The Aquarium Vet

e-quarist Course®

Dr Rob Jones

'The Aquarium Vet'

Welcome to The Aquarium Vet e-quarist course®.

In this document you will find the Contents page as well as a few pages from each Module to give you an insight into the course.
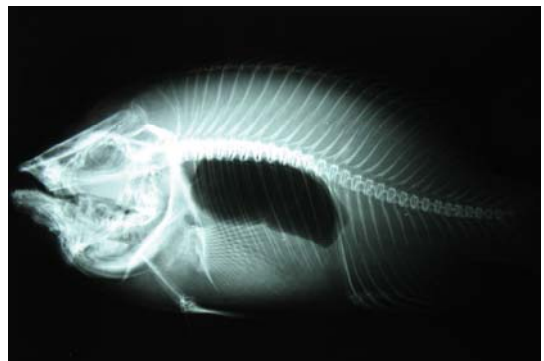
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Module One

Anatomy and Physiology

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Contents

Section of Chapter 3 - Cardio-vascular and respiratory systems

Figure 59 - Discus (*Symphysodon spp.*) – gill monogeneans (*Dactylogyrus sp.*) a very common discus problem (40 times magnification). Monogeneans were previously called flukes. Note air bubble with black circular outline. Air bubbles are often seen and are not pathological.
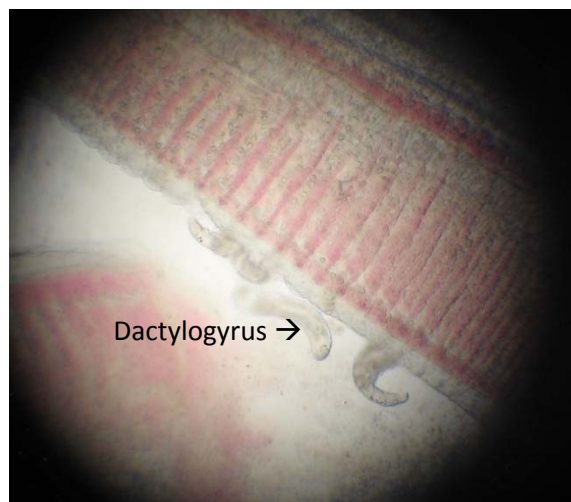

Figure 60 - same case as figure 59 Discus (*Symphysodon spp.)* (100 times magnification).

The next two photographs are of gills when prepared for histology. Basically in this process the tissues are fixed in 10% buffered formalin, sliced very thinly and then stained with Haematoxylin and Eosin (H&E) as the standard stain.
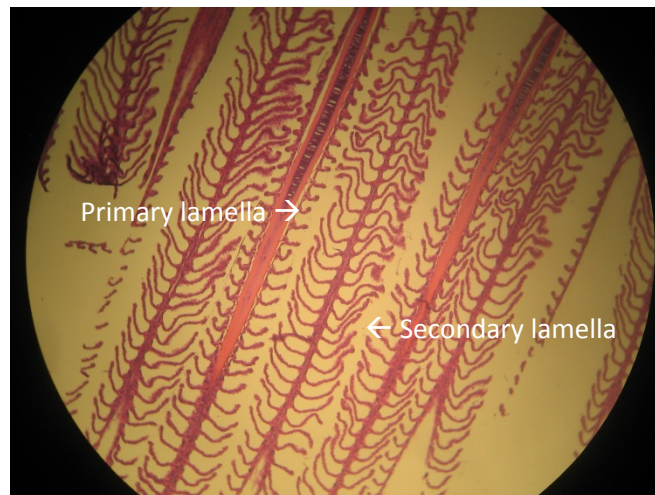
Figure 61 - Barber Perch (*Caesiperca razor)* normal gills (100 times magnification)
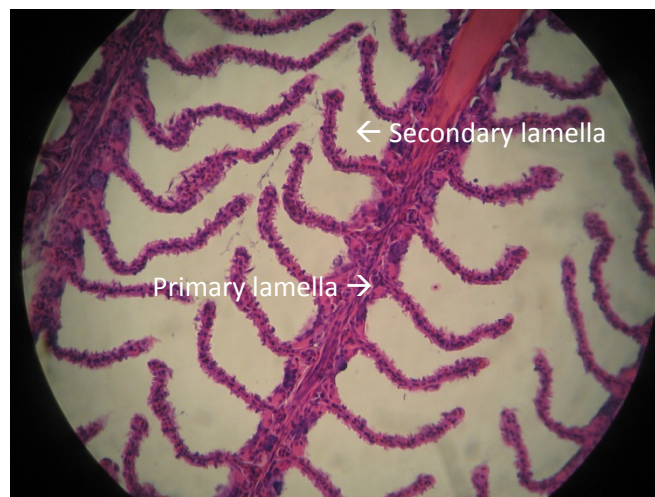

Figure 62 - Barber Perch (*Caesiperca razor*) normal gills (400 times magnification)

Fish suffocate out of water because the secondary lamella collapse and hence the surface area available for oxygen diffusion is reduced to only a fraction of what it is in the water. Catfish (Siluridae) and eels (Anguillidae) will survive for slightly longer because they have thicker secondary lamellae which are further apart than other species and so are less likely to collapse out of the water.

Oxygen is extracted from water by a process called **diffusion**. As previously described, diffusion is the movement of a substance from an area of high concentration of that substance to an area of lower concentration. It is a passive process i.e. no energy is used in the process as compared to an active process which uses energy.

Gills operate under a **counter current mechanism** i.e. the blood passing through the gills flows in the opposite direction to the water passing over the gill surface. This maximizes the possible oxygen transfer, as the blood that contains the least amount of oxygen meets water

that has had some oxygen removed, but still has a higher concentration. The reverse also occurs - blood that has already received some oxygen comes in contact with water that is fully laden with oxygen and therefore at a higher concentration.

Percentage of oxygen concentration in the water

100	80	60	20

Water flow over the gills →

← Blood flow through the gills

100	75	50	25	0

Percentage of oxygen concentration in the blood (haemoglobin)
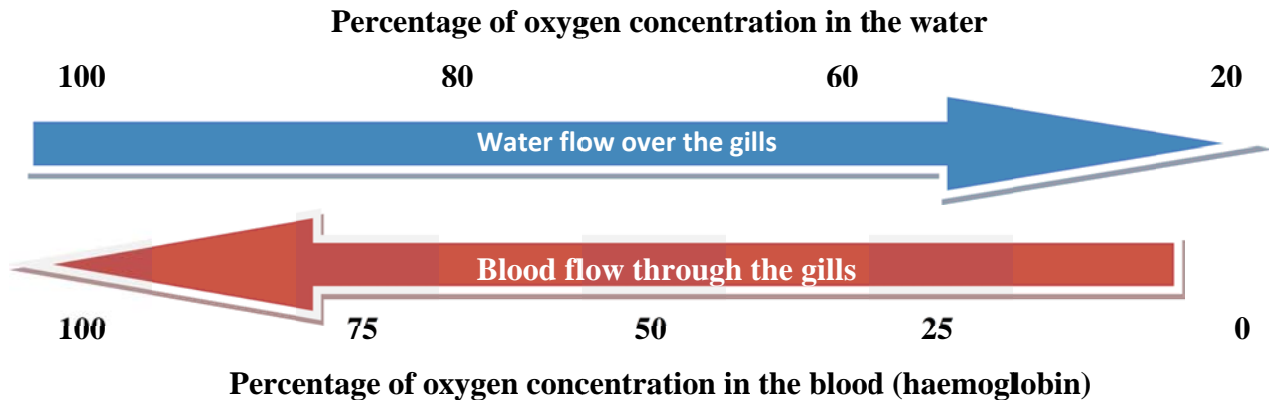
Figure 63 – Diagram of the counter-current mechanism in the gills

In teleosts, the distance between the water and the red blood cells (circulating within the gill capillaries) varies between 1 and 5 μm. A micrometre (μm) is one thousandth of a millimetre. The more active the species of fish, the smaller is this distance. This greatly assists with oxygen diffusion etc. However, it is also a very short distance for pathogens to cross and enter the fish. The gills are therefore a major source of entry for pathogens resulting in infection. Pathogens are disease-causing organisms such as bacteria and viruses and are covered in more detail in Unit 5 – Diseases and Treatments.

The oxygen requirement of the fish varies with the level of activity for example resting versus active swimming. The area of gills used can vary by a factor of up to six at maximum metabolic rates. This occurs due to an increased number of secondary lamellae being perfused with blood to their maximum capacity. Another alteration that can occur is to change the angle of gill arches to increase oxygen uptake – the adductor muscles are responsible for this. These processes are all under the control of the Autonomic Nervous System (ANS) and, as such, are involuntary. The ANS is discussed further in Section 9 – Nervous system and Sensory organs.

The process of oxygen extraction from the water requires a very high energy requirement compared to mammals breathing air. Up to 10% of the oxygen extracted by fish is in fact used for ventilation purposes – driving the buccal and opercular pumps or the swimming required for ram ventilation and the blood circulation involved. Remember the oxygen actually enters via diffusion which is a passive (no energy involvement) process. Water that is too low in oxygen (usually warm temperatures and still water) may cause respiratory distress and may even lead to death.

The rate of oxygen diffusion across the gills varies with two factors –

- Surface area of the gills – the rate is **directly** proportional to the surface area of the gills. An increase in the surface area leads to increased diffusion.
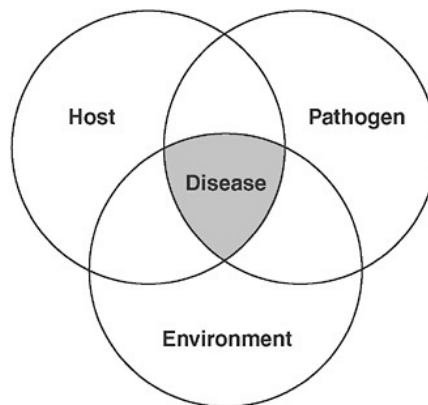
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Module Two

Disease Concepts and Diagnostics

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Contents of this Unit

Section of Chapter 5 - Diagnostic tests

5.1 Skin scrape

This is one of the most common procedures carried out on live fish to assist in a diagnosis, and an essential part of each and every post-mortem. There is no minimum size limit of live fish upon which this procedure may be performed. I have performed it succesfully upon 2.5 cm Neon Tetras (*Paracheirodon innesi*).

Equipment required –
- Microscope slides – the frosted ended ones are always better quality
- Coverslips
- Scalpel or scissors
- Water – freshwater or seawater (see below) with a dropper
- Microscope
- Digital camera or attachment

Figure 27 - Equipment required for skin scrape

The fish is restrained either in a net or plastic bag and the area of the body to be scraped is then lifted out of the water momentarily so that the sample collected is not washed away. In some cases a second person will help to make the process easier.

Usually a small area on the side of the body is targeted, although the area at the base of the fins is recommended by some. Using the back of a scalpel blade or a scissor blade, a **gentle** scrape is taken and the resulting sample – mainly mucous - is placed on a microscope slide, add a few drops of water (see below) and then a coverslip is placed in position ready for

examination. This is then called a **wet preparation** (or **wet prep**) and is a standard technique used for many other samples as well.

Figure 28 - Taking a skin scrape from an Australian Snapper (*Chrysophrys auratus*)

Figure 29 - Placing the skin scrape material on the slide
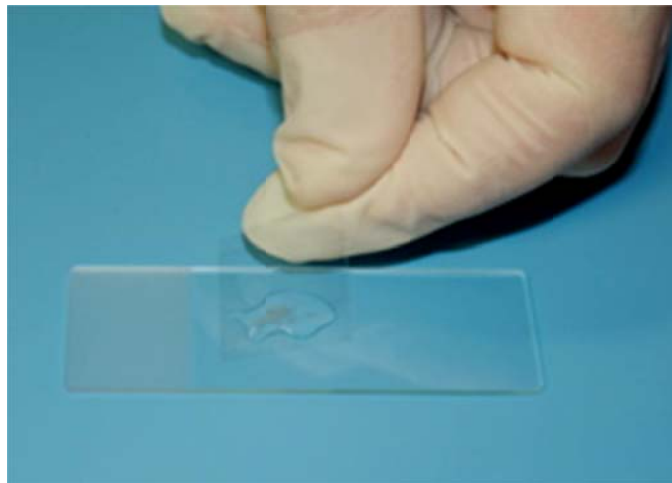
Figure 30 - Adding the water to the slide

Figure 31 - Placing the cover slip

IMPORTANT NOTE

The type of water added to the sample on the microscope slide is very important. For freshwater fish it is important to use freshwater (distilled water is ideal) and for marine fish use seawater (preferably the water the fish has been in). For freshwater fish the use of tap water (which may contain high chlorine levels) is not ideal.

If it is a marine fish parasite that you are examining and it is placed in fresh water, there will be an osmotic pressure on the parasite, and the parasite may die (no motility) and may even rupture and not be seen. Remember salt water baths are used in freshwater fish to remove parasites. The reverse applies for freshwater fish. Movement of parasites is critical in making a diagnosis.

Examine initially at 40 X (low power) and then at 100 X and finally 400 X (called high dry). Generally with wet preparations, do not use the oil immersion (1000X). The eye pieces have a 10 X magnification and so by choosing different lenses the two must be multiplied together. Thus the 4 x lens piece with the eye pieces (10 X) gives 40 X magnification (4 x 10 = 40). Similarly the 40 X lens with the eye pieces gives 400 X magnification (40 x 10 = 400). See Appendix Two for Microscope Use and Care.

Refer back to Unit One for the section on skin and the pictures that show normal scales etc. (figures 26 to 32 inclusive).

Scan the entire sample at low power (40 X) before going to higher powers for a better examination. Look for movement and then the type of movement if present i.e. ciliated or flagellated. These terms refer to the main two different types of protozoans which are discussed in more detail in Unit 5 – Diseases and Treatments. See back to Figure 14 which shows two *Ichthyophthirius multifilis* (which is ciliated) trophonts from a skin scrape (times 40 magnification).

