http://sustain.no/projects/globalpop

The IPY project Global POP

International School Education

Protocol: DIOXINs in local fish

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Content

Introduction to Global POP Protocol

The purpose of this protocol is to investigate the level of dioxins and dioxin-like compounds such as some PCBs and PAHs in fish used for local consumption in various countries. Since fish will be caught at various sites, both near industrial areas and in more remote, less populated areas, we do not know which levels to expect to find. Hence, this project will give us a broad screening of levels of toxic chemicals in fish used for consumption.

- **Phase I: Fish sampling and preparation**
- **Phase II: Analysis by bioassay**
- **Phase III: Evaluating results**
- **Phase IV: Writing**

Phase I, III and IV are done at the school, while phase II is done at NILU in Norway. There will be an option for the schools to do some of the preparation of the fish if they have the proper facilities and equipment. There will be a full description of the various steps on the project web page, suitable for teaching purposes as well as a multimedia presentation of the methodology.

Short description of each phase:

Phase I: Sampling
The schools are given the task to identify and sample a suitable fish based on certain criteria; the most important being that the fish is common as food item. Schools record and write down important parameters on a datasheet, then prepare samples for the chemical analysis by cutting, and finally packing fillets of the fish and send these to NILU. Schools with access to laboratory facilities, will have the opportunity to do parts of the preparation of the fish sample. The sampling is to be done by all involved schools in the period October 2007-February 2008.

Phase II: Chemical analysis
NILU analyses the samples as they arrive and submits the results to the respective schools.

Phase III: Evaluating results
Each school will be given specific tasks in evaluating their results. This will include comparison with the results from the other schools and scientific literature, as well as evaluating dietary limits.

Phase IV: Reporting
The schools should then write a project report describing what was done, reporting the levels of toxins found, and evaluate how these findings compare with the same species from other regions. The report is then submitted to the web page: http://sustain.no/projects/globalpop/.

The IPY Project Global POP

Protocol: DIOXINs in local fish

Phase I: Fish sampling

The Phase I of the protocol, fish sampling consists of catching fish, recording data, preparing high quality samples, and collecting biological data. The fieldwork entails aqcuiring suitable fish, recording sampling locations and taking photos. Sample preparation and recording of biological data may also be done in the field or at the school. The packing and freezing of the samples should be carried out adhering to guidelines provided in this protocol, thus ensuring that samples are processed in a scientifically correct way before shipment to NILU in Norway. The recording of data is to be done by filling out a datasheet, see Appendix 1.

1 Objectives

Schools should find a good representative fish for this project, prepare samples in a scientifically correct manner, and record all relevant information useful for the evaluation of the results. It is very important that the ID of the sample follow the sample when packing and the sample ID is to be entered in the datasheets for each of the 3 fish. The ID should indicate the name of the species, the number 1, 2 or 3, and what kind of tissue that has been sampled. Examples: trout-1-muscle, trout-2-muscle, trout-3-muscle or salmon1-muscle, salmon2-muscle or salmon3-muscle.

2 Field work

The schools are to identify a suitable local fish, preferably a fish that is common to eat, and either catch it themselves using ordinary fishing equipment, or get it from local fishermen. The fish must be fresh when retrieved, and the sample preparation should preferentially be prepared in the field or at school the same or the subsequent day (store refrigerated).

Farmed fish should only be considered if it is too difficult to get hold of wild fish , and if fish farming is a very important source for food in your local community. All fields of the datasheed should be completed also in these cases.

2.1 Which fish and location to choose

The selection of fish should be made adhering to the following criteria

Species	There is no requirement of certain fish species, however preferentially it should be the most common fish type used for consumption in your community.
Sample type	The purpose of fish sampling is to have a representative part of the fish. We focus on fillets (muscles) of the fish since this part is the most common to eat. Choose a different organ of the fish if this is a very important food item for your community.
Size	Choose a fish of a similar size to what is commonly used for consumption, or try to catch 2-3 years old fish. If possible, check with local expertise what this age corresponds to in terms of fish length and body weight.
Number	Get at least 5 fishes of same species. 3 samples are to be used for sample preparation, and it is nice with 1-2 extra specimen for testing the protocol. Please use your own equipment on the test fish and use new and clean equipment for sample preparation.

Fishing equipment	Use ordinary available fishing tackle as rod, line, net etc. Keep the equipment as clean as possible.
Type of water	Fish from both freshwater and salt water may be used.
Surroundings	The fish should be caught in an area where fish are commonly caught for consumption

2.2 Fill out datasheet

Please ensure that you gather the necessary information needed to complete the relevant parts of the Datasheet. The one page Datasheet is to be used when performing the protocol for each of the 3 fish. Use a pencil to fill in the Datasheet during fieldwork outside (in case of rain). Datasheet for each fish should be placed into zip-locked plastic bags before shipping them together with the samples. Take photos when performing the various parts of the protocol!

