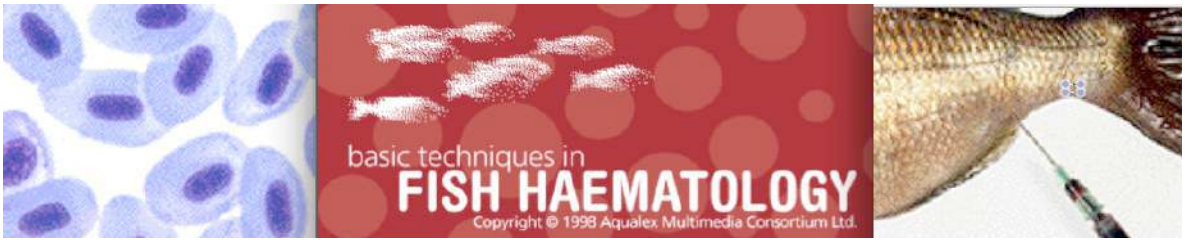
ONLINE eLEARNING UNIT

3 modules

Fish Blood Constituents/Collection of Blood/Haematological Techniques

in

English/French/Spanish/Greek/Norwegian/
Polish/Hungarian/Turkish/Galician



MODULE 1
FISH BLOOD CONSTITUENTS

These modules were written, compiled and designed for use a distance/online learning module that can be used in tutor-led blended learning or in self-directed learning which can take place anywhere, at any time. Further information on the AQUALEX Fish Health Toolset can be found in the Appendix to this module.

MODULE 1
FISH BLOOD CONSTITUENTS

Entry level

No entry level requirements for free access e-learning users

Entry level for blended learning courses to be set by course tutors

For the user

On completion of this module you will be able to:

- ✓ Understand the structure, function, origin and occurrence of red and white fish blood cells
- ✓ Recall details of the structure of red and white blood cells
- ✓ Recognise visual presentations of red and white blood cells
- ✓ Identify all types of red and white blood cells
- ✓ Name all types of red and white blood cells shown in microscope examinations
- ✓ Describe specific functions of individual red and white blood cells.

You will be able to:

- ✓ Include this in your EUROPASS, including EUROPASS Digital Credentials (https://europa.eu/europass/en/europass-digital-credentials) This will also help you to draw up your EUROPASS CV.
- ✓ Include these skills in browsing the ESCO list of skills, competences and knowledge, while searching for job opportunities throughout Europe. (https://ec.europa.eu/esco/portal/home)

Details on the **Toolset basic language syllabus** are at the end of this module, after the Module Glossary.

For the evaluator/assessor/teacher

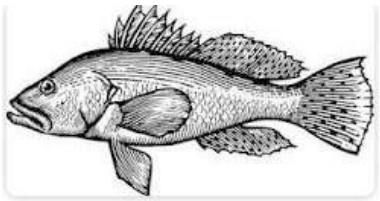
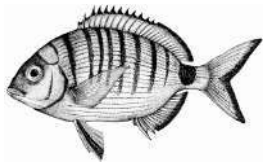
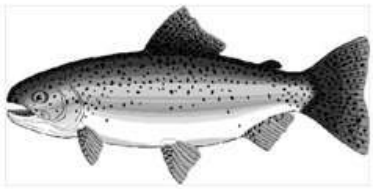
This module is equivalent to EQF Intermediate Level, requiring the student

- ✓ to have basic general knowledge of the subject
- ✓ to be able to recall general information
- ✓ to be able to explain factual knowledge.

Introduction to the module

The regular monitoring of fish blood is a very useful diagnostic tool in establishing the health status of fish farm stocks. This eLearning Unit is concerned with the basics of fish haematology. It comprises 3 modules: constituents of fish blood (**Module 1**); shows examples of the different techniques used in taking blood samples (**Module 2**); and in the examination of examining blood samples (**Module 3**).

Every effort has been made to ensure that comparable data from representative species are consistently presented. Therefore reference will be made throughout to the same species of fishes when indicating figures and proportions of blood cell volumes and constituents. These species are shown below.

Sea Bass (*Dicentrarchus labrax*)	
White Bream (*Diplodus sargus*)	
Saupe (*Sarpa salpa*)	
Rainbow Trout (*Oncorhynchus mykiss*)	

Nevertheless, it has not proved possible to keep strictly to these species because of the difficulty of obtaining blood profiles for single species of fish. Also, different authors have reported varying figures for the same species. In many cases, this variation accurately reflects the innate variation in blood parameters between fish of different size, age, and health status and also fish at different stages of sexual maturity. Where data for one of the above-named species are not available, different, though still relevant, examples have been given.

Definitions of all terms highlighted in bold can be found in English in the glossary at the end of the module; ***multilingual definitions*** *can be accessed in the AQUALEX FISH HEALTH ONLINE TOOLSET* http://www.aqualex.org/index.php/glossaries

BLOOD

Tissue composed of circulatory cells in a liquid medium, plasma, or in the case of some invertebrates, haemolymph. The role of blood is to carry oxygen, food-materials and excretory products through the body to different organs and tissues. It usually contains respiratory pigments. Blood is circulated by the muscular action of vessels or specialised organs (hearts).

PLASMA

Plasma is approximately 90% water and 10% dissolved organic and inorganic compounds.
The organic materials include **proteins** such as globulins (alpha, beta, gamma and immunoglobulins), **albumins**, **clotting factors** and **antibodies**.
Serum is a straw-coloured liquid which remains after blood has clotted. Its composition is therefore the same as that of plasma, but without the clotting factors.

CELLS

Blood cells of fishes are categorised into two main groups.
Erythrocytes (red blood cells)
Leucocytes (white blood cells)

Erythrocyte

The term erythrocytes refers collectively to those nucleated blood cells which carry the red-pigmented protein **haemoglobin**. Erythrocytes differ from leucocytes, and their primary function is in the transport of gases throughout the body.

Leucocyte

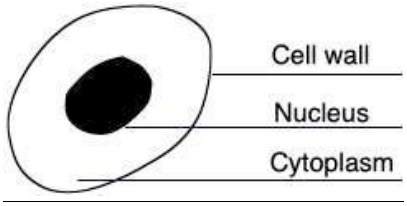
The term leucocytes refers collectively to those non-pigmented, nucleated blood cells whose primary function is to combat infection and in some cases to **phagocytose** and digest debris. They differ from erythrocytes, in that they leave the vascular system to carry out their tasks by passing through the walls of small blood vessels.

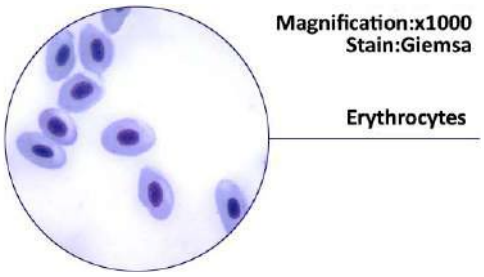
ERYTHROCYTES (red blood cells)

Structure

Fish erythrocytes are elongated, elliptical, cells with an oval, centrally-located nucleus.

Mature fish erythrocyte





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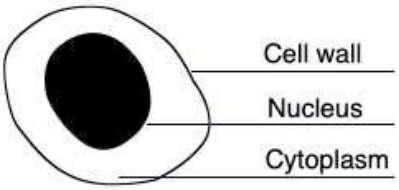
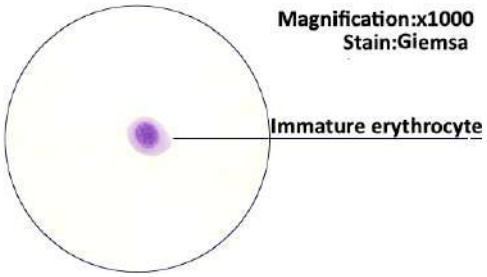
Microscope view with labels, stain and magnification(¨1000)

Immature erythrocytes are called **polychromatocytes.**

Immature fish erythrocyte





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Microscope view with labels, stain and magnification(¨1000)

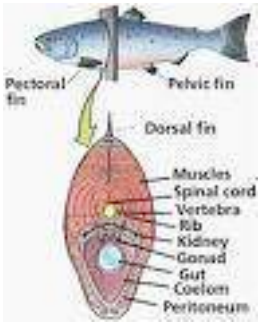
Function

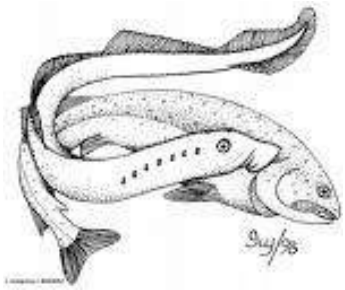
Erythrocytes function in the transport of oxygen and, to a much lesser degree, carbon dioxide. Molecular oxygen is carried on molecules of **haemoglobin** in the cells.
Haemoglobin is a large protein which (except in the primitive jawless fishes) consists of four smaller protein subunits, two alpha chains and two beta chains, each of which carries one molecule of oxygen. These peptides are bound together by four haeme rings, and an iron atom is bound in the centre of each haeme ring.

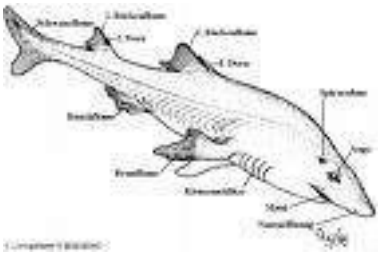
Origin

Erythrocytes are formed from embryonic cells in the peripheral blood, called erythroblasts. These erythroblasts undergo successive **mitoses**, and develop increasing amounts of haemoglobin, finally giving rise to fully differentiated erythrocytes.
In the more advanced bony fishes, Osteichthyes, the **kidney** is where **erythropoieses** takes place, while in the more primitive jawless fishes, Agnatha, and cartilaginous fishes, Chondrichthyes, the blood is a major site of erythrocyte synthesis.

Osteichthyes **Agnatha** **Chondrichthyes**







Occurrence

Erythrocytes are the predominant blood cell type in fish.
There is considerable variation in their numbers in different species of fish. There may also be variation within species, depending on health status.
For example, in Rainbow Trout (*Oncorhynchus mykiss*), erythrocyte count may vary from 0.77 to 1.58 x 10^6cells /mm^3.

Percentage of fish blood cells accounted for by erythrocytes	
Sea Bass (*Dicentrarchus labrax*)	96.5% of blood cells
White Bream (*Diplodus sargus*)	96..5% of blood cells
Saupe (*Sarpa salpa*)	98% of blood cells

LEUCOCYTES (white blood cells)

Leucocytes fall into **four** main categories: **granulocytes, lymphocytes, monocytes** and **thrombocytes**, each with their own specific features and it is therefore difficult to provide a detailed description of leucocyte features without referring to each cell type individually.
In fish, the overall number of leucocytes varies in number; for example, the normal range of lymphocytes, the predominant type of leucocyte, in the salmonid, Rainbow Trout (*Oncorhynchus mykiss*), is between 7.8 and 20.9 x 10^3 cells per mm^3.

White blood cells account for a small proportion of all circulating blood cells.

Percentage of fish blood cells accounted for by leucocytes	
Sea Bass (*Dicentrarchus labrax*)	3.5% of blood cells
White Bream (*Diplodus sargus*)	3.5% of blood cells
Saupe (*Sarpa salpa*)	2% of blood cells

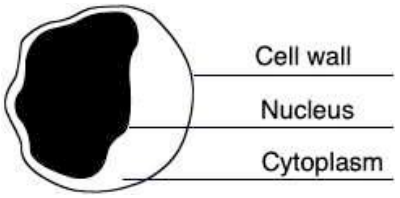
In higher vertebrates such as mammals, formation of white blood cells is restricted to bone marrow, the spleen and the lymph nodes. In fishes, organs such as the **kidney**, **spleen** and **thymus** take part in **haematopoeisis**.

GRANULOCYTES

Structure

These cells are have a distinctive structure and are sometimes referred to as Polymorphonuclear (PMN) leucocytes. The cytoplasm contains numerous fine granules.
Granulocytes are assigned to different sub-populations depending on the staining characteristics of these granules in smear preparations with histological dyes (Romanowsky dyes).
In fish, granulocytes are of three types: **neutrophils** and **eosinophils** are the most common while **basophils** are much rarer. It is thought that basophilic granulocytes are not found in salmonids.

Granulocyte



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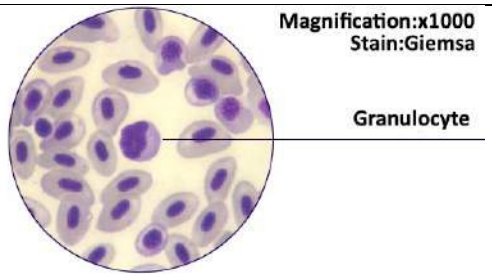


Microscope view with labels, stain and magnification(¨1000)

Function

Granulocytes are involved in non-specific defence mechanisms, i.e. they respond to the presence of foreign material in the body but do not recognise specific **antigens**. These cells migrate to parts of the body where invasion occurs and destroy the foreign particles by **phagocytosis** or by direct killing known as the cytotoxic response. This process is termed the **inflammatory response.**

Origin

Granulocytes are formed from embryonic cells called granuloblasts in the haemopoietic tissues of the **kidney**.

Occurrence

Granulocytes may account for 4-60% of leucocytes in fish, and there is considerable **variation in the numbers** of granulocytes present in different species of fish.
Granulocytes are thought to account for 1-9% of the total white blood cells in juvenile Rainbow Trout (*Oncorhynchus mykiss*).
It should be noted that different authors have reported varying figures for the same species.

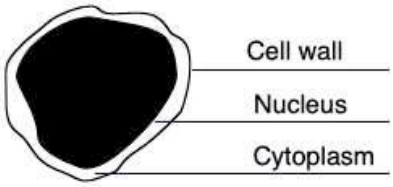
Percentage of white blood cells/all blood cells accounted for by granulocytes		
	White blood cells	All blood cells
Sea Bass (*Dicentrarchus labrax*)	8%	0.28%
White Bream (*Diplodus sargus*)	28%	0.98%
Saupe (*Sarpa salpa*)	12%	0.24%

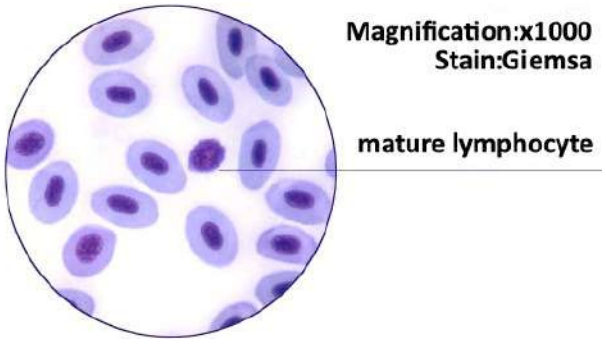
LYMPHOCYTES

Structure

Lymphocytes are spherical cells.

Mature lymphocyte





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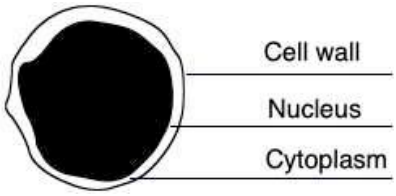
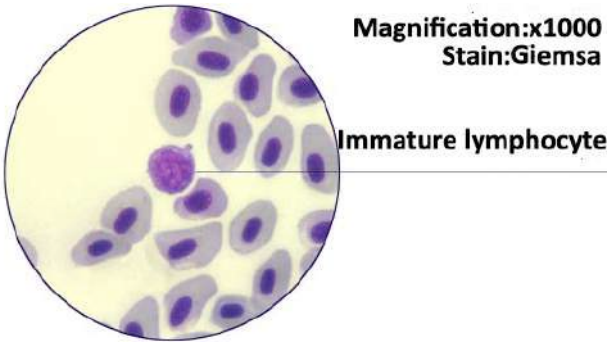
Microscope view with labels, stain and magnification(¨1000)

Larger **immature lymphocytes** are sometimes also seen.

Immature lymphocyte





Line drawing

Microscope view with labels, stain and magnification(¨1000)

Function

There is some evidence that fish lymphocytes are differentiated into at least two functional sub-populations with functions similar to those of **B** and **T lymphocytes** in mammals. The function of lymphocytes is to mediate the **humoral** and **cellular immune response**.
On making contact with foreign material (**antigen**) there is a proliferation of lymphocytes, which then secrete large quantities of **immunoglobulin** antibody**.**

Origin

Thymocytes which originate in the **thymus** give rise to T-lymphocytes. Large numbers of small lymphocytes are also found in the **kidney** and **spleen** and it is thought that circulating lymphocytes are of two types: T-cells of thymic origin, and B-cells which are believed to originate in the kidney.

Occurrence

The proportion of leucocytes which are lymphocytes may be as high as 85% in some fish species. Lymphocytes comprise 89- 98% of leucocytes in juvenile Rainbow Trout (*Oncorhynchus mykiss*).
BUT it is difficult to estimate the numbers of lymphocytes in fish blood accurately as it is often difficult to distinguish them from thrombocytes.

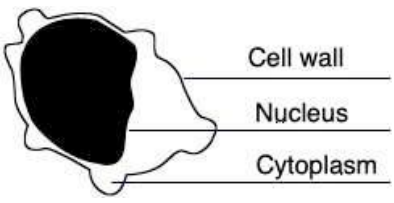
Percentage of white blood cells/all blood cells accounted for by lymphocytes		
	White blood cells	All blood cells
Sea Bass (*Dicentrarchus labrax*)	90%	3.15%
White Bream (*Diplodus sargus*)	70%	2.45%
Saupe (*Sarpa salpa*)	88%	1.76%

MONOCYTES

Structure

Monocytes are large cells with a large nucleus, occupying between one third and one half of the cell. The cytoplasm contains small scattered granules.

