

# Procedural Guideline No. 6-3

## Specimen collection, preservation and storage

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### Background

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A reference set of specimens for each SAC will provide an important permanent record of the species recorded. Specimen collections provide an important quality control mechanism to ensure consistent identification. Specimens preserved should include any unusual or rare species, species found outside their known distributional limit, specimens of doubtful identification and species of uncertain taxonomic status. A collection of the more common species would also be useful for training and familiarisation of field staff on repeat visits.

### Purpose

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To provide a consistent, permanent record of species from a site and a reference for future work. This can aid the monitoring of long-term changes in community structure.

### Advantages

- Provides a permanent record of species at a site
- Provides a reference collection to refer and monitor long-term change
- Can be done alongside other faunal collection, e.g. quantitative sampling of sediment biotopes

### Disadvantages

- Requires a high level of expertise in identification
- Requires room for storage and cataloguing
- Hand sorting of animals from sediment and debris is time-consuming and can result in damage of specimens
- Uses toxic chemicals
- To gain a comprehensive list of species for an area will require a considerable amount of time and money since the task is quite labour intensive

### Logistics

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#### Equipment

Table 1 gives a general list of equipment that will be needed for specimen collection and preservation. However, further specialist equipment may be needed depending on the type of substrata being surveyed, and whether the site is intertidal or subtidal.

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1 English Nature, Northminster House, Peterborough, PE1 1UA.

2 Joint Nature Conservation Committee, Monkstone House, City Road, Peterborough, PE1 1JY.

<i>Field</i>	<i>Laboratory</i>
Containers: bottles, tubes and bags	Chemicals for relaxing, fixing and preserving
Collecting instruments	Compound microscope
Sediment corers	Dissection microscope
Dishes	Mounted needle/tweezers
Lenses	Labelling material
Photography equipment	

For further details on equipment please refer to Lincoln and Sheals (1979).

## Personnel

Personnel will need specialist identification skills and may need to be accredited, e.g. NMBAQC for sediment specimens.

## Method

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### Field collection

#### *Intertidal macrofauna collection*

Collections should be made during a spring tide. Care should be taken to examine the shore thoroughly as many organisms retreat into crevices at low tide, especially when this coincides with daylight.

Procedures for rock substrata:

- Non-sessile animals which are large enough to see by eye or hand lens are picked off by hand or blunt forceps
- Sessile animals should be taken with the small piece of rock or seaweed to which they are attached
- Displace the seaweed to reveal motile fauna
- Turn over boulders to expose the fauna beneath them
- Collect a variety of seaweed to be examined thoroughly at the laboratory
- Animals in rock crevices may be induced to release by squirting with a weak solution of formalin, or if necessary obtained by breaking open the crevice with crowbar
- Collect fauna in rock pools using a fine meshed hand net and a wide-bore pipette
- Use a long handled scrape net for steep rock faces and other relatively flat surfaces

For further information on collecting fish in rockpools see Wilding *et al.* (2000a).

Procedures for sediment substrata:

- Turn over larger cobbles and boulders to expose the fauna beneath them
- Dig out or core sections of sediment for sieving back at the laboratory
- Use cores collected for community sampling to eliminate repetition and reduce the quantity of material removed from the shore

Puddle sediment in sieves as swirling of sediment will damage delicate specimens.

#### *Meiobenthic infauna collection*

Sediment substrata:

- When tide is out dig a small pit in sediment (2–3m) with a number of channels radiating from it for a distance of 2–3m. Allow water to accumulate in the pit and then filter off free swimming animals with a fine sieve (62µm pore diameter)
- Take a small core of sediment to sample less active and sessile meiofauna. A number of techniques can be used for separating the animals (see Lincoln and Sheals 1979 for details)

### *Subtidal benthic fauna collection*

*Rocky substrata* The same methods as detailed above for intertidal rock substratum can be used by divers to remove fauna and flora from submerged rock. The use of anaesthetic to displace organisms may be widely employed in the subtidal zone since time is of greater importance and this may facilitate faster collection. Divers may also wish to use a 'slurp gun' (Lincoln and Sheals 1979) to suck fauna out of crevices.

*Sediment substrata* Divers may employ suction sampling to collect sediment infauna (see Rostron 2000). Epifauna and flora can be hand-collected but the diver will be limited to flora and relatively slow-moving or sessile fauna.

Dredging and trawling can be employed to collect epifauna remotely (see Wilding *et al.* 2000b) although individuals are often damaged by mechanical abrasion.

Grabs and corers can be employed to collect infauna remotely (see Thomas 2000).

### *Plankton collection*

To ensure that a cross-section of the plankton community in an area is sampled, sampling should occur at several different depths. If a thermocline is present then sampling should occur both above and below the thermocline. Either a tow-net or Hansen net can be used to collect a general plankton sample. Consideration should be given to the mesh size of the plankton net with regard to what minimum body size of plankton is to be sampled.