2.3 Sample preparation in the field or at school:

All schools should do the sampling in the period between October 2007 and February 2008. Equipment emphasized below in bold are the most important tools needed for the sample preparation.

Equipment
Please, acquire or purchase the necessary equipment for sample fileting such as disposable laboratory gloves, disposable scalpels, and forceps from your local pharmacy or relevant school laboratory suppliers. Please, document by photos and written material for later web report that clean equipment (disposable scalpels etc.) is used and the sampling procedure is followed for sampling of the filet of the 3 fish.

Balance(s) for total body weight of fish
Square (angle iron) or similar equipment for measuring total length of fish
Disposable latex or plastic laboratory gloves,
One disposable scalpel for each fish *(Important: should be unused and clean, if possible rinse with (HPLC graded) cyclohexane before use)*
Aluminum foil (plastic free foil),
Field datasheet for each fish sample
A large pair of forceps for each fish,
A pair of scissors for dissecting, to determine gender and maturity
A knife and maybe one small pair of forceps for retrieving otoliths
Camera
Paper, pencil, permanent pen
2 ziplock plastic bags for each fish; one for the sample and one for field datasheet

Note: Students should not alone attach or change scalpel blades due to the very sharp blades. The teachers are responsible for attaching scalpel blades to the scalpel handlers.

It is very important not to mix the equipment used for sample preparation of the 3 fish. If necessary, mark the 3 scalpel handlers (and the large forceps) with fish1, fish2, fish3, respectively. You can write on some tape and put it on parts of the equipment that will not be in contact with the fish samples.

The sample preparation can be performed at the day of the field work at the sampling site. Remember to have the datasheet and camera available out in the field. Also, remember to measure the total body weight (and the total length) before cutting the samples.

We would prefer that the samples are prepared the same day the fish are caught. If this is not possible or practical, keep the fish cold in the refrigerator or frozen to the next day. The filleting may actually be easier when the surface layer of the muscle tissue is half frozen. The students can test the protocol by using some additional "test fish", but remember not to use the same scalpels (and large forceps) that are to be used for preparation of the 3 fillet samples.

Cover all areas that will be in contact with the fish (like cutting boards and balances) with aluminum foil and change after each fish. If the samples are prepared of frozen or partially frozen fish, be expedient when preparing the samples and immediately transfer the packed and marked samples to the freezer (-20 °C) to avoid water and fat loss which might occur during the melting process.

Sample preparation should preferably be performed at the sampling site to avoid unnecessary transport that may contaminate the surface layer of the fillet. **Remember to measure the weight of the fish before cutting the fillets**. If transported to the school before sample preparation, cover a clean stainless steel bucket or a similar container with aluminum foil, wrap aluminum the foil around each fish and put them into the bucket.

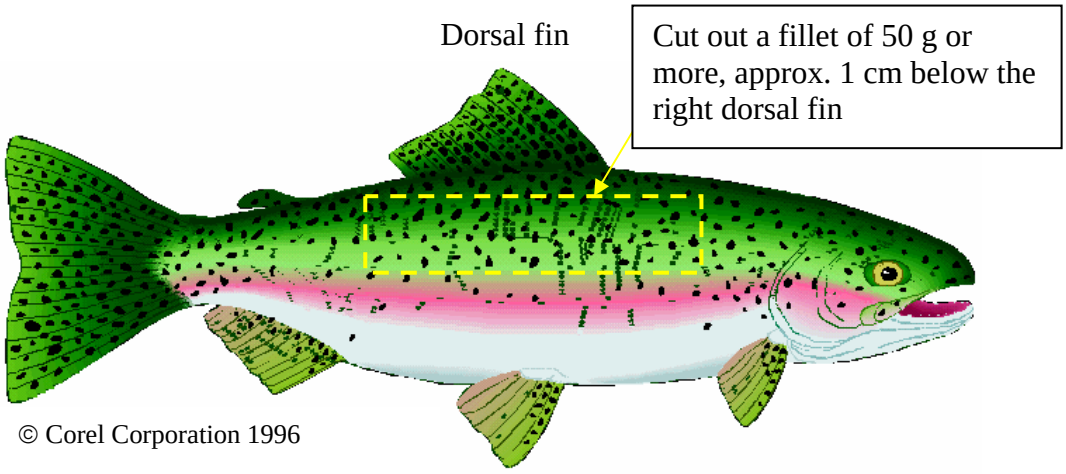
2.4 Procedure for sample preparation

DO NOT MIX THE EQUIPMENT FOR THE 3 FISH!

