2022 Science Week eDNA MidCoast waterway survey

Sampling Procedure

This event is funded by National Science Week grant 2022 and
is supported by the Commonwealth Government.

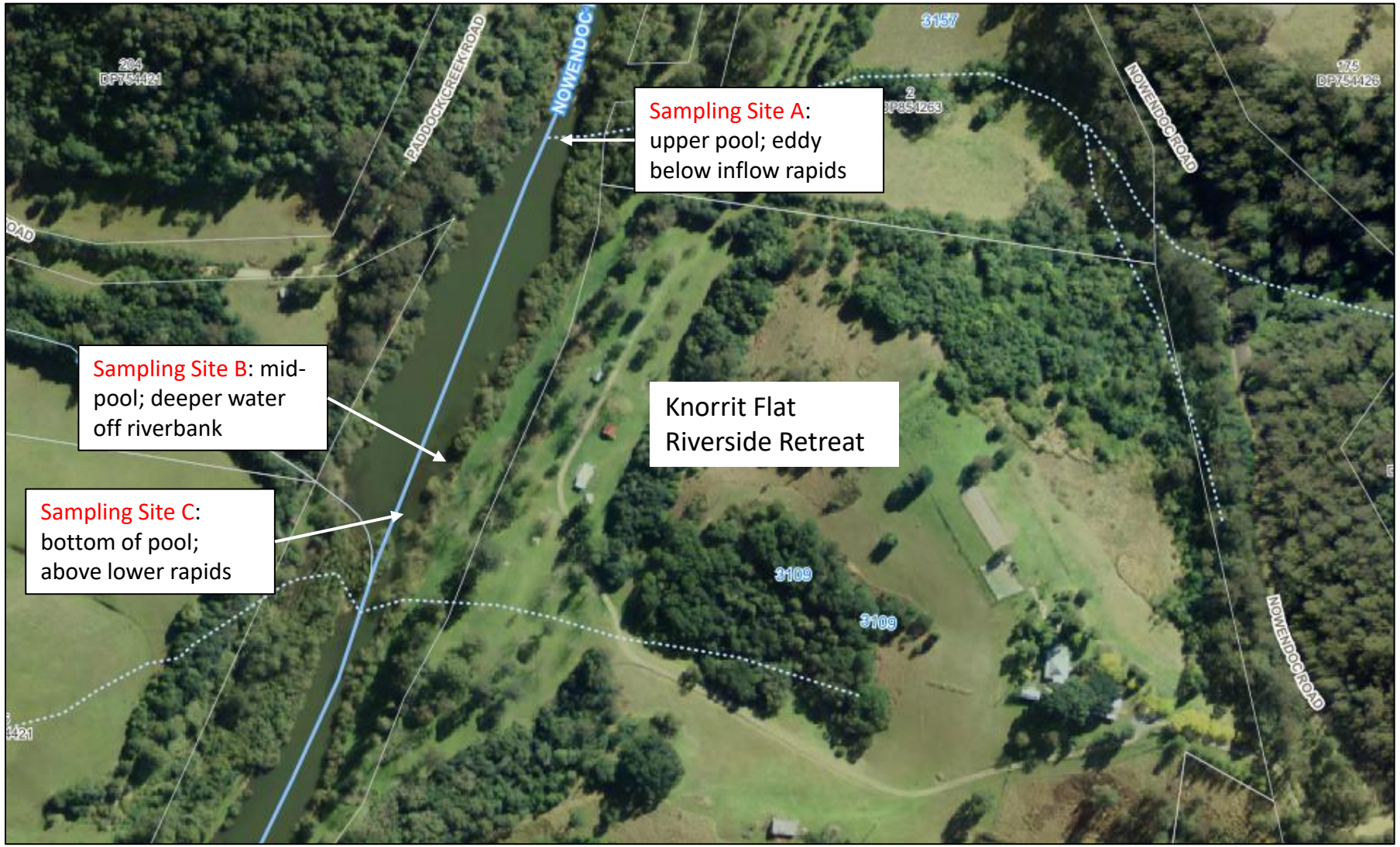
To view this procedure online, please see the following video with Dr Josh Griffiths from enviroDNA for Odonata's Great Platypus search:

https://www.youtube.com/watch?v=30G16kOFN7U&t=248s

1. Site confirmation & safety

A. You need to select the waterway you wish to sample (eg. Nowendoc River)
B. You need to select the pool or area you wish to sample (eg. Pool at Knorrit Flat Riverside Retreat pool (above Paddock Creek junction)
C. Within this pool / area, select three different sites: “A, B and C”
D. To assist how you determine your sampling sites, please see the next slides
E. Describe each site in terms of location and habitat on the data sheet and online survey
F. Once you have decided on your waterway, pool / area and three sites, ensure your access to the waterway is safe and keep an eye on children at all times – do not let them enter the water