Monocyte



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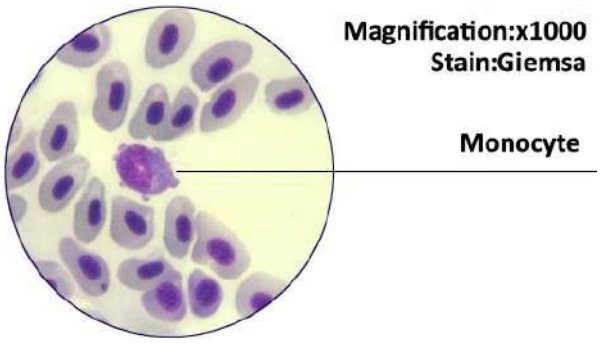
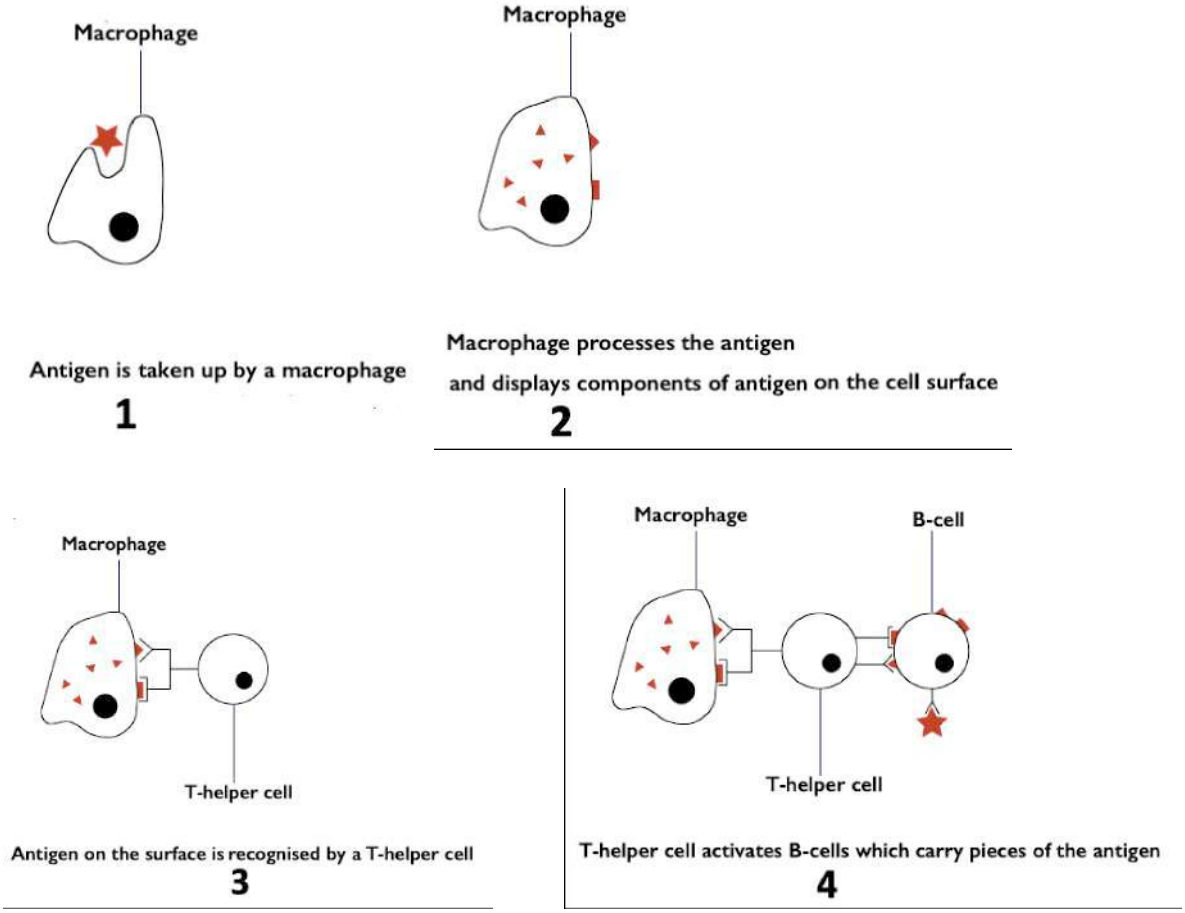


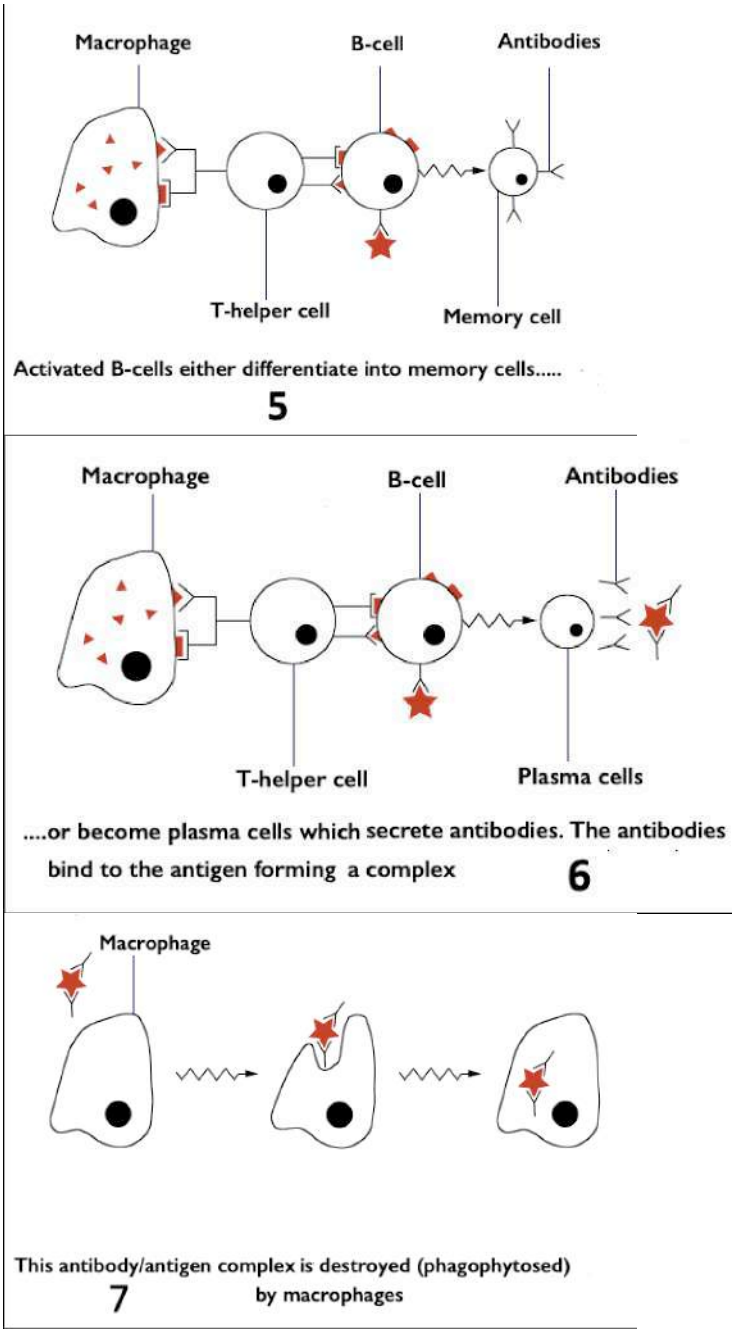
Microscope view with labels, stain and magnification(¨1000)

Function

Monocytes are the precursors of **macrophages**. Monocytes function by responding to infection. Thus monocytes like granulocytes play an important role in non-specific immunity and the **inflammatory response**.
However monocytes are much more phagocytic than granulocytes. Animated infographic here: infographic here
https://youtu.be/B-46wsm9uec

Function





Origin

Monocytes are also present in large numbers in the **kidney**, suggesting that they are derived from stem cells in this organ.

Occurrence

In fish, the blood contains variable numbers of monocytes, but generally the numbers are low. For example in Plaice they account for only 0.1% of the total number of leucocytes. In Catfish however they are more numerous, making up 1-8% of the leucocytes. Monocytes are considered to be quite rare in the circulating system in Rainbow Trout (*Oncorhynchus mykiss*).

Percentage of white blood cells/all blood cells accounted for by monocytes		
	White blood cells	All blood cells

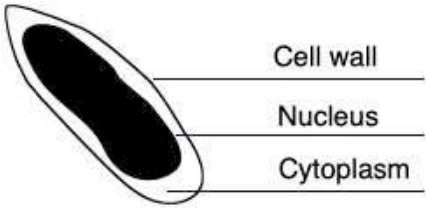
Sea Bass (*Dicentrarchus labrax*)	2%	0.07%
White Bream (*Diplodus sargus*)	2%	0.07%
Saupe (*Sarpa salpa*)	0%	0%

THROMBOCYTES

Structure

There are four morphological forms of thrombocytes which are commonly seen: **oval, spindle-shaped, spiked** and **fragmented**. The **oval or spindle-shaped** cells are thought to be the normal forms of thrombocytes *in vivo*, and these are easily confused with lymphocytes because they look very similar. Thrombocytes also sometimes appear in preparations as **spiked** or **fragmented** forms.

Oval thrombocyte



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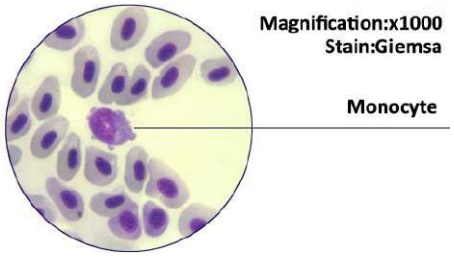
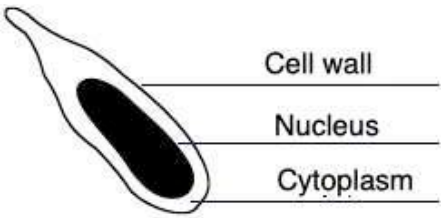


Microscope view with labels, stain and magnification(¨1000)

Spindle thrombocyte



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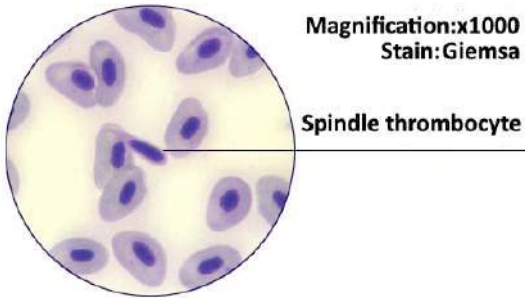
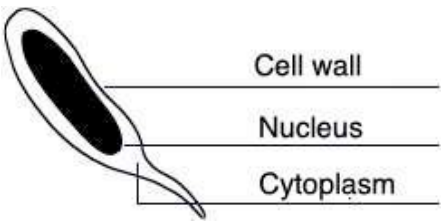
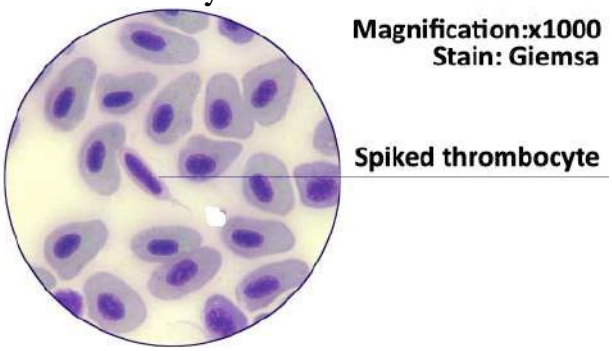


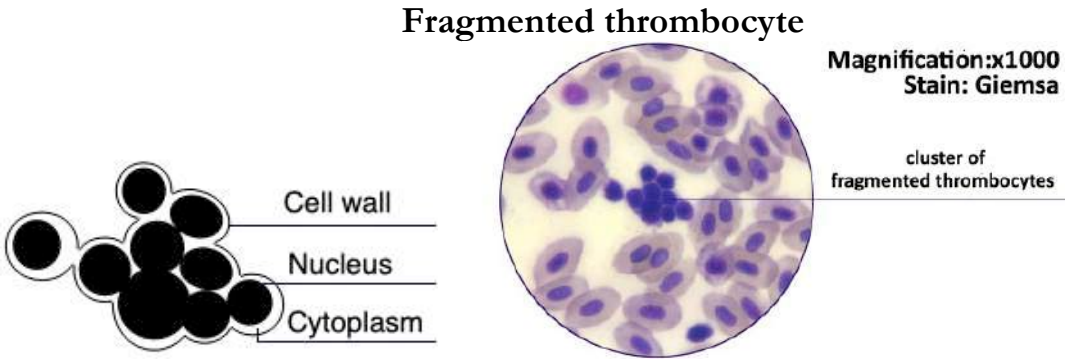
Microscope view with labels, stain and magnification(¨1000)

Spiked thrombocyte





Line drawing Microscope view with labels, stain and magnification(¨1000)



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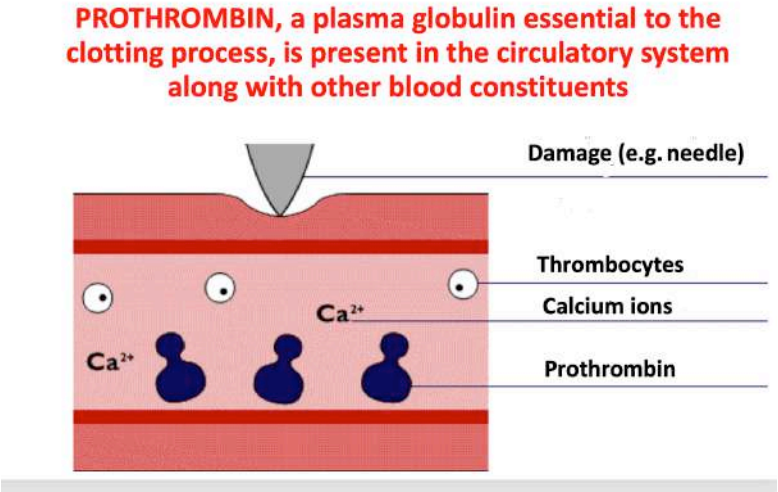
Function

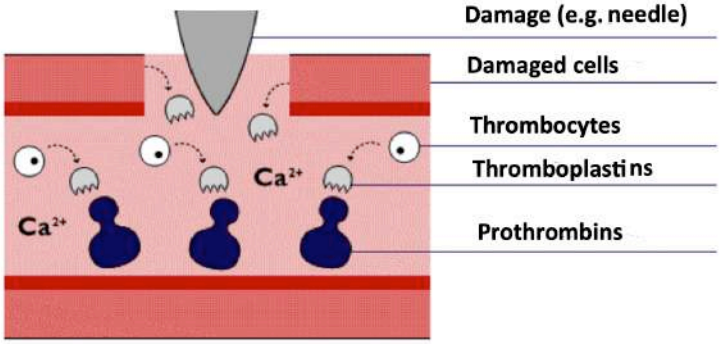
Thrombocytes play a part in the **clotting of blood**. Their activities during clotting have been observed *in vitro*.

Clotting of blood animation https://www.youtube.com/watch?v=0--Wm7ye22Q
Illustrations below
1.



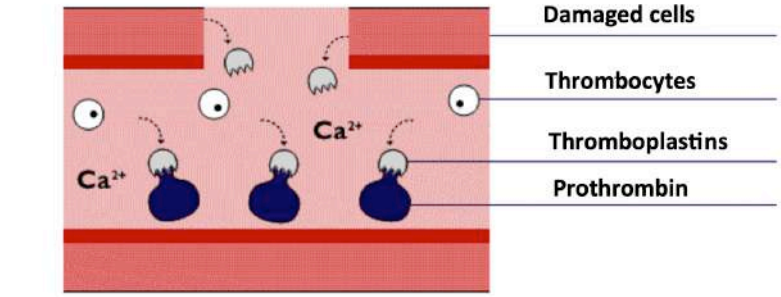
2.

PROTHROMBIN splits into several fragments, one of which is thrombin, by the actions of thromboplastins



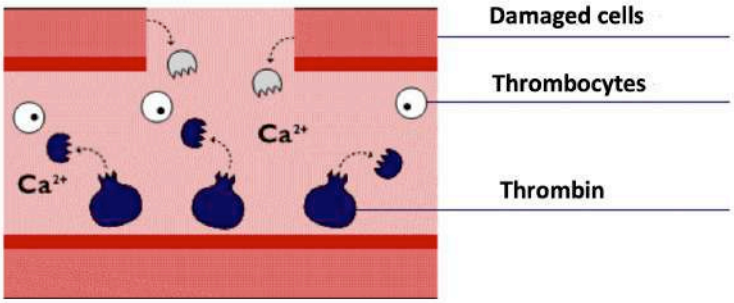
3.

When blood cell is ruptured, damaged cells and thrombocytes release THROMBOPLASTINS



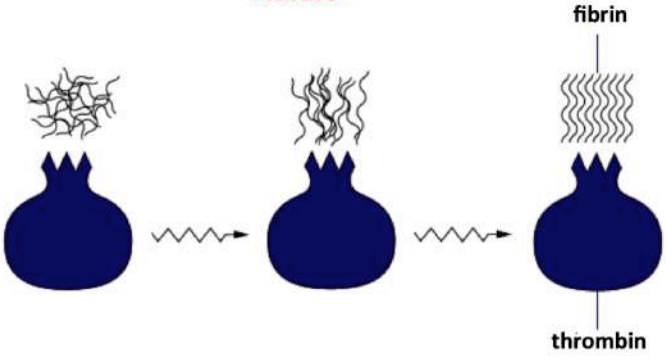
4.

When thromboplastins and calcium ions are present PROTHROMBIN is converted to THROMBIN



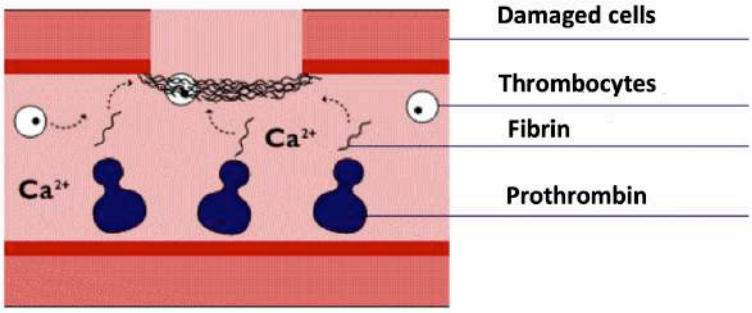
5.

Thrombin then reacts with soluble FIBROGEN in the plasma to form FIBRIN



6.

FIBRIN and cell elements form the blood CLOT which constricts into a compact mass



Origin

The source of thrombocytes remains to be established. Some workers suggest that they derive from the same cells as lymphocytes, and others that they are actually descended from small lymphocytes. However, thrombocytes are seen in large numbers in the **spleen** suggesting that the main source of these cells is splenic tissue.

Occurrence

In mammals, thrombocytes are the precursors of cells called platelets, which circulate in the blood and are responsible for clotting. Mammalian thrombocytes are seldom found in the circulation. In fish however, unlike mammals, thrombocytes circulate in the blood.
There is disagreement over the numbers of thrombocytes present in the peripheral blood of fish, which could arise because of the ease with which they can be mistaken for lymphocytes.