1. Measure **total body weight** of the fish before you do anything else. Use gloves. Avoid touching the fish where the fillet is to be cut.

2. The **total length** (the distance from the most anterior part of the head to the tip of the longest caudal fin ray) may be measured after filleting if there is a risk of contamination. Use squares, angle iron or similar equipment to measure the total length correctly (see Appendix).

3. ***For all fish species***: cut a fillet (50 g or more) beneath the right dorsal fin. The size of the fillet is dependent on the size of the fish. If the fish is very small, take a large part, or cut out fillets on both sides. **Remove the skin of the fillet, either before or after filleting**. Use clean forceps and clean gloves. Use a new scalpel blade (and pair of forceps) for each fish, and immediately transfer the sample to aluminum foil and close properly.



4. On top of the closed foil packed sample, put a **pencil written sheet** with sample **ID, Name of School and Date** and wrap it all with more aluminum foil so that the sample is fully covered. **Remember, one ID per fish**. Write the sample ID, Name of School and Date on a ziplock plastic bag with a permanent pen before putting the foil packed sample into it. Put the packed sample into another extra ziplock bag to avoid leakage. Carefully check that the sample is fully covered by foil and

make sure that the sample is not directly in contact with the plastic. Close the plastic bags properly. Do this for each of the 3 samples. Immediately put the samples in the freezer. **The samples (3 fish fillets) should be kept frozen at –20^{o} C before sending. Do not forget to put the freezing elements into the freezer when (or before) you freeze the samples.**

5. Open the fish with a pair of scissors, identify the gonads if they are visible and measure the length. Try to determine the gender of the fish, male or female. You can use binocular (stereo) lenses (for instance Leitz x 5-50) to check if you can find oocytes (egg particles) or not in the gonade.

6. Optional learning activity which should be carried out under close supervision of teachers(s): Try to sample the otoliths. Be careful with the knife! If you are going to do an age determination later on, store the otoliths in ethanol or glycerol. Scales on the fish exterior can also be used for age determination, see example further down.

2.5 Documentation

For practical projects of this kind it is desirable to have hands-on documentation both of the field work and of work done at school. Use a camera (e.g. a pocket disposable camera) to document the various steps of the project.

The following should be camera documented:

- Sampling site
- Fishing (or of fishermen/women)
- Biological data – the internal organs
  - Fish lying next to measuring tape
  - Fish being weighed
  - Cutting of fillet
  - Female/male (gonads)
  - Preparation of sample
- Packing and marking of samples

2.6 Shipment of the fish samples

Put each of the sample plastic bags with foil-packed fish samples into an appropriate (lunch) box. Place the box and the cooling or freezing elements in a large padded envelope (or, alternatively, in a bigger box) to keep the samples at as low a temperatures as possible during transport. If there is sufficient space, place all 3 plastic bags into the large envelope/box. Complete the electronic version of the field datasheets at the project web site: http://sustain.no/projects/globalpop/ as soon as possible. Please, do not loose these data. The large envelope or box with the 3 samples and accompanying datasheets are now ready to be shipped to NILU.

Shipping of samples

NILU recommends that each school finds out which service to use for shipment; for instance regular mail, DHL, TNT or similar courier services.

Address: NILU, Norwegian Institute for Air Research
Instituttveien 18
2007 Kjeller
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Datasheet (obligatory task)

Fill in the datasheet with a pencil during fishing and preparation

Name of school		
Address		
E-mail (teacher/class)		
Sampling method in field		
Date of sampling		
Name of sampling site		
Site type		
Latitude and longitude (GPS investigation)	NS	
	EW	
Nearest city/town/village		
Near industry (if, which industry)		
Fish species		
Local name of species		
Latin name of species		
ID of fish sample		
Total body weight of fish (in whole grams)		
Total length of fish (in mm)		
Female/Male/Unknown		
Sampled otoliths (set X)		
Sampled scales (set X)		
Immature/Mature/Spent		
Length of gonad (if measured)		
Weight of gonad (if measured)		
General or unusual observations (for example if there is a large scar on the fish, tumors, heavy parasite load, odd colouring etc.)		