### *Special considerations for algae collection*

It is important to collect a representative sample of specimens (seasonally), being careful to collect the entire plant (including holdfast) as well as representative plants from various habitats, noting information about the habitat for the label.

Crustose coralline algae should be taken with the rock they are attached to, so as to prevent damage to the specimen (further information on coralline algae collection can be found at <http://www.botany.uwc.ac.za/clines/>).

### Laboratory preservation of fauna

Many fauna are highly contractile and need to be relaxed with an anaesthetic before death and fixation. Anaesthetisation should not be prolonged since it can cause tissue breakdown.

The process of fixation stabilises the proteins in tissue so that after death and subsequent treatment, the tissues generally retain the form they held when alive. The required fixative varies between organisms and also depends on what the tissue will be used for. The treatments given below are for specimens which are to be preserved whole and held in a collection.

Preservation by fluid allows the material to be stored indefinitely without seriously distorting the specimen or destroying the tissue. It is not a substitute for fixation and should be considered as a post-fixation process.

Table 2 summarises typical treatments for invertebrate anaesthetisation, fixation and preservation; for a more comprehensive list of taxa and further information see the National Museum of Natural History, Smithsonian Institution (<http://nsmnhwww.si.edu/iz/usap/usapspec.html>)

Table 2 Typical treatments for invertebrate anaesthetisation, fixation and preservation

General taxa	Specific taxa	Relaxing agent	Fixative solution	Wash solution	Final solution preservative
Porifera	–	n/a	10% formalin sea water buffered by methenamine	70–80% EtOH change twice	70–80% EtOH
Cnidaria	Anthozoa	MgCl <sub>2</sub> (c. 7%)	6–10% phosphate buffered formalin	30%, 50%, 70% EtOH	70% EtOH
	Hydrozoa	MgCl <sub>2</sub> (c. 7%)	4% phosphate buffered formalin	30%, 50%, 70% EtOH	70% EtOH
Bryozoa	–	MgCl <sub>2</sub> (c. 7%)	5% phosphate buffered formalin	30%, 50%, 70% EtOH	70% EtOH
Annelida	Polychaeta, Oligochaeta and Hirudinea	MgCl <sub>2</sub> (c. 7%)	10% phosphate buffered formalin in seawater	30%, 50%, 70% EtOH	70% EtOH
Crustacea	Decapoda and other larger crustaceans	MgCl <sub>2</sub> (c. 7%) or oil of cloves	5–10% phosphate buffered formalin in seawater or 75% EtOH	50%, 70% EtOH	70% EtOH
	Ostracoda, Copepoda, Branchiopoda and Amphipoda	MgCl <sub>2</sub> (c. 7%)	4–10% phosphate buffered formalin in sea water or 70% EtOH (Ostracoda only)	–	70% EtOH
Mollusca	Bivalvia	MgCl <sub>2</sub> (c. 7%)	10% phosphate buffered formalin or 70% EtOH	30%, 50%, 70% EtOH	70% EtOH
	Gastropoda	MgCl <sub>2</sub> (c. 7%)	10% phosphate buffered formalin	30%, 50%, 70% EtOH	70% EtOH
	Polylacophora and Monoplacophora	MgCl <sub>2</sub> (c. 7%)	10% phosphate buffered formalin	30%, 50%, 70% EtOH	70% EtOH
Echinodermata	Ophiuroidea	MgCl <sub>2</sub> (c. 7%)	70–75% EtOH	n/a	70% EtOH
	Holothuroidea, Asteroidea and Echinoidea	MgCl <sub>2</sub> (c. 7%)	70–75% EtOH	n/a	70% EtOH
	Crinoidea	MgCl <sub>2</sub> (c. 7%)	90% EtOH (hold arms downwards)	–	70% EtOH
Urochordata	Ascidacea	MgCl <sub>2</sub> (c. 7%)	10% phosphate buffered formalin	n/a	70% EtOH

## Preservation of flora

Specimens can be preserved either through desiccation and pressing or by placement in a preservative solution. However, the first two steps are the same for both procedures.

- Fixation: colour of specimens is best preserved by fixing in 3–5 % buffered formalin seawater away from direct sunlight. Deterioration of algae occurs quickly and therefore it is best to carry out fixation in the field.
- Preparing the specimen: specimens should be rinsed free of any sand/debris using tap water, artefacts removed which are not part of the specimen, and holdfasts split if they are too thick to be pressed.

### *Drying specimens*

- Pressing fleshy seaweeds: spread seaweed out in a tray of fresh clean seawater. Note details in pencil of location, collector, date and identification if possible, on stiff white cartridge paper of suitable size. Float specimens onto paper, arrange plant and remove paper from tray, allowing excess water to drain away. Cover plant with an absorbent liner, and place between dry blotting paper or newspaper and compress in a seaweed press. Change drying paper after 24 hours and again after 2–3 days. Specimens should be dry after about 1 week.
- Coralline algae: soak fixed specimens in 40% glycerin in 3% buffered formalin seawater. Then dry and place in box.