Disagreement
In juvenile Rainbow Trout (*Oncorhynchus mykiss*), thrombocytes account for 1-6% of total leucocytes.
The ratio of lymphocytes to thrombocytes in Rainbow Trout has been measured as 25:1; however for the same species a ratio of 2:1 has also been measured. Conversely a ratio of lymphocytes to thrombocytes in Plaice *(Pleuronectes platessa)* of 1:3 has been reported.
For the percentage of leucocytes comprised of thrombocytes in the Carp (*Carassius auratus),* a figure of 70% has been obtained by some researchers, while others have given a figure of just 3-13%.

UNIT SPECIFIC GLOSSARY

Protein
Complex, naturally-occurring, polymers comprised of amino acids, joined by peptide linkages.

Albumins
Group of several small proteins, forming a large part of the plasma protein content. They are responsible for the transport of free fatty acids.

Clotting factors
Proteins in the blood which are involved in the complex process of converting soluble fibrinogen into fibrin.

Serum
The fibrinogen-free fluid fraction of blood or haemolymph. In vertebrates, the blood is first allowed to clot (i.e. fibrinogen is converted to insoluble fibrin) and then centrifuged to remove blood cells and fibrin.

Haemoglobin
Red-pigmented protein occurring in the blood cells of vertebrates and elsewhere in the animal kingdom. Haemoglobin combines readily with oxygen to form oxyhaemoglobin responsible for transferring oxygen through the blood system.
Haemoglobin is made up of the colourless protein globin, and the red-yellow pigment haeme which contains iron. Oxygen is transported in combination with the ferrous iron of the haeme.

Phagocytosis
The intracellular uptake of solid particles by cells, either for nutritional purposes (e.g. food), or in the case of phagocytes such as macrophages and granulocytes as a defence mechanism (foreign bodies).

Kidney
Organ of excretion and water regulation in vertebrates, consisting of numerous nephrons and their blood supply. The kidney also functions in formation of blood cells in some animals including the bony fishes.

Erythropoeisis
The term **erythropoeisis** refers to the formation of red blood cells, or erythrocytes.

Spleen
Organ comprising a mass of lymphoid tissue in the mesentery; unlike lymph nodes it is interposed in the blood circulation.

Thymus
A paired lymphoid gland situated dorso-laterally in the gill chamber. The site of T-lymphocyte production, it is regulated by hormones produced by thymic epithelial cells.
In fish (and in all vertebrates) the thymus gradually atrophies after the onset of sexual maturity but does not completely disappear.

Haematopoeisis
Haematopoeisis is a general term referring to the formation of all types of blood cells, a process occurring in the haematopoeitic tissue.

Neutrophil
A leucocyte having no affinity for acid or basic dyes, but stainable by neutral dyes. The most abundant type of leucocyte; they are able to move out of the blood and into the tissues of the body to engulf bacteria wherever they invade.

Eosinophilic structure
A special white blood cell (polymorphonuclear leucocyte) that can be stained with acid dyes such as eosin. These cells are involved in destruction of internal parasites and in the modulation of allergic inflammatory reactions.

Basophil
A substance or tissue element (e.g. white blood cell) showing an affinity for basic dyes; (e.g. granulocytes that can digest micro-organisms). Their numbers are normally very low in blood. There is some question as to whether such cells are present in fish.

Antigen
Any agent which can elicit an immune response. Antigen may refer to an individual macromolecule or to a homogeneous or heterogeneous population of antigenic macromolecules. A given antigen usually contains a number of sites where combination with various antibodies may occur (determinants). An antigen may be soluble (e.g. microbial toxins, extracts) or particulate. The most effective antigens are proteins and polysaccharides. The surface of a microorganism typically consists of repeating patterns of antigens, and the classification of some groups of microorganisms is based on differences between the antigens of different strains.

Inflammatory response
The reaction of the tissues to injury or presence of any antigen characterised clinically by heat, swelling, redness and pain and pathologically by vaso-dilation, hyperaemia, accumulation of leucocytes, exudation of fluid and deposition of fibrin. This reaction is termed the inflammatory response.

B Lymphocytes function
B-LYMPHOCYTES: White blood cells of the immune system derived from bone marrow and involved in the production of **antibodies**.

T Lymphocytes function
T-LYMPHOCYTES: Lymphoid cells whose development depends on the presence of the thymus; responsible for cell mediated immunity.

Humoral response
Any immune response mediated by antibodies in the circulation.

Cellular immune response
Any immune response mediated by the cellular components of the immune system.

Antibody
A specific immunoglobulin molecule produced by vertebrates in response to an antigen; Antibodies are produced by B-lymphocytes and plasma cells and exhibit specific binding properties for the antigen which has caused their production; confers protection against infective agents exhibiting this antigen.

Immunoglobulin
Glycoproteins having the ability to bind antigens with a high degree of specificity.

MacrophageA cell capable of ingesting bacteria, foreign particles, and other cells. There are (i) microphages (neutrophil leucocytes) which are mobile and (ii) macrophages which are immobile or sessile (e.g. endothelial cells, mono- and histocytes).

APPENDIX

AQUALEX Fish Health Toolset: the story

WHY create the Toolset?

We, the AQUALEX Multimedia Consortium (AMC), decided to create the AQUALEX Fish Health Toolset so that basic essential factual knowledge about certain aspects of **fish health** (important for European aquaculture) could be combined with fast-track multi-lingual learning. Successful EU competitive projects had given us the impetus (and the funds) to create a wide range of individual multilingual materials: 3 multilingual Glossaries in 7 languages, language learning units at 3 levels in 10 languages, and fish health course materials also in 10 languages. We brought these separate elements together first as a Concept, then as a working Toolset freely available as **multilingua**l **units/modules** in user-friendly online learning formats.

WHAT is covered in the Toolset

Both the species and the fish health aspects covered result from several European-wide needs analyses which targeted industry and academic users including VET providers. These are:

- Basic Techniques for Fish Haematology
- Fish Health Management Manual
- Aquatic Pathology for rainbow trout, carp, sea bass and turbot
- Fish, shellfish and crustacean meristics (in construction)

HOW to use the Toolset

The Toolse**t** links three different **content areas** and two **language level**s (Beginner: CEFR A1 &A2 and Basic: CEFR A1 & A2). Because the Fish Health content is freely accessible online, you can find the information/content you need, whenever and wherever you need it, whether studying or in the workplace. You can access the **Fish Management Toolset** and try it out via the following link http://www.aqualex.org/index.php/pescalex-courses,

The **3 updated multilingual glossaries (AQUALEX, PESCALEX, MARPOL)** provide high-quality terms and definitions of, respectively, aquaculture, fish diseases and marine pollution terminology (3500 items variously in English, French, German, Greek, Spanish, Italian, Norwegian, Polish, Hungarian, Turkish, Swedish and Galician). Unique multilingual access at a single click. http://www.aqualex.org/index.php/glossaries

The Toolset**'s** language **learning modules** are very helpful for workers in aquaculture, because they exist in languages important in the European aquaculture industry (English, French, Spanish, Norwegian, Greek, Polish, Portuguese, Hungarian, Turkish, Swedish and Galician). The modules can be accessed online at http://www.aqualex.org/index.php/multilingual-esp-language-courses. Syllabus details are given below**.**

WHEN to use the Toolset

The Toolset helps you to communicate in your chosen language through the online modules. Its unique multilingual combinations give you the chance to learn a language

while also acquiring essential basic **Fish Health conten**t in English, French, Spanish, Norwegian, Greek, Polish, Portuguese, Hungarian, Turkish, Swedish and Galician.

A final note

How NOT to use the Toolset

The AQUALEX Toolset materials should not be regarded or used either wholly or in part as a comprehensive fish health manual. There are many such specific and easily accessible publications which are both reliable and comprehensive.

How to use the AQUALEX online language lessons

http://www.aqualex.org/index.php/multilingual-esp-language-courses

The AQUALEX language lessons are designed for **COMPLETE BEGINNERS in English**, whose first/native languages are French, Spanish, Greek, Norwegian, Polish, Portuguese, Swedish, Hungarian, Turkish and Galician.
These online language learning lessons work well as a support for tutor-led blended courses. They will help complete beginners in each language to learn basic grammar points and important aquaculture keywords in English.
They are NOT a complete online course. They simply give basic grammar points within a vocational context (aquaculture). The online format is designed to give beginners a chance to understand and handle simple sentence with essential keywords.
Because English is still the most popular choice for a second language, English grammar points are explained, in English and in the user's language. Where the user language is different from English (i.e., French masculine and feminine nouns), then explanations of the user language structures are given in both English and the user language.

- Level 1 lessons (14) for beginners with no previous knowledge of the target language.
- Level 2 lessons (10) for those with some knowledge, though still at the basic level.
- You can enter each level via the dropdown menus at the top of the page.
- You can enter the lessons (English, French, Galician, Greek, Hungarian, Norwegian, Polish, Portuguese, Swedish, Turkish) by clicking on the country flags as shown.

Each lesson is organised into TEXT, GRAMMAR, HOMEWORK and ASSESSMENT with clickable menus at the top of each page. The ASSESSMENT section is not available to the general public.

- The TEXT menu contains the lesson itself, which may have 5 pages.
- Each page of the lesson can be entered via the NEXT and PREVIOUS live links at the bottom of the page.
- The GRAMMAR section can be entered via the top dropdown menu. Each page of the grammar can be entered via the NEXT and PREVIOUS live links at the bottom of the page.
- The HOMEWORK section cannot be entered until all the TEXT and the GRAMMAR pages have been viewed, in order to consolidate language acquisition.
- All TEXT pages are inter-changeable in all AQUALEX languages, by clicking on the country flag at the bottom of the page.
- **BUT** Grammar and Homework sections are specific to each language and do not have an interactive function.

*TEXT pages also contain **audio material** which can be heard by running the mouse over the words on the page. Click on live items to hear the term as recorded by native speakers (make sure you have the necessary software for this facility (i.e., Quicktime Player).*

In Level 1 you learn:

The use of

- ✓numbers (lesson 1)
- ✓definite article (lesson 1)
- ✓indefinite article the/a/an (lesson 2)
- ✓demonstrative pronouns this/that/these/those (lesson 2)
- ✓singular/plural nouns (lessons 1,3)
- ✓irregular plurals (lessons 3.4)
- ✓countable /uncountable nouns (lesson 6)
- ✓agreements (subject/verb) (lessons 11-13)

how to make statements (lesson 1)

- ✓how to make negative statements (lesson 2)

how to make simple measurements

- ✓temperature (lesson 9)
- ✓length, breadth, width, height (lesson 10)
- ✓volume (lesson 10)

the use of prepositions

- ✓of place (lesson 8)
- ✓of time (lesson 9)

- ✓names of days (lesson 13)
- ✓names of months (lesson 14)

In Level 2 you learn:

- ✓more adjectives and adverbs (lesson 2)
- ✓comparisons (lesson 3)
- ✓pronouns (lesson personal, relative)4)
- ✓Imperative (lesson 5)
- ✓past tense (lesson 6)
- ✓future tense (lesson 7)
- ✓conditionals (lesson 7)
- ✓modals/gerundive (lesson 8)
- ✓passive (lesson 9)
- ✓many, some, few, a lot of, more (lesson 6)

the use of verbs

- ✓is, are (lesson 1)
- ✓has, have (lesson 2)
- ✓this is, there are (lessons 2, 3)
- ✓present tense (forms and functions) (lessons 11, 12, 13)

- ✓how to ask and answer questions (lesson 2)
- ✓true/false response (lesson 4)
- ✓how to tell the time (lesson 6)

Language attainment levels

Level 1 (CEFR) levels A1, A2)

The priority for many **first-time language learners** is to understand and convey simple but vital pieces of information (i.e., keywords) in a new language. The AQUALEX online language lessons in English, French, Spanish, Greek, Norwegian, Polish, Hungarian, Turkish, Portuguese, Swedish and Galician are designed to allow complete beginners to build on their native language knowledge of familiar items in the workplace/laboratory, in a step-by-step visual presentation with audio input. This method gives them a chance to fast-track their learning, at their chosen time and at their own speed.

Level 2 (CEFR levels B1, B2)

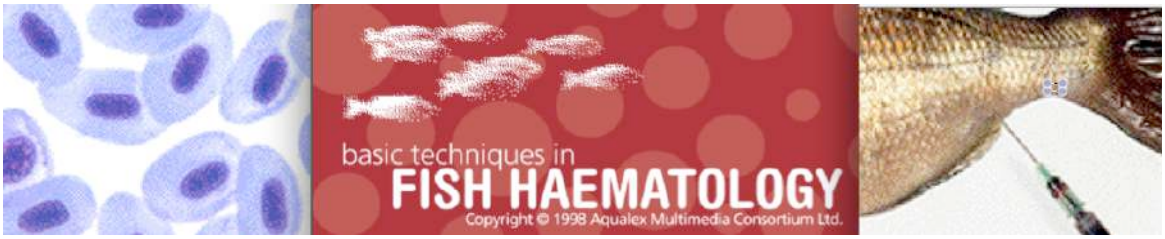
Having picked up the first essentials in a user-friendly way, **students or workers** requiring vocationally relevant fish health information can progress at their own pace of learning through the Toolset Fish Health multi-lingual course materials (shown above) in English, French, Spanish, Greek, Norwegian, Polish, Hungarian, Turkish and Galician This can be done online at http://www.aqualex.org/index.php/multilingual-esp-language-courses

Level 3 (CEFR levels C1, C2)

For the seasoned practitioner, Ph.D. student or academic, the AQUALEX Toolset contains two **multi-lingual aquaculture and fish diseases glossaries** in English, French, German, Spanish, Italian, Greek, Norwegian, Polish, Hungarian, Turkish and Galician. These online resources present high-level information and detailed definitions in the accepted academic format.

ONLINE eLEARNING UNIT

3 modules
Fish Blood Constituents
Collection of Blood
Haematological Techniques
in
English/French/Spanish/Greek/ Norwegian/
Polish/Hungarian/ Turkish/Galician



MODULE 2
COLLECTION OF FISH BLOOD

The module was written, compiled and designed for use as a distance learning module that can be used in tutor-led blended learning or in self-directed learning which can take place anywhere, at any time.

More detailed information on the AQUALEX Fish Health Toolset can be found in the Appendix to this module.

MODULE 2
COLLECTION OF FISH BLOOD

Entry level

- No entry level requirements for free access e-learning users
- Entry level for blended learning courses to be set by course tutors

For the user

On completion of this module you will be able to understand how to collect blood by:

i) severing the caudal peduncle
ii) puncturing the caudal vessel
iii) performing a cardiac puncture
iv) performing a dorsal aorta puncture

You will be able to include this in your EUROPASS, including EUROPASS Digital Credentials (https://europa.eu/europass/en/europass-digital-credentials) This will also help you to draw up your EUROPASS CV.

You will be able to include these skills in browsing the ESCO list of skills, competences and knowledge, while searching for job opportunities throughout Europe. (https://ec.europa.eu/esco/portal/home)

For the evaluator/assessor/teacher

This module is equivalent to EQF Intermediate Level, requiring the student to: have basic general knowledge of the subject, be able to recall general information, and to be able to explain factual knowledge. Further details in the Appendix to this module.

Introduction

The regular monitoring of fish blood is a very useful diagnostic tool in establishing the health status of fish farm stocks.
There are several different techniques which can be used to collect blood samples from fish. Blood may be taken by using the following methods.

1. Severing the caudal peduncle
2. Puncturing the caudal vessel
3. Cardiac puncture
4. Dorsal aorta puncture.

These are four of the commonest techniques for extraction of blood samples from fish. **T**here are more advanced techniques:

- puncturing the common cardinal vein (duct of Cuvier)
- cannulisation, which allows repeated extraction of blood from a single fish through a cannula which is surgically inserted, usually through the gills of the fish.

Generally whenever blood samples are taken, the tubes and syringes to be used should be treated with an **anticoagulant** such as heparin to prevent clotting.

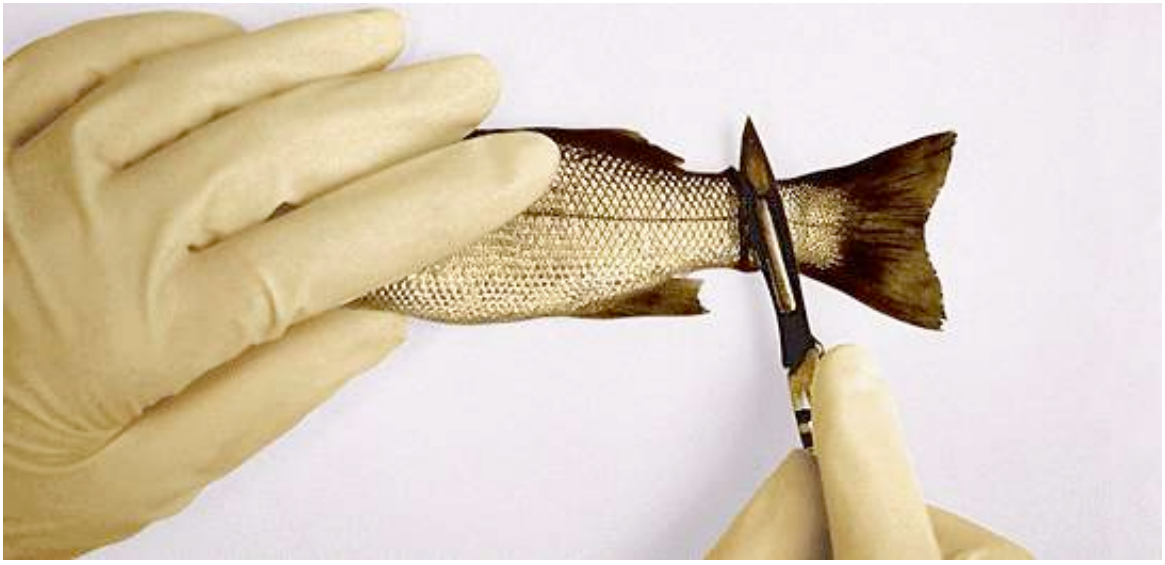
But when serum is required this is not necessary as the blood is first allowed to clot (i.e. fibrinogen is converted to insoluble fibrin) in tubes and is then centrifuged to remove blood cells and fibrin.

Severing Caudal Peduncle: 1.1

This sampling technique is suitable for smaller fish (eg. less than 10 cm), and in many cases may be the only option because the blood vessels are small and blood volumes are very low. It should be noted that it is sometimes quite difficult to collect blood efficiently using this method. As coagulation tends to occur rapidly, the blood sample must be collected immediately after the tail is severed.
In this procedure the fish is killed to obtain the sample (the term “sacrifice” is used to describe this act).