Appendix 2 Learning activities

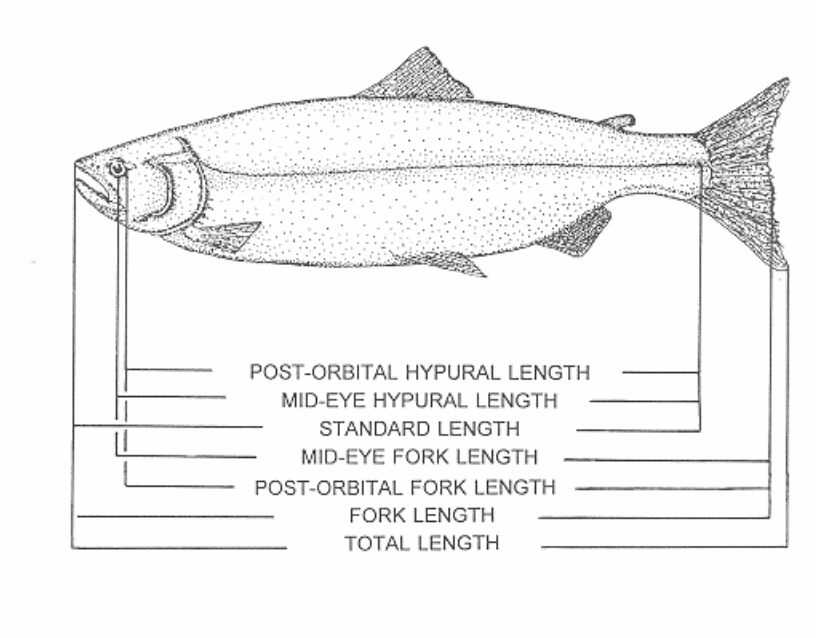
DISSECTION is an optional task, but it is a nice learning activity for the pupils. The measurements of fish length and weight are mandatory tasks, and it can be very useful to know the gender and maturity of the fish.

Optional: Explain external morphometric characteristics (scales, fins, lateral line, and chin barbell on the fish). Dissect the fish and identify organs in the body cavity (liver, reproductive organs, heart, swim bladder, stomach). Cut open the stomachs and identify contents. Focus on what this can tell you about the fish as well as its prey species. Extract otoliths (the balance organs of the fish, which also reveal age structure) from the base of the brain (students can look at these under a microscope).

