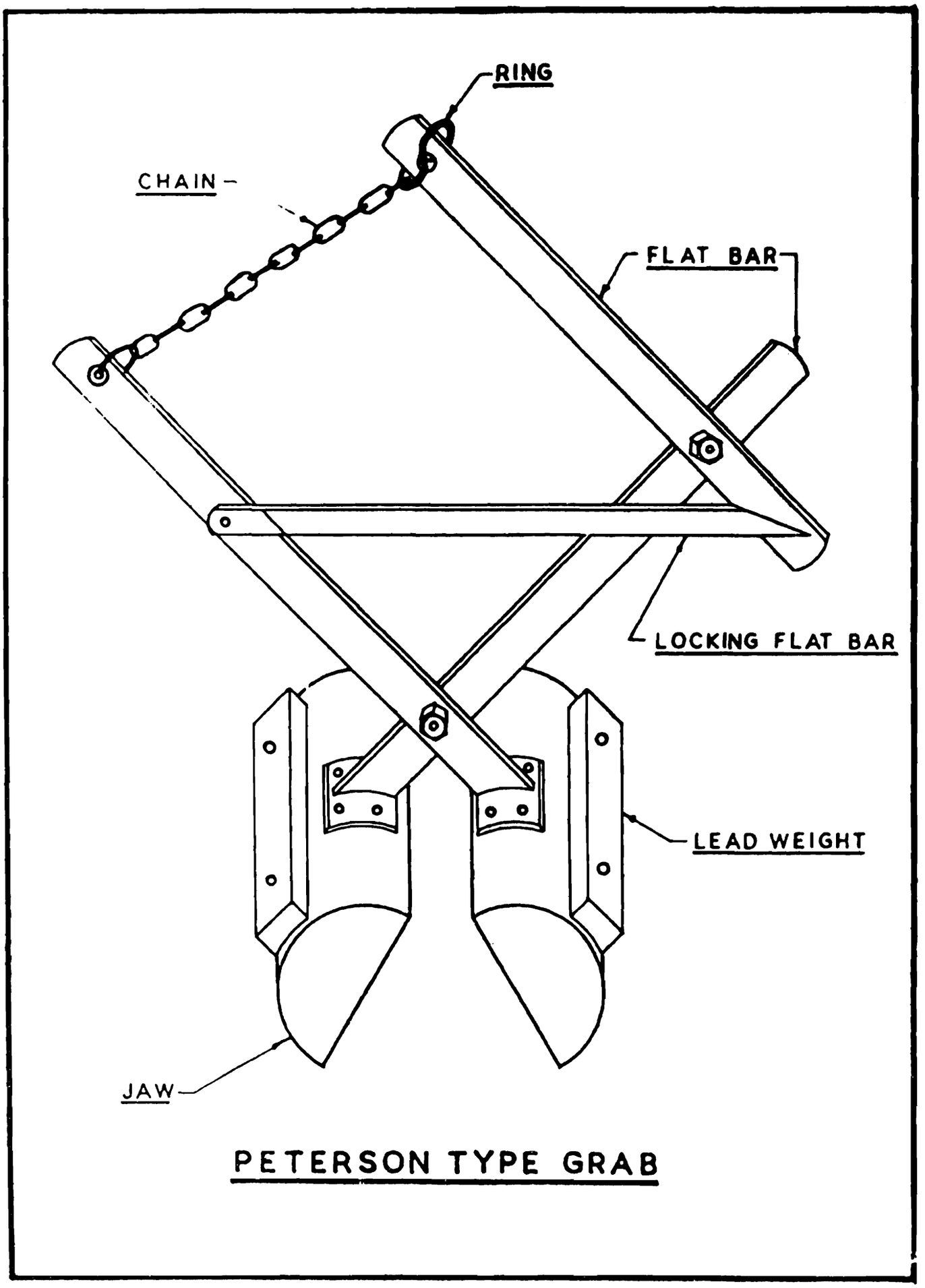

Fig. 4 Peterson grab (Modified, 8 Kg. light weight model)