Figure 32 - Large monogeneans from a Yellowtail Kingfish (*Seriola lalandi lalandi*). A = head with hooks (haptors); B = microscope slide preparation showing monogenean in the circle; C = tail end (A and C are times 40 magnification).
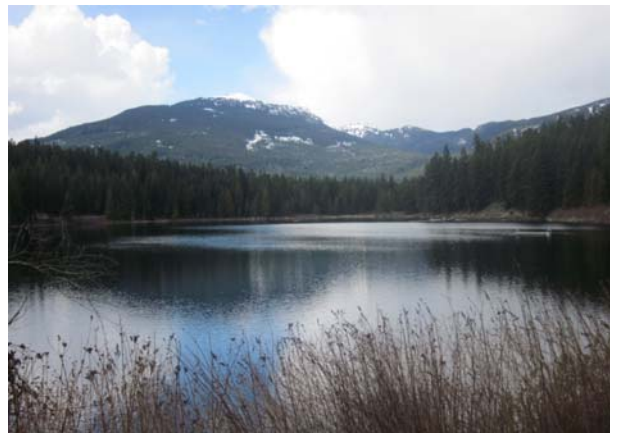
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Module Three

Water Chemistry and Quality

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Contents of this Unit

Section of Chapter 7 – The Nitrogen Cycle

Ammonia is produced by fish as the end product of protein (nitrogen) metabolism. This is covered in more detail in Unit 6 – Nutrition and Reproduction. Ammonia is predominantly excreted across the gills directly into the water (covered in Unit 1 – Anatomy and Physiology). Only a very small amount is excreted by the kidneys into the urine.

The other sources of ammonia in water are from fish faeces (small amount), dead fish, decaying plant matter and any uneaten food which can be broken down by bacteria to ammonia.

Ammonia is colourless and has no odour unless present in very high levels in water. In water ammonia is present in two forms (see chemical equation below) –

- **ammonia** (NH_3) – undissociated or non-ionised or free
- **ammonium** ion (NH_4^+) - dissociated or ionised or bound

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$
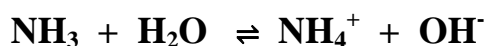
The ammonium ion is called ionised because it has a positive charge (it is a cation).

The non-ionised molecule of **ammonia** (NH_3) is **very** toxic to fish, while the **ammonium** ion (NH_4^+) is relatively non-toxic. As a comparison, the non-ionised molecule of **ammonia** (NH_3) is approximately 100 times more toxic to fish than the **ammonium** ion (NH_4^+). This is because NH_3 is far more capable of diffusing across the gill membrane than the ammonium ion (NH_4^+).

The majority of measurement techniques are of **total ammonia nitrogen** (TAN) which is a combination of both forms. This is discussed later in this section in more detail. Fortunately there is a process (the nitrification process) that breaks down ammonia into a less toxic product called nitrate.

Nitrification Process

The nitrification process uses bacteria. There are two main groups of bacteria -

- Autotrophic bacteria – cellular carbon is obtained from inorganic sources such as carbon dioxide (CO_2)

- Heterotrophic bacteria – cellular carbon is obtained from organic sources

Ammonia (NH_3) is very toxic, however through a two-step process called nitrification it is first converted to nitrite (NO_2^-) and then to the less toxic nitrate (NO_3^-).

| Ammonia | | Nitrite | | Nitrate |
| NH_3 / NH_4^+ | → | NO_2^- | → | NO_3^- |

Fig 25 – the Nitrification process

This process of nitrification is undertaken by a variety of bacteria and requires oxygen – **it is an aerobic process**. In aquariums, this occurs in a biological filter or biofilter. The biofilter requires a large surface area for the bacteria to attach to, a good oxygen supply and the nutrients (ammonia and nitrite).

Figure 26 – Common biofilter media (large surface areas)

I have simplified the following two equations as it is the concept and not the exact chemistry that is critical to our understanding. The first step involves the addition of oxygen to ammonia (actually it is the ammonium ion form) as follows -

$$NH_4^+ + O_2 \rightarrow 2NO_2^- + 5H^+ + H_2O$$
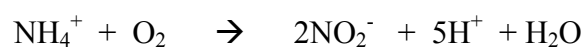
NB. The two sides are not balanced as I have simplified the actual equation that occurs. Also note the production of hydrogen ions (H^+), which as previously discussed will cause the pH to decrease. This is a major issue in closed aquaria.

In freshwater nitrite (NO_2^-) is produced from ammonia by two main groups of bacteria - *Nitrosomonas marina*-like and *Nitrospira* both of which require oxygen (aerobic bacteria). They are often called Ammonia Oxidising Bacteria (AOB). Previously these were only considered as *Nitrosomonas sp.*. The nitrification process in freshwater is most active in the pH range of 7 to 8 and at a temperature range of $25 – 33^oC$. In fact below a pH of 6 many of the bacteria cease to function and the biofilter becomes inefficient.

The second step (once again simplified) involves the addition of oxygen to nitrite as follows -

$$2NO_2^- + O_2 \rightarrow 2NO_3^-$$
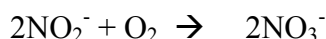
Nitrate (NO_3^-) is produced from nitrite by *Nitrospira*-like bacteria in freshwater which are also aerobic. They are often called Nitrite Oxidising Bacteria (NOB). Previously these were only considered as *Nitrobacter sp.* Nitrate is much less toxic to fish and much higher levels can be tolerated before problems arise.

The nitrification process also requires an adequate level of alkalinity. This is due to the hydrogen ion (H^+) production that occurs as a by-product of the process. If the alkalinity is less than 20 mg/L, then the nitrifying bacteria will not function.

If you are having trouble remembering the nitrification process, then there is a word association. The nitrate is the last of the three stages because the nit**rate** comes l**ate.**

High levels of organic carbon compounds can promote heterotrophic bacteria which then compete with the nitrifying bacteria.

The process of nitrification is a high oxygen demand process. For every 1 kilogram of NH_4-N converted to nitrate (NO_3-N) there is a requirement of a little over 4 kilograms of oxygen (O_2) to complete the process. As such an oxygen level (DO) of less than 80 % saturation may impede the efficient function of the biofilter.

Biofilters in general are discussed in more detail in Unit 4 – Life Support Systems. However, one important point to make here is that whenever cleaning out a biofilter, it is important to preserve the bacteria. Hence, it is advisable to not use tap water to rinse the filter media. This is because tap water may contain chlorine or chloramines which could destroy the nitrifying bacteria in the filter and reduce its efficiency. Use some of the tank water and then discard the water.

In the past decade there have appeared many commercial preparations of "starter" bacteria which are designed to fast track the establishment of biofilters. They certainly do assist, but regular water testing is still imperative. The same applies with the use of ammonium chloride (NH_4Cl) to feed a maturing biofilter before adding fish. Adding 0.9 grams of ammonium chloride per 100 litres of water will achieve a level of 3 ppm (total ammonia) – see the calculations below.

High levels of ammonia and nitrite, rather than acting as an extra food source, actually have a negative impact on the biofilter bacteria and will kill the bacteria. This is important if using ammonium chloride as discussed above. In this scenario, the total ammonia nitrogen (TAN) (expressed as NH_3-N) should be always be less than 5 ppm (mg/l).
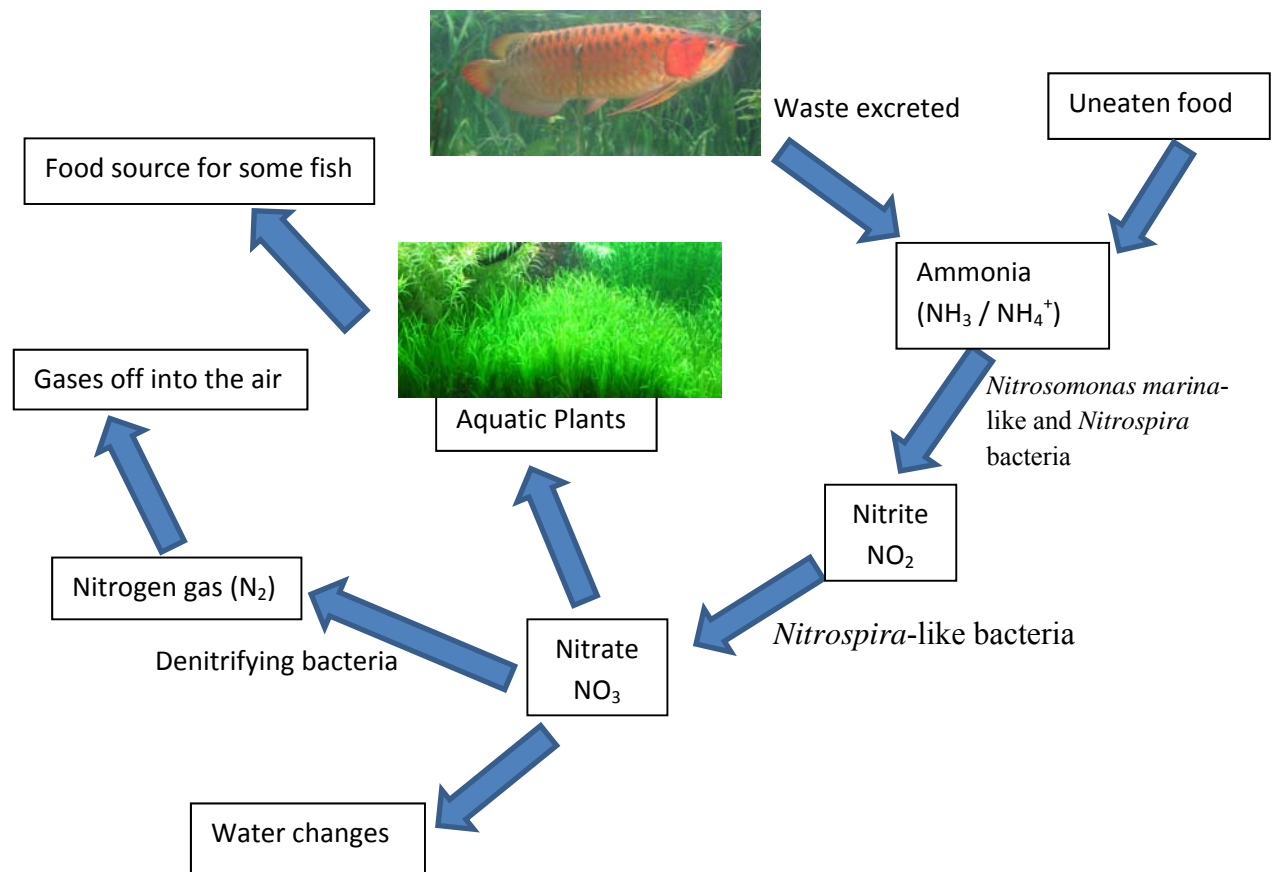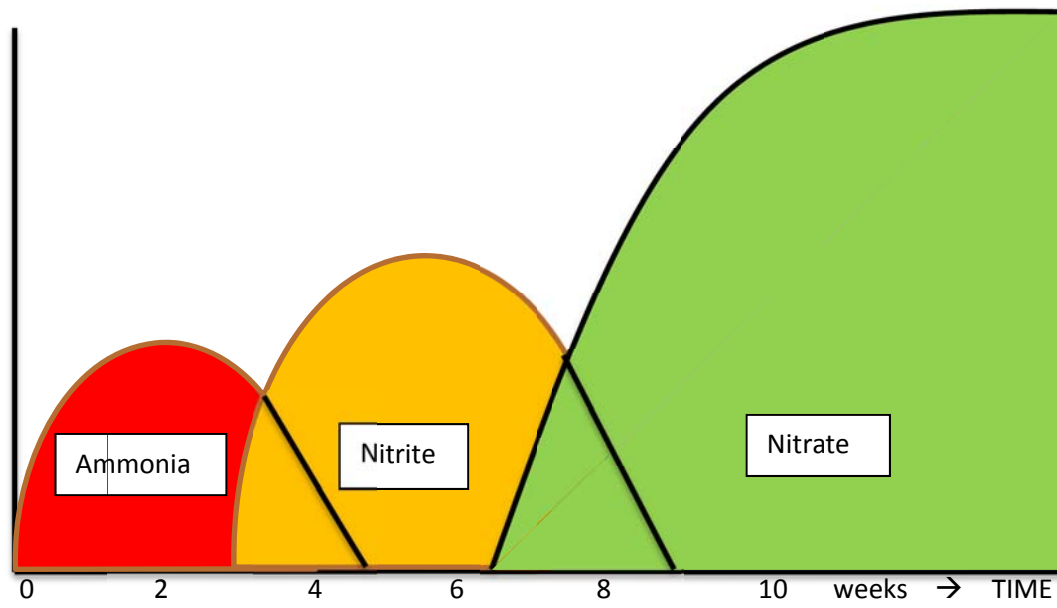

Figure 27 - The Nitrogen Cycle in freshwater

Figure 28 – The nitrification cycle in freshwater with a peak of ammonia, followed by nitrite and finally nitrate (approximate times)

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Module Four

Life Support Systems

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Section of Chapter 11– Ultraviolet Radiation

There are two main agents used for water sterilisation and pathogen reduction in aquarium systems. The first is ultraviolet radiation (UV) and the second is ozone which is discussed in the next section. A advantage of UV is the lack of any toxic residuals compared to ozone.

Disease transmission is covered in greater detail in *Unit 5 - Diseases and Treatments*. Briefly there are two means of transmission of pathogens – horizontal and vertical. Horizontal refers to the transmission of a pathogen within a generation i.e. from one fish to another whereas vertical transmission refers to transmission from one generation to another via eggs or semen. Horizontal transmission mainly occurs through the water, although hands and nets can be other important ways of spreading disease. Generally we are only considering horizontal transmission when we are discussing UV and Ozone.

Louis Pasteur (1822 – 1895) first recognised that sunlight was germicidal. However, it was not until 1893 that Marshall Ward showed that it was the UV radiation that was the cause of this phenomenon.

As discussed in the previous section, ultraviolet (UV) radiation forms part of the electromagnetic spectrum. UV radiation has a spectrum wavelength of 10 – 400 nm.

Within the UV spectrum there are several sub-groups (see also Figure 61 over the page):

1. UV type A – spectrum range of 315 – 400nm and is normal UV light or blacklight
2. UV type B – spectrum range of 290 – 315nm, usually associated with sun-tanning and the skin's formation of Vitamin D
3. UV type C – spectrum range of 200 – 290nm, is germicidal and used for disinfection
4. UV Vacuum – spectrum range of 10 – 200nm. Although vacuum UV is germicidal it rapidly dissipates in water and is therefore not practical for disinfection purposes

While UV A and B have some germicidal properties, we are mainly dealing with UV C in this discussion. UV C is largely blocked from reaching the earth's surface by the ozone layer.