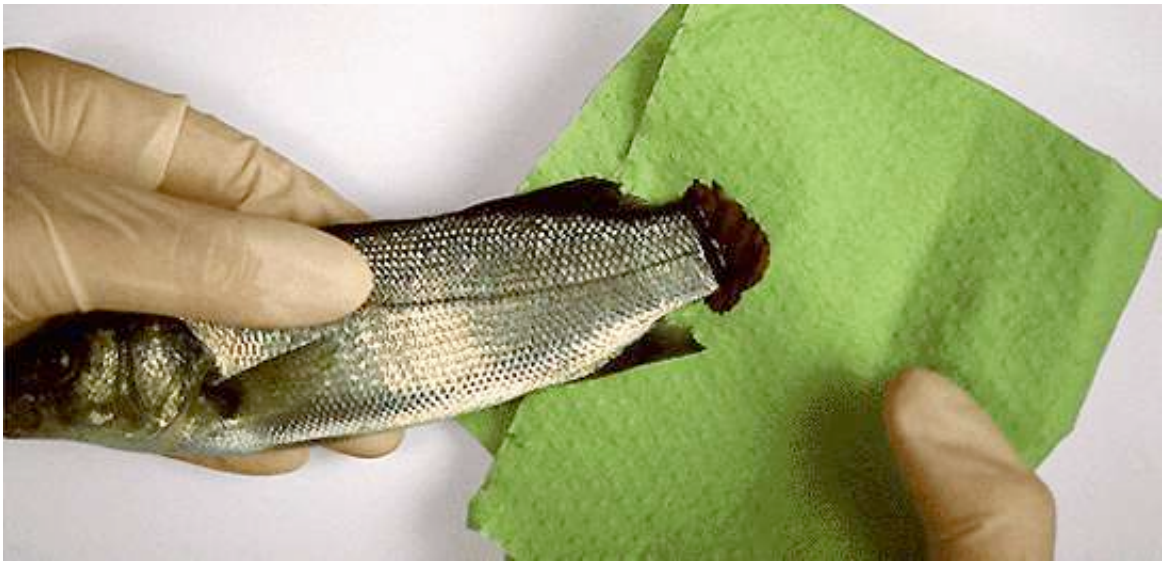
Sacrifice the fish by administering an overdose with an anaesthetic solution.

Severing Caudal Peduncle: 1.2



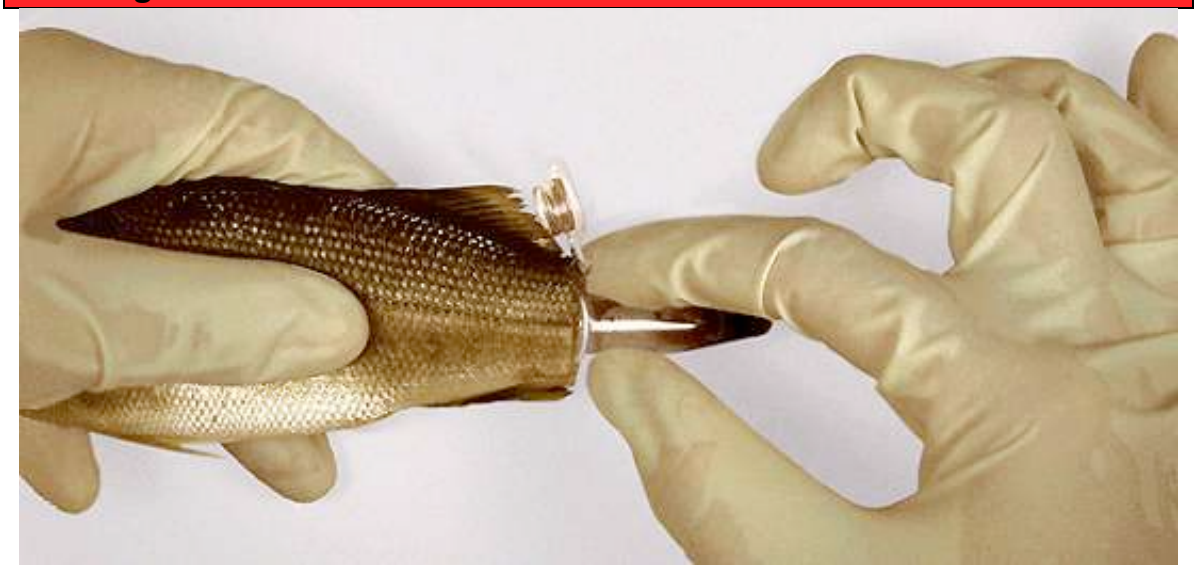
Cut dorsoventrally through the caudal peduncle.

Severing Caudal Peduncle: 1.3



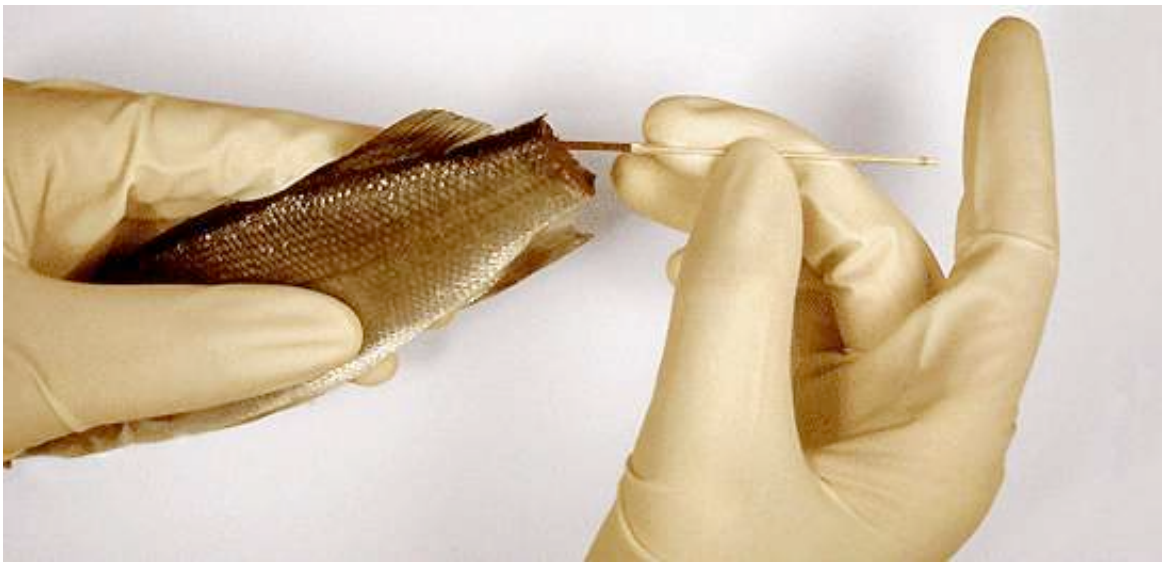
Wipe the caudal area with absorbent tissue to avoid contamination with mucus and water.

Severing Caudal Peduncle: 1.4



Place a heparinised collection tube or capillary tube at the end of the caudal vessel which you have just cut. (The type of tube chosen to collect the blood will depend on which haematological technique is to be carried out on the sample).

Severing Caudal Peduncle: 1.5



In the case of a capillary tube allow the tube to fill by means of capillary action.

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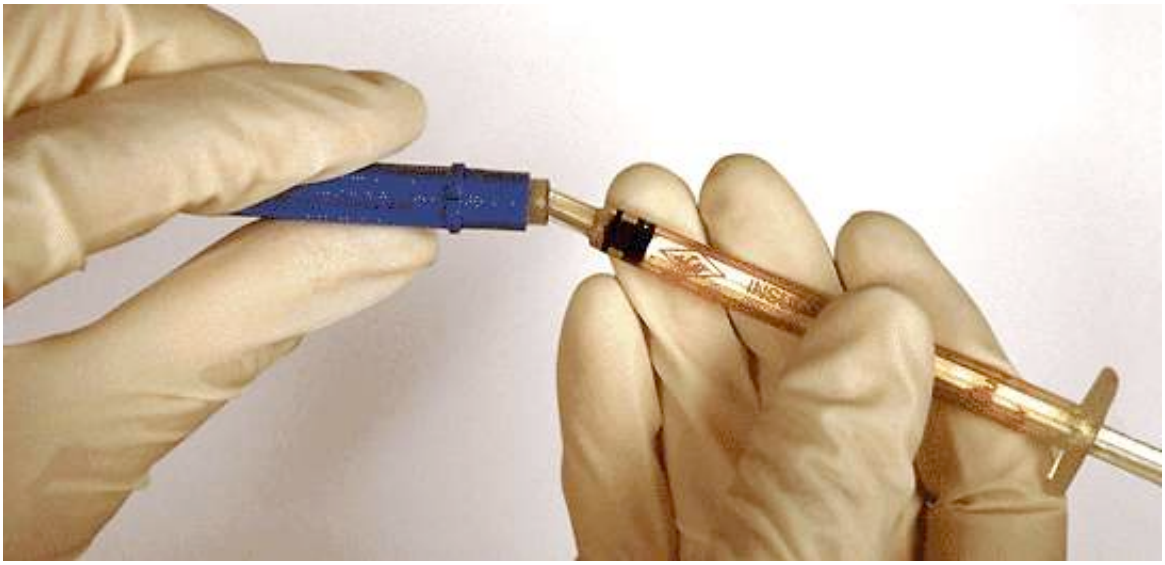
Puncture of Caudal Vessel: 2.1

This is a method which can be used repeatedly to take blood samples from larger fish (generally more than 10 cm long).

From 0.5 to 1 ml of blood can be drawn from fish of 200g every week without causing high mortalities or serious debilitation.

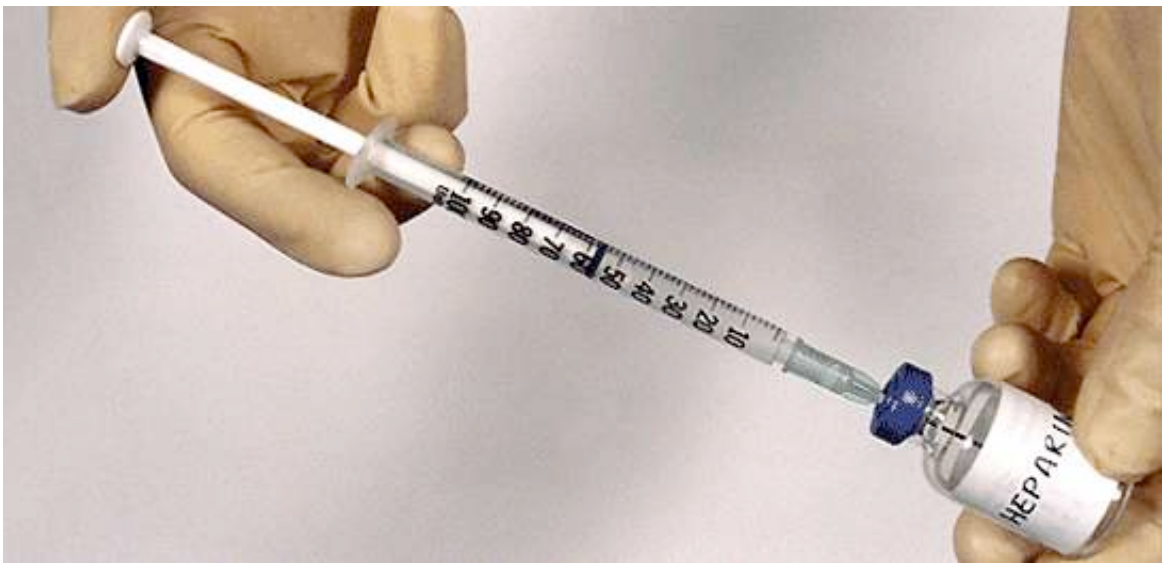
Render the fish unconscious in an anaesthetic solution .

Puncture of Caudal Vessel: 2.2



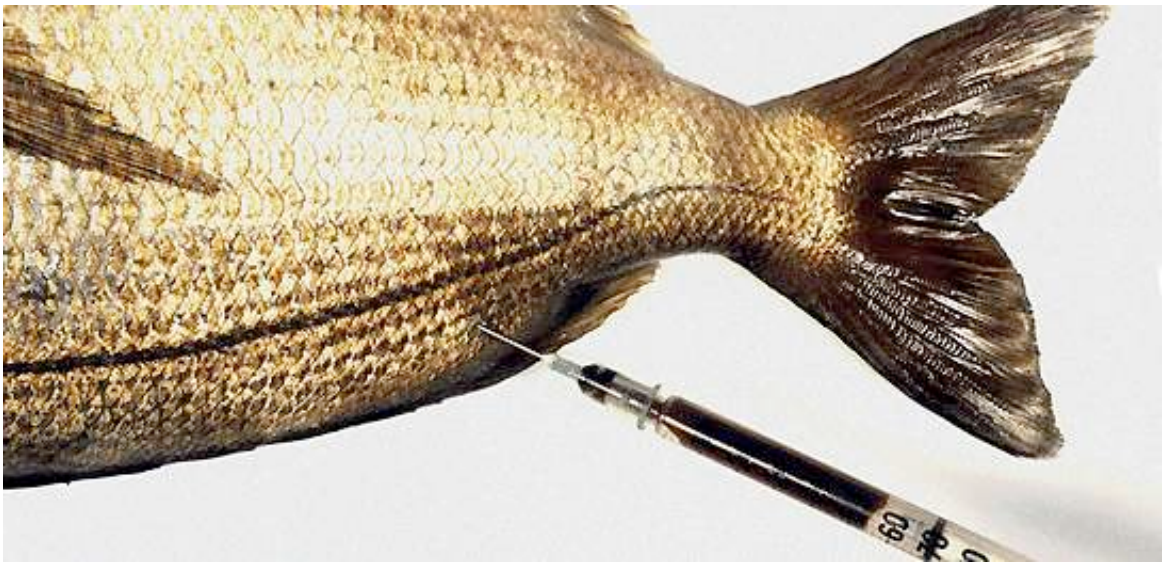
Attach the needle to the syringe.

Puncture of Caudal Vessel: 2.3



Flush out the syringe with heparin solution. Leave about 50-100 ml of solution at the base of the syringe. In practice, after flushing, sufficient anticoagulant is retained within the needle to prevent the clotting of the blood sample.

Puncture of Caudal Vessel: 2.4



Insert the needle on the mid-ventral line behind the anal fin. Push the needle into the musculature until the spinal column (backbone) is reached.

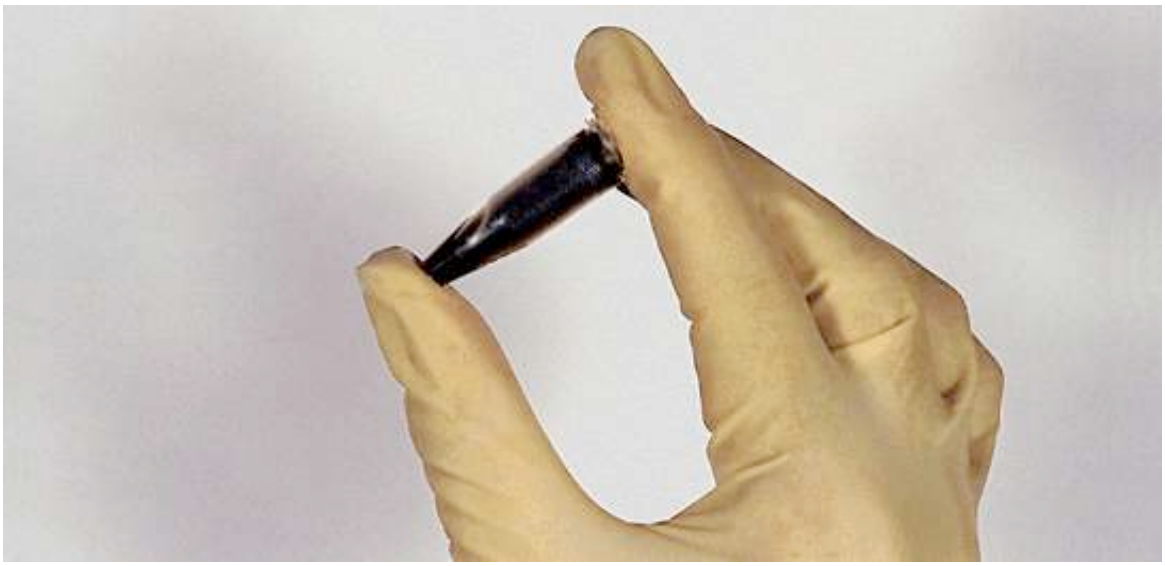
Keeping a steady vacuum on the syringe, slowly withdraw the needle until blood enters the syringe. This procedure may require a number of trial attempts to perfect the technique.

Make sure that no air bubbles are allowed to enter.

Puncture of Caudal Vessel: 2.5

Slowly and carefully withdraw the needle and syringe completely from the fish. Rotate the syringe gently and remove the needle and empty the contents of the syringe into a tube held on ice. The tube should be held at a slight angle as the blood is emptied in, allowing the fluid to flow down the side of the container.

Puncture of Caudal Vessel: 2.6



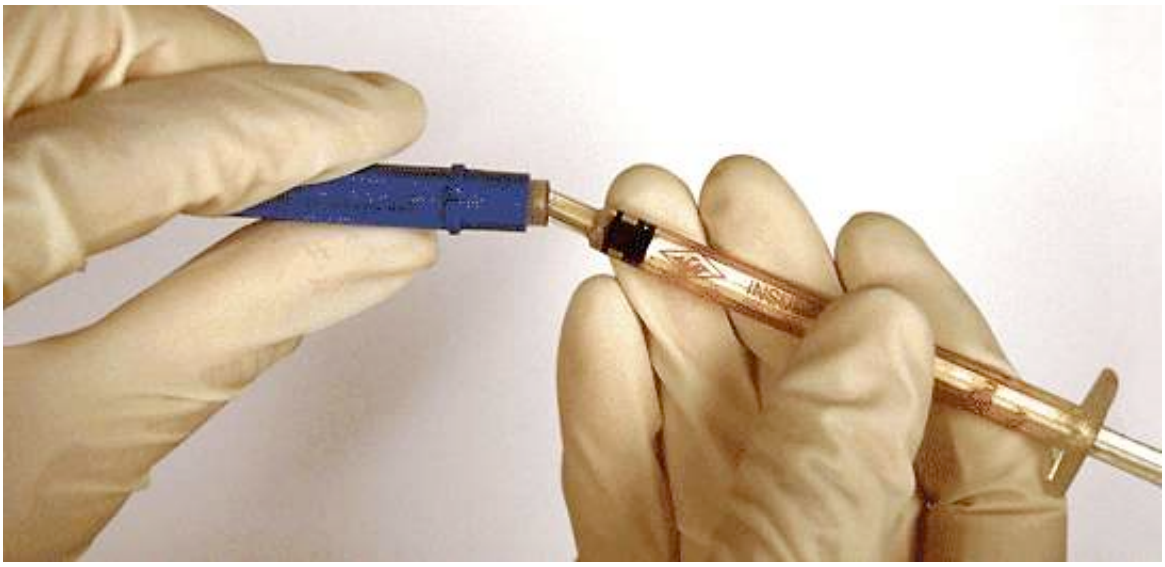
Mix the contents by inversion. This is done by gently turning the tube upside down.