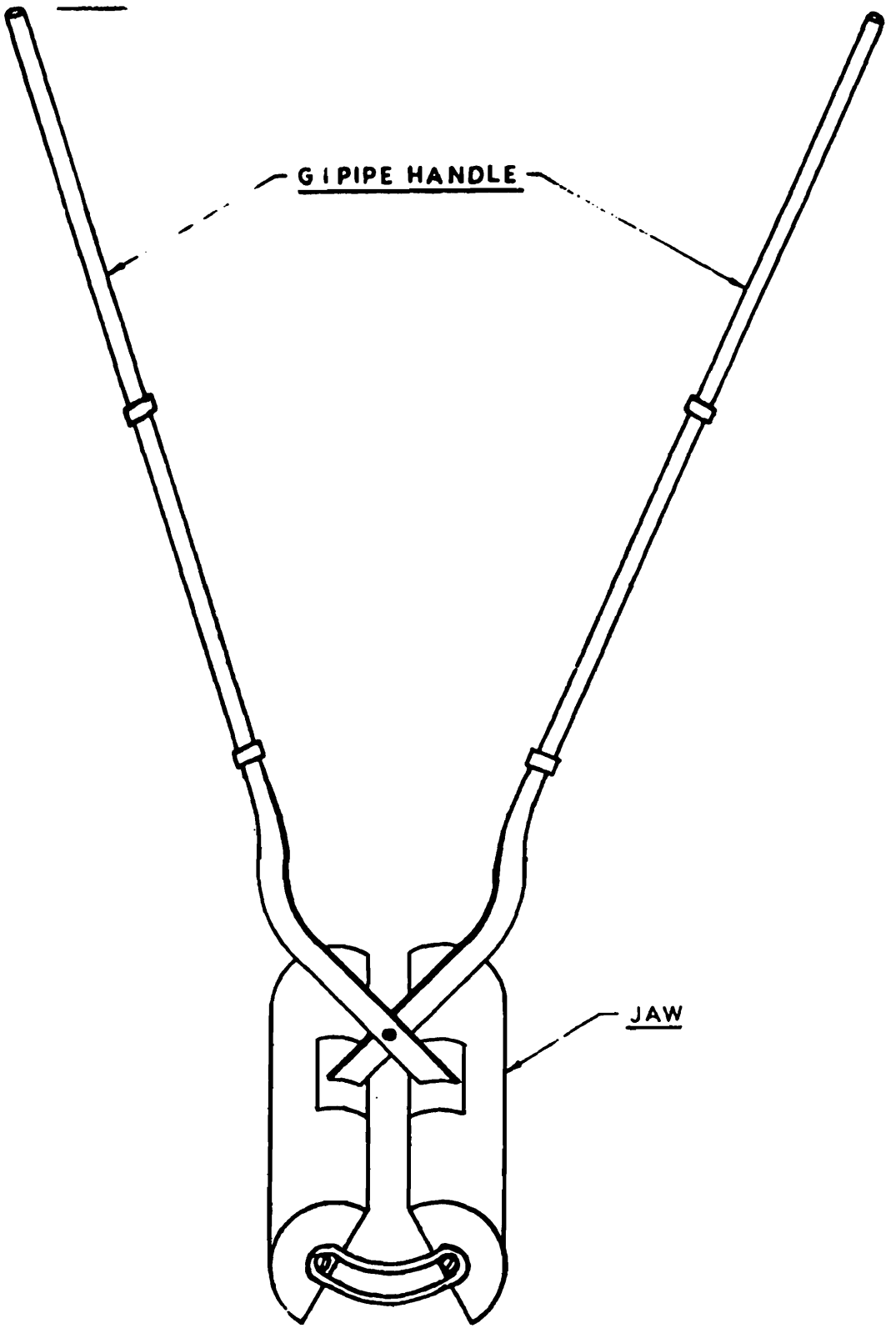

Fig. 5 Grab (Modified version of van veen hand grab model where the length of the hands can be incresed up to 10 feet)