However, it is important to remember that the sun produces UV rays, and that in outdoor exhibits the sun's UV rays will actually have some water disinfection properties. The amount will depend on the clarity of the water and the absence of clouds. UV rays are also absorbed by glass and plastic.
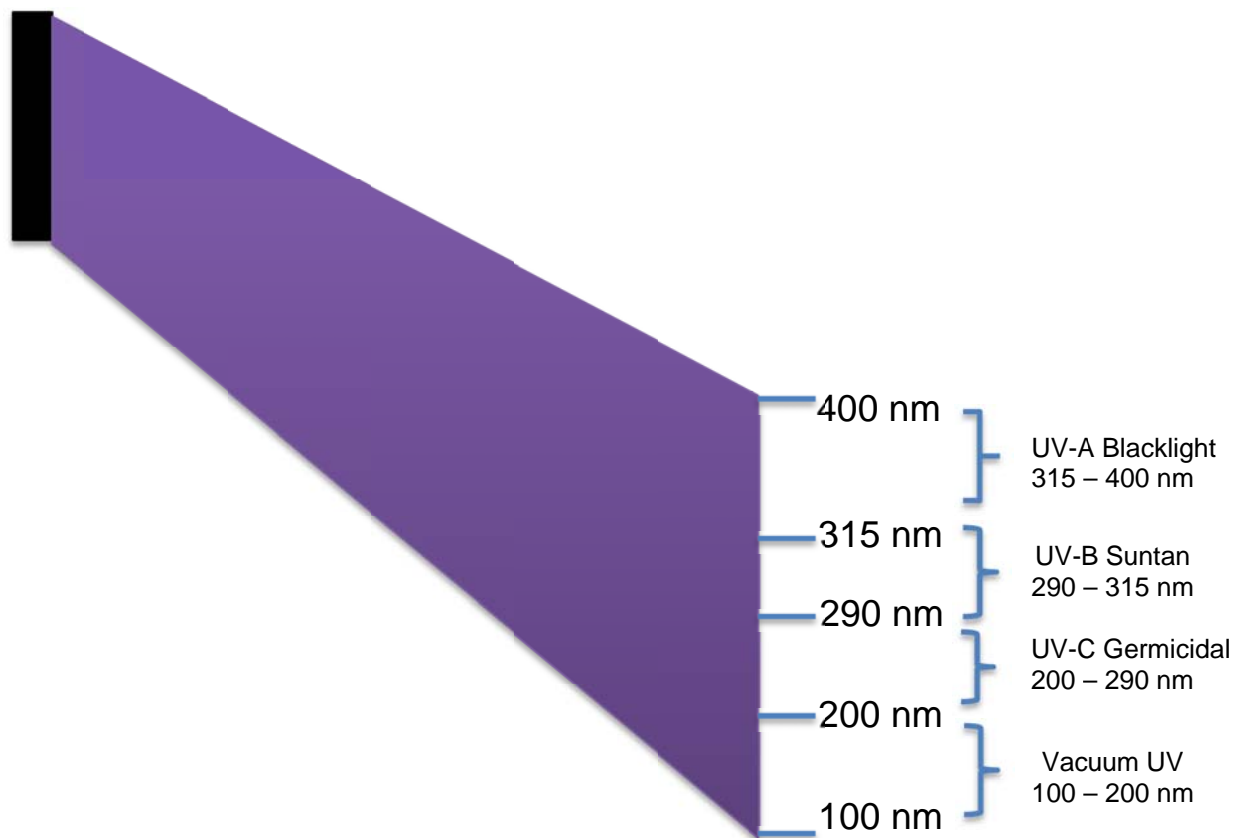
Figure 61 - Various UV subtypes

Effects of Ultraviolet Radiation

Ultraviolet (UV) radiation kills pathogens by inactivating the **Deoxyribonucleic acid** (DNA) or **Ribonucleic acid** (RNA) that is present within the nucleus of cells. This prevents the pathogens from multiplying and thus attacking a host (the fish). **DNA absorbs UV C most effectively at the 260nm wavelength**. At 280nm wavelength, some UV is absorbed into the aromatic rings of some amino acids which damages proteins within the cells. This will also have a negative effect on the cells and cause death.

The effect of UV C is different to chemical disinfectants which generally act only by damaging cell structures, such as the cell wall, and interfering with the pathogen's metabolism.

Some organisms have the ability to repair the damage caused by UV C to DNA and RNA. Depending on the pathogen this may increase the dose of UV needed to completely inactivate the pathogen. Normal light can assist with DNA repair after UV exposure and thus preventing access to light will increase the kill rate. Whilst this will be rarely practical, it is an option worth considering in certain circumstances.

It should also be noted that high doses of UV will remove chlorine and chloramines. To reduce chloramines a dose in the range of 60 to 200 mWs/cm2 (mJ/cm2) is required. An explanation of these dose levels is presented later in this section.

Artificial Production of Ultraviolet Radiation

UV radiation is usually produced in a mercury (Hg) vapour lamp. An electrical current passes through the mercury vapour and excites the electrons in the mercury atom. This excess energy (**photons**) is then released as UV radiation of a specific wavelength, dependent on the gas (in most cases mercury) and the electrical voltage used.

UV lamps are housed within lamp sleeves that are tubes of quartz silica. The sleeve is open at both ends to allow for water flow. The distance between the exterior of the lamp and the interior of the lamp sleeve is usually about 1cm (1/4 inch).
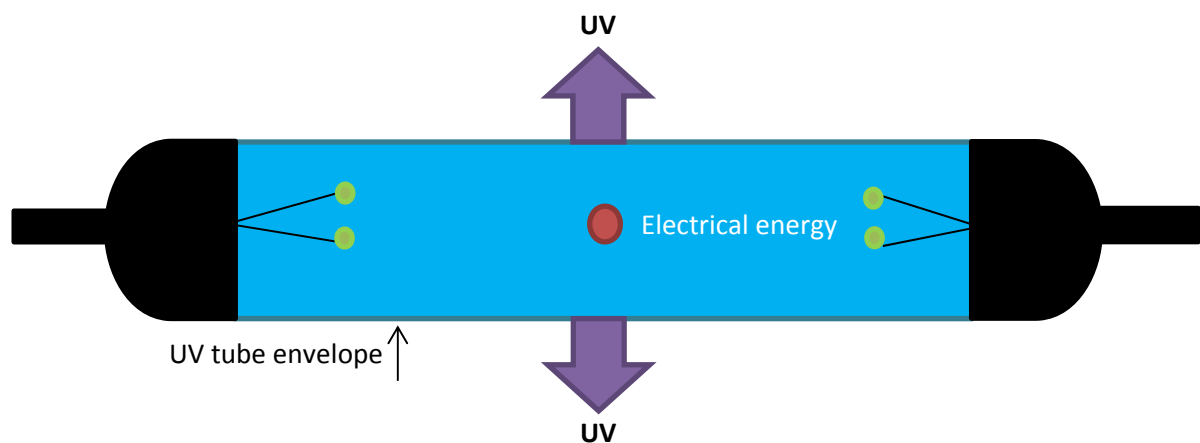

Figure 62 – Diagram showing the production of UV radiation

There are generally two types of UV units available:

1. **Low pressure**

Low pressure UV units operate at a low vapour pressure of mercury and a moderate temperature of about 40°C (104°F). About 95% of the UV produced has a wave length of 253.7nm (monochromatic). See figure 64. Note that this is very close to the wavelength at which DNA absorbs UV C most effectively (260 nm). These units are generally inexpensive to purchase compared to medium pressure units. The output of low pressure UV units ranges from 40 to 280 watts, with up to 100 watts per metre of lamp length. At this range, some DNA repair can occur influenced by exposure to light and the enzyme photolyase.

Low pressure UV units convert a greater percentage of their energy input to producing UV C than a medium pressure UV unit (see below).

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Module Five

Diseases and Treatments

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Section of Chapter 9 – Protozoans

With protozoans there are many species, some of which are parasitic and some which are not. I have divided them into those that are predominantly external parasites (ectoparasites) and those that are predominantly internal (endoparasites).

9.1 External Protozoans

The external parasites are those that affect the gills, skin and fins of the fish, but can at times burrow into the skeletal musculature.

9.1.1 White Spot or Ich

Agent (Pathogen): The most common external parasite in fish is the protozoan commonly known as white spot or Ich. There are two main species:

- *Ichthyophthirius multifilis* – freshwater Ich
- *Cryptocaryon irritans* – marine Ich

Both are large, being up to 1mm (1000 μm), and are slowly motile ciliates with almost a rolling movement. *Ichthyophthirius multifilis* often has a crescent-shaped nucleus visible ("smiley face") which is not seen in *Cryptocaryon irritans*.

They are common in the environment and most systems will harbour these ciliates in very low numbers and outbreaks usually occur because of the presence of a stressor (remember the three circles).

Life Cycle: Both the freshwater and marine species have similar life cycles.

Understanding the various stages of the life cycle is crucial to knowing how this affects the success of treatments etc. Figure 46 illustrates the *I.multifilis* life cycle.

The four stages are:

- **Trophont** – this stage lives on the fish (see Figure 47 and 48). It can reside on the gills where it causes irritation and the production of excess mucous. It mainly resides on the skin where it buries under the epithelium and grows to become quite large – up to 1 mm and is the white spot that is visible to the naked eye and gives the common name of the disease.
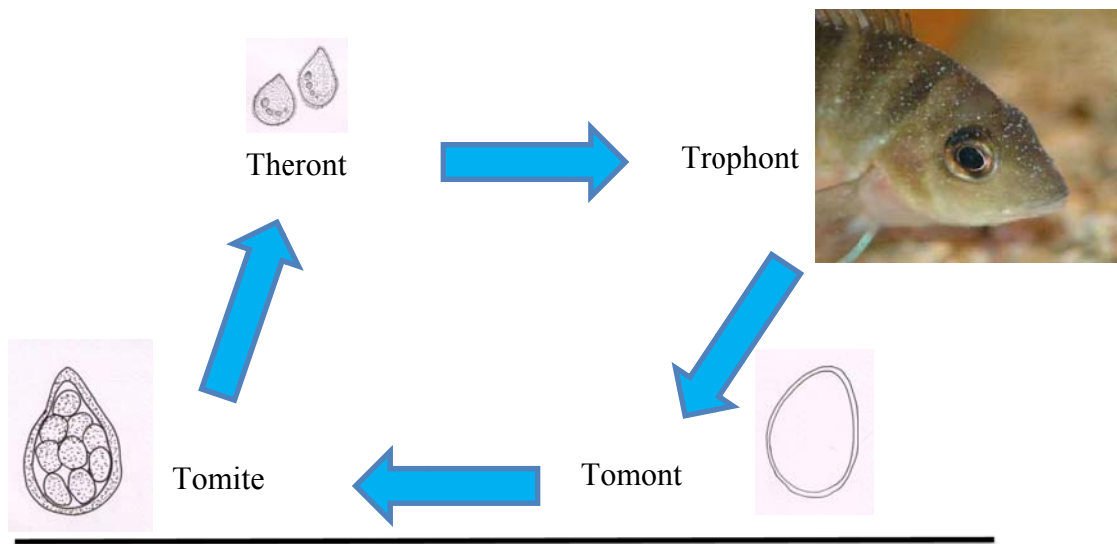

Figure 48 - Life cycle for the protozoan *Ichthyophthirius multifilis*. The dark line at the bottom represents the substrate of the tank.
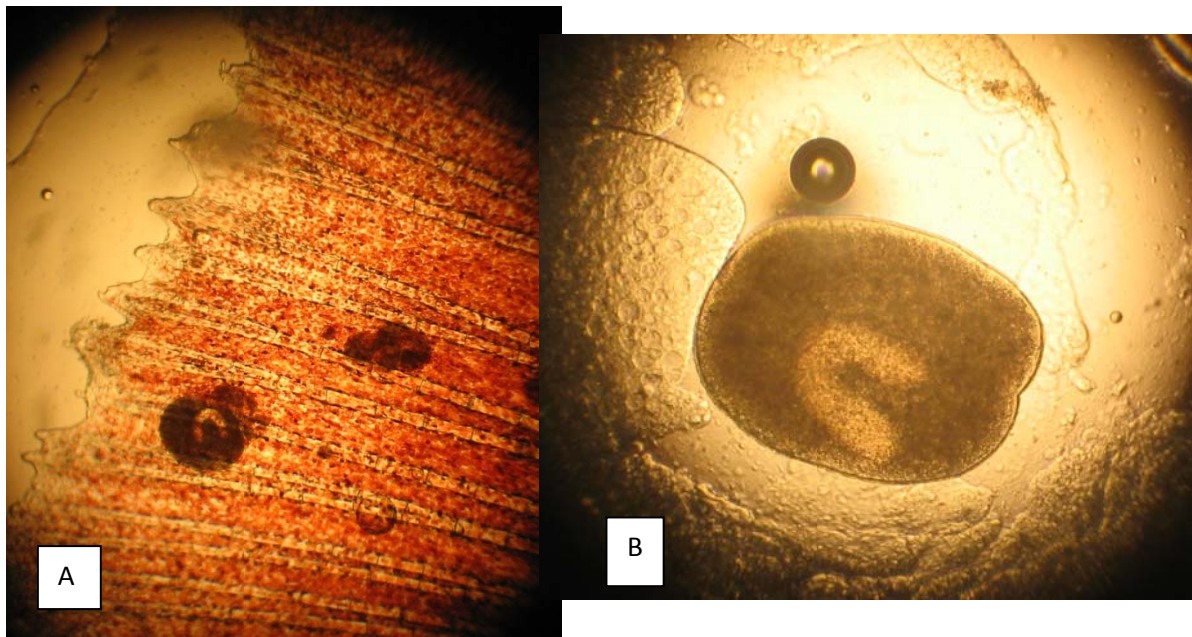

Figure 49 - Ichthyophthirius multifilis trophonts from an infection in a Murray Cod (Maccullochella peelii) A = Fin clip (40 X magnification); B = Skin scrape (400 X magnification). Note the "smiley face"nucleus.

- **Tomont** – this stage develops after the trophont falls off the fish and encysts on the substrate including the gravel, rocks and sides of the tank. Inside this cyst stage there is a rapid multiplication process so that each tomont rapidly develops to contain up to 256 tomites.

- **Tomite** – these are the very small stages that reside inside the tomont. This rapid multiplication stage happens in as short as 2-3 days and is responsible for the very rapid build-up of numbers that can occur in a white spit outbreak. After only 1-2 days the tomites break out and become free swimming as the theront.

- **Theront** – this is the infective stage that is free-swimming in the water and then attaches to the fish and buries into the epithelium and produces the trophont. This infective stage only survives 3-4 days without a host.