1 Length of fish (mandatory task)

Source: Fisheries and Oceans Canada

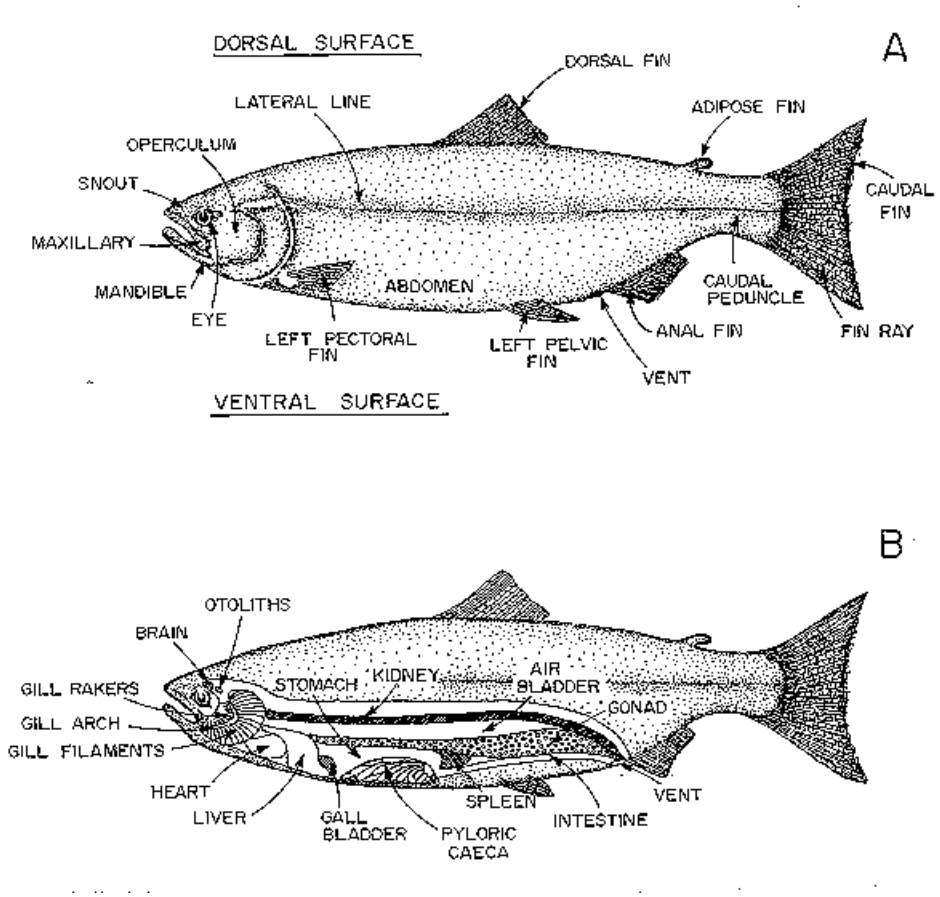
Measure the TOTAL LENGTH of the fish



2 Anatomical Features of a Typical Salmonid

Source: Fisheries and Oceans Canada

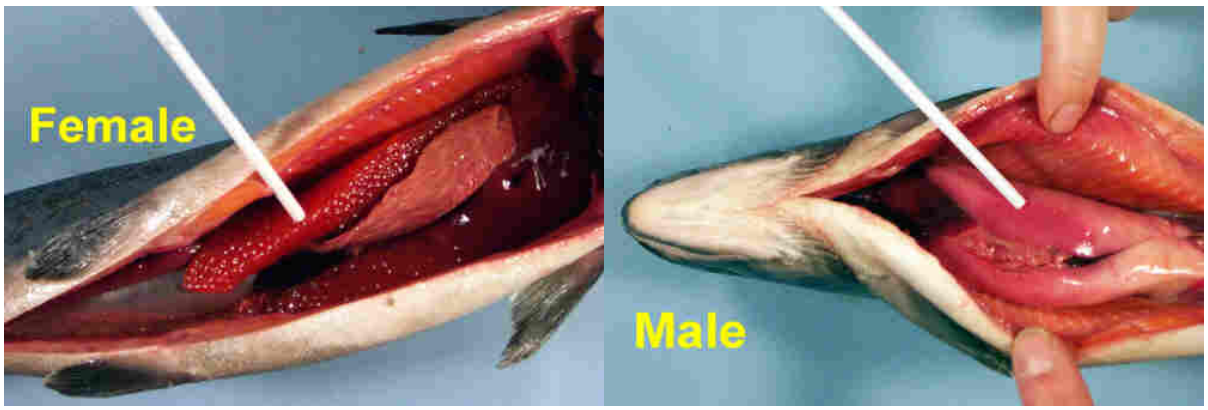
Locate the liver, gonad and otoliths on figure B.



3 Female/male (mandatory task)

The gender/sex is fairly easy to determine in sexually mature fish. In most fish species the female fish has yellow or orange ovaries where one may find eggs. The eggs may be from the size of tiny small corns until 5 mm in diameter. The testicles of the male fish are usually less colorful and the content is more homogenous in structure. You do not need to detemine the sex for younger fish where the gonads hardly can be seen.

For illustrative examples see "SALMONIDS IN THE CLASSROOM: SALMON DISSECTION" http://www.pskf.ca/sd/



4 Schematic outline of the maturity, salmonids (mandatory task)

Use this figure to estimate the maturity of the fish.

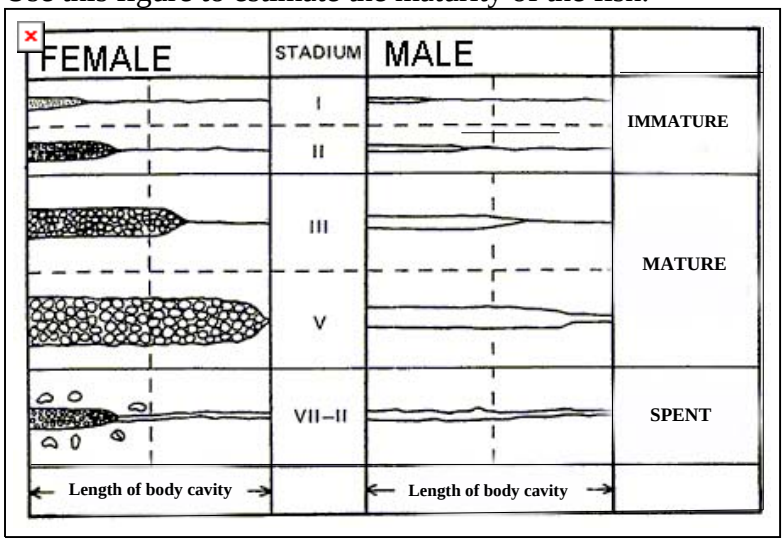


Figure adopted from
http://miljolare.uib.no/fagstoff/vann/artikler/kompendier/fiskekompendiet/kjonnsmodning.php
and translated into English

