Fish Ecology protocol

Aims of the Fish Ecology protocol are to:

- determine the species spatial distribution (total abundances and biomass) according to environmental factors to develop habitat modelling, community studies and ecosystem modelling
- to gather individual measurements for studying sex and populations differences
- to collect specimens for identification combining molecular and morphology techniques.

1. Ichthyoplankton

All the larval stages

Specific work needs to be done on *Pleuragramma antarcticum* for studying their survival conditions.

a. Early life stages

The larval stages are according to Koubbi et al. (1990).

Eggs / Embryo: spawning to hatching

Stage 1: yolk-sac larvae, hatching to complete absorption of yolk sac.

Stage 2: preflexion larvae: complete yolk sac absorption to start of notochord flexion.

Stage 3: flexion larvae: start of notochord flexion to completion of notochord flexion.

Stage 4: transition larvae: completion of notochord flexion to start of metamorphosis (postflexion done). All fin rays started to formed.

Juvenile: completion of fin ray development and start of squamation to attainment of sexual maturity

b. Pleuragramma antarcticum

i. Morphometrics, diet contents
   30 specimens per net in 5% buffered seawater.

ii. Bar-coding
   70% alcohol for bar-coding identification (at least 10 specimens per developmental stages per species).

iii. Histology
   15 specimens per net in bouin-holland.

iv. Biochemistry
   All the remaining specimens in liquid nitrogen or –80°C.

c. Other species

For the other species, specimens will be preserved:

- in 5% buffered seawater formalin for morphology, identification and diet content studies,
- in 70% alcohol for bar-coding identification (at least 10 specimens per developmental stages per species). Pigments and morphologic description has to be done immediately after preservation following the larval base database (Table I). Drawing and Pictures are necessary for each stage. Description of morphology and pigments are according to Russell (1976) or can be found in the Ichthyoplankton information system of Alaska Fisheries science center http://access.afsc.noaa.gov/ichthyo/use.cfm .

Table I: Exemple of morphologic and pigmentation informations needed for identification of fish larvae http://www.larvalbase.org/

Larvae Information Summary for *Champsocephalus gunnari*							
Main Ref: 41550							
Yolk-sac larvae							
	max	min	mod	Ref.			
Length at birth (mm)							
Preanal L. % TL							
Place of development							
Larval area	Atlantic Antarctic						
Yolk-sac		Ref:					
Yolk		Oil globules					
Post larvae							
Striking feature	teeth clearly visible						
Striking shape lateral		dorsal					
Striking feature	teeth clearly visible						
Shape of gut	triangular						
Gas bladder early		late					
Spinal armature early		late					
Pigmentation early							
Rows on tail	dorsal + ventral row						
Other melanophores on tail	no other melanophores						
Melanophores on head + trunk	melanophores on trunk						
Rows on tail	dorsal + ventral row						
Other melanophores on tail	no other melanophores						
Melanophores on head + trunk	melanophores on trunk						
Peritoneum	with row of melanophores						
Pectorals	normal						
Pelvics	normal (i.e. small or absent)						
Eyes circular, without ventral extension to the orbit; pectoral fin base not stalk-like; pectoral fin base normal and does not extend ventrally below mid-abdomen level; teeth obvious, small in pre-flexion larvae, long and canine-like in later larvae; small pelvic fin in early larvae; a single dorsal and ventral row of melanophores present.							
	L 1st feeding	Ref.	Months of presence of larvae				
max			○ Jan	○ Feb	○ Mar	○ Apr	
min			○ May	○ Jun	○ Jul	○ Aug	
mod			○ Sep	○ Oct	○ Nov	○ Dec	
Water parameters Metric characters							

List of Metric Characters of Solea solea			
Pre-flexion Ref: 38			
(% of reference length)	max	min	literature
Preanal length	51.2	46.5	48.8
Prepectoral length	26.6	29.4	28
Preorbital length	7.9	6.9	7.4
Diameter of eye	5.9	7.4	6.7
Depth at eye	25.1	29.1	27.1
Depth at pectorals	34.3	44.1	39.2
Depth at anus	13.2	11.1	12.2