IMPORTANT NOTE

I always use a word association to help me remember things that are not always easy to remember.
Applying this method to these life stages -

- Trophont lives on the fish – because it contains the sound f (ph)
- Tomont lives in the substrate contains the letter m for mud
- Tomite which are very small (the "wee little mites")
- Theront free-swimming stage contains the letter r because it roves around looking for a new fish

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Clinical Signs: The clinical signs vary depending on the severity of the infection. It is possible to see the white spots (the trophonts) with the naked eye. However, it is not possible to make a diagnosis of Ich based upon the presence of white spots as there are several other problems that can appear as white spots (previously discussed under lymphocystis in Section 5).

There will usually be flashing due to the irritation caused by the trophonts burying themselves into the epithelium of the skin. Death occurs due to osmoregulatory issues as the trophonts leave the skin, effectively leaving a hole, as well as the effect that many may have draining nourishment from the fish. There also appear to be different subspecies as some will

mainly infect the gills and cause an increased respiratory rate, with others predominantly on the skin. Death commonly follows due to irritation of the gills, mucous production and respiratory failure.

Diagnostic Tests: Diagnosis is readily made by a skin scrape or a gill clip which shows large fairly slowly moving ciliates. The pictures below show many *Cryptocaryon irritans* present in a gill clip taken at post-mortem.

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Module Six
Nutrition and Reproduction

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NUTRITION SECTION

Contents

Vitamins and Minerals

Following on from the macronutrients (protein, carbohydrate, fat) we will now look at the micronutrients. The micronutrients include vitamin and minerals, which though required in much smaller quantities compared to the macronutrients, still play vital roles in the body function.

Vitamins

Vitamins are organic compounds, required in many metabolic reactions within the body – ranging from general metabolism to normal growth and reproduction. When not present i.e. a deficiency, then a variety of conditions can occur, depending which vitamin is deficient. Due to the large number of fish species that we deal with, accurate required vitamin levels are not available for most species. The required levels are usually therefore extrapolated from species that have been studied, which are often aquacultured species such as members of the salmonid family.

Most vitamin deficiencies show a variety of signs, which are often vague. It is rare to be able to make a diagnosis from just looking at the fish signs or pathology tests. The signs can also vary from one species of fish to another. Some of the vitamin deficiencies are very unlikely to occur with normal diets. Often, only by formulating a diet, deficient in a specific vitamin, has a deficiency been demonstrated.

Confirming a diagnosis of a vitamin deficiency is not easy as testing is expensive. The first step is to rule out other causes of the problem. If there appear to be none, and a dietary issue is suspected, then review the manufacturing of the diet as well as the handling and storage of the diet after leaving the manufacturer. Additional vitamin / mineral supplementation (avoiding the fat-soluble vitamin excess that is possible) may be trialled to see if it resolves the problem.

There are two main groups of vitamins:

- fat-soluble vitamins which include vitamins A, D, E and K
- water-soluble vitamins which are predominantly the vitamin B group and vitamin C

As they can accumulate in the body, it is possible to overdose on fat-soluble vitamins and problems can arise. Fat-soluble vitamins are measured in International Units (I.U.), except for vitamin K; while water-soluble are measured in milligrams (mg).

It is very difficult to overdose with the water-soluble vitamins. However they are the ones that are most at risk of being destroyed by a variety of means as described below.

Water-soluble vitamins can be leached from certain diets when they are placed in water. In a study by Pannevis and Earle (1994) a substantial percentage of Vitamin B_{12}, choline, pantothenic acid and vitamin C were lost within 30 seconds of the commercial flake diet entering the water.

> ## IMPORTANT NOTE
>
> **Any vitamin supplement should have low levels of fat-soluble vitamins, as they can accumulate in the body and be toxic**

Fat-Soluble Vitamins

Vitamin A

Plants produce pigments called **carotenoids** which range from yellow to red in colour, and some of which have a Vitamin A-like activity. Carotenoids are simple molecules containing carbon, hydrogen and sometimes oxygen. There are two groups:

- xanthophylls (oxygen containing)
- carotenes (non-oxygen containing)

The carotenoids are important for colouration in fish. In Chapter 5 on Food Types, they will be examined in more detail. For now, refer back to *Module One – Anatomy and Physiology* to the explanation of Chromatophores (lipophores) in Chapter One.

B-carotene produces two molecules of retinol when it undergoes hydrolysis. Retinol is often referred to as the active form of vitamin A. Vitamin A is required for cell membrane production, eyesight, bone development and reproduction. Retinol (as part of the compound rhodopsin) is an essential part of the photochemical reaction that occurs in the retina at the back of the eye that results in vision.

A deficiency of Vitamin A can therefore cause vision issues, as well as poor growth rates and reduced fertility. Other signs can include exophthalmos, skin and fin haemorrhages and deformed opercula.

Vitamin A, like other fat-soluble vitamins, is stored in the liver if there is a dietary excess present. There is thus a considerable amount of vitamin A present in the livers of fish and so

if intact fish, non-eviscerated, are fed then no or minimal vitamin A supplementation is required. In fact, over-supplementation can produce hypervitaminosis A, resulting in spinal deformities.

Both B-carotene and retinol are sensitive to oxidation in diets. Hence, additional vitamin A should be, incorporated into commercial fish feeds. The general recommended minimal nutritional requirement for vitamin A is 1000 to 2500 I.U. per kilogram of food.
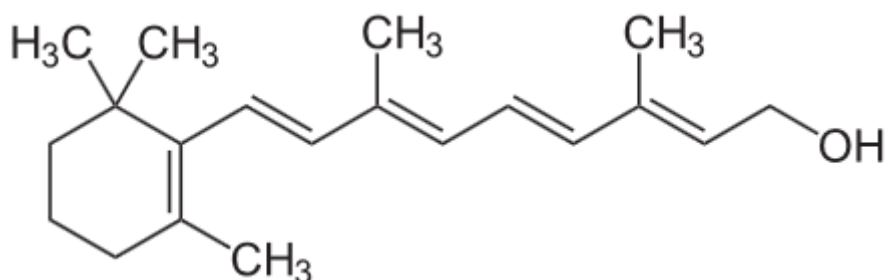

Figure 13 - Vitamin A (retinol) (from https://commons.wikimedia.org/wiki).

Vitamin D

Vitamin D occurs in two forms ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3). Fish utilize Vitamin D3 more efficiently and so rarely is Vitamin D2 discussed.

Cholecalciferol (vitamin D3) is absorbed from the intestine and in the liver is converted to 25-hydroxycholecalciferol and this in turn is converted to 1, 25-dihydroxycholecalciferol (the active hormone) in the kidney. The hormone 1, 25-dihydroxycholecalciferol is responsible for maintaining calcium and phosphorus blood levels in the body, by altering the rate of intestinal absorption.

Calcium levels are essential for muscle function and so a vitamin D deficiency can lead to tetany (involuntary muscle seizures). Bone ash content (calcium dependent) is also reduced.

Plants contain no vitamin D. Additional vitamin D (usually via fish oil) should be, incorporated into commercial fish feeds The general, recommended minimal nutritional requirement for vitamin D is 500 to 2400 I.U. per kilogram of food.
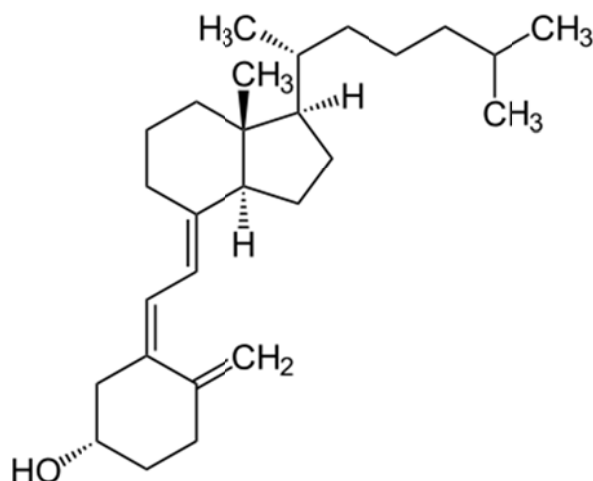
Figure 14 - Vitamin D3 (cholecalciferol) (from https://commons.wikimedia.org/wiki).

Vitamin E

Vitamin E refers to a group of compounds, called tocopherols, with alpha- or α-tocopherol having the greatest vitamin E activity. The major role of Vitamin E is as a metabolic antioxidant preventing oxidation reactions of the unsaturated **phospholipids** in cell membranes, as a free radical scavenger and in the synthesis of **prostaglandins**.

Ethoxyquin, a synthetic anti-oxidant used in food preservation, also has some Vitamin E activity. Thus, the use of ethoxyquin may reduce the level of vitamin E needed in the diet.

Vitamin E is essential for optimal reproduction, i.e. good ovarian and testicular development, good muscular function, the circulatory, nervous and immune systems. Stress can increase the requirement for vitamin E.

A vitamin E deficiency often shows up as a muscular dystrophy. Dystrophy is a degeneration or wasting away of a tissue (in this case the muscle) due to disease or a nutritional issue. Vitamin E can also cause reduced fertility and reproductive capability. Other signs can include anaemia and fatty liver.

Alpha-tocopherol is found in most plant seeds and dietary supplementation is usually needed. In commercial feeds with high levels of polyunsaturated fatty acids (PUFAs), such as fish oils, additional vitamin E is necessary to act as an anti-oxidant. The general, recommended minimal nutritional requirement for vitamin E is 30 to 50 I.U. per kilogram of food.

As vitamin E is fat-soluble, it is possible to overdose and a hypervitaminosis E may actually induce a vitamin K deficiency (see next page) because of competition for uptake in the gut.
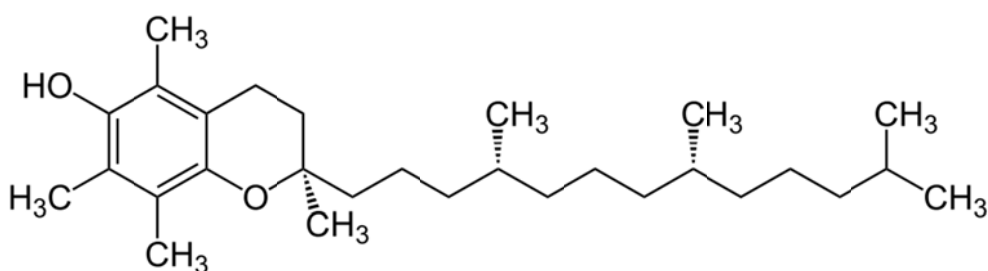
Figure 15 - Vitamin E (alpha-tocopherol) (from https://commons.wikimedia.org/wiki).

Vitamin K

Vitamin K is essential for blood coagulation (clotting). Several of the clotting factors, including prothrombin and proconvertin) require vitamin K for their synthesis in the liver. Hence, a deficiency can lead to spontaneous haemorrhage with prolonged clotting times and death. The coagulation process was discussed in *Module One – Anatomy and Physiology*.

The general, recommended minimal nutritional requirement for vitamin K is 10 mg per kilogram of food. Vitamin K_3 (menadione) is a synthetic form that is usually added to feed as it has a higher vitamin K effect than the naturally occurring vitamin K_1 (found in green plant leaves) and vitamin K_2 (found in fish meal).

Water-Soluble Vitamins

The B group of vitamins includes:

- Vitamin B1 – Thiamine
- Vitamin B2 – Riboflavin
- Vitamin B3 – Niacin
- Vitamin B5 – Pantothenic acid
- Vitamin B6 – Pyridoxine
- Vitamin B7 – Biotin
- Vitamin B9 – Folic acid
- Vitamin B12 – Pantothenic acid

REPRODUCTION SECTION

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Module Seven

Elasmobranchs

Dr Rob Jones
'The Aquarium Vet'

Contents

Section of Chapter 2   2.2 Osmoregulation

Fish face problems, in comparison to terrestrial animals, because they are immersed in water of a different salinity to their internal body osmolality. In addition, their respiratory apparatus (gills) has a large surface area and the gill capillaries are usually a distance of less than 10μm from the water. The principles of teleost osmoregulation have been covered in Chapter 8 of *Module One – Anatomy and Physiology* and will not be covered again here. It may be worth spending a few minutes re-reading that chapter. The review by Hammerschlag (2006) of elasmobranch osmoregulation is worth reading if you are interested in more detail than the following summary.

Teleosts produce ammonia as an end-product of protein metabolism. All elasmobranchs (except for the Potamotrygonidae family) are ureotelic in that they produce urea as the end-product of protein metabolism. Other ureotelic organisms are land (adult) amphibians and mammals. This metabolic process occurs in the liver.

Marine Elasmobranchs

Ninety percent of elasmobranch species live predominantly in a marine environment. The critical difference between marine teleosts and marine elasmobranchs is that the teleosts are hypotonic to sea water, while the elasmobranchs are in fact slightly hypertonic. Normally sea water has a salinity of approximately 35 parts per thousand (ppt). The internal salinity of a teleost is between 9 and 10 ppt while for an elasmobranch it is 36 to 37 ppt.

Elasmobranch ion levels (sodium, chloride and potassium) are only slightly higher than teleosts and so this is not the main reason for the higher osmolality of elasmobranchs. In elasmobranchs, the osmolality is mainly due to high levels of two non-ionic substances (see Figure 58):

- urea
- tri-methylamine oxide (TMAO)

TMAO accumulates in elasmobranchs via two mechanisms. Firstly, TMAO is present in fish and invertebrates that are consumed by elasmobranchs and is absorbed and retained following digestion of the food. TMAO is present in cold water teleosts, where it has been shown to possess anti-freeze properties, and in deep water fish to assist with pressure stabilization.
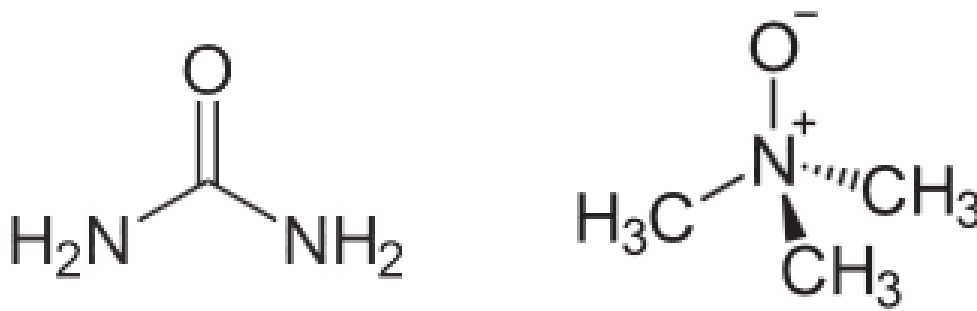
The second mechanism is that many elasmobranchs have the ability to synthesize TMAO. This involves the enzyme trimethylamine oxidase. The amount of TMAO that arises from the two sources varies enormously with the species of elasmobranch.