Cardiac Puncture: 3.1

This method can be used to take regular blood samples from larger fish (generally more than 10 cm).

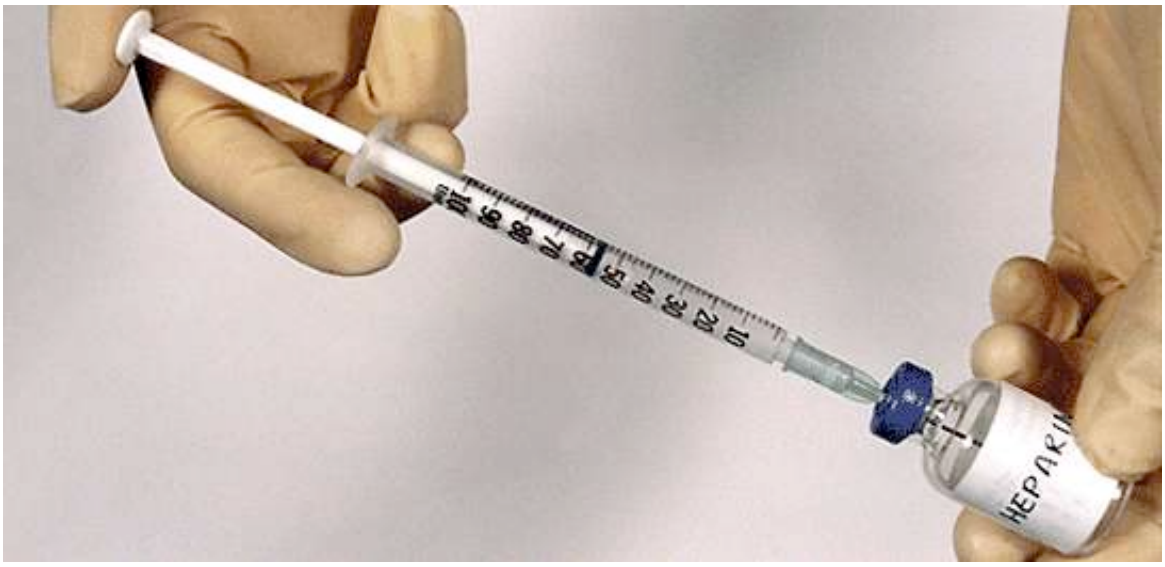
Render the fish unconscious in an anaesthetic solution.

Cardiac Puncture: 3.2



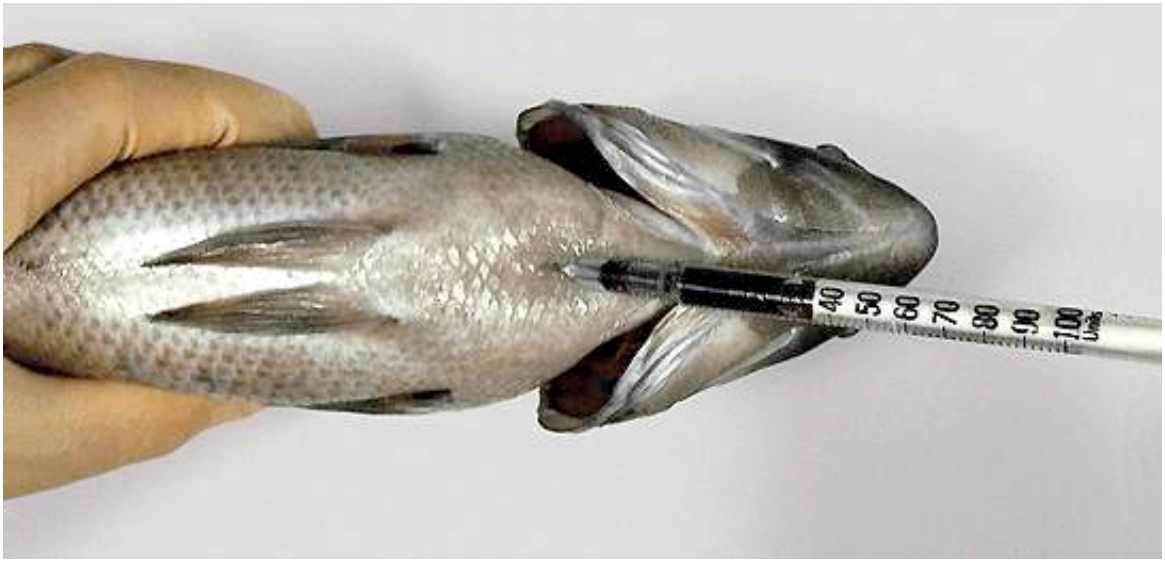
Attach the needle to the syringe.

Cardiac Puncture: 3.3



Flush out the syringe with heparin solution. Leave about 50-100 ml of solution at the base of the syringe. In practice, after flushing, sufficient anticoagulant is retained within the needle to prevent the clotting of the blood sample.

Cardiac Puncture: 3.4

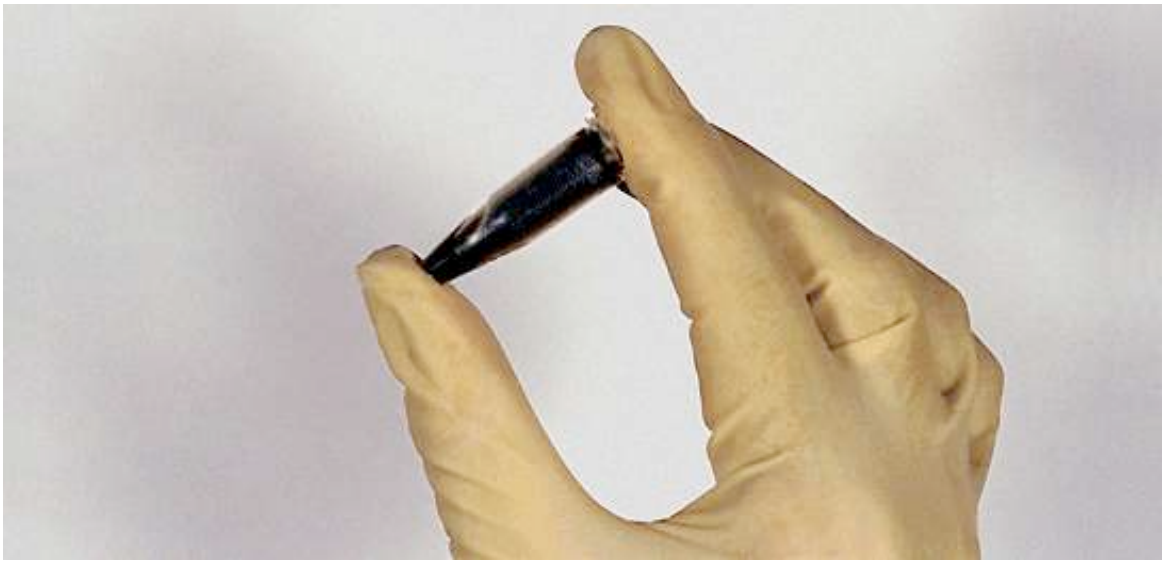


Hold the fish with the ventral side on top (uppermost).
Insert the needle vertically, midway between the anterior bases of the pectoral fins.
Apply negative pressure on the plunger until blood enters the syringe.
Slowly withdraw the syringe completely from the fish.

Cardiac Puncture: 3.5

Rotate the syringe gently and remove the needle and empty the contents of the syringe into a tube held on ice. The tube should be held at a slight angle as the blood is emptied in, allowing the fluid to flow down the side of the container.

Cardiac Puncture: 3.6



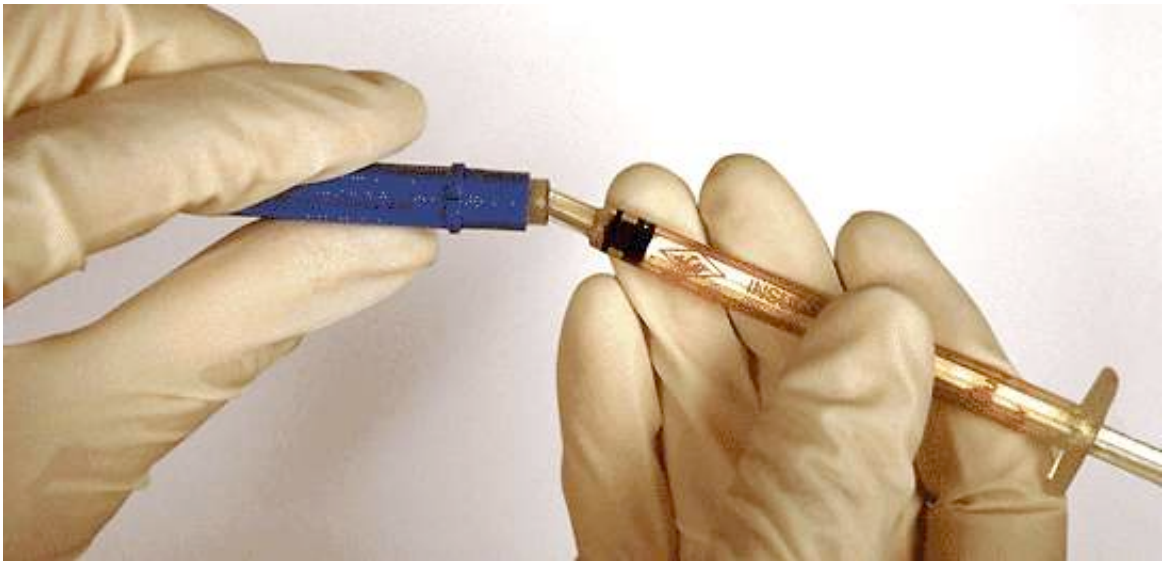
Mix the contents by inversion. This is done by gently turning the tube upside down.

Dorsal Aorta Puncture: 4.1

This method can be used to take regular blood samples from larger fish (generally more than 10 cm).

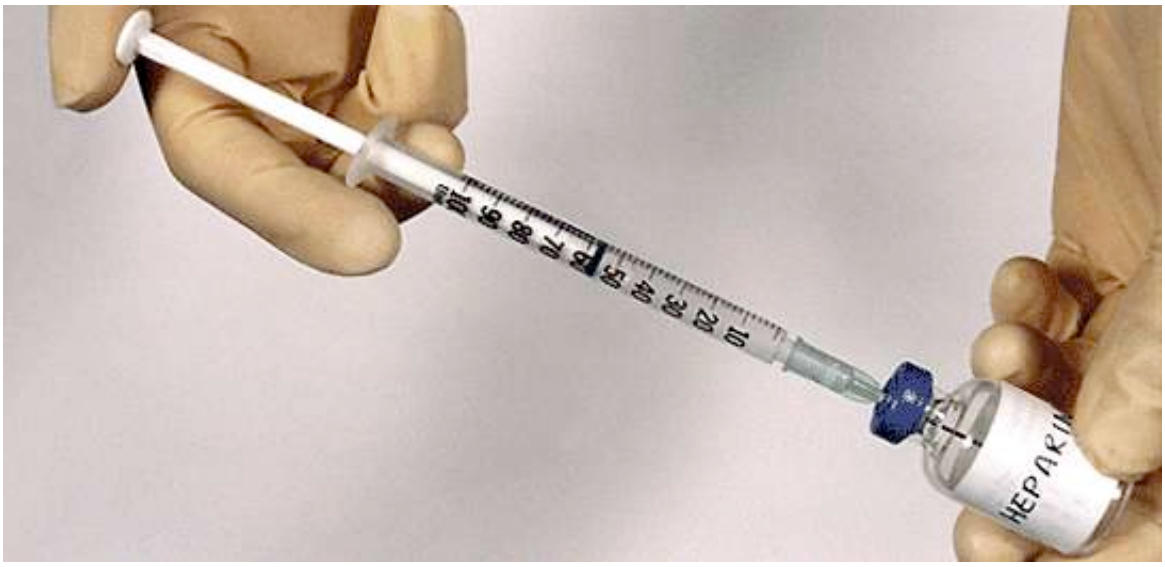
Render the fish unconscious in an anaesthetic solution.

Dorsal Aorta Puncture: 4.2



Attach the needle to the syringe.

Dorsal Aorta Puncture: 4.3



Flush out the syringe with heparin solution. Leave about 50-100 ml of solution at the base of the syringe. In practice, after flushing, sufficient anticoagulant is retained within the needle to prevent the clotting of the blood sample.

Dorsal Aorta Puncture: 4.4

Insert the needle along the midline of the fish, below the backbone (spinal column), until the backbone is reached.

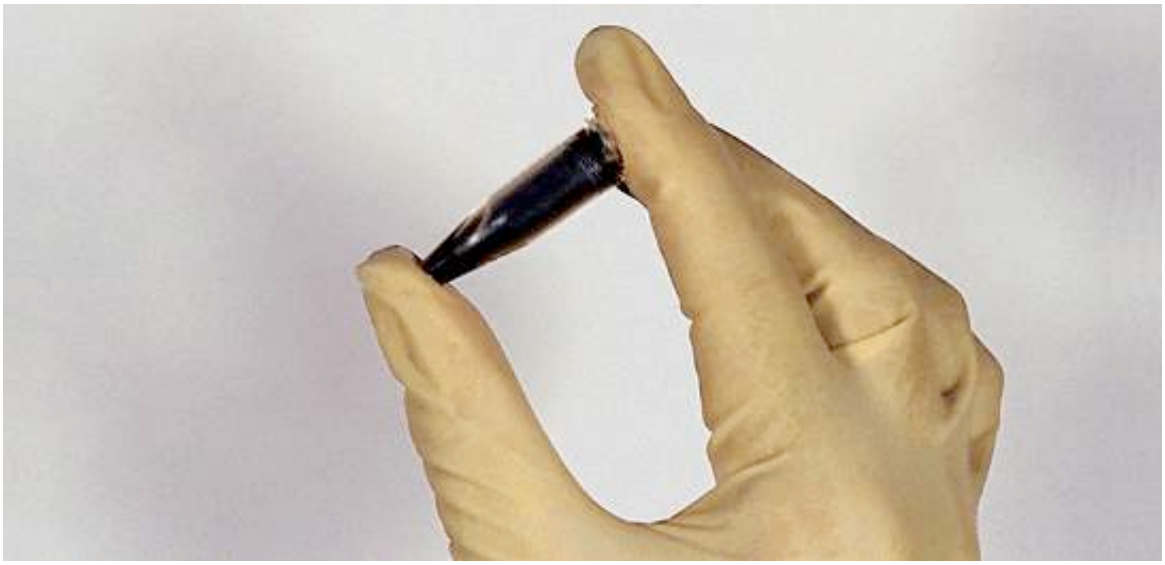
Apply negative pressure to the syringe until blood enters the syringe.

Slowly withdraw the syringe completely from the fish.

Dorsal Aorta Puncture: 4.5

Rotate the syringe gently and remove the needle and empty the contents of the syringe into a tube held on ice. The tube should be held at a slight angle as the blood is emptied in, allowing the fluid to flow down the side of the container.

Dorsal Aorta Puncture: 4.6



Mix the contents by inversion. This is done by gently turning the tube upside down.

UNIT SPECIFIC GLOSSARY

Coagulation

The process of changing into a clot, by which an organic liquid solidifies, e.g. the clotting of blood.

Anaesthetic

In the case of fish, chemicals used to relax and facilitate handling, surgery and spawning procedures. Commonly used agents include tricane methane sulfonate (MS-222), benzocaine and quinaldine which are widely used in handling freshwater fish. Ethylenglycol-monophenylether is an anaesthetic agent more suitable for marine fish species. It is usually administered by immersing fish in a bath solution.

Caudal Peduncle

The relatively thin posterior section of the body to which the caudal fin is attached; the region between the base of caudal fin and the base of the last ray of the anal fin.

APPENDIX

AQUALEX Fish Health Toolset: the story

WHY create the Toolset?

We, the AQUALEX Multimedia Consortium (AMC), decided to create the AQUALEX Fish Health Toolset so that basic essential factual knowledge about certain aspects of **fish health** (important for European aquaculture) could be combined with fast-track multi-lingual learning. Successful EU competitive projects had given us the impetus (and the funds) to create a wide range of individual multilingual materials: 3 multilingual Glossaries in 7 languages, language learning units at 3 levels in 10 languages, and fish health course materials also in 10 languages. We brought these separate elements together first as a Concept, then as a working Toolset freely available as **multilingua**l **units/modules** in user-friendly online learning formats.

WHAT is covered in the Toolset

Both the species and the fish health aspects covered result from several European-wide needs analyses which targeted industry and academic users including VET providers. These are:

- Basic Techniques for Fish Haematology
- Fish Health Management Manual
- Aquatic Pathology for rainbow trout, carp, sea bass and turbot
- Fish, shellfish and crustacean meristics (in construction)

HOW to use the Toolset

The Toolse**t** links three different **content areas** and two **language level**s (Beginner: CEFR A1 &A2 and Basic: CEFR A1 & A2). Because the Fish Health content is freely accessible online, you can find the information/content you need, whenever and wherever you need it, whether studying or in the workplace. You can access the **Fish Management Toolset** and try it out via the following link http://www.aqualex.org/index.php/pescalex-courses,

The **3 updated multilingual glossaries (AQUALEX, PESCALEX, MARPOL)** provide high-quality terms and definitions of, respectively, aquaculture, fish diseases and marine pollution terminology (3500 items variously in English, French, German, Greek, Spanish, Italian, Norwegian, Polish, Hungarian, Turkish, Swedish and Galician). Unique multilingual access at a single click. http://www.aqualex.org/index.php/glossaries

The Toolse**t's** language **learning modules** are very helpful for workers in aquaculture, because they exist in languages important in the European aquaculture industry (English, French, Spanish, Norwegian, Greek, Polish, Portuguese, Hungarian, Turkish, Swedish and Galician). The modules can be accessed online at http://www.aqualex.org/index.php/multilingual-esp-language-courses. Syllabus details are given below**.**

WHEN to use the Toolset

The Toolset helps you to communicate in your chosen language through the online modules. Its unique multilingual combinations give you the chance to learn a language

while also acquiring essential basic **Fish Health conten**t in English, French, Spanish, Norwegian, Greek, Polish, Portuguese, Hungarian, Turkish, Swedish and Galician.