### *Preserving specimens in liquid*

- Fleshy specimens should be preserved in 70% EtOH after the fixative has been rinsed off with tap water.
- Coralline algae should be preserved in 70% ethanol and 10% glycerol.

The National Museum of Natural History, Smithsonian Institution (<http://www.nmnh.si.edu/botany/projects/algae/Alg-CoPr.htm>) comprehensively covers further information on the different preservatives and general information on algae collection and preservation. For specific information on coralline algae preservation refer to <http://www.botany.uwc.ac.za/clines/colpres.htm> .

## Data analysis

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Use standard taxonomic guides.

## Accuracy testing

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The accuracy of the collection depends on the correct identification of the specimens. This in turn depends on the experience of the identifier and also the care taken with preparing specimens. Quality of specimens can be affected by the method of extraction, transportation and laboratory processing.

## QA/QC

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To ensure that the quality of specimens collected is high, collectors should be made aware of the importance of removing an intact specimen. To this end, sufficient training should be given in the use of equipment for the removal of specimens, e.g. the 'slurp gun'. Care should be taken when sieving fauna from sediment and large rocks and fauna should be removed before the sediment is agitated. This should reduce damage to specimens.

Correct use of chemicals is important for the preservation of intact specimens. Workers should be aware that fixative solutions that are too weak will not protect tissue adequately and similarly preservative solutions must be strong enough to prevent rotting. Containers should be checked to ensure that they are airtight since neglect of this can cause specimens to go dry and rot.

Identification of specimens and labels should be checked by an experienced individual. If the identifier is unsure which species the specimen belongs to, this should be noted on the label and sent to someone who can identify it.

## Labelling

Specimens should be labelled directly with name and code. All specimens returned to the MNCR for deposition in museums should be properly labelled, in indian ink and on paper suitable for storage in alcohol. Temporary field labels should be retained with the specimen.

- Species name and authority (according to Howson and Picton 1997)
- Determinor
- Date determined
- Collector
- Date collected
- Location (site name)
- Area (including county or region)
- OS grid reference or latitude and longitude
- Height or depth collected (in metres from chart datum)
- Habitat details (e.g. under boulder; clean shell gravel)

Where possible, specimens should be identified to species level. Where the identification is uncertain, this should be indicated, with notes on the label where appropriate.

## Storage

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Wet specimens are best kept in glass airtight containers, although glass jars with ground glass stoppers, or Copenhagen glass jars with plastic caps are very expensive. Small specimens can adequately be stored in glass soda vials with airtight plastic caps. Larger specimens can be stored more cheaply in polystyrene screw cap jars (particularly suitable for fieldwork, as these are light and non-breakable), though alcohol tends to evaporate from these with time.

## Health and safety

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The use of chemicals in the field should be limited and COSHH procedures should be followed. Many of the chemicals used in the preservation process may be listed as environmentally hazardous. It is suggested that all work with chemical solutions should be conducted in a fume cupboard, and that lab personnel wear appropriate eye protection, gloves and a chemical apron. Follow COSHH procedures.

## References

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- Howson, C M and Picton, B E (1997) *The species directory of the marine fauna and flora of the British Isles and surrounding seas*. Ulster Museum and The Marine Conservation Society, Belfast and Ross-on-Wye.
- Lincoln, R J and Sheals, J G (1979) *Invertebrate animals – Collection and Preservation*. British Museum (Natural History) and Cambridge University Press.

### References to other procedural guidelines in this volume

- Procedural Guideline No. 3-9: Thomas, N (2000) Quantitative sampling of sublittoral sediment biotopes and species using remote-operated grabs.
- Procedural Guideline No. 3-10: Rostron, D M (2000) Sampling marine benthos using suction sampling.
- Procedural Guideline No. 4-3: Wilding, T A, Gibson, R N and Sayer, M D J (2000) Sampling benthic and demersal fish populations on sediments.
- Procedural Guideline No. 4-4: Wilding, T A, Gibson, R N and Sayer, M D J (2000) Sampling fish in rockpools.

## Related websites

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<http://www.botany.uwc.ac.za/clines/> Coralline algae – methods of preservation and collection

<http://www.nmnh.si.edu/botany/projects/algae/Alg-CoPr.htm>) National Museum of Natural History, Smithsonian Institution – algae collection and preservation techniques

<http://nmnhwww.si.edu/iz/usap/usapspec.html> National Museum of Natural History, Smithsonian Institution – invertebrate specimen processing procedures

<http://www.nhm.ac.uk> Natural History Museum, London

<http://www.ulstermuseum.org.uk> The Ulster Museum – see reference to Howson and Picton above.