Plasma urea levels usually contribute about 30% of plasma osmolality. Urea is needed for normal cell function in elasmobranchs. Because the blood urea level is so high and the sea water level is lower, there is an outward diffusion gradient across the gills. Various aspects of gill structure and function prevent massive losses of urea across gill membranes.

The kidney plays a major role in maintaining high serum urea levels by reabsorbing most of the urea from the urine. Less urea is lost via the kidneys than occurs across the gills.

Euryhaline elasmobranchs that move into estuaries and freshwater (lower salinity), have both increased urine output (can increase up to fifty fold) and also higher urea and ion (sodium, chloride, magnesium and sulphate) excretion that act as a compensatory mechanisms. One of the most well recognized species that does this is the bull shark (*Carcharhinus leucas*) which can exist in freshwater for long periods of time. Another group are the sawfish (family Pristidae). These elasmobranchs attain an internal salinity of about 23 ppt. Another compensatory mechanism is decreased urea production via the liver.

Anorexia in elasmobranchs potentially leads to lower urea levels due to a reduced protein intake. This decreased intake is compensated for, by a decrease in the renal clearance (removal) of urea and thus preservation of the body urea levels. Obviously, this can only occur for so long and then, osmoregulatory issues will develop.

Following death, it is the high urea and TMAO tissue levels that cause elasmobranchs to smell so quickly. This is compounded by the presence of enzymes and bacteria in the tissues that break down the urea to ammonia.

Ureolytic bacteria from the *Vibrio* genus have been identified in various organs, including the liver, spleen and kidney (Knight, Grimes and Colwell 1988). These bacteria presumably play a role in regulating elasmobranch tissue urea concentrations. For this reason, culturing a

Vibrio bacteria from these organs, during a necropsy needs to be interpreted very carefully as these can be normal bacteria and not pathogenic (Mylniczenko *et al.* 2007).

The higher osmolality of elasmobranchs compared to their environment, causes a slight water influx to occur from the surrounding sea water. This extra water is then removed via the kidneys through urine production, which occurs at a slightly higher level than marine teleosts. The excess salt levels caused by the sea water influx are dealt with by the rectal (or salt) gland.

The rectal (or salt) gland is a small, elongated structure that opens into the rectum near the cloaca. It is a salt-secreting organ that appears glandular (see Figure 59) and predominantly excretes excess sodium (Na^+) and chloride (Cl^-) ions. This is an active ion pump system that requires energy to work. A peptide hormone, vasoactive intestinal peptide, appears to be responsible for controlling salt secretion via the rectal gland.
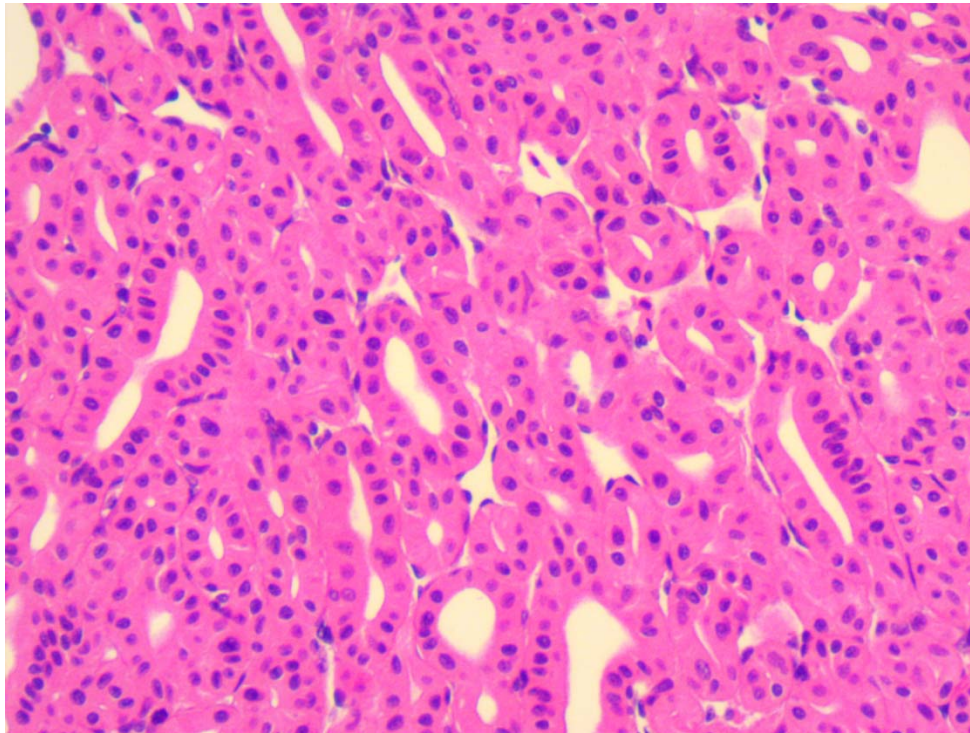
Figure 59 – Histology of rectal gland from an Australian swell shark (*Cephaloscyllium laticeps*) x 100

The rectal glands of euryhaline elasmobranchs moving from salt to fresh water have been shown to decrease in weight and length (Hammerschlag 2006).

Marine elasmobranchs can drink but generally do not take in much water. Some elasmobranch species however, can control their drinking rate to regulate their osmolality in response to rapidly changing exposures to salinity
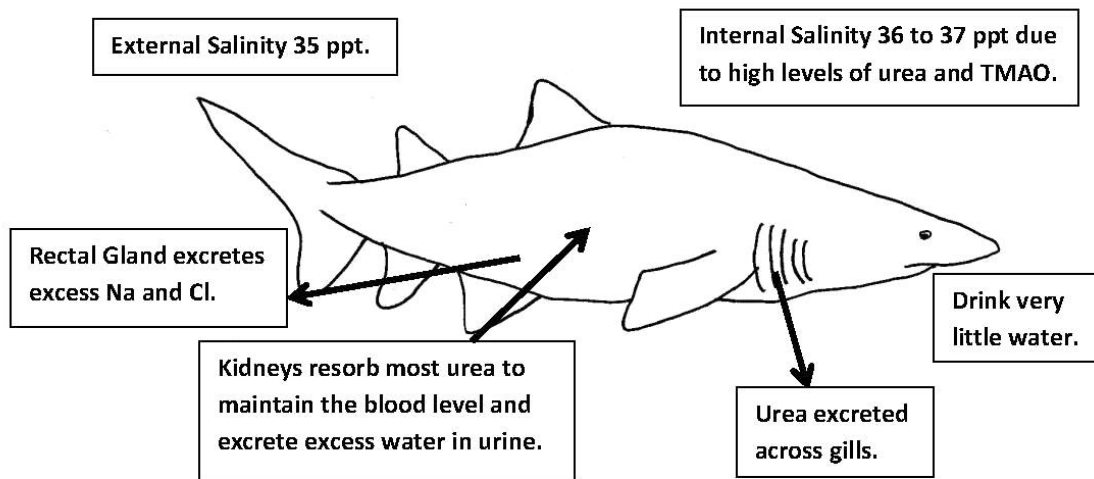
Figure 60 – Diagram summarizing marine elasmobranch osmoregulation.

The coelacanth (*Latimeria chalumnae*) for many years was considered extinct until 1938 when a specimen was found in South Africa. If you want some fascinating reading, there are various books on the re-discovery of the coelacanth. Interestingly, coelacanth osmoregulation is very similar to elasmobranchs with high levels of urea and TMAO.

Figure 61 - Coelacanth fish (courtesy of Wiki Commons).

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Module Eight

Cephalopods and Crustaceans

CEPHALOPOD SECTION - CONTENTS

3. Cephalopod Husbandry

Recently many invertebrates, primarily cephalopods, have received increased attention regarding animal welfare in captive conditions. Cephalopods in particular are a focus of this discussion due to their advanced neuro-sensory system. It has become generally accepted that cephalopods should be accorded the same considerations that vertebrates receive regarding husbandry protocols in aquariums or laboratory (Vidal, 2014).

Here we will look at:

- Enclosure Design
- Habitat Design
- Nutrition
- General Maintenance
- Enrichment
- Transport

3.1 Enclosure Design

Enclosure details are important to consider when holding cephalopods in captivity for display or breeding.

NAUTILUS

Nautilus should not be kept in a shallow tank since in the wild they have been found to vertically migrate (Ward *et al.* 1984). Providing them with enough vertical space in aquaria allows them to ascend and descend in the water column when desired.

A vertical habitat such as real or faux rockwork gives them something to hold onto which is a frequently observed behaviour. Avoid adding décor with gaps similar to the width of a nautilus shell. Nautilus are not very maneuverable swimmers and can occasionally get wedged between décor gaps that are the width of their shell. See Figure 36.

**Figure 36 -** **Wedged nautilus between enclosure décor. Photo by Bret Grasse, Monterey Bay Aquarium.**

Moderate to low water flow is preferred so Nautilus are not pushed around by aggressive current or are swept onto the discharge screen.
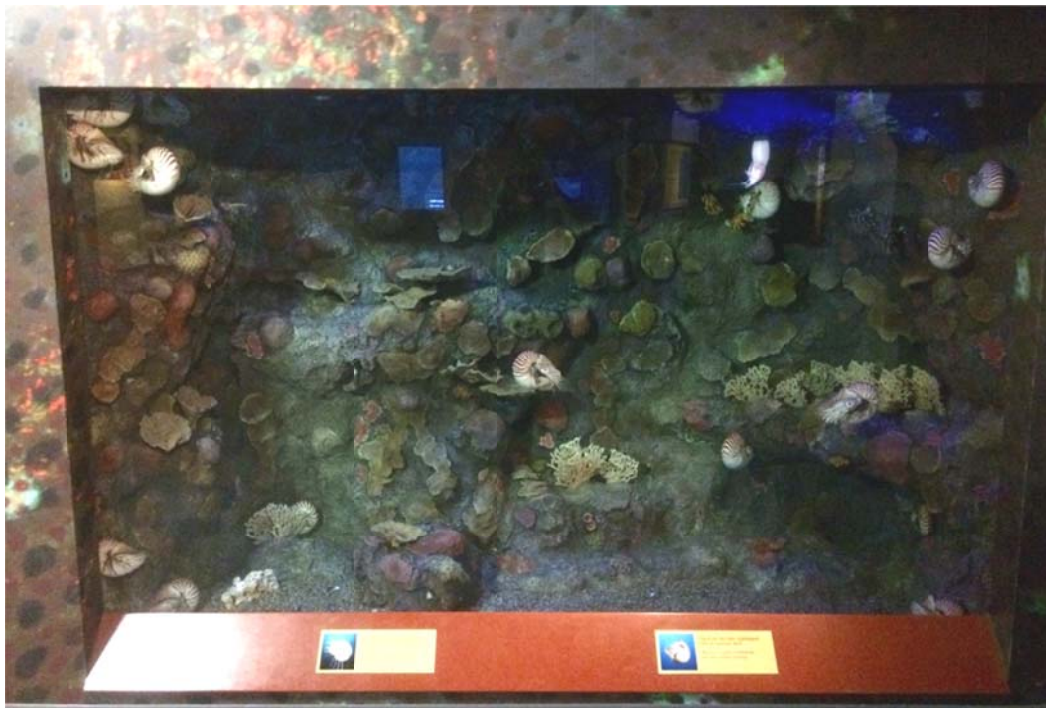
Figure 37 - Nautilus display tank at the Monterey Bay Aquarium. Photo by Bret Grasse, Monterey Bay Aquarium.

CUTTLEFISH

Cuttlefish require different enclosure considerations. A primary health issue of cuttlefish in captivity is mantle abrasion and lacerations. The epidermal damage occurs from jettisoning into tank walls or other rigid objects in the tank. The caudal mantle tip is particularly vulnerable in cuttlefish because the layer of muscle and skin overlying the posterior tip of the cuttlebone is quite thin. As a result, focally extensive, deep ulcerative **dermatitis** and **cellulitis** develop (Hanley *et al*., 1999). With severe enough trauma fractures of the cuttlebone can occur. See figure 38.
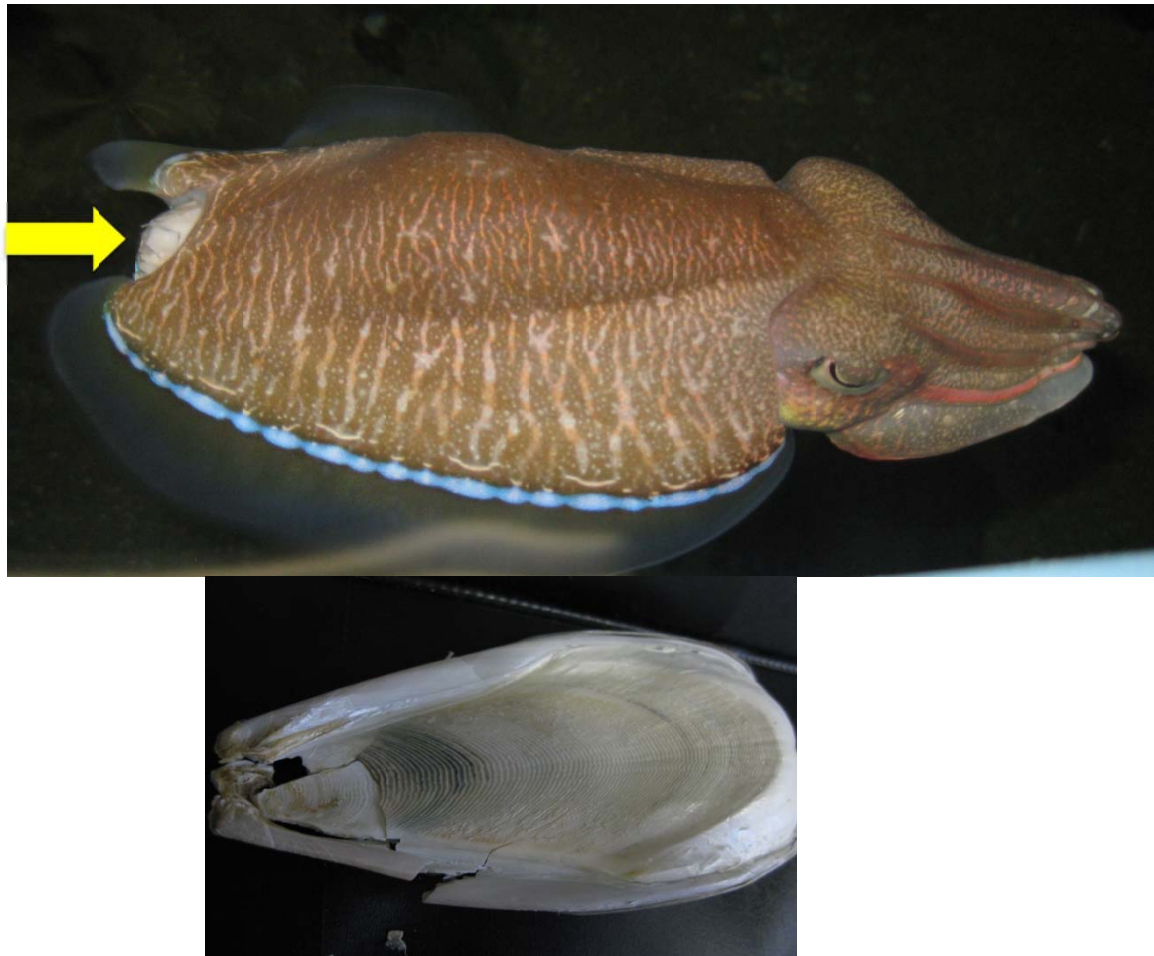

Figure 38 - Top: Mantle laceration on pharaoh cuttlefish (*Sepia pharaonis*) (arrow); Bottom: Fractured cuttlebone from impact against hard surface. Photos by Bret Grasse, Monterey Bay Aquarium.