5 Gonads, otoliths and scales (optional)

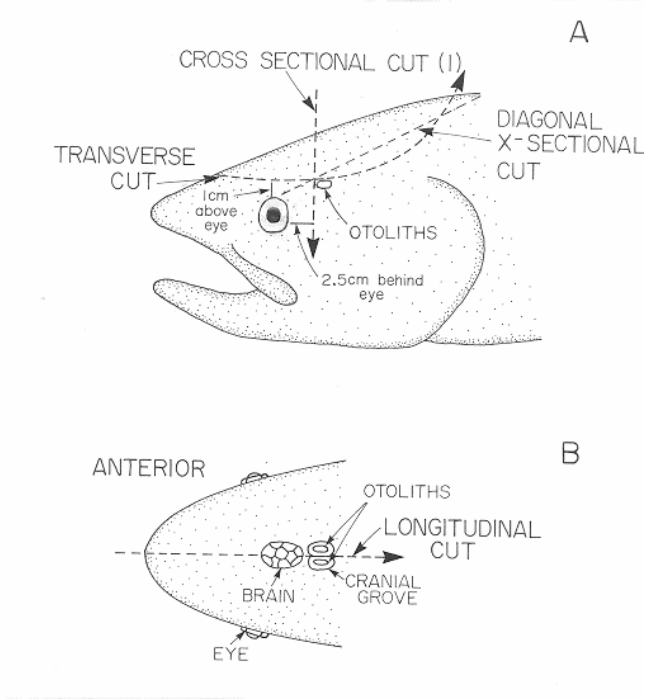
The removal of gonads and otoliths is done after you have sampled the fillet. This part does not require sterile equipment since neither otoliths nor gonads are used in chemical analysis of organic pollutants. A knife may be better than a scalpel for the dissection of the fish head. Use small forceps to remove the otoliths from the cranial grooves.

The gonad of female fish is the ovary (hard roe/spawn) and the testis (milt) of male fish. The maturation stage (length and weight of gonads) and age (otoliths, scales) will provide important information for use in scientific evaluation and comparison of POP levels in fish. Otoliths may be difficult to locate and to remove, and the gonads may be absent if the fish is very young. If the gonads are very small, a small letter balance may be necessary for determining the weight. Weighing of the gonads is therefore optional but please do measure the length of the gonads with a ruler if they are visible. Gonads of immature fish appear as thin ribbons of tissue only a few centimeters in length with almost no volume. As the fish grows and matures the gonads elongate and the testes and ovaries become easily distinguishable. The ovaries have a granular appearance (developing eggs) in comparison to the testes, which will appear smooth and whiter in color than the ovaries. The ovaries eventually take on a red or light orange color while the testes will appear translucent to white. Use the Maturity figure in the Appendix to estimate if the fish is immature, mature or spent and mark the sample datasheet with Mature, Immature or Spent, also use the code I-V if you can determine maturity to this level of precision . Generally, the fish is approximately mature if the ovaries or testis fill up more than the half of the body cavity.

5.1 Otolith location and removal in salmon

Source: Fisheries and Oceans Canada

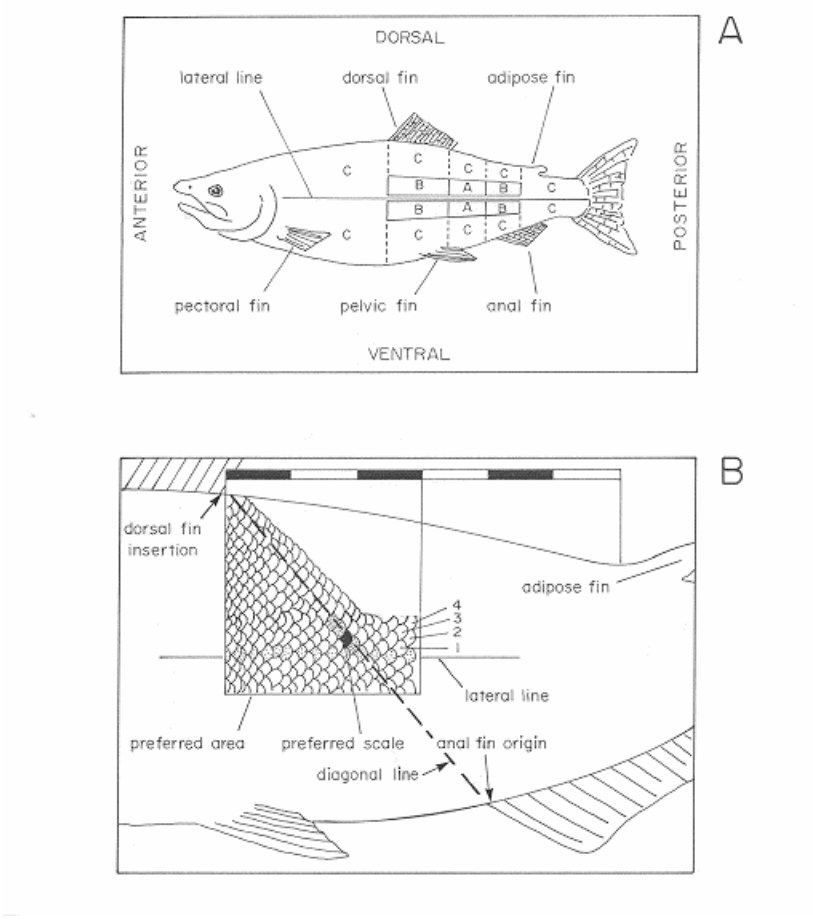
Otolith removal; **A)** the 3 common cuts used to remove the paired otoliths from the cranium; and, **B)** the otoliths are located in cranial grooves directly behind the brain.