A final note

How NOT to use the Toolset

The AQUALEX Toolset materials should not be regarded or used either wholly or in part as a comprehensive fish health manual. There are many such specific and easily accessible publications which are both reliable and comprehensive.

How to use the AQUALEX online language lessons

http://www.aqualex.org/index.php/multilingual-esp-language-courses

The AQUALEX language lessons are designed for **COMPLETE BEGINNERS in English**, whose first/native languages are French, Spanish, Greek, Norwegian, Polish, Portuguese, Swedish, Hungarian, Turkish and Galician.
These online language learning lessons work well as a support for tutor-led blended courses. They will help complete beginners in each language to learn basic grammar points and important aquaculture keywords in English.
They are NOT a complete online course. They simply give basic grammar points within a vocational context (aquaculture). The online format is designed to give beginners a chance to understand and handle simple sentence with essential keywords.
Because English is still the most popular choice for a second language, English grammar points are explained, in English and in the user's language. Where the user language is different from English (i.e., French masculine and feminine nouns), then explanations of the user language structures are given in both English and the user language.

- Level 1 lessons (14) for beginners with no previous knowledge of the target language.
- Level 2 lessons (10) for those with some knowledge, though still at the basic level.
- You can enter each level via the dropdown menus at the top of the page.
- You can enter the lessons (English, French, Galician, Greek, Hungarian, Norwegian, Polish, Portuguese, Swedish, Turkish) by clicking on the country flags as shown.

Each lesson is organised into TEXT, GRAMMAR, HOMEWORK and ASSESSMENT with clickable menus at the top of each page. The ASSESSMENT section is not available to the general public.

- The TEXT menu contains the lesson itself, which may have 5 pages.
- Each page of the lesson can be entered via the NEXT and PREVIOUS live links at the bottom of the page.
- The GRAMMAR section can be entered via the top dropdown menu. Each page of the grammar can be entered via the NEXT and PREVIOUS live links at the bottom of the page.
- The HOMEWORK section cannot be entered until all the TEXT and the GRAMMAR pages have been viewed, in order to consolidate language acquisition.
- All TEXT pages are inter-changeable in all AQUALEX languages, by clicking on the country flag at the bottom of the page.
- **BUT** Grammar and Homework sections are specific to each language and do not have an interactive function.

*TEXT pages also contain **audio material** which can be heard by running the mouse over the words on the page. Click on live items to hear the term as recorded by native speakers (make sure you have the necessary software for this facility (i.e., Quicktime Player).*

In Level 1 you learn:

The use of

- ✓numbers (lesson 1)
- ✓definite article (lesson 1)
- ✓indefinite article the/a/an (lesson 2)
- ✓demonstrative pronouns this/that/these/those (lesson 2)
- ✓singular/plural nouns (lessons 1,3)
- ✓irregular plurals (lessons 3.4)
- ✓countable /uncountable nouns (lesson 6)
- ✓agreements (subject/verb) (lessons 11-13)

how to make statements (lesson 1)

- ✓how to make negative statements (lesson 2)
- ✓how to ask and answer questions (lesson 2)
- ✓true/false response (lesson 4)
- ✓how to tell the time (lesson 6)

how to make simple measurements

- ✓temperature (lesson 9)
- ✓length, breadth, width, height (lesson 10)
- ✓volume (lesson 10)

the use of prepositions

- ✓of place (lesson 8)
- ✓of time (lesson 9)

- ✓names of days (lesson 13)
- ✓names of months (lesson 14)

In Level 2 you learn:

- ✓more adjectives and adverbs (lesson 2)
- ✓comparisons (lesson 3)
- ✓pronouns (lesson personal, relative)4)
- ✓Imperative (lesson 5)
- ✓past tense (lesson 6)
- ✓future tense (lesson 7)
- ✓conditionals (lesson 7)
- ✓modals/gerundive (lesson 8)
- ✓passive (lesson 9)

- ✓many, some, few, a lot of, more (lesson 6)

the use of verbs

- ✓is, are (lesson 1)
- ✓has, have (lesson 2)
- ✓this is, there are (lessons 2, 3)
- ✓present tense (forms and functions) (lessons 11, 12, 13)

Language attainment levels

Level 1 (CEFR) levels A1, A2)

The priority for many **first-time language learners** is to understand and convey simple but vital pieces of information (i.e., keywords) in a new language. The AQUALEX online language lessons in English, French, Spanish, Greek, Norwegian, Polish, Hungarian, Turkish, Portuguese, Swedish and Galician are designed to allow complete beginners to build on their native language knowledge of familiar items in the workplace/laboratory, in a step-by-step visual presentation with audio input. This method gives them a chance to fast-track their learning, at their chosen time and at their own speed.

Level 2 (CEFR levels B1, B2)

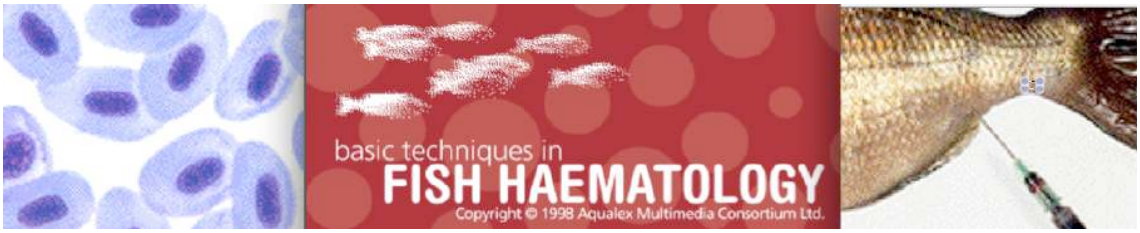
Having picked up the first essentials in a user-friendly way, **students or workers** requiring vocationally relevant fish health information can progress at their own pace of learning through the Toolset Fish Health multi-lingual course materials (shown above) in English, French, Spanish, Greek, Norwegian, Polish, Hungarian, Turkish and Galician This can be done online at http://www.aqualex.org/index.php/multilingual-esp-language-courses

Level 3 (CEFR levels C1, C2)

For the seasoned practitioner, Ph.D. student or academic, the AQUALEX Toolset contains two **multi-lingual aquaculture and fish diseases glossaries** in English, French, German, Spanish, Italian, Greek, Norwegian, Polish, Hungarian, Turkish and Galician. These online resources present high-level information and detailed definitions in the accepted academic format.

ONLINE eLEARNING UNIT

3 modules
Fish Blood Constituents
Collection of Blood
Haematological Techniques
in
English/French/Spanish/Greek/ Norwegian/
Polish/Hungarian/ Turkish/Galician



MODULE 3
HAEMATOLOGICAL TECHNIQUES

The module was written, compiled and designed for use as a distance learning module that can be used in tutor-led blended learning or in autonomous learning.
Definitions of all terms highlighted in bold can be found in the attached module glossary or in the multi-lingual Glossaries in the AQUALEX FISH HEALTH ONLINE TOOLBOX (http://www.aqualex.org/index.php/multilingual-esp-language-courses)

Module 3
HAEMATOLOGICAL TECHNIQUES

Entry level

No entry level requirements for free access e-learning users

Entry level for blended learning courses to be set by course tutors

For the user

On completion of this module you will be able to:

- ✓ Understand techniques for the examination of fish blood samples
- ✓ Know different methods used to estimate haemoglobin concentration
- ✓ Name different methods of obtaining erythrocyte cell counts
- ✓ Recognise advantages and disadvantages of each method
- ✓ Name different methods of obtaining leucocyte cell counts
- ✓ Know how to prepare a blood smear on a microscope slide
- ✓ Discuss uses of staining in differential cell counts

You will be able to:

- ✓ Include this in your EUROPASS, including EUROPASS Digital Credentials (https://europa.eu/europass/en/europass-digital-credentials) This will also help you to draw up your EUROPASS CV.
- ✓ Include these skills in browsing the ESCO list of skills, competences and knowledge, while searching for job opportunities throughout Europe. (https://ec.europa.eu/esco/portal/home)

Details on the **Toolset basic language syllabus** are at the end of this module, after the Module Glossary.

For the evaluator/assessor/teacher

This module is equivalent to EQF Intermediate Level, requiring the student

- ✓ to have basic general knowledge of the subject
- ✓ to be able to recall general information
- ✓ to be able to explain factual knowledge.

General Introduction

The regular monitoring of fish blood is a very useful diagnostic tool in establishing the health status of fish farm stocks. This unit shows examples of the different techniques used in examining blood samples (**Module 3**).

Unit 1: Fish Blood Constituents
Unit 2: Collection of Blood
Unit 3: Haematological techniques

Introduction to the module

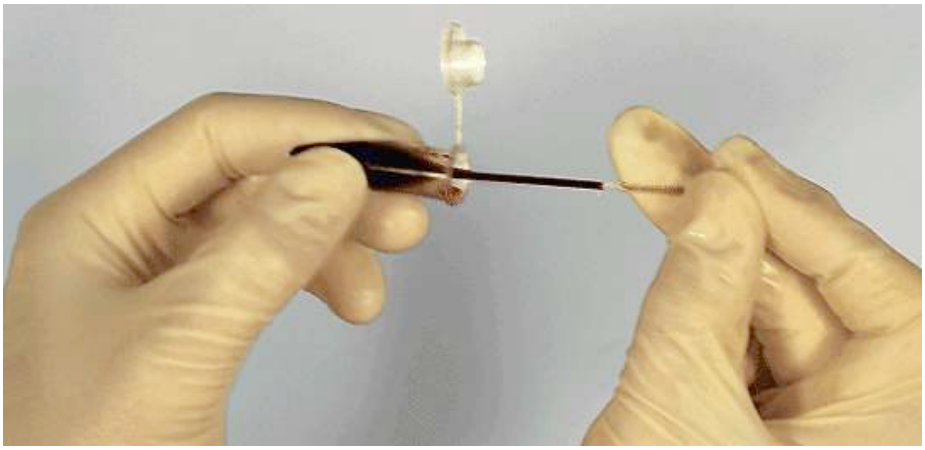
The regular monitoring of fish blood is a very useful diagnostic tool in establishing the health status of fish farm stocks.

Several techniques are used to investigate the various properties of fish blood.

1. Haematocrit
2. Haemoglobin Determination
3. Erythrocyte Count
4. Leucocyte Count
5. Differential cell counts

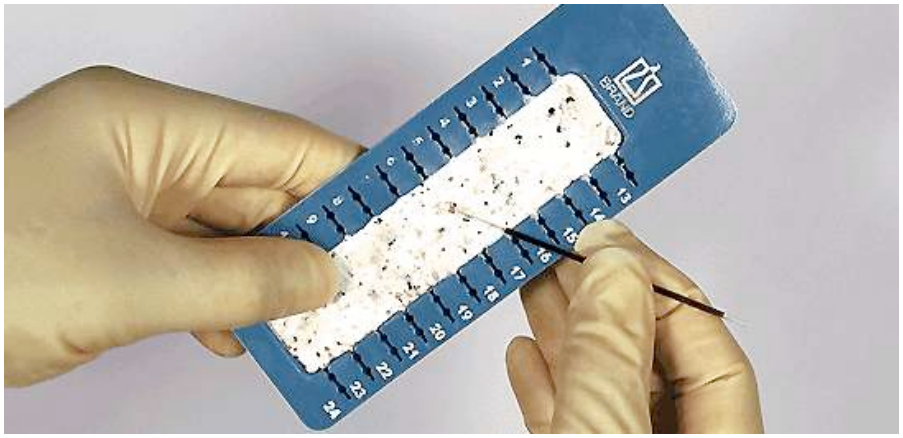
Haematocrit: 1.1

A haematocrit is a method which is used to determine of the volume of packed cells in the blood. The haematocrit will vary, depending on the health and physiological condition of the individual fish.



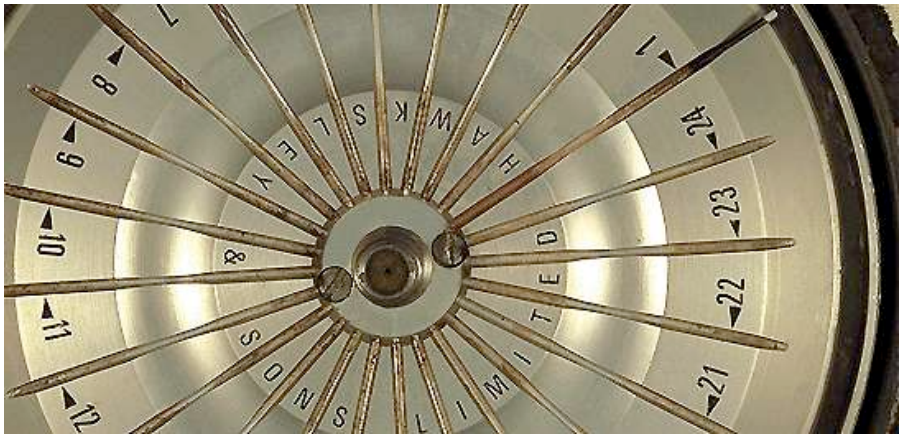
Touch a heparinised capillary tube to the blood sample and allow it to fill three-quarters full.

Haematocrit: 1.2



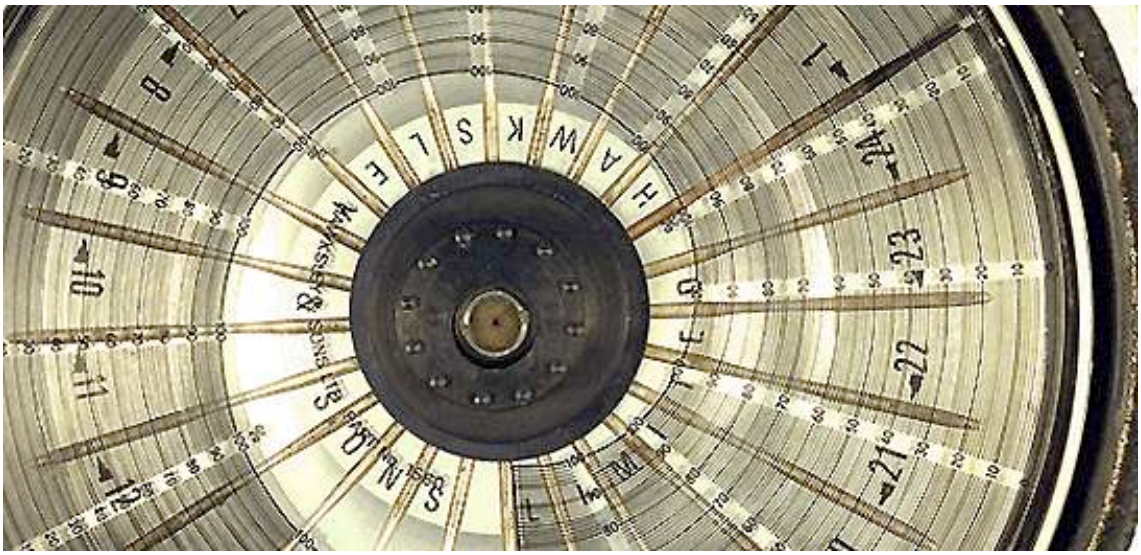
Seal the end of the tube with a suitable sealant (eg. plasticene).

Haematocrit: 1.3



Centrifuge the capillary tube for 5 minutes at 10.500 rpm in a micro haematocrit centrifuge.

Haematocrit: 1.4



Remove the cover from the centrifuge and measure the volume of the capillary tube occupied by packed cells using a rotoreader.

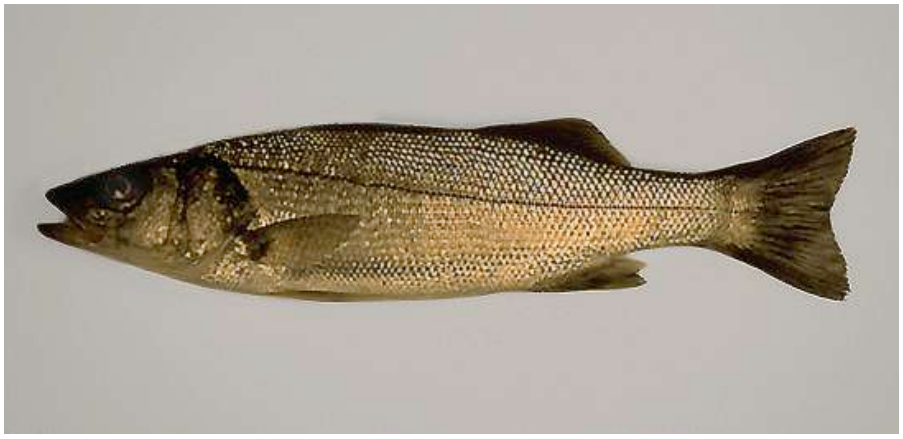
This is a transparent plate showing measurements, which is placed over the haematocrit tube in the centrifuge allowing packed cell volume to be read. The relative numbers of red cells and white cells may also be estimated as the white cells will appear above the red cells as a 'buffy coat'.