This is more common with sub-adults and adults than it is with hatchlings. High stocking densities can further perpetuate this issue due to increased interactions. For public displays, rounded tank walls may reduce direct impacts and resulting mantle damage. In holding, this mantle damage can be further reduced or prevented with the use of soft-sided tanks.

There are multiple ways to achieve this through the use of soft material like plastic or polyethylene sheeting. Figures 39 and 40 illustrate one design using PCV, visqueen polyethylene plastic, and zip ties. It is important to build the soft-sided insert so that the cuttlefish cannot jump over the top or slip through the bottom and become trapped in the narrow space against the tank wall. It's advantageous to put a screen in the plastic at the air/water interface to allow skimming discharge of oils and other organics. Jump guards or lids are good practice to prevent cuttlefish from jettisoning out of their enclosure.
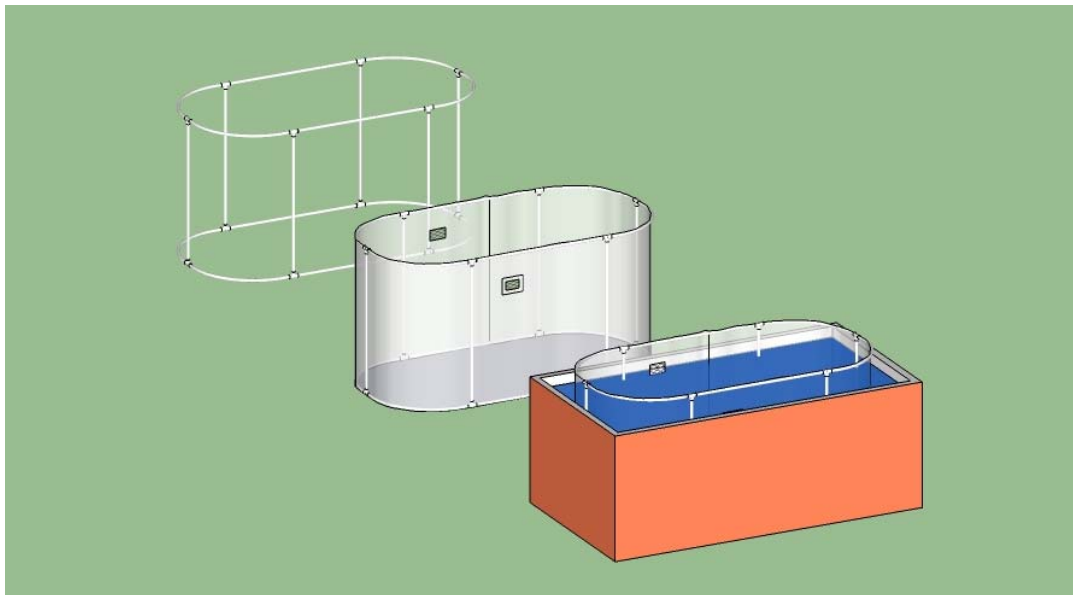
Figure 39 - Soft-sided tank prototype, Sketchup model by Bret Grasse, Monterey Bay Aquarium.
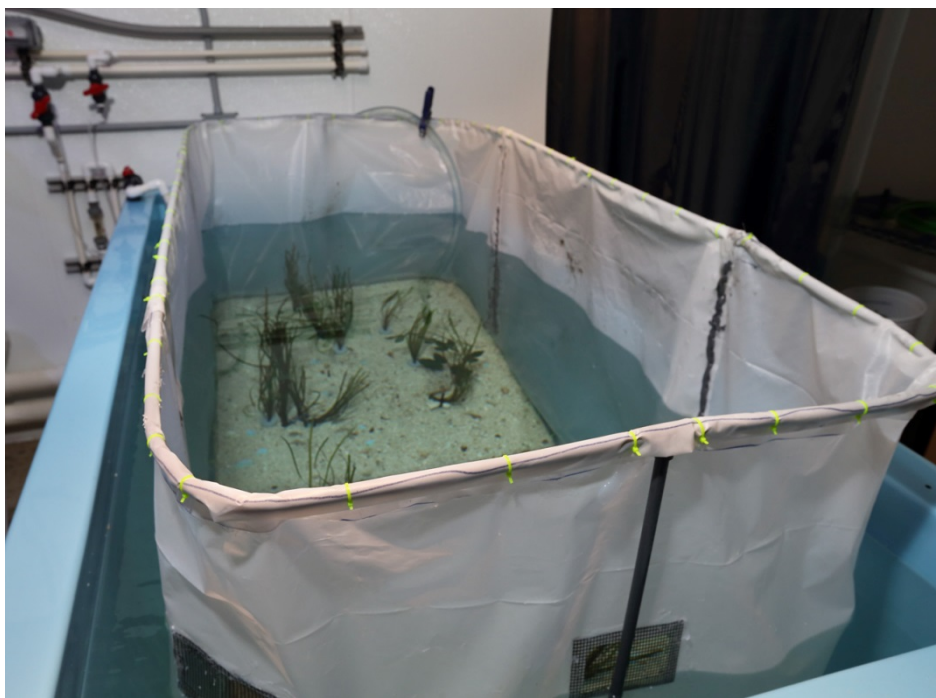
Figure 40 - Soft-sided tank. Photo by Bret Grasse, Monterey Bay Aquarium.

SQUID

Squid don't necessarily require a soft-sided tank since they lack the rigid cuttlebone inside a cuttlefish. Enclosures with rounded edges can minimize direct impacts and reduce potential damage to the squid. It is also advisable to eliminate any unnecessary sharp objects or rough surfaces that may cause harm to a mobile cephalopod. Many squid and cuttlefish species can jump out of their enclosures using jet-propulsion. It is important to prevent this through the use of jump guards or lids.

CRUSTACEAN SECTION - CONTENTS

2.2 Moulting

Growth can only occur by a complete shedding of the exoskeleton. The process is called moulting or **ecdysis**. In the larval stages (discussed in Chapter 6) moults occur every 24 hours or less. As the crustaceans enlarge and become adults, this frequency gradually decreases and they will usually only moult once or twice a year.

There are four stages to the moult cycle:

1. Pro-ecdysis (pre-moult) during which there is an increase in food reserves and an increase in haemolymph calcium (Ca^{2+}) level due to altered gut function and resorption from the cuticle. Chitinase and protease enzymes, produced by the epidermis, start to break down the endocuticle. The body begins to swell via the uptake of water through the gills and gut. This is often visible at the caudal end of the carapace where it joins the abdomen.

2. Ecdysis is the actual loss of the old cuticle. The old cuticle is called the **exuvia** (plural exuviae) and is often eaten by the crustacean that has just moulted or other crustaceans in order to recycle nutrients, especially calcium.

3. Post-ecdysis (post-moult) the new cuticle is still soft. It stretches to the increased size of the crustacean. There can be 20% or more increase in size within the first few days following the moult. This size increase is partly due to tissue growth (during the inter-moult period) but is also partly due to water uptake after the moult. A new endocuticle is produced and hardening occurs over a week. The more mature the crustacean is the less of a percentage increase in body size there is with each moult.

4. Intermoult – the period before the next ecdysis. This time varies with the life stage of the crustacean. Tissue growth is continuous during this period even though there is no visible growth.

The post-ecdysis stage is often important from a reproductive viewpoint (see Chapter 6) and is a very vulnerable period. Crustaceans that are moulting need to be isolated from other crustaceans so that they are not, predated upon while they are soft. Fortunately, crustaceans often cease feeding before a moult and this can be the cue to isolate the individual. It usually takes a week for the next exoskeleton to harden sufficiently so that there is not a risk. Improper moults are a major issue in aquariums and are, discussed in Chapter 8.

It is essential to ensure that there is sufficient room for the moulting crustacean to be able to exit backwards from its old exoskeleton (see Figure 14). If the area that the crustacean is in is too small then there may be an inability to moult correctly.

During moults, damage to claws may be, reversed such that the biting surfaces are rejuvenated. It is possible that, any missing appendages (limbs), may be regrown in full or partly with a moult. If partly it may take two to three moults for a full regrowth.

See Figure 14 over the page for some photographs of a successful Japanese Spider Crab (*Macrocheira kaempferi*) moulting.

Figure 14 - Japanese spider crab (*Macrocheira kaempferi*) undergoing a moult. A = carapace is lifting up and the crab (bright red) is starting to move out backwards; B = crab is almost completely out of its old exoskeleton; C = final stage with soft crab in the background and exuvia in the foreground.

The moulting process is under the influence of the endocrine system.

Situated in the eyestalk is the X-organ- sinus gland-complex. It produces several hormones, including:

- Crustacean hyperglycaemic hormone
- Gonad-inhibiting hormone
- Vitellogenesis-inhibiting hormone
- Moult-inhibiting hormone

The Y-organ is a narrow strip of tissue near the anterior branchial (gill) chamber. This epithelial endocrine gland produces a hormone called **ecdysone**. Ecdysone is a steroidal prehormone, which after its release is converted to a 20-hydroxy ecdysone, which is the active moulting hormone. Secretion of ecdysone is blocked by the neurohormone, moult-inhibiting hormone, which is produced by the eyestalk complex.

These hormones will be, discussed further in Chapter 6 on Reproduction.

## *2.3 Internal Anatomy*

Muscles and Movement

Crustaceans have "skeletal" muscle similar to teleosts in that it is striated. The muscles attach to the inside of the exoskeleton. Movement of the appendages and tail can occur because the exoskeleton is divided into separate sections by a thin non-chitinised, flexible cuticle.

In prawns and lobsters the majority of the abdominal section is comprised of "skeletal" muscle. A rapid contraction of these muscles and the ventro-flexion of the abdomen and tail fan, create the fast backward movement characteristic of prawn and crayfish mobility.

Respiratory System

As with fish, gills are the gas exchange organs and sit in branchial chambers on either side of the cephalothorax. The gills are highly vascularized, sac-like outgrowths at the base of the walking legs (pereiopods). The carapace extends laterally and ventrally to cover the gills. Unlike teleosts with four gill arches, the number of gills in crustaceans can vary with the species.

Water circulation is produced by an appendage on the second maxilliped (the "gill beater"). Water is drawn up in the gaps between the thoracic appendages into the branchial chamber, through the gill filaments and then leaves anteriorly. Look at the arrows in Figure 15 below. This water movement enables a counter-current mechanism to enhance oxygen uptake, as happens in teleost gills. Water flow rates of 1000 ml/kg/minute have been recorded in decapods.
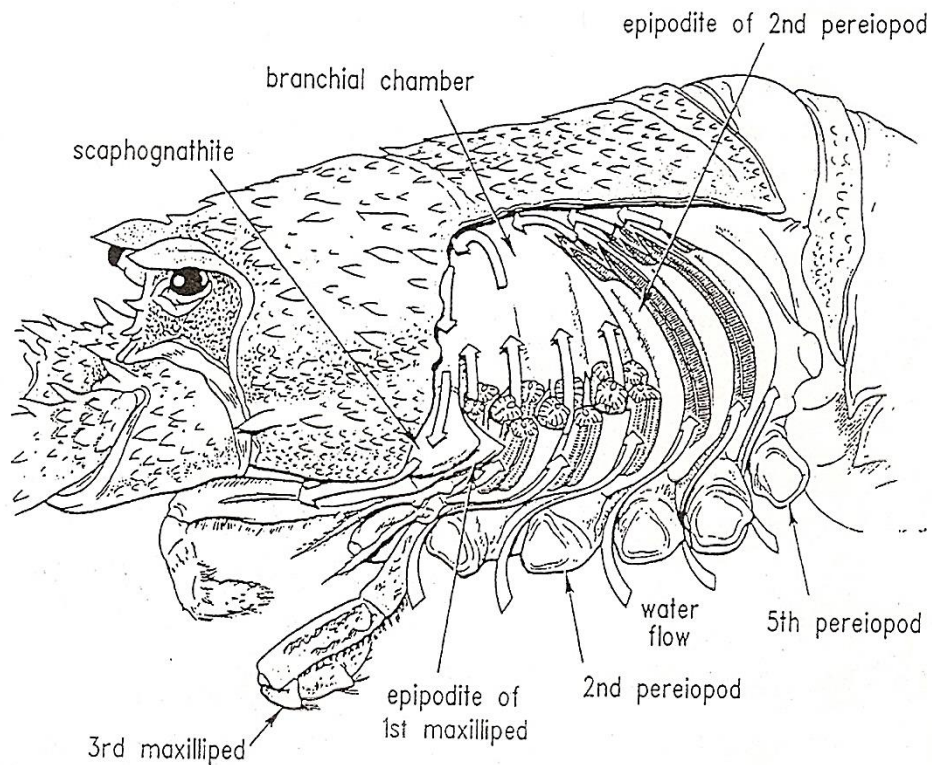
Figure 15 - Diagram showing the position of crustacean gills and water flow. The lateral aspect of the carapace has been removed (courtesy Roxanna Smolowitz, DVM, Roger Williams University).