1. Site confirmation & safety



Waterway to sample: Nowendoc River

Area to sample: Pool at Knorrit Flat Riverside Retreat pool (above Paddock Creek junction)

1. Site confirmation & safety

Waterway to sample: Bobin Creek

Area to sample: Pool above 3rd crossing on Bobin Creek Road

Sampling Site A: upper pool; eddy below inflow rapids

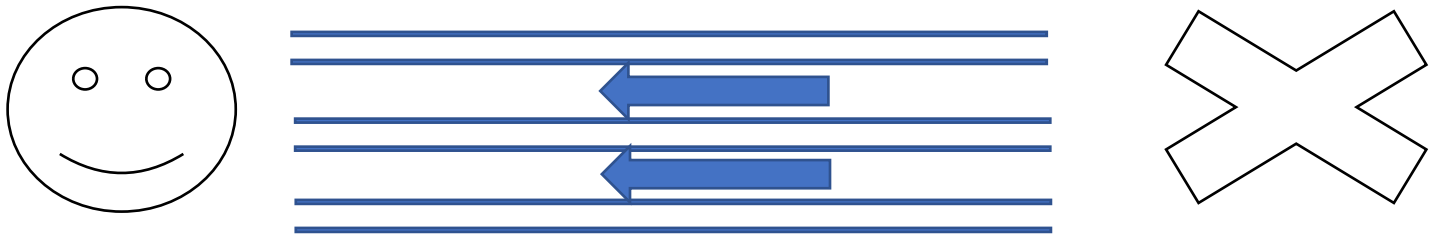
Sampling Site B: mid-pool; deeper water off riverbank

Sampling Site C: bottom of pool; above crossing

2. Please do not enter the water to take the samples

Some important considerations:

- You want to select 3 sites within your chosen waterway where the water is not turbid – full of suspended sediment, debris, or algae
- You want to take a water sample upstream from yourself (orient yourself downstream from the water sample): water should be flowing in the direction of your syringe to your body, to minimise contamination



You – downstream of water sample

Direction of water flow

Take water sample upstream of yourself

3. Your eDNA Sample pack (provided to you):

1. 1 x large zip-lock bag
2. 3 x 50 ml syringes – one for each site “A, B or C” within your chosen waterway
3. 3 x disc filters – one for each site “A, B or C” within your chosen waterway – please label using permanent marker
4. 3 x 3 ml syringes filled with 1 ml of preservative – syringes are capped – one for each site “A, B or C” within your chosen waterway
5. 1 x permanent marker
6. Set of gloves
7. Small zip-lock bags – one for each site labelled “A, B or C” within your chosen waterway
8. Data sheet
9. These instructions!

4. Decide on the roles of people involved:

A. For a smooth sampling procedure, please assign clear roles and responsibilities to all members of your team before sampling commences, such as:
   i. Who will record information on data sheet – included volume filtered – the data scribe
   ii. Who will label the disc filters as A, B or C – corresponding to the zip-lock bag they have come from
   iii. Who will take water samples using the 50 ml syringe and gloves, and report to scribe how much water was filtered (ml) per site
   iv. Who will filter the water samples using the disc filter and gloves, & air-dry the filter
   v. Who will un-cap the 1ml syringes and fill the disc filter with preservative and cap the opposite end of the disc filter – for School groups, this will be done by the teacher leading the group
   vi. Who will put the disc filter full of preservative with a cap on one end and the attached 1ml syringe on the other end in the corresponding zip-lock bag and seal it
   vii. Who will complete the online google doc form: https://forms.gle/RrzCM1a1JasTnE2a7
   viii. Who will return the samples to MidCoast Council within the 2 week timeframe

5. Record sample information

A. Using the data sheet provided in eDNA sample packs, please record much information as you can before commencing sampling!
B. Important – please record the volume of water filtered at each site within your chosen waterway

6. Begin sampling! - Gloves

A. Put gloves on

7. Sampling procedure – label filter disc

A. With gloves on, remove a filter disc from its packaging and using the permanent marker, label it as either A, B, or C (keep it with its corresponding bag)
B. Only open the filter at its corresponding site. Do not open a filter that will not be used at that site
C. Only label each filter at the site (“A, B or C”) which sampling will occur at immediately before sampling commences. This will ensure the filter is not exposed for longer than necessary
D. Please do not touch the inlet or outlet of the filter disc

8. Sampling procedure – 50 ml syringe

A. Remove one 50 ml syringe from its packaging
B. At your site, select your first sampling area where you will not enter the waterway to minimise contamination with your own DNA in the water
C. Also try to ensure the water body you will sample from is not too cloudy or turbid with suspended solids or algae if possible – these will clog the filter before enough volume is filtered and eDNA captured
D. Ensure you are downstream of where you will put the syringe into the water to take the water sample from – again, to minimise contamination from yourself! Your syringe should be upstream from you when sampling – or the direction the water is flowing in should flow from the syringe to yourself
E. Draw water through the syringe until it is full
F. Remove any air bubbles by inverting the syringe and tapping it with your finger so the air bubble rises to the top of the syringe outlet where you can then release the air bubble by slowly and gently pressing on the syringe base
G. To keep track of the amount of volume filtered at each site, it may be easier to begin on 50 ml