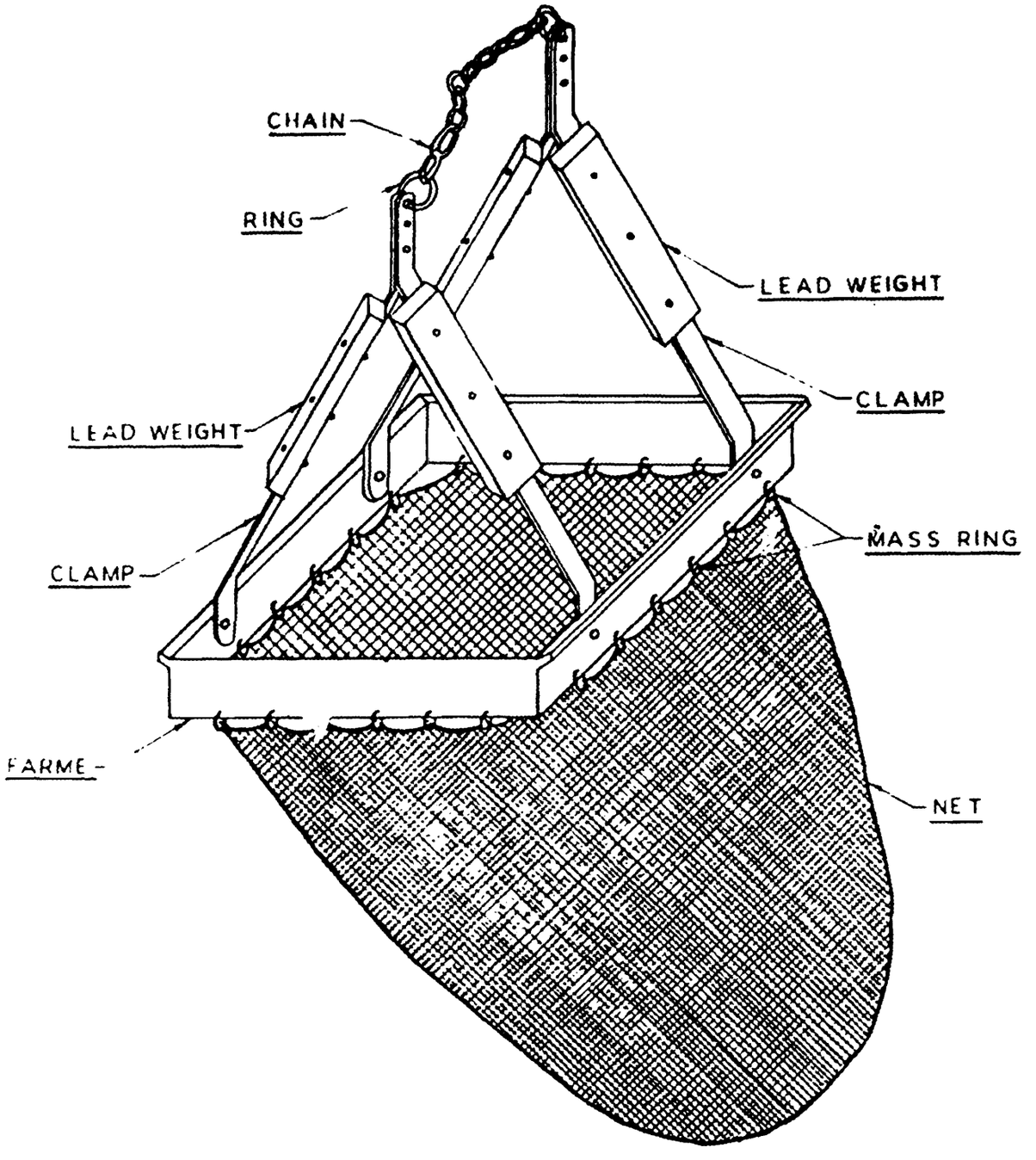

Fig. 6 Dredge (light weight model)