Each gill has a central axis with a series of paired lateral branches, positioned at right angles, and which run off along its length. Each branch then has several filaments that which usually bifurcate and are again at right angles off the branch.

The gills are covered by thin layer (2 – 4 μm) of flexible cuticle. As with teleosts, pillar cells are an essential part of the architecture. The haemolymph circulates through the gills to absorb oxygen and then deliver it to the various tissues.
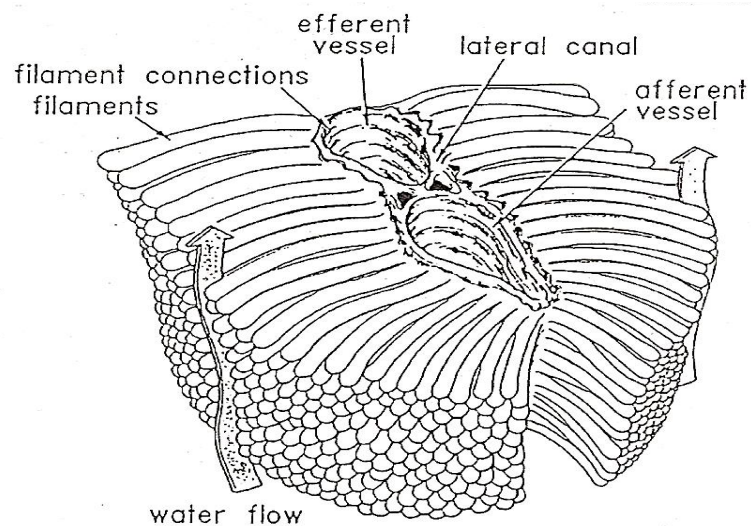
Figure 16 – Diagram of crustacean gills at a higher power (courtesy Roxanna Smolowitz, DVM, Roger Williams University).
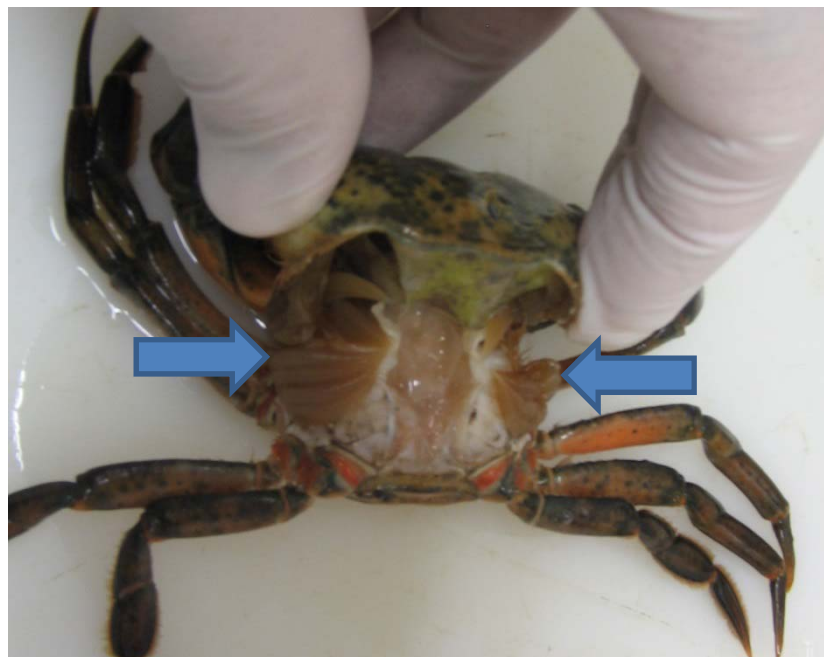
Figure 17 - Crab necropsy. Removing the carapace and exposing the gills (arrows).

As with teleosts, crustaceans are predominantly **ammonotelic**. Urea represents less than 20% of the total Nitrogen (N) excretion in both marine and freshwater species. Ammonia predominantly arises as a waste product of protein metabolism. The gills play a vital role in the excretion of ammonia in aquatic species. The mode of branchial ammonia excretion is still, not fully understood and appears to be a combination of passive process (no energy use) as well as an active process using transport molecules. In terrestrial species, the antennal gland (see osmoregulation below) is the likely ammonia excretory organ. The toxic effect of ammonia (and the other nitrogenous compounds) is, examined in Chapter 4 (Water Quality).

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Module Nine

Sea Jellies

Contents

3.2 Exhibit / Display Design

Anyone interested in the history of jelly displays, or even aquariums in general, should read *A Fascination for Fish: Adventures of an Underwater Pioneer*. Written in 2003 by David Powell it details the establishment of the internationally famous Monterey Bay Aquarium in California, USA. David was the first Curator at MBA and one section of the book details their jelly displays.

The following are the most common exhibit / display designs:
- **Kreisel (planktonkreisel)**
- **Stretch Kreisel**
- **Pseudo-Kreisel**
- **Cylinder**
- **Modified Box**

The planktonkreisel, or kreisel, is the standard bearer for displaying gelatinous zooplankton. It was first conceptualized in Germany by Wolf Greve (1968, 1970, 1975) and was

redesigned for use aboard ships by Hamner (1990). It was then first used in public aquariums at the Monterey Bay Aquarium (Sommer 1992, 1993 and Raskoff *et al*. 2003).

The kreisel was the beginning of sea jelly husbandry at most aquariums. Now most jelly aquarists tend to use pseudo-kreisels. We will examine the various tanks for jellies in depth. By the end of this reading you should be able to calculate the flow of a kreisel and determine its worthiness as an enclosure for jellies.

The Kreisel

The kreisel is a flat-sided, circular aquarium, capped with a removable lid to complete the circle at the top of the tank (see Figure 19 below). When viewing the kreisel from the front, water is driven by two pumps, typically in a counterclockwise direction, forming a gyre where water is moving fastest at the edges and slowest in the middle of the tank. The goal of the kreisel is to keep the jellies away from the edges of the tank and centered in the slower moving water. Faster flow at the edges pushes the jellies to the center. Ideally the aim is to maximize the time jellies spend swimming against a gentle current and minimize the time they contact tank surfaces and screens, as repeated contact can lead to significant damage.
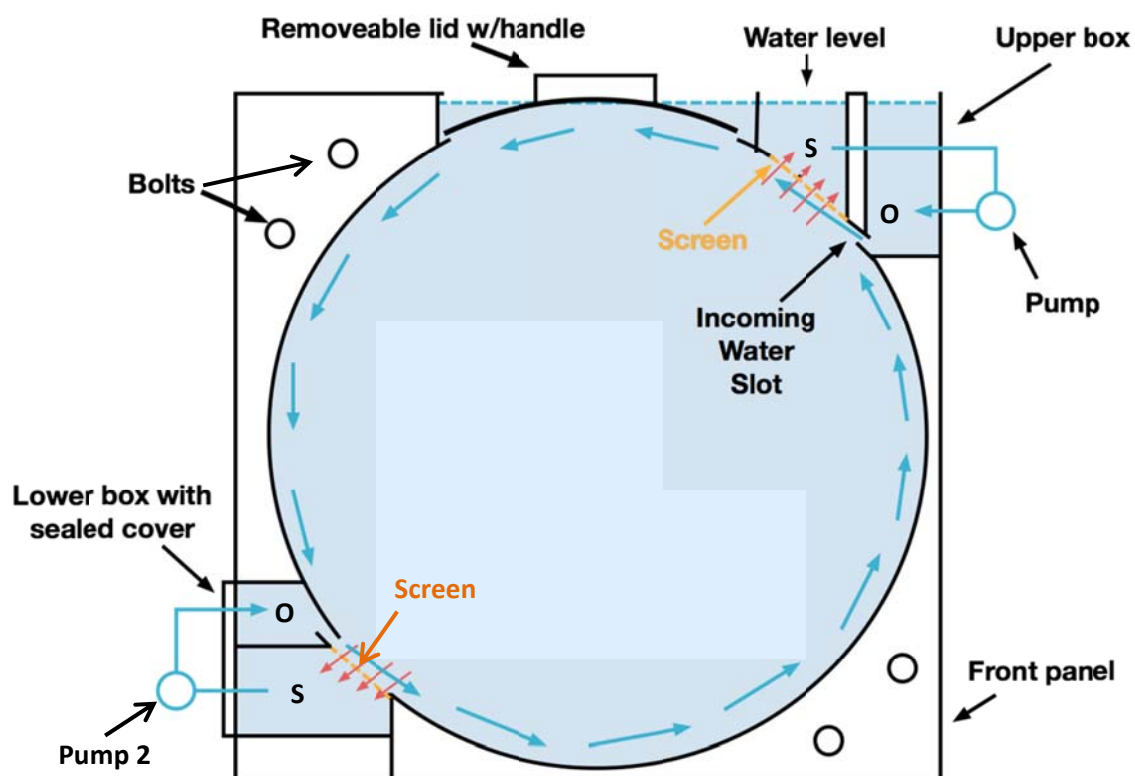

Figure 19 - The standard kreisel tank designed at the Monterey Bay Aquarium, a modified version of Hamner 1990. (Arrows indicate water flow; O = outlet; S = suction). Figure by Wyatt Patry.

Traditionally a kreisel has two water supply boxes each attached to a separate pump. Each is divided into two sections: a supply side and a suction side that the pump draws from. The suction side is screened off with fine soft mesh to prevent jellies from getting sucked into the pump. The supply side is provided with water from the pump output and forced into a chamber (can be sealed on top) with a narrow slit opening allowing water to exit the chamber along the side of the tank in a 'laminar' flow. The exit slit is typically filled with corrugated (or channeled) plastic sheet material to drive the water in a laminar flow pattern (see Figure 20 below). The outlet flows directly over the screen (suction) to prevent the sea jellies getting stuck in the suction (refer back to Figure 19 for the flow pattern). Hence, water is pushed faster along the side of the tank in a flat 'blade' so velocity is higher on the sides, diminishing as you reach the center of the tank (center of the gyre).
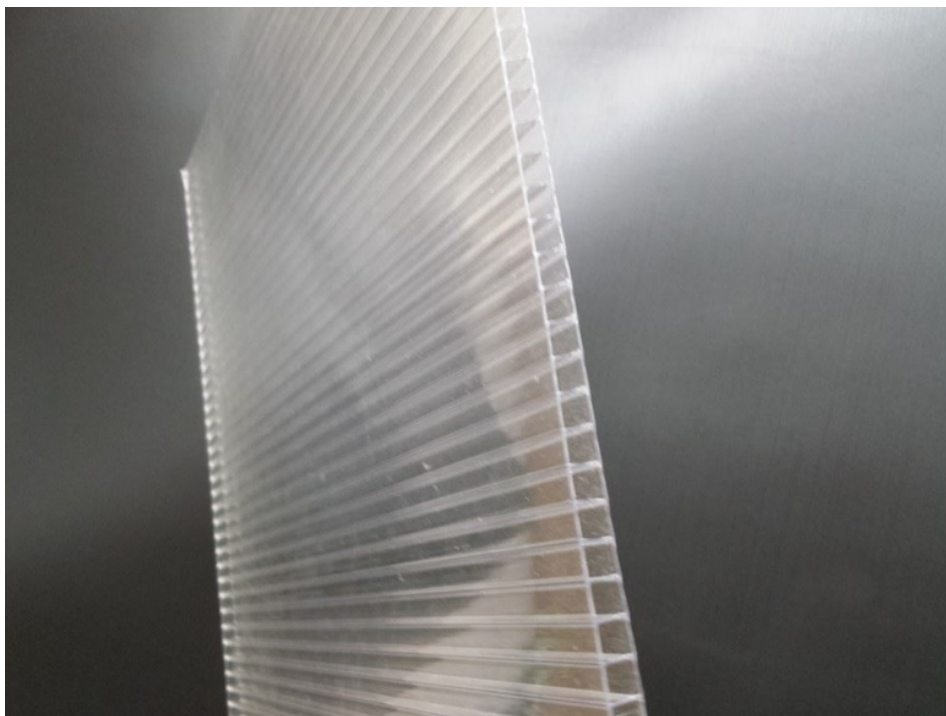
Figure 20 - Corrugated plastic material, commonly used in greenhouses, fills the slots of the kreisel box.

Early versions of the kreisel designed for use aboard ships had a 'chimney' to help prevent water sloshing out the top however this made accessing the tank extremely difficult to work in and is typically left out of newer designs.

Another important structural aspect of kreisels are the through bolts. Whilst adhesive is used, it should be supported by these bolts which help keep the two halves of the kreisel from breaking apart. In a catastrophic failure, if there are no bolts to hold the two pieces together then the halves will separate and fall apart (see Figure 21 over the page).

Figure 21 - A catastrophic failure of a large stretch kreisel because no through bolts were used in its construction.

In this example, the lower acrylic seam failed causing approximately two metres (6.6 feet) of water pressure to begin pouring out the bottom. This applied pressure to the back plate and eventually the entire back half of the tank split off. The back half fell backwards, damaged plumbing on the wall and causing a major flood, pieces landed on and damaged the sump and chiller. Had through bolts been installed during or after construction of this stretch kreisel, the tank would have simply leaked through the failed seam instead of actually splitting in half. **Always use through bolts.**

The Stretch Kreisel

The stretch kreisel is exactly as it sounds, a kreisel tank that is stretched out so that its width is roughly twice its height, resulting in two counter-acting gyres, one side running counter clockwise and the other side running clockwise. The goal of the stretch kreisel is to create a central upwelling flow where jellies can pulse against the current, this works particularly well for Sea Nettles. See Figure 22 over the page.