8. Sampling procedure – 50 ml syringe

- To keep track of the amount of volume filtered at each site, it may be easier to begin on 50 ml – as shown below

Image still from: The Great Australian Platypus Search: eDNA Water Sampling Tutorial and Safety Video with Dr Josh Griffiths

9. Sampling procedure – attach syringe to filter disc & filter water

A. Once air bubbles have been removed, attach the 50 ml syringe to the wider inlet tube of the filter disc (the side with Whatman written on it) by screwing them together until secured

B. Slowly press the base of the syringe to push the water through the filter disc – do not do this quickly or force the water through if you feel too much resistance

C. The water that is leaving the syringe via the outlet tube is filtered water, and is fine to enter the waterway you have sampled

D. The DNA we are interested in has been captured by the white filter paper inside the filter disc

E. Once you have emptied one volume of the syringe, report this value to your data scribe

F. Repeat this procedure steps 8-9 – refilling the same syringe with more water - the more water filtered, the more DNA captured, until no more water can be gently pushed through the filter disc

G. The filter disc will begin to change colour from white to brown and it will become clogged, making it harder to continue pushing water through it

H. You will begin to feel resistance when pushing the syringe when the filter disc becomes clogged, do not force the water through if resistance has increased so much that water no longer is easily pushed through

I. Ensure you keep track of the volume of water filtered and report this to the data scribe

J. Remove the 50 ml syringe from the filter disc – this 50 ml syringe should not be used at any other site for this project – but you still need it for the next step – part 10

9. Sampling procedure – attach syringe to filter disc & filter water

Image still from: The Great Australian Platypus Search: eDNA Water Sampling Tutorial and Safety Video with Dr Josh Griffiths

10. Sampling procedure – air dry the filter

A. After removing the 50 ml syringe from the filter disc, fill the 50 ml syringe with air by drawing in air, instead of water
B. Attach the 50 ml syringe to the disc filter in the same manner in step 9 – to the wider inlet tube of the filter (with Whatman written on it)
C. Gently push the air through the filter to expel any remaining water inside the filter disc housing
D. Once there is no more water leaving the filter disc, remove the 50ml syringe – do not use this syringe for sampling any other site for this project
E. Do not put the filter disc on the ground – if required, you can place it back into the packaging it came from to conduct the next step, or hold it, but do not touch the inlet or outlet tubes

10. Sampling procedure – inserting preservative into the filter

A. This step should only be conducted by an adult for school groups
B. Take a 3 ml syringe filled with 1 ml of preservative from the corresponding zip-lock bag
C. Take the cap off the 3 ml syringe
D. Attach the 3 ml syringe to the filter disc in the same manner as used with the 50 ml syringe – to the wider inlet tube side of the filter disc with Whatman on it
E. Push the preservative through the filter – some may dribble out of the syringe disc via the outlet tube – this is OK
F. The preservative will ensure that DNA will not degrade between sampling and getting the filters to the lab for extraction and sequencing
G. Leave the 3 ml syringe attached to the side of the filter with Whatman on it and then cap the opposite side of the filter with the cap that was on the 3 ml syringe: see over

11. Sampling procedure: end of sampling

A. Leave the 3 ml syringe attached to the wider inlet tube side of the filter with Whatman on it and then cap the opposite side of the filter outlet with the cap that was on the 3 ml syringe. This is how the filter will be returned:

Image still from: The Great Australian Platypus Search: eDNA Water Sampling Tutorial and Safety Video with Dr Josh Griffiths

12. Packaging the eDNA sample

A. Label the corresponding zip-lock bag with key information such as Group name, waterway, sampling location, date, time, volume filtered, habitat sampling location and type “A, B or C”, GPS location etc
B. Put 3 ml syringe attached to the labelled filter disc that is full of preservative and the opposite end is capped into the corresponding zip-lock bag.
C. So, “A” labelled filter disc should be returned to “A” labelled zip-lock bag.
D. Seal the zip-lock bag and put back into larger zip-lock bag, ready to be returned to Mat Bell at MidCoast Council within the two-week timeframe

13. Continue with remaining samples

- Repeat steps 5-12 for sample “B” and “C”
- Put all 3 zip-lock bags “A, B & C” into the larger zip-lock bag and label with your group name and contact details

15. Return samples to XYZ

- Please return samples to XYZ:
- Contact details:
  - Name: Mat Bell
  - Phone:
  - Email:
  - Returning location: MidCoast Council Yalawanyi Ganya 2 Biripi Way TAREE
  - By when: 19th MAY 2022

15. Need any help at all?

- We are here to assist you take, record or deliver samples:

- For help, please contact: