Collection instructions

Introduction

I am a student at Victoria University of Wellington, studying for an MSc in Marine Biology. In my study, I am collecting DNA from kahawai (*Arripis trutta*) with the goal of determining how many genetic stocks exist in NZ waters. From the material collected in the field, I will be extracting DNA and amplifying it using the Polymerase Chain Reaction, or PCR method. This technique involves creating many millions of copies of DNA by controlled heating and cooling of the sample in the presence of free amino acids. This allows tiny samples to create measurable amounts of DNA within an hour or so, which is then "read" in a computer controlled sequencer. This machine provides a readout of the actual sequence of DNA in the sample.

In addition to investigating the stock structure of kahawai, I am interested in determining the limits of the four members of the family Arripidae. These are:

Species	Details
Kahawai or Australasian salmon (*Arripis trutta*)	Believed to extend right around NZ and around the southern coast of Australia. Grows to approx 80cm.
Kermadec kahawai (*Arripis xylabion*)	Range is from the Kermadecs south to approximately the Bay of Plenty. *A. xylabion* can be identified by measuring the top lobe of the caudal (i.e., tail) fin. If this is >30% of the length of the body of the fish, the specimen is *A. xylabion*. If this fin lobe is <30%, the specimen is *A. trutta*. Can grow larger than *A. trutta*.
Western Australian Salmon (*Arripis truttacea*)	Confined to southern Western Australia. Can grow larger than *A. trutta*.
Tommy Ruff or Australian herring (*Arripis georgianus*)	From southern Australia. The smallest member of the family.

It is possible that each of these fish visit NZ, albeit very rarely in the case of *A. truttacea* and *A. georgianus*. It is also possible that *A. xylabion* and *A. truttacea* are actually sub-species rather than full species.

General Instructions

Only small samples are needed for the study. Clippings need only be approx ½ cm x ½ cm. After collecting the sample and placing it in the tube, please complete the columns in the collection sheet. Measure or estimate the fork length[1] of the fish, and record this in the row of the collection sheet that corresponds with the tube number. Record additional details such as where on the fish the sample was taken. Record the location the fish was captured on the return sheet. This need not be specific. Something like "4nm offshore from Whakatane" is more than sufficient. You can also record any other details you feel are pertinent. There is also a column for "assumed species" on the return sheet. If you are unsure which member of the Arripidae family you have, just record "kahawai".

Live fish – to be returned to the water

If you are taking a clipping from a live fish that you intend to release, take a small clipping, approx ½ cm x ½ cm, from the trailing edge of the dorsal or anal fin. The fin rays are soft in these areas, and the material can be easily clipped off with a pair of scissors.

Record which fin the material was collected from as this will allow me to determine which fin gives better results after I run the genetic tests on the samples.

[1] Fork length is measured from the nose of the fish to the centre of the V of the caudal (tail) fin. This is the easiest and most consistent length measurement to perform.

Dead fish

If the fish you are collecting the material from is dead, it is still perfectly acceptable to take a fin clip as outlined for live fish. However, there is a much better chance of collecting viable DNA from muscle tissue. Cut a small section of meat from the gut cavity or from a fillet, preferably with a small piece of skin attached. Again, this only needs to be very small. Additionally, collect a couple of scales and place them in the collection tube with the flesh sample. I can use these to age the fish, and when combined with the fork length information, can build up and idea of how fish age relates to length.

Sample Record Sheet

Tube No.	Date Collected	Collected by	Location	Assumed Species	Fork Length	Notes
A0001	22nd Nov 08	Brenton	Wellington Harbour	Kahawai (*A. trutta*)	37cm	Dead fish. Sample taken from filet. Two scales also collected for aging.
A0002	"	"	Wellington Harbour	Kahawai	27cm	Live fish, returned to water. Fin clip taken from trailing edge of anal fin
A0003	25th Nov 08	Brenton	90 Mile Beach	Kermadec kahawai (*A. xylabion*)	29cm	Live fish, returned to water. Fin clip taken from trailing edge of anal fin.

Please note: "Ditto" marks are fine on the return sheet, as are estimates of location and fork length.

After you have finished collection

While the tubes need no special treatment other than keeping them out of the sun as much as possible, they do store better if kept cool. Please keep them in the fridge until you are ready to send them back to me. Also, the tubes contain 70% ethanol. While the volume is tiny, it is flammable. Avoid smoking near the tubes while they are open….

After you have a sample in each of the tubes, simply place them back in the ziplock bag, re-wrap them in the bubble wrap, place them in the courier bag and send them back to me.

And thank you so much for your assistance. I am working on putting a website together so I can provide regular updates to everyone who has helped with this project.

Also, if you think these instructions could be clearer, please feel free to let me know! My home email address is brenton.hodgson@clear.net.nz

Regards,

Brenton Hodgson
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