Haemoglobin Determination: 2.1

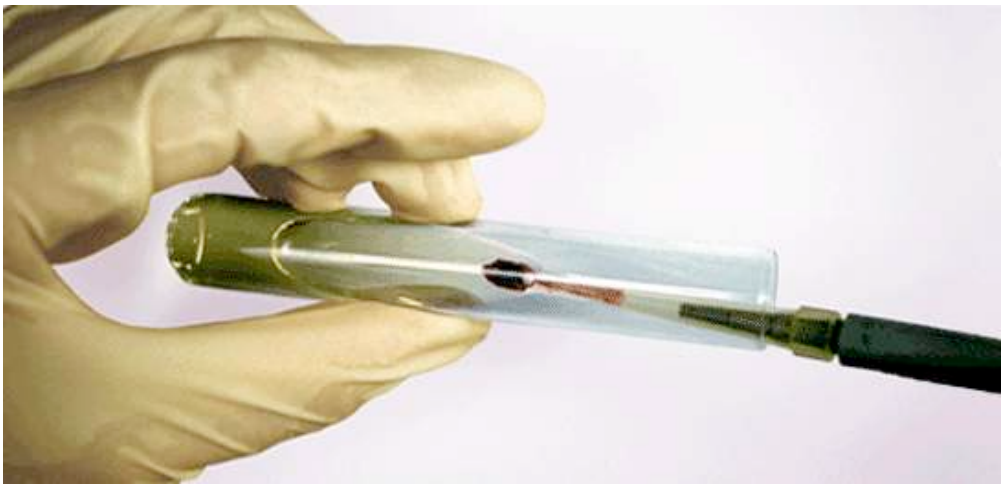
Calculating the concentration of haemoglobin in the blood is a rapid method of detecting disease conditions in fish, including **anaemia.**

The precision of various methods for estimating haemoglobin concentration may differ considerably. The cyanohaemoglobin method has been the standard method used in haematological studies for a number of decades. However the alkaline haematin D-575 method has been adapted for studies in fish haematology and it has been reported that the cyanohaemoglobin method may yield lower haemoglobin concentrations than those obtained with the alkaline haematin D-575 method. It has been suggested that the cyanohaemoglobin method may have other disadvantages; primarily that the reagent contains cyanide which is toxic, and that the end-product is light labile.

For the purposes of this module, the cyanohaemoglobin method, as the most widely used procedure in determining haemoglobin concentration in fish (and in other animals), is described.



Haemoglobin Determination: 2.2



Using a pipette add a sample of 20 ml of blood to 5ml of Drabkin's solution in a test tube and mix thoroughly.

DRABKIN'S SOLUTION:

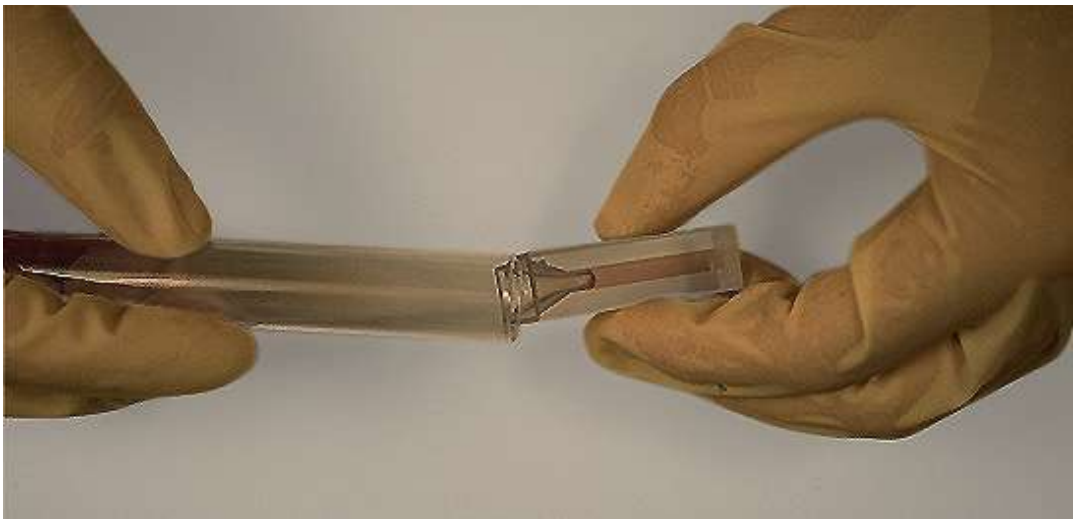
200 mg Potassium ferricyanide

50 mg Potassium cyanide

140 mg Potassium dihydrogen phosphate

Distilled water made up to 1 Litre

Haemoglobin Determination: 2.3



Place approximately 2ml of the resulting solution into a cuvette, and read the absorbance values in a spectrophotometer at 540nm.

Erythrocyte count: 3.1

Obtaining a count of erythrocytes in fish blood is a useful tool because of the association of abnormal red blood cell counts with disease conditions.

There is a great deal of variation between laboratories in the procedures employed to carry out blood cell counts. Diluting solutions which may be used include Hendricks diluting solution (1:200 dilution of blood), and formal citrate solution (1:200 dilution of blood). Blaxhall and Daisley's method for red blood cell counts outlined here is widely used. The same method may be used to carry out leucocyte counts, although the procedure employed for counting and calculating occurrence of the cells is different.

Make a 1:50 dilution of blood in Dacies fluid.

Dacies fluid:

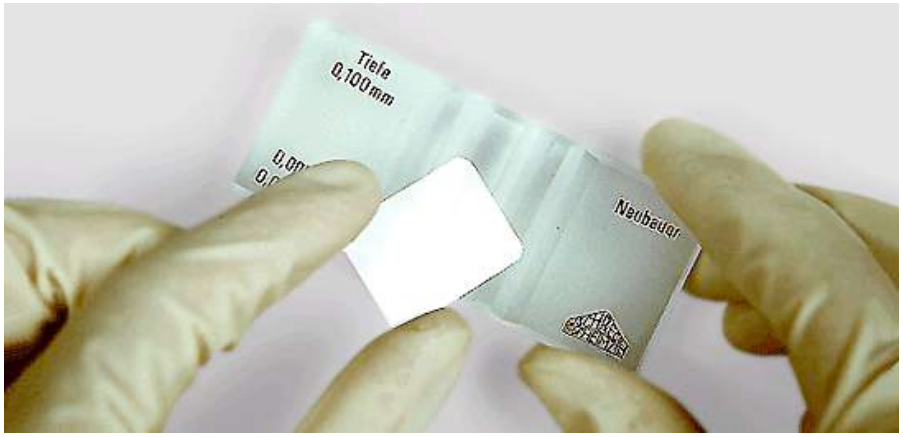
Formaldehyde:	10 ml
Trisodium citrate:	31.3g
Brilliant cresyl Blue:	1.0g
Distilled water	1 litre

Dacies fluid should be filtered immediately before use.

Erythrocyte count: 3.2

Mix the diluted blood by tilting the sealed tube gently to avoid destroying the cells.

Erythrocyte count: 3.3

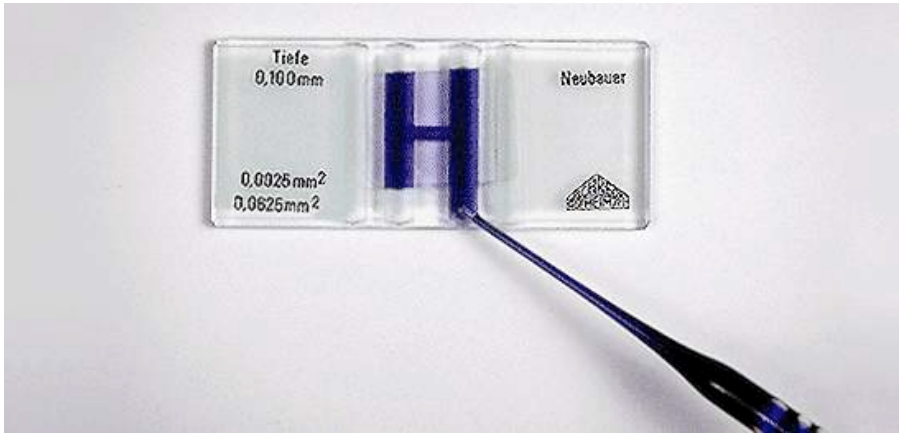


Place a cover slip over a Neubauer haemocytometer.

This is a special type of slide which is designed to act

as a blood cell counting chamber.

Erythrocyte count: 3.4



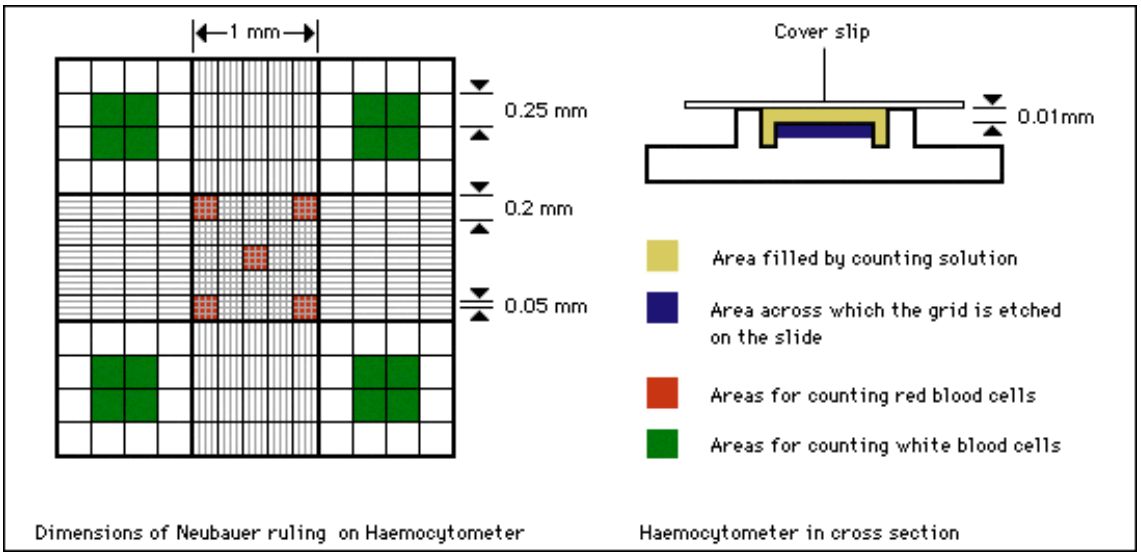
Draw some of the diluted blood solution into a Pasteur pipette

and touch the tip of the pipette to the edge of the cover slip.

Capillary action will draw the diluted blood into the chamber.

Erythrocyte count: 3.5

Erythrocytes are a great deal more numerous than leucocytes in fish. In order for an erythrocyte count to be considered statistically accurate a minimum of 200 cells (but preferably 400-500) must be counted. The total area counted here (0.02mm^3 at a dilution of 1:50) should be sufficient for an accurate count to be obtained.



Place the haemocytometer under the microscope.

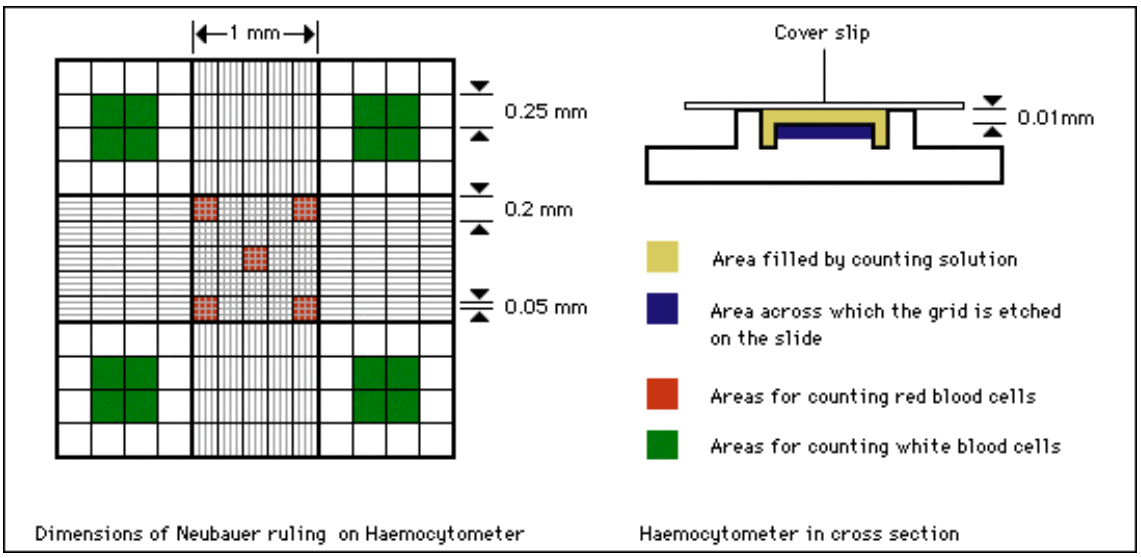
Count the erythrocytes occurring in five small squares at the centre of the grid, a total area of 0.02mm^3 (1/50 of 1mm^3).

The dilution is 1:50, therefore the number of cells occurring per mm^3 may be calculated as follows:
Number of cells occurring per mm^3 =
Number of cells counted in 0.02mm^3 X 50 (area counted) X 50 (dilution).

Leucocyte count: 4.1

There is a great deal of variation between laboratories in the procedures employed to carry out blood cell counts. Shaw's counting solutions may be used to estimate leucocyte occurrence (making a 1:200 dilution of blood).

Blaxhall and Daisley's method outlined here for white blood cell counts is widely used. The same method may be used to carry out erythrocyte counts, although the procedure employed for counting and calculating occurrence of the cells is different.



Make a 1:50 dilution of blood in Dacies fluid.

Dacies fluid:

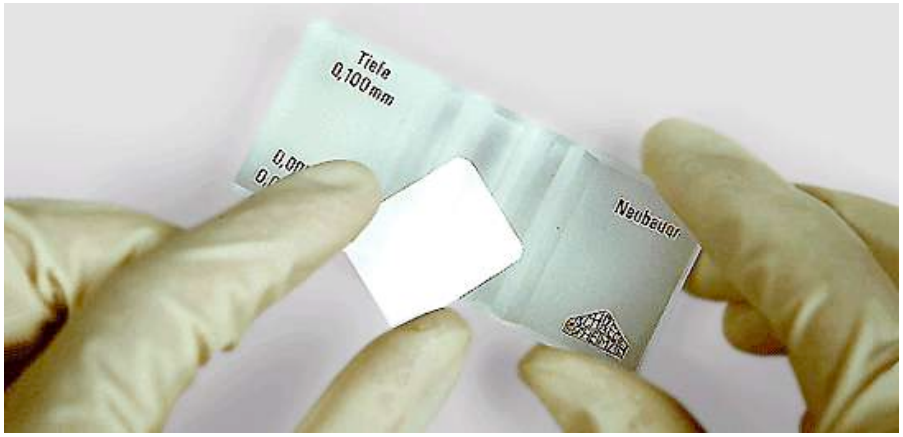
Formaldehyde:	10 ml
Trisodium citrate:	31.3g
Brilliant cresyl Blue:	1.0g
Distilled water	1 litre

Dacies fluid should be filtered immediately before use.

Leucocyte count: 4.2

Mix the diluted blood by tilting the sealed tube gently to avoid destroying the cells.

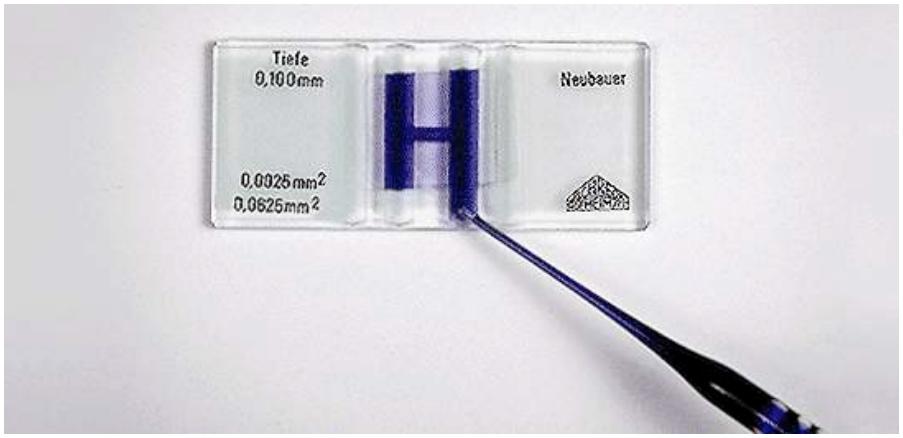
Leucocyte count: 4.3



Place a cover slip over a Neubauer haemocytometer.

This is a special type of slide which is designed to act as a blood cell counting chamber.

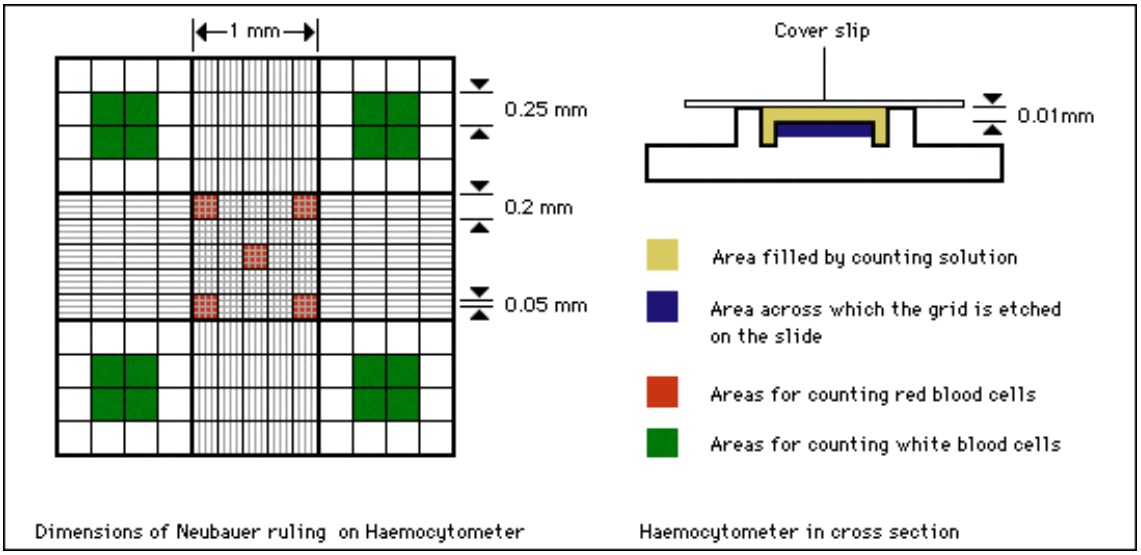
Leucocyte count: 4.4



Draw some of the diluted blood solution into a Pasteur pipette and touch the tip of the pipette to the edge of the cover slip. Capillary action will draw the diluted blood into the chamber.

Leucocyte count: 4.5

Leucocytes are not nearly as abundant as erythrocytes in fish. In order for a leucocyte count to be considered statistically accurate a minimum of 200 cells (but preferably 400-500) must be counted. The total area counted here (0.1mm^3, at a dilution of 1:50), should be sufficient for an accurate count to be obtained.

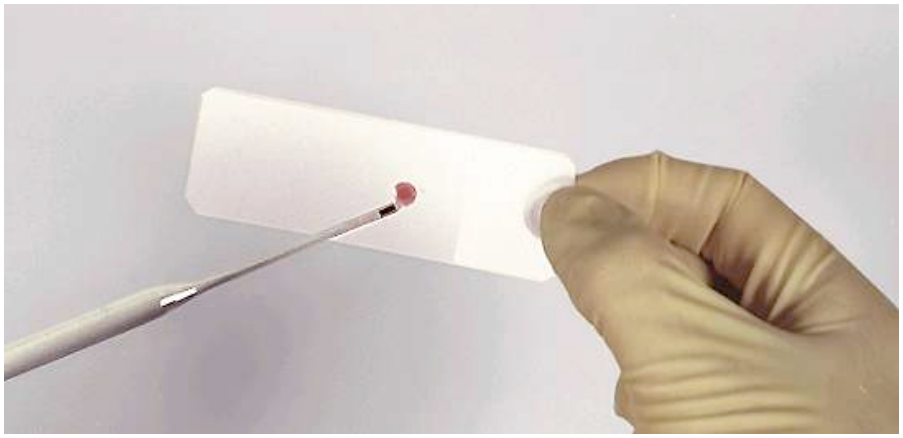


Place the haemocytometer under the microscope.
Count the leucocytes occurring in the four corner squares marked on the grid, a total area of 0.1 mm^3.

The dilution is 1:50, therefore the number of cells occurring per mm^3 may be calculated as follows:
Number of cells occurring per mm^3 =
Number of cells counted in 0.1mm^3 X 10 (area counted) X 50 (dilution).

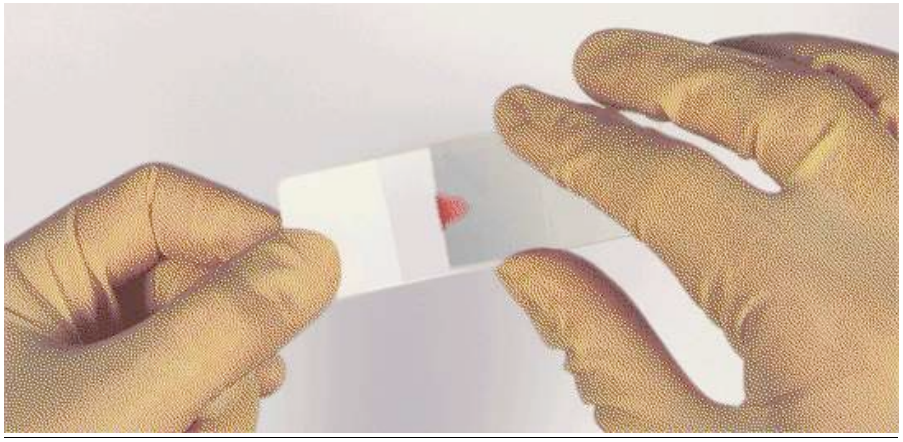
Differential Cell Counts: 5.1

Microscopic examination of different cell types as revealed by differential staining can be carried out by making a blood smear. The purpose of this method is to obtain a single layer of cells on a microscope slide, which is then fixed and stained in the appropriate dyes. Different types of cell (erythrocytes, lymphocytes, etc.) and components within the cell, take up different stains, and this allows cells to be identified and counted.



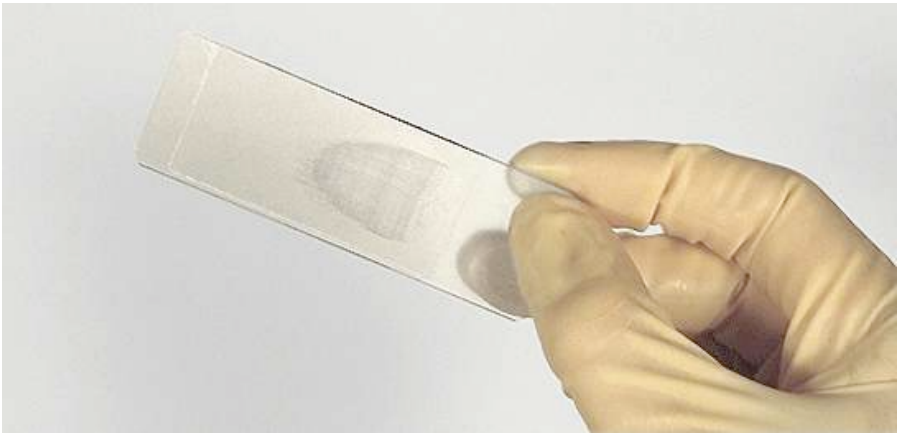
Place a small drop of blood on one end of a clean dry microscope slide. Use a slide which has been cleaned in detergent and stored in absolute methanol or ethanol. Place this slide on a table top.

Differential Cell Counts: 5.2



Hold the smearing slide at an angle of 35^0 to 40^0 to the other slide and allow the drop of blood to flow along the interface between the slides. Push the smearing slide firmly along the microscope slide, away from the drop of blood in order to avoid distorting or crushing the blood cells, maintaining uniform pressure, to create a thin film of blood.

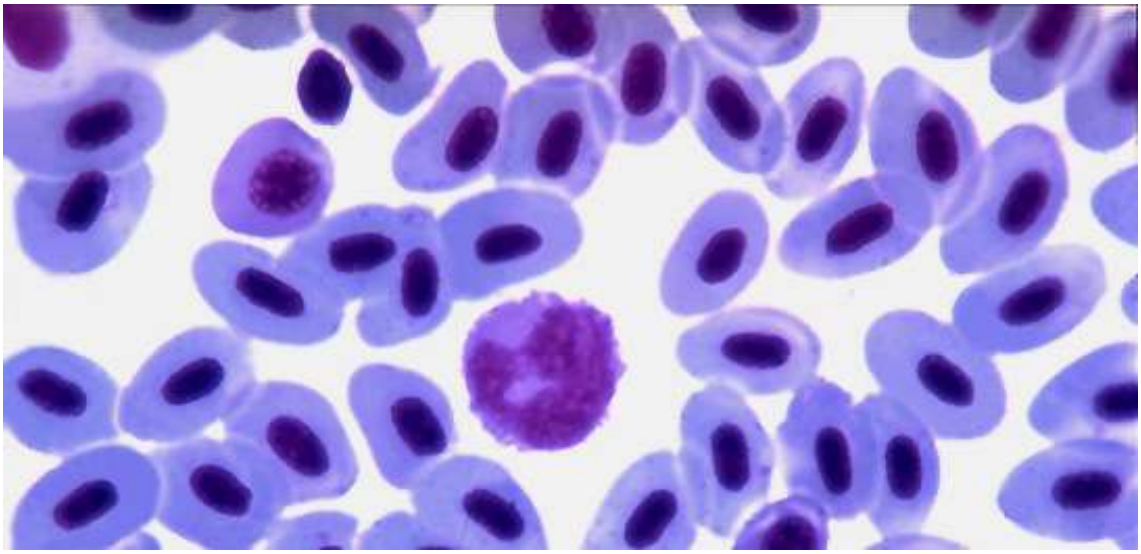
Differential Cell Counts: 5.3



Dry the slide rapidly in air and fix for 1-2 min. in 95% methanol.
The smear can be stored for some weeks before staining, if needed.

Differential Cell Counts: 5.4

MAY-GRUNWALD/GIEMSA STAIN: Eosine-methylene blue, solution modified for microscopy. This is among the more widely used solutions used in histological staining of blood smears. Different types of cell (erythrocytes, lymphocytes, etc.) and components within the cell, are distinguishable from one another by their colour and shape when viewed under a microscope.



Erythrocytes: Round/oval shaped cells with a centrally situated nucleus and a well defined outline. Stain pink/brown with May-Grunwald/Giemsa.

Lymphocytes: Round cells with large round nucleus. Considerable size range. Nucleus stains a deep red/violet colour, while the cytoplasm is either a dark blue ring or not visible.

Neutrophils: Round/oval cells with granulated cytoplasm and eccentric nuclei, which may appear two- or three- lobed. Nucleus stains a deep red/violet colour, while the cytoplasm is either a dark blue ring or not visible.

Eosinophils: Round cells with a round, sometimes eccentrically located nucleus. Nucleus stains pink/orange. Cytoplasm stains pale pink with abundant red/pink shining granules.

Monocytes: Large cells. Cytoplasm sometimes shows extended processes giving the cells an irregular outline. Nucleus stains dark blue/purple with pale blue cytoplasm.

Thrombocytes: Cells may be round, oval, elongate or spindle shaped, and they tend to appear singly or in clusters. Large nucleus. Nucleus stains deep purple, and cytoplasm remains unstained.

Basophils: The occurrence of basophils in fish blood is disputed. If they do occur it is possible that they do not stain sufficiently with May-Grunwald/Giemsa to be recognised.

Differential Cell Counts: 5.5

Prepare the stain as directed by the manufacturer/supplier.
Place the blood smear (which has been air dried and fixed in methanol) in the stain for five minutes.
Wash the slide and allow to dry.

UNIT SPECIFIC GLOSSARY

Anaemia

A condition characterised by a deficiency of functional haemoglobin, packed cell volume and/or erythrocytes. The more important types of anaemia in fish are: normocytic anaemia caused by acute haemorrhaging, bacterial and viral infections, and metabolic diseases resulting in red cell destruction; microcytic anaemia due to chronic haemorrhaging, e.g. caused by external parasites, iron deficiency and the deficiency of certain haematopoietic factors; and macrocytic anaemia (e.g. absence of juvenile cells; too many mature cells) resulting from an increase in haematopoietic activity in the spleen and kidney.

Spectrophotometer

A device that measures reflectance of visible light, or absorbance, as a function of wavelength, thus allowing analysis of colour or comparison of luminous intensities. When light is passed through a solution of a known constituent in a spectrophotometer it measures the absorbance of the solution. This may then be compared to the absorbance of that constituent in a solution of known concentration, thus allowing the concentration of the solution to be determined.
The haemoglobin concentration of the blood sample can be calculated from a curve prepared from known standards.

Drabkin's solution

200 mg Potassium ferricyanide

50 mg Potassium cyanide

140 mg Potassium dihydrogen phosphate

Distilled water made up to 1 Litre

Dacies fluid

Formaldehyde:	10 ml
Trisodium citrate:	31.3g
Brilliant cresyl Blue:	1.0g
Distilled water	1 litre

Dacies fluid should be filtered immediately before use.

Haemoytometer

This is a specialised microscope slide with one or two etched grids and a thick coverslip. It was originally designed to estimate concentrations of blood cells, but it is now widely used in aquaculture to estimate concentrations of planktonic organisms in small sub-samples of water.
There are different types of haemocytometer, one of which is the Neubauer haemocytometer used here.

APPENDIX

AQUALEX Fish Health Toolset: the story

WHY create the Toolset?

We, the AQUALEX Multimedia Consortium (AMC), decided to create the AQUALEX Fish Health Toolset so that basic essential factual knowledge about certain aspects of **fish health** (important for European aquaculture) could be combined with fast-track multi-lingual learning. Successful EU competitive projects had given us the impetus (and the funds) to create a wide range of individual multilingual materials: 3 multilingual Glossaries in 7 languages, language learning units at 3 levels in 10 languages, and fish health course materials also in 10 languages. We brought these separate elements together first as a Concept, then as a working Toolset freely available as **multilingua**l **units/modules** in user-friendly online learning formats.

WHAT is covered in the Toolset

Both the species and the fish health aspects covered result from several European-wide needs analyses which targeted industry and academic users including VET providers. These are:

- Basic Techniques for Fish Haematology
- Fish Health Management Manual
- Aquatic Pathology for rainbow trout, carp, sea bass and turbot
- Fish, shellfish and crustacean meristics (in construction)

HOW to use the Toolset

The Toolse**t** links three different **content areas** and two **language level**s (Beginner: CEFR A1 &A2 and Basic: CEFR A1 & A2). Because the Fish Health content is freely accessible online, you can find the information/content you need, whenever and wherever you need it, whether studying or in the workplace. You can access the **Fish Management Toolset** and try it out via the following link http://www.aqualex.org/index.php/pescalex-courses,

The **3 updated multilingual glossaries (AQUALEX, PESCALEX, MARPOL)** provide high-quality terms and definitions of, respectively, aquaculture, fish diseases and marine pollution terminology (3500 items variously in English, French, German, Greek, Spanish, Italian, Norwegian, Polish, Hungarian, Turkish, Swedish and Galician). Unique multilingual access at a single click. http://www.aqualex.org/index.php/glossaries

The Toolse**t's** language **learning modules** are very helpful for workers in aquaculture, because they exist in languages important in the European aquaculture industry (English, French, Spanish, Norwegian, Greek, Polish, Portuguese, Hungarian, Turkish, Swedish and Galician). The modules can be accessed online at http://www.aqualex.org/index.php/multilingual-esp-language-courses. Syllabus details are given below**.**

WHEN to use the Toolset

The Toolset helps you to communicate in your chosen language through the online modules. Its unique multilingual combinations give you the chance to learn a language

while also acquiring essential basic **Fish Health conten**t in English, French, Spanish, Norwegian, Greek, Polish, Portuguese, Hungarian, Turkish, Swedish and Galician.

A final note

How NOT to use the Toolset

The AQUALEX Toolset materials should not be regarded or used either wholly or in part as a comprehensive fish health manual. There are many such specific and easily accessible publications which are both reliable and comprehensive.

How to use the AQUALEX online language lessons

http://www.aqualex.org/index.php/multilingual-esp-language-courses

The AQUALEX language lessons are designed for **COMPLETE BEGINNERS in English**, whose first/native languages are French, Spanish, Greek, Norwegian, Polish, Portuguese, Swedish, Hungarian, Turkish and Galician.
These online language learning lessons work well as a support for tutor-led blended courses. They will help complete beginners in each language to learn basic grammar points and important aquaculture keywords in English.
They are NOT a complete online course. They simply give basic grammar points within a vocational context (aquaculture). The online format is designed to give beginners a chance to understand and handle simple sentence with essential keywords.
Because English is still the most popular choice for a second language, English grammar points are explained, in English and in the user's language. Where the user language is different from English (i.e., French masculine and feminine nouns), then explanations of the user language structures are given in both English and the user language.

- Level 1 lessons (14) for beginners with no previous knowledge of the target language.
- Level 2 lessons (10) for those with some knowledge, though still at the basic level.
- You can enter each level via the dropdown menus at the top of the page.
- You can enter the lessons (English, French, Galician, Greek, Hungarian, Norwegian, Polish, Portuguese, Swedish, Turkish) by clicking on the country flags as shown.

Each lesson is organised into TEXT, GRAMMAR, HOMEWORK and ASSESSMENT with clickable menus at the top of each page. The ASSESSMENT section is not available to the general public.

- The TEXT menu contains the lesson itself, which may have 5 pages.
- Each page of the lesson can be entered via the NEXT and PREVIOUS live links at the bottom of the page.
- The GRAMMAR section can be entered via the top dropdown menu. Each page of the grammar can be entered via the NEXT and PREVIOUS live links at the bottom of the page.
- The HOMEWORK section cannot be entered until all the TEXT and the GRAMMAR pages have been viewed, in order to consolidate language acquisition.
- All TEXT pages are inter-changeable in all AQUALEX languages, by clicking on the country flag at the bottom of the page.
- **BUT** Grammar and Homework sections are specific to each language and do not have an interactive function.

*TEXT pages also contain **audio material** which can be heard by running the mouse over the words on the page. Click on live items to hear the term as recorded by native speakers (make sure you have the necessary software for this facility (i.e., Quicktime Player).*

In Level 1 you learn:

The use of

- ✓numbers (lesson 1)
- ✓definite article (lesson 1)
- ✓indefinite article the/a/an (lesson 2)
- ✓demonstrative pronouns this/that/these/those (lesson 2)
- ✓singular/plural nouns (lessons 1,3)
- ✓irregular plurals (lessons 3.4)
- ✓countable /uncountable nouns (lesson 6)
- ✓agreements (subject/verb) (lessons 11-13)

how to make statements (lesson 1)

- ✓how to make negative statements (lesson 2)
- ✓how to ask and answer questions (lesson 2)
- ✓true/false response (lesson 4)
- ✓how to tell the time (lesson 6)

how to make simple measurements

- ✓temperature (lesson 9)
- ✓length, breadth, width, height (lesson 10)
- ✓volume (lesson 10)

the use of prepositions

- ✓of place (lesson 8)
- ✓of time (lesson 9)

- ✓names of days (lesson 13)
- ✓names of months (lesson 14)

In Level 2 you learn:

- ✓more adjectives and adverbs (lesson 2)
- ✓comparisons (lesson 3)
- ✓pronouns (lesson personal, relative)4)
- ✓Imperative (lesson 5)
- ✓past tense (lesson 6)
- ✓future tense (lesson 7)
- ✓conditionals (lesson 7)
- ✓modals/gerundive (lesson 8)
- ✓passive (lesson 9)

- ✓many, some, few, a lot of, more (lesson 6)

the use of verbs

- ✓is, are (lesson 1)
- ✓has, have (lesson 2)
- ✓this is, there are (lessons 2, 3)
- ✓present tense (forms and functions) (lessons 11, 12, 13)

Language attainment levels

Level 1 (CEFR) levels A1, A2)

The priority for many **first-time language learners** is to understand and convey simple but vital pieces of information (i.e., keywords) in a new language. The AQUALEX online language lessons in English, French, Spanish, Greek, Norwegian, Polish, Hungarian, Turkish, Portuguese, Swedish and Galician are designed to allow complete beginners to build on their native language knowledge of familiar items in the workplace/laboratory, in a step-by-step visual presentation with audio input. This method gives them a chance to fast-track their learning, at their chosen time and at their own speed.

Level 2 (CEFR levels B1, B2)

Having picked up the first essentials in a user-friendly way, **students or workers** requiring vocationally relevant fish health information can progress at their own pace of learning through the Toolset Fish Health multi-lingual course materials (shown above) in English, French, Spanish, Greek, Norwegian, Polish, Hungarian, Turkish and Galician This can be done online at http://www.aqualex.org/index.php/multilingual-esp-language-courses

Level 3 (CEFR levels C1, C2)

For the seasoned practitioner, Ph.D. student or academic, the AQUALEX Toolset contains two **multi-lingual aquaculture and fish diseases glossaries** in English, French, German, Spanish, Italian, Greek, Norwegian, Polish, Hungarian, Turkish and Galician. These online resources present high-level information and detailed definitions in the accepted academic format.