5.2 Preferred areas for scale removal for salmonids

Source: Fisheries and Oceans Canada

A) area A is the primary preferred area; area B is the second preferred area if no scales in area A; and, area C is the non-preferred area. B) Close up of the preferred area with the preferred scale in solid black. It is located 2 rows up from the lateral, on a diagonal from posterior the dorsal fin insertion to the origin of the anal fin.


A
DORSAL
lateral line
dorsal fin
adipose fin
ANTERIOR
POSTERIOR
C
B
A
pectoral fin
pelvic fin
anal fin
VENTRAL
B
dorsal fin
insertion
adipose fin
4
3
2
1
lateral line
preferred area
preferred scale
anal fin origin
diagonal line

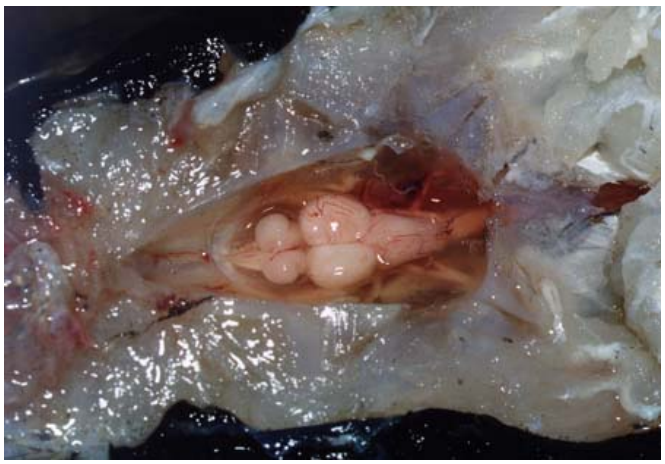
5.3 Dissection of cod head – location and removal of otoliths

1. The head is ready to be examined

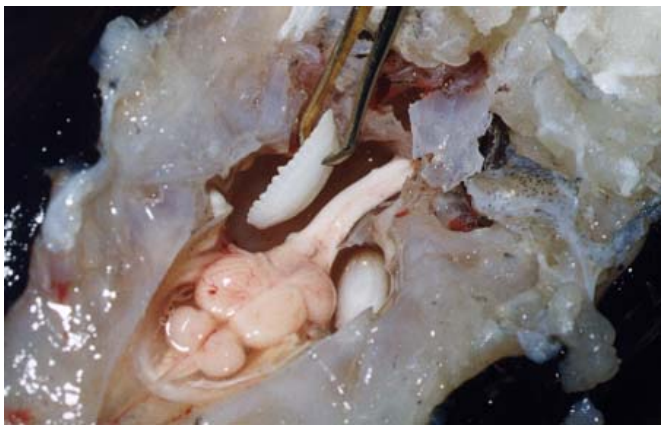
2. Cut thin slices of the forehead from the eyes and backwards

3. The first cut

4. After 2-3 thin slices one can see the brain



5. The brain with membranes and fluid



6. Otoliths are part of the fish vestibular apparatus and reside in the cranial cavity. They are composed of calcium carbonate and protein and are formed by the process of biomineralization.
Otoliths function as sound receptors and are also used by the fish for balance and orientation.
Otoliths can provide useful information on age, growth rate, life history, recruitment, and taxonomy.

Adopted from http://www.miljolare.uib.no/fagstoff/vann/artikler/dyr/marint/torskehode.php (in Norwegian)

6 Recommended web links for additional learning

We recommend you to use Wikipedia to search for most topics. Please, be aware that not all topics necessarily are complete with facts. **http://en.wikipedia.org/wiki/Main_Page**

6.1 Arctic links

UNEP Key Polar Centre and the Arctic
http://polar.grida.no/arctic.cfm

AMAP- the Arctic Monitoring and Assessment Programme
www.amap.no

Links to education within the Arctic theme
· Arctic Theme page- Education
http://www.arctic.noaa.gov/education.html

· **UNEP, Persistent Toxic Substances, Arctic**
http://www.chem.unep.ch/pts/regreports/Arctic%20full%20report.pdf
Main page: http://www.chem.unep.ch/pts/default.htm