Reference length (mm)	
early:	4.42
flex:	6.13
late:	7.85
Type:	SL

Flexion			
(% of reference length)	max	min	literature
Preanal length			
Prepectoral length			
Preorbital length			
Diameter of eye			
Depth at eye			
Depth at pectorals			
Depth at anus			

Reference length (mm)	
early:	
flex:	
late:	
Type:	

Post-flexion			
(% of reference length)	max	min	literature
Preanal length			
Prepectoral length			
Preorbital length			
Diameter of eye			
Depth at eye			
Depth at pectorals			
Depth at anus			

Reference length (mm)	
early:	
flex:	
late:	
Type:	

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2. Adult fish

a. Total biomass and abundances

Total biomass and abundance of each species for each net needs to be done after each sample.

30 individuals minimum per net per species will be used for morphometry, determination of the reproductive stage and eventually otoliths and diet contents. These specimens has to be numbered individually.

b. Morphometrics

Total length (TL), Standard length (SL) and Total weight (TW) needs to be collected for a minimum of 30 specimens per species and net.

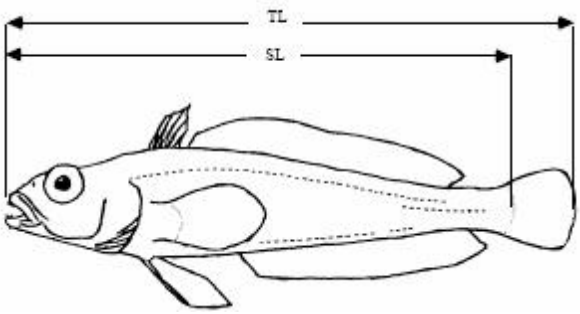

Figure 1: Morphometrics (from CCAMLR). Standard body length measurements of fish: TL - Total Length is from the most anterior part of the snout to the most posterior part of the caudal fin when this fin is extended along the length of the body; SL - Standard Length is from the most anterior part of the snout to the end of the vertebral column (usually marked by a vertical groove in the caudal peduncle when it is flexed).

Each specimen has to be labelled and a picture taken (head on the left, mouth closed, all fins deployed) with a vertical and horizontal scale. Morphometrics will be used to compare populations or doing ecomorphological studies using Truss network or Thin Plate Spline analysis. The specimens used for bar-coding or population genetics should be photographed the same way.
These morphometric techniques are based upon taking co-ordinates of homologous points (points that you can easily recognise on each fish) such as beginning or end of fins, position of the eye, operculum, mouth,…(figure 2).

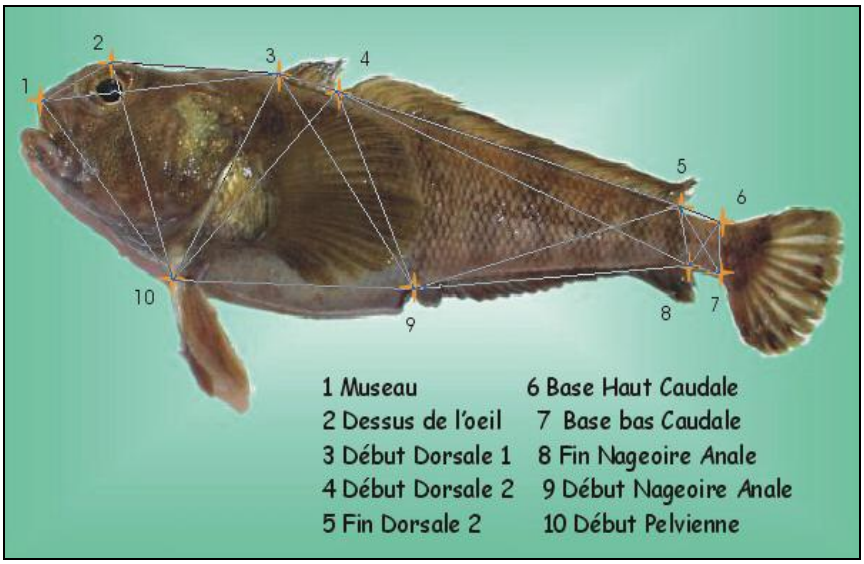

Figure 2 : exemple of truss network and definition of homologous points for a Nototheniidae.