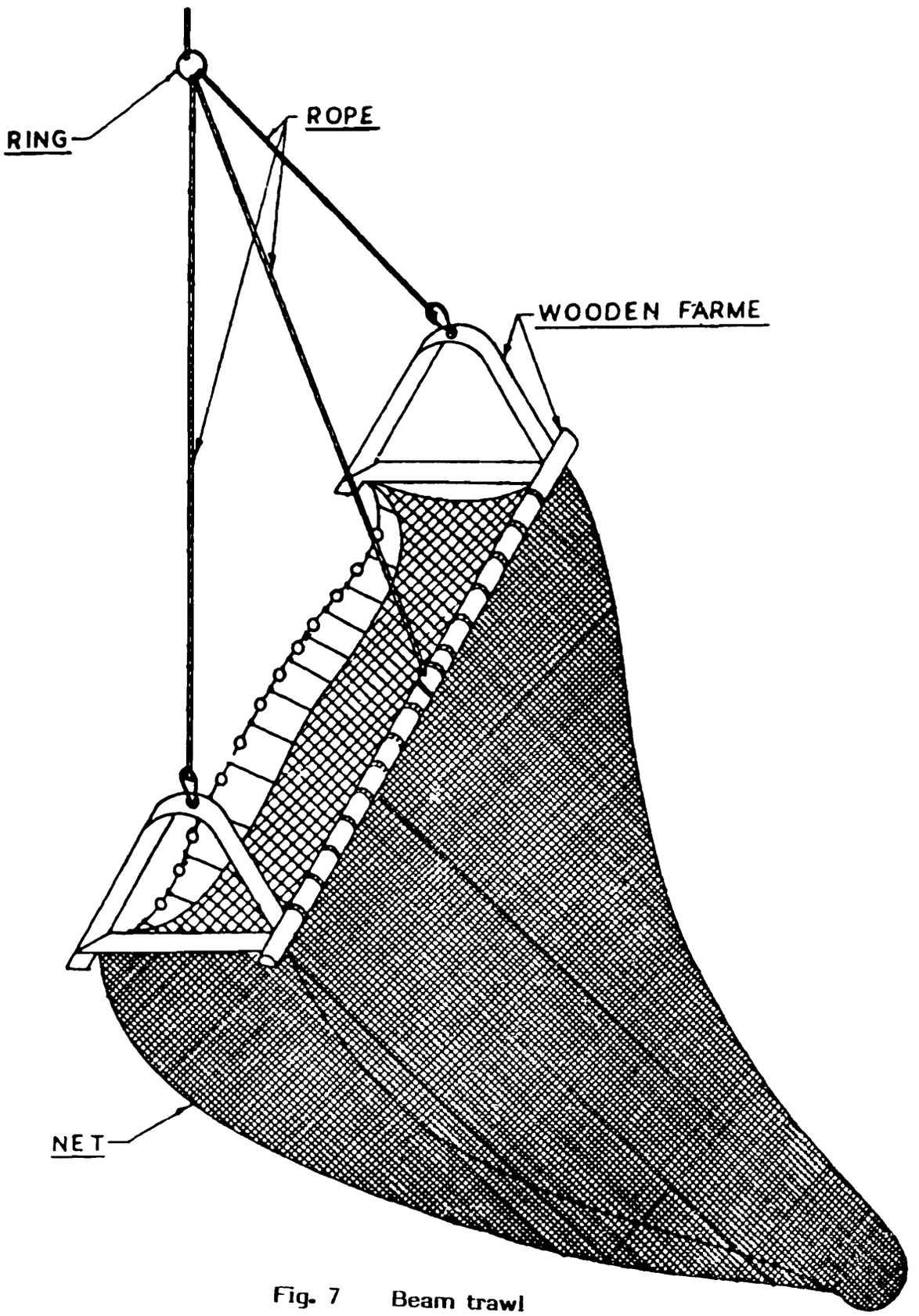

Fig. 7 Beam trawl

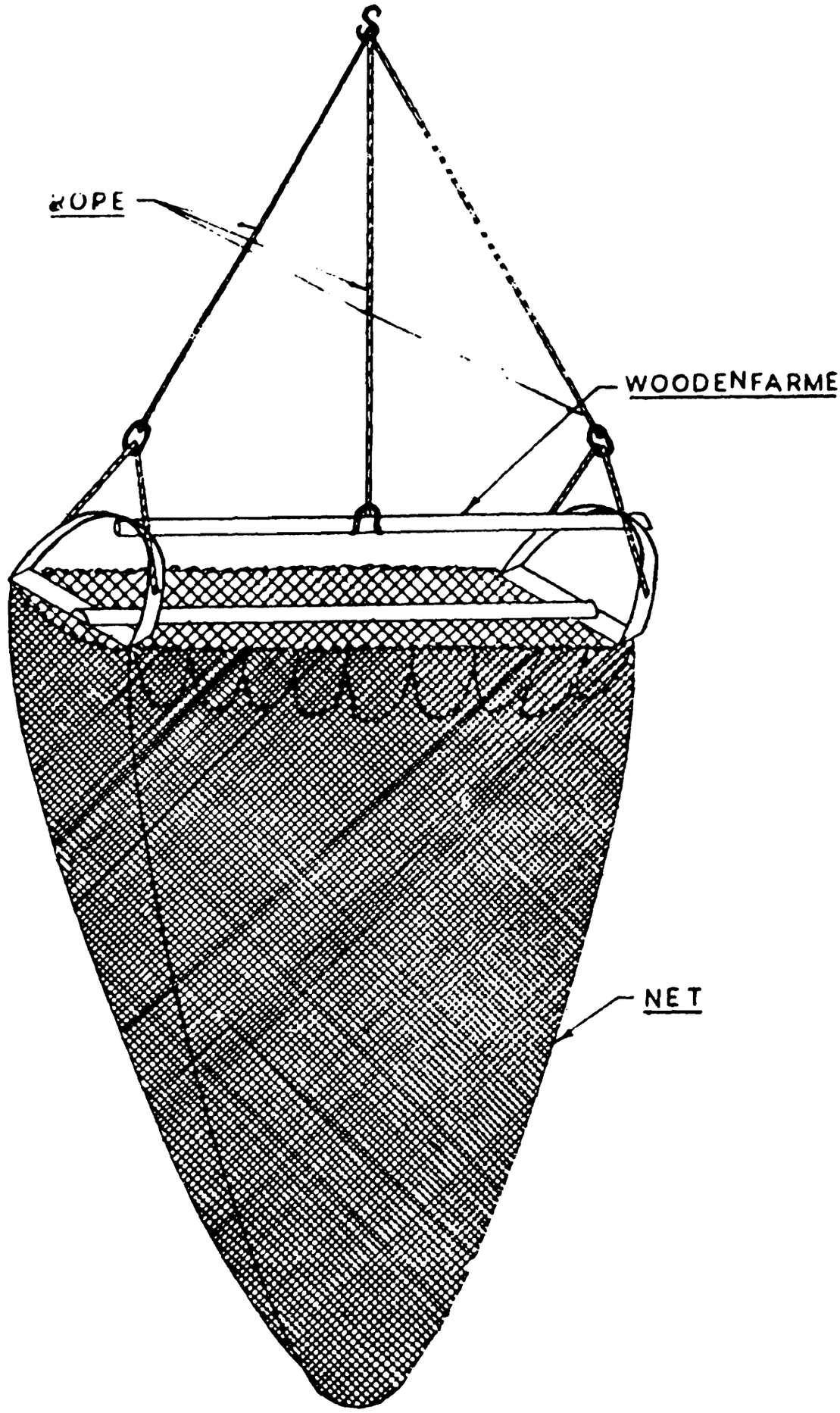

Fig. 8 Agassiz trawl

EBS/ZSI/BERHAMPUR Sample No. Locality Date	EBS/ZSI/BERHAMPUR Sample No. Locality Date
EBS/ZSI/BERHAMPUR Sample No. Locality Date	EBS/ZSI/BERHAMPUR Sample No. Locality Date

Fig. 9

ZOOLOGICAL SURVEY OF INDIA ESTUARINE BIOLOGICAL STATION, BERHAMPUR
SURVEY, 19
LOCALITY DATE COLL.

