Physical and chemical assessment

Version: February 2018

Sampling and preparation for stable isotope analyses of biota and sediment

1 Purpose and scope

This document outlines the methods for sample collection, preservation and storage of samples prior to transport and delivery to a laboratory for carbon or nitrogen Stable Isotope Analysis (SIA). It does not cover collection of water samples.

Methods described in this document should be discussed with the analytical laboratory staff prior to sample preparation in order to determine specific requirements for that laboratory. The methods have been adapted from the *Stable Isotope Analysis Protocol* from the Australian Rivers Institute, Griffith University.

Further information on methods and issues associated with SIA are provided in Section 8. These documents should be consulted prior to developing a SIA monitoring program.

2 Associated documents

Physical and chemical assessment: Background information on stable isotope analyses

Sampling design and preparation – Record keeping including taking field photographs and videos

Refer also to other relevant documents within this manual, which outline methods for sediment sampling, fish sampling, macroinvertebrate sampling (but **DO NOT preserve the samples with ethanol**) and chlorophyll *a* sampling.

3 Health and safety

Before following the methods contained in this document, a detailed risk management process (identification, assessment, control and review of the hazards and risks) must be undertaken. All work carried out must comply with the Queensland Work Health and Safety legislative obligations.

4 Permits and approvals

A general fisheries permit is required for all work that involves 'fish' as defined in the *Fisheries Act 1994*. Note that early life stages such as eggs, spat or spawn of fish are considered as fish under the Act. Under the *Animal Care and Protection Act 2001*, prior approval in writing from an Animal Ethics Committee is required for the use of animals for scientific purposes. All work carried out must comply with Australian Code for the Care and Use of Animals for Scientific Purposes (National Health and Medical Research Council 2013).

Note: Some crustaceans (e.g. crabs, prawns and lobsters) are considered fish under the *Fisheries Act 1994* and therefore relevant permits and approvals need to be sought.

If a boat is to be used for research and/or commercial purposes, consideration must be given as to whether a Certificate of Survey or Marine Safety (scientific research and educational activities) Exemption is required.

Permits and approvals may be required to conduct activities involving animals, plants and/or in protected areas (for example National Park/Regional Park, State Forest or State Marine Park). See *Permits and approvals* for more information on requirements.

5 Skills, training and experience

Staff using this method should have previous training and experience in sampling protocols specific to sediments, plants, algae or fish depending on which sample type is to be used.

Previous training and experience in sampling for stable isotope analysis and a sound knowledge of the underlying theory is preferable. Some suggested reading is provided in section 8.

6 Equipment

Equipment and materials specific to this sampling method are included in (but not limited to) Appendix 1. Additional equipment may be needed depending on sample type (e.g. whether sampling vegetation, phytoplankton, animals or sediments).

7 Procedure

7.1 Sample collection

Obtaining a clean sample at the time of collection can save a substantial amount of SIA processing time in the laboratory. Aim to collect a clean sample of the material of interest by excluding (or picking out) potential contaminating material. If possible, rinse the sample when it is collected. All sample materials collected should be placed into clean, clearly labelled bags (e.g. labelled with site, sample type, sample date and replicate number).

7.1.1 Vegetation sampling

SIA can be carried out on terrestrial, semi-aquatic and aquatic plants. Samples are frozen after collection. For terrestrial vegetation (trees, shrubs, grasses etc.) leaves are typically sampled.

Different types of aquatic plants and algae may be sampled and processed for SIA including:

- floating macrophytes
- rooted submerged aquatic macrophytes
- filamentous algae and other attached visible algal material (on snags, rocks, mats of algae on sediments).

After collection, remove any particulate organic matter (POM) and sediment from the sample prior to freezing. This can be done in a number of ways:

- float the material off the sample
- hand pick the sample
- sieve the sample

Epiphytes are also commonly collected for SIA. Epiphytes are small plants without roots that grow on other plants. If they are available at the time of sampling, the best method of collection is by scraping them from the plant leaf using a scalpel, rinse and store in clean clip-seal bags prior to freezing.

All samples must be labelled correctly with details on the site where the samples were collected, time, date and sampler and given a unique identifier. In addition, record the type of sample collected, details on the part of the plant, preparation undertaken, preservation and sampler name.

7.1.2 Phytoplankton sampling

Water samples should be collected into clean containers and processed as soon as possible. The amount of water required depends on the chlorophyll content of the water. Sample collection and processing should be undertaken as outlined in the *Chlorophyll a sample collection methods* document. Glass fibre filters used in the collection of chlorophyll *a* samples for SIA should be pre-combusted and pre-weighed before use. Zooplankton and large detritus can be removed from the sample by pre-filtering water through a 100-200 μm mesh sieve, or

by removing large pieces with forceps.

All samples must be labelled correctly with details on the site where the samples were collected, time, date and sampler and given a unique identifier. This information must be recorded. In addition, record the type of sample collected, preparation undertaken, preservation and sampler name in a notebook or equivalent.