Arctic Environmental Atlas
http://maps.grida.no/arctic/

Arctic maps
http://www.athropolis.com/links/maps.htm

Online Map Creation
http://www.aquarius.geomar.de/omc/omc_intro.html

Arctic theme page
http://www.arctic.noaa.gov/maps.html

Arctic Council
http://www.arctic-council.org

WWF Arctic bulletin
http://www.ngo.grida.no/wwfap/core/publications/arctic_bulletin.html#ab

http://sustain.no/projects/globalpop

6.2 Fish biology links

SALMONIDS IN THE CLASSROOM: SALMON DISSECTION
http://www.pskf.ca/sd/

Classroom Salmon Dissection
http://www.sf.adfg.state.ak.us/region2/ie/sicc/dissectn.cfm#external%20anatomy
http://www.sf.adfg.state.ak.us/region2/ie/sicc/pdfs/anatomy.pdf

Fishbase
http://www.fishbase.org/home.htm
http://64.95.130.5/search.php

Salmon dissection game
http://library.thinkquest.org/05aug/00548/DissectionGame.html

Salmon: From Wikipedia, the free encyclopedia
http://en.wikipedia.org/wiki/Salmon
Cod: From Wikipedia, the free encyclopedia
http://en.wikipedia.org/wiki/Cod

6.3 Dioxins and POPs links

What are dioxins?
http://www.greenfacts.org/en/dioxins/l-2/dioxins-1.htm
http://en.wikipedia.org/wiki/Dioxin
http://www.ejnet.org/dioxin/

AMAP
http://www.amap.no
i) Click on: Assessment Results (left margin), Arctic Pollution 2002
ii) Click on: Resources & Projects

UNEP/GPA -What Are POPs?
http://www.chem.unep.ch/gpa_trial/default.htm

Northern Contaminants (credit to Inuit Tapiriit Kanatami)
Short and good overview on contaminants, bioaccmulation and transport:
http://www.itk.ca/environment/contaminants-about.php
http://www.itk.ca/environment/contaminants-sources-pathways.php
http://www.itk.ca/environment/contaminants-wildlife-humans.php
http://www.itk.ca/environment/contaminants-health-risks.php

The 12 POPs under the Stockholm Convention
http://www.pops.int/documents/pops/default.htm

The Arctic Council
http://www.arctic-council.org/index.asp

US EPA- Pollutants/Toxic-Dioxins
http://www.epa.gov/ebtpages/pollutants.html
http://www.epa.gov/ebtpages/pollchemicalsdioxins.html

6.4 Transport links

Pathways of contaminants to the Arctic
http://maps.grida.no/go/graphic/pathways_of_contaminants_to_the_arctic

Dominating air currents
http://maps.grida.no/go/graphic/dominating_air_currents1

The Link between Persistence and Long-Range Transport
http://www.cefic.be/icca/pops/en/pops1002.htm
Persistence *is the ability of a chemical to stay unchanged in the environment for a long time.*
Half-life *is the time it takes for half of the amount of chemical to be removed from the environment.*
Long-Range Transport *is the potential for a chemical to travel from its point of release to a remote region, typically hundreds of kilometres distant*

Transboundary Arctic Contaminants
http://www.nativescience.org/html/contaminants.html

Arctic Pollution: How Much is Too Much?
http://www.carc.org/pubs/v18no3/1.htm

6.5 Some IPY resources and research projects with focus on POPs

The International Polar Year
http://www.ipy.org/

IPY- Opportunities for Teachers and Students
http://www.ipy.org/index.php?/ipy/detail/teacher_opportunities/

IPY-EDUCATORS
http://www.ipy.org/index.php?/ipy/audience/C27/

COPOL
http://www.copol.net/
http://www.ipy.no/prosjekter/COPOL

BEARHEALTH
http://www.biologi.no/bearhealth.htm
http://www.ipy.no/prosjekter/BearHealth

BIRDHEALTH
http://www.birdhealth.nl/

http://sustain.no/projects/globalpop

http://www.ipy.no/prosjekter/Birdhealth

INCATPA: INterContinental Atmospheric Transport of Anthropogenic Pollutants to the Arctic http://www.ipy.org/index.php?/ipy/detail/incatpa/

EBESA: Environmental, Biological, and Ecological Studies in Antarctica
http://www.ipy.org/index.php?ipy/detail/ebesa_environmental_biological_and_ecological_studies_in_antarctica/

POLARCAT
http://www.polarcat.no
http://www.ipy.no/prosjekter/POLAR-CAT

ATMOTROLL
http://www.ipy.no/prosjekter/AtmoTroll