Fig. 10

ZOOLOGICAL SURVEY OF INDIA
ESTUARINE BIOLOGICAL STATION
BERHAMPUR

Plankton Tow Sample No.
General Location
Lat. Long.
Duration of Tow
Mesh No. Mouth Diam.
Temp. at meters
Collector Date
Ship
Remarks

Fig. 11

ZOOLOGICAL SURVEY OF INDIA
ESTUARINE BIOLOGICAL STATION
BERHAMPUR

Nekton (Trawl) Sample No.
General Location
Lat. Long.
Net used
Duration of trawling
Depth of Trawl
Bottom depth
Collector Date
Ship

Fig. 12

ZOOLOGICAL SURVEY OF INDIA
ESTUARINE BIOLOGICAL STATION
BERHAMPUR

Benthic Sample No.
General Location
Lat. Long.
Type of Gear
Depth
Nature of Bottom
Collector Date
Ship
Remarks

Fig. 13

EBS/ZSI/BERHAMPUR GENUS SPECIES LOCALITY REG. NO.	EBS/ZSI/BERHAMPUR GENUS SPECIES LOCALITY REG. NO.
EBS/ZSI/BERHAMPUR GENUS SPECIES LOCALITY REG. NO.	EBS/ZSI/BERHAMPUR GENUS SPECIES LOCALITY REG. NO.

Fig. 14

ZOOLOGICAL SURVEY OF INDIA
ESTUARINE BIOLOGICAL STATION
BERHAMPUR

Order
Family
Genus
Species
Locality
Coll. Date
Reg. No. Deg. by

Fig. 15

ZOOLOGICAL SURVEY OF INDIA
ESTUARINE BIOLOGICAL STATION
BERHAMPUR

REG. NO. GROUP
NAME
LOCALITY
DATE COLL. COLL.
DATE DET. DET.
NO. SIZE

Fig. 16

ZOOLOGICAL SURVEY OF INDIA,.
ESTUARINE BIOLOGICAL STATION,
BERHAMPUR

Phylum
Class
Order
Fam.
D : Dry/W : Wet

Genus
Species

Sl. No.	Reg. No.	Locality	Date of Coll.	Collector/ Donor	Remarks

Fig. 17

- FROM -
ZOOLOGICAL SURVEY OF INDIA
ESTUARINE BIOLOGICAL STATION
HILL PATNA BERHAMPUR (GM)
760 005 ORISSA INDIA

Q.M.S.
R.M.S.
LATE FEE PAID
BOOK POST
UR. CERTI. POST
REGD. POST A.D.

REG. PARCEL A.D.
UR. PL. CERTI.
RECORD DELI.
REG. AIR MAIL
SURFACE MAIL
PRINTED MATTER

Fig. 18

ZOOLOGICAL SURVEY OF INDIA
ESTUARINE BIOLOGICAL STATION, BERHAMPUR

Fig. 19

ZOOLOGICAL SURVEY OF INDIA
ESTUARINE BIOLOGICAL STATION,
BERHAMPUR

Negative No. **Print**

Fig. 20
(Front Side)

Details of Photograph

Loc. **Dist.** **State**
Lat. **Long.** **Alt.** **Meters**
Date **Coll. Station No.**
Photo by
Description
Animals
Remarks

Fig. 21
(Back Side)

ZOOLOGICAL SURVEY OF INDIA
ESTUARINE BIOLOGICAL STATION
BERHAMPUR

N. No. Technical Details
Film Used Exposure Date
Exposure Film Stop Filter
Lighting Hour Camera Facing
Camera Lens