c. Reproduction

Maturity stage of Pleuragramma antarcticum, all Notothenioids and Electrona antarctica needs to be done on 30 individuals per net samples.

*** Macroscopic observations**

Maturity stages scale is from recommandations of CCAMLR for all fishes (Table II). 5 maturity stages are determined.

*** Gonado Somatic Index**

GW: gonad weight (g)

EW: Eviscerated weight (g)
Calculation of GSI (Gonado Somatic Index)%: (GW*100)/EW

*** Fecondity**

Fecondity measurements has to be done on gravid females (stage 4).

Weight the gonad : GW

Weight 1/20 of the gonad. The sample has to be done in the middle of these one. Count the eggs.

d. Diets

Diet contents should be weighted and preserved in 5% buffered seawater formaline.
Stomach repletion should be indicated
0 for empty
1 less than 25% of the volume
2 25 - 50%
3 50 - 75 %
4 75 - 100%

References :

Koubbi P., Duhamel G. & P.Camus, 1990. Early life stages of Notothenioidei (Pisces) from the Kerguelen Islands. *Cybium*, 14(3) : 225-250.

CCAMLR: http://www.ccamlr.org/pu/e/e_pubs/om/toc.htm
Russell, F.S., 1976. The eggs and planktonic stages of British marine fishes.. Academic Press, London, UK. 524 p.

Table II: Maturity stage

Maturity stage	Description
Females	
1. Immature	Ovary small, firm, no eggs visible to the naked eye
2. Maturing virgin or resting	Ovary more extended, firm, small oocytes visible, giving ovary a grainy appearance
3. Developing	Ovary large, starting to swell the body cavity, colour varies according to species, contains oocytes of two sizes
4. Gravid	large, filling or swelling the body cavity, when opened large ova spill out
5. Spent	Ovary shrunken, flaccid, contains a few residual eggs and many small ova
***Electrona antarctica* females**	
1. Immature	Ovaries small and transparent, membrane thin. Maturity index no higher than 1.5%. Oocytes small and transparent having a diameter from 0.25 to 0.3 mm; visible to the naked eye. Oocytes the size of protoplasm and oogonia are visible in histological preparations.
2. Developing	Initially and repeatedly maturing fish. Ovaries more extended yellowish in colour, membrane thin and semi-transparent. Opaque ovarian cells visible - diameter 0.3 to 0.7 mm. Maturity index from 1.5 to 7%.
3. Mature	Ovaries maximum size, yellow in colour, opaque. Maturity index 11 to 14%. As oil droplets and protein granules blend, oocytes become transparent and ovaries become semitransparent. The larger oocytes have a diameter of 1 to 1.2 mm. Apart from the larger and often semi-transparent cells, opaque cells with a diameter up to 0.5 mm are visible.
4. Gravid	Gravid stage.
5. Spent	Appears similar to maturity stage 3, the difference here being a wrinkled and somewhat thicker membrane and also the presence of remaining mature water-filled oocytes in the ovarian cavity.
Males	
1. Immature	Testis small, translucent, whitish, long, thin strips lying close to the vertebral column
2. Developing or resting	Testis white, flat, convoluted, easily visible to the naked eye, about 1/4 length of the body cavity
3. Developed	Testis large, white and convoluted, no milt produced when pressed or cut
4. Ripe	Testis large, opalescent white, drops of milt produced when pressed or cut
5. Spent	Testis shrunk, flabby, dirty white in colour