7.1.3 Animal sampling

Animals can be rinsed and frozen whole for processing later. If the animal is of substantial size (e.g. a large fish), measure standard length (Figure 1) and total weight of the fish before removing any tissue samples. When collecting samples from large fish for SIA:

- collect muscle (flesh) samples from the fish at the time of collection rather than transporting the entire animal
- collect any other samples such as gut contents, otoliths (for diet and aging purposes), or the liver (for shorter term diet indication) that are required
- bag and label each tissue sample separately for each individual fish.

Very small fish can be sent to the laboratory whole. It is recommended a number of small fish from a sampling site are sent to the laboratory so samples can be pooled if necessary.

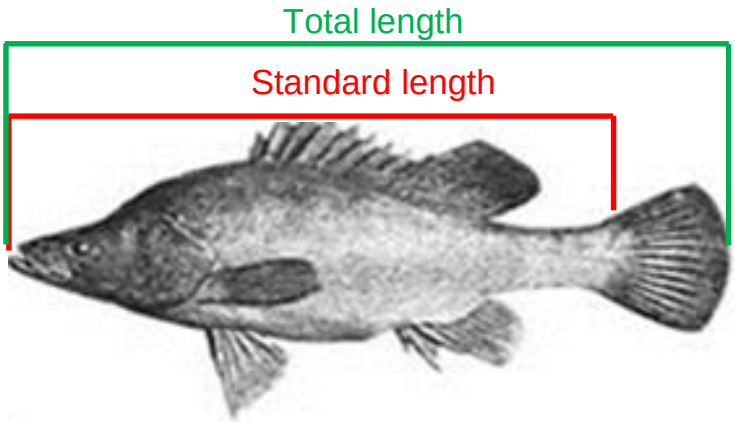

Figure 1: Fish schematic, showing total and standard length measurements

Some smaller animals such as worms and nematodes need to have the contents of their guts emptied.

1. Place animals in filtered clean water (freshwater or seawater) in a labelled watertight plastic bag.
2. Let sit for between 12 and 24 hrs to evacuate gut.
3. Rinse samples in de-ionised water.
4. Freeze samples as soon as possible

If this is not possible, rinse and freeze the animals; their guts can be removed later.

All samples must be labelled correctly with details on the site where the samples were collected, time, date and sampler and given a unique identifier. This information must be recorded. In addition, record samples collected, preparation undertaken, preservation and sampler name in a notebook or equivalent.

7.1.4 Sediment sampling

Sediment sampling should be used when sampling for sediment sources and deposited sediment for SIA. Sediments should be collected as outlined in the *Collection and preservation of sediment* document. Sediment samples can be size fractionated in the laboratory into different particle sizes for isotopic analysis of each size fraction if required. Selected size fractions for analysis would be defined by the aim of the study.

If suspended sediment is being studied, sampling equipment such as time integrated samplers (Phillips et al. 2000) or auto-samplers (see *Water quality sampling using automated sampling equipment* document) can be used.

For SIA sediment collection:

- Large pieces of organic matter, such as leaves and twigs, should be removed in the field with sieves or forceps.
- Coarse particulate organic matter (CPOM) >1.0mm and/or fine particulate organic matter (FPOM) <1.0mm should be collected using a series of sieves, as each size fraction contains different organic detritus which will be targeted during SIA analysis.
- Collected sampled soil and/or sediment should be bagged into clean snap-lock bags, labelled and put on ice.

Because of the high variability of C and N content in sediment samples, repeat/replicate analyses may be required. It is important therefore to collect and provide as much sample as possible for subsequent runs if required. If composite samples are collected, ensure that they are completely homogenised before sending to the laboratory.

All samples must be labelled correctly with details on the site where the samples were collected, time, date and sampler and given a unique identifier. This information must be recorded. In addition, record samples collected, preparation undertaken, preservation and sampler name in a notebook or equivalent.

7.2 Storage

After collection, all samples must be frozen immediately (to -18ºC or lower) or stored on ice (do not use dry ice) until they can be frozen. Samples must remain frozen until processed. Chemical preservation and/or incorrect storage of samples (see below) may affect isotopic composition (e.g. Kaehler and Pakhomov 2001; Arrington and Winemiller 2002; Sarakinos et al. 2002).

Note: Preservatives **must not** be used on samples undergoing SIA.

7.3 Quality assurance and quality control

All storage containers must be clean, air tight and clearly labelled. Cross-contamination of samples must be avoided by using a clean set of processing equipment for each sample. Chain of custody documentation and clear and accurate sample data sheets must be provided to the laboratory.

7.4 Sample submission

Sample submission details should be discussed directly with the laboratory.

7.5 Variation to method

Different laboratories will have different requirements and the operating technician should be contacted prior to project commencement to determine any specific needs. Variations may include measurement of other isotopes such as oxygen, hydrogen or strontium and the inclusion of water samples for isotope analysis.

8 References and additional reading

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Appendix 1

Table 1: Equipment checklist

Equipment	✓
A note book or equivalent for recording data.	
Distilled water	
Sample submission sheets	
Storage containers and bags	
Permanent, water proof marker	
Sieves	
Latex gloves (powder free)	
Aluminium pie dishes	
Analytical balance	