A stretch kreisel typically includes two supply boxes on each side; however more recent

versions feature simplified supply boxes only at the top corners of the tank. Just like the kreisel, the outflow slits are filled with corrugated plastic material (refer back to Figure 20).
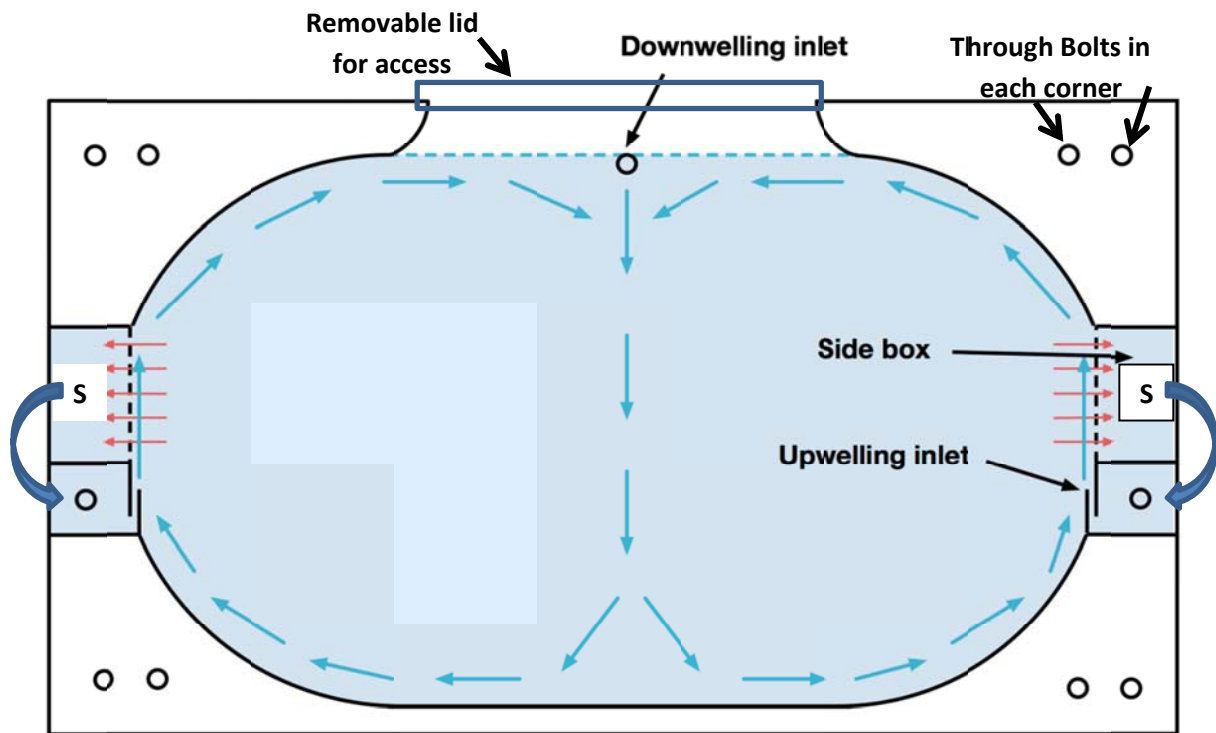

Figure 22 - The original stretch kreisel developed at the Monterey Bay Aquarium (Raskoff *et al.* 2003). This tank has three separate inputs of water. The down-welling inlet has since been discontinued and the upwelling inlets reversed using a spray bar at the top. (Blue arrows inside the kreisel indicate water flow; red arrows indicate water going through the screens; O = outlet; S = suction; large blue arrows on each side represent pumps and indicate the water direction). Figure by Wyatt Patry.

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Module Nine

Echinoderms

Contents

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Module Nine

Corals

Contents

1. Anatomy and Physiology

2.1 Hard Corals

An understanding of coral anatomy is important to enable proper identification of the type of coral, diagnose and treat coral disease, and for providing the correct husbandry management required for a particular species. To tackle this sometimes confusing topic, we will divide anatomy into two categories: the skeleton and the live animal.

2.1.1 Coral Skeleton Anatomy

A significant feature used to class coral by taxonomists is the calcium carbonate ($CaCO_3$) skeleton. Coral skeletons can range from a very simple single polyp species, such as disc corals in the genus *Fungia*, to a very large colony comprised of thousands of individual polyps that are connected to each other via a matrix of canals within the calcium carbonate skeleton. The basic skeletal structure of a single polyp is the **corallite** (refer to Figure 9 below) which is essentially a tube that the living polyp sits in. The coral skeleton is produced by the living polyp discussed in further detail in Section 2.1.2.
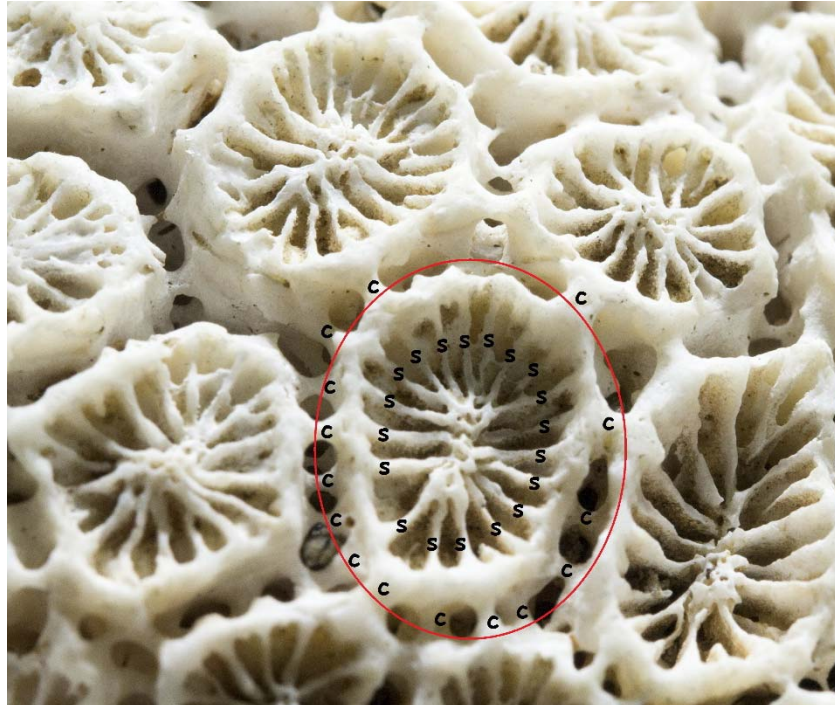
Figure 9 - Coral Skeleton which has distinct septae walls. The basic skeletal structure the corallites is outlined in the red circle. The septae are labelled with "s" and the costae are labelled "c" (courtesy of Shutterstock).

Each corallite is divided by a defined corallite wall which separates **septae** from **costae** and from other corallites. The visible portion of the corallite is the **calice**, which can vary in morphology ranging from indented, flush, or raised in relation to the colony skeleton surface. The wall of the calice can be grouped into three main morphologies which are used in the classification of a coral: shared corallite walls, separate walls, and indistinct walls (refer to Figure 10 below).

Figure 10 – A = coral with separate corallite walls; B = coral with shared corallite walls (courtesy of Shutterstock).

Within the corallite calice walls are vertical partitions called **septae** which provide support to the mesenteries. Vertical partitions which cross over the wall are called **costae**. The term **septocostae** is

used to describe the vertical partitions in species with undistinguished walls and distinction between septae and costae cannot be made.

The wall of the corallite extends into the skeleton forming a tube shape skeletal structure that the coral polyp sits in (see Figure 11 below). Within the tube of the corallite are the **columella**, a tangled mass of intertwined septa. The living polyp within the corallite, has radial mesenteries between the septa and columella which increase the surface area of the body cavity and aid in digestion. In colonial corals the individual corallites are joined together by a series of structural plates called the **coenosteum**.
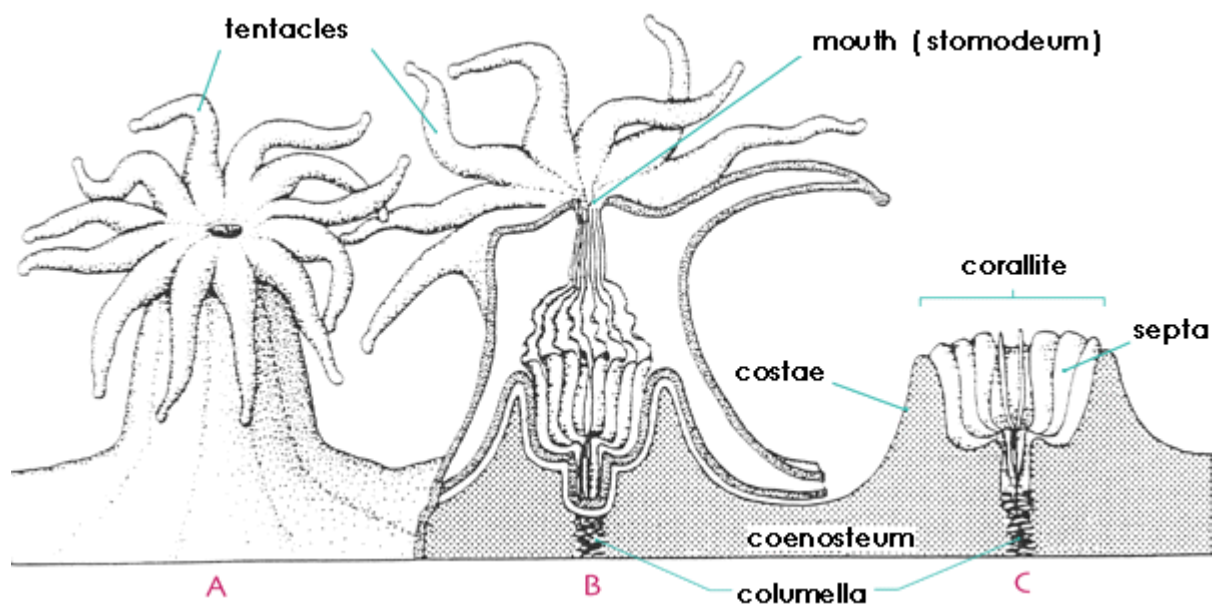
Figure 11 - Coral skeletal structure in association with the living tissue of an individual polyp. A = Polyp; B = Cross section of a polyp and of the skeleton; C = Skeleton (Corallite) only. Source: A Coral Reef Handbook: A Guide to the Geology, Flora, and Fauna of the Great Barrier Reef, edited by Patricia Mather and Isobel Bennett 1993.

2.1.2 The Living Coral Anatomy

An individual coral is called a polyp and is relatively simple in anatomy. The polyp is essentially a tubular sac composed of a tissue made up of three layers:

- **epidermis** = the outer cellular layer (referred to as the ectodermis in some literature)
- **mesoglea** = the thin middle layer
- **gastrodermis** = the inner cellular layer

See Figure 12 over the page.
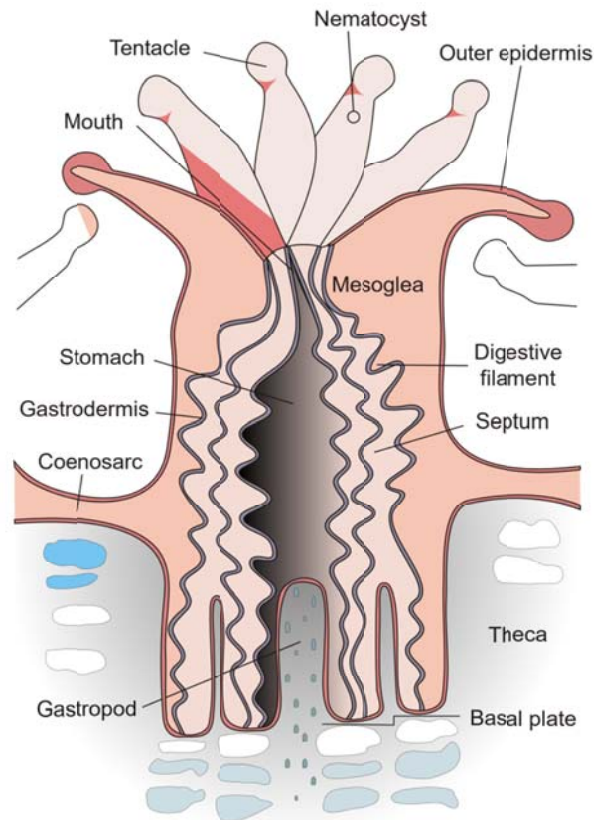
Figure 12 - Schematic diagram of coral polyp anatomy comprised of three layers in the shape of a tube. Source Wikimedia Commons.

The **epidermis (**outer layer of the polyp), contains several specialized cell types that serve several functions. **Cnidocytes** are cells involved in food capture and defence. Additional cell types include mucous producing cells. The mucous coats the polyp and allows removal of sediment from the polyp surface, capture of food and protection from pathogens. Cells that make up the epidermis are also lined with microcilia that help move food particles to the mouth and waste and sediment away from the mouth. In stony corals the epidermis at the base of the polyp is called the **calicoblastic epithelium** which secretes the calcium carbonate ($CaCO_3$) skeleton.

Pigments are also present within the epidermis. **Fluorescent pigment proteins** are produced in the epidermal cells in spherical granules. The fluorescent proteins produced include blue, cyan, green and yellow pigments and are responsible for some of the blue, green, and pink fluorescent colours observed in corals. The overall coloration observed is also largely dependent on the symbiont zooxanthellae pigments which are discussed further in Section 2.3.

The fluorescent pigment proteins provide a photobiological system for regulating the light environment of coral host tissue and play a role in both low and high light intensity. Under low light, the fluorescent proteins may enhance light availability and assist the zooxanthellae with photosynthesis. At the other end of the spectrum, with excessive sunlight fluorescent proteins are photoprotective. This is achieved by dissipating excess energy at wavelengths of low photosynthetic activity and by reflecting visible and infrared light (Salih *et al*. 2000).

In aquariums and zoos the visible colour of a coral is affected by several factors:

- spectrum of light
- UV exposure
- microelements

Certain colours, such as fluorescent red or orange, which are not visible in the daylight spectrum become visible under actinic lighting. Mixes of light sources with different spectrums, along with different lighting systems, will produce varying effects on the colours of the same or similar corals.

As discussed above many of the fluorescent pigments in the wild assist with photo-protection, particularly due to ultraviolet (UV) light exposure. Corals exposed to a larger amount of UV will produce larger amounts of the protective pigments. Most LED light units used for corals do not produce UV, and glass covers on metal halide units reflects the UV produced and so low to no UV is available in the aquarium setting. It is common for corals with bright colours to adapt to the lower UV-A and UV-B conditions within the aquarium by losing their colour pigments as they are no longer required.

The second layer is the **mesoglea** which is a very thin layer between the epidermis and the gastrodermis. This layer is predominantly an acellular gelatinous connective tissue layer. The mesoglea interacts with **desmocytes** (anchor cells) to attach the living polyps' tissue to the corallite skeleton. Reproductive organs also develop within the mesoglea layer.

The third layer is the **gastrodermis** which lines the interior of the polyp's body. The gastrodermis is made up of different cell types which are involved in digestion and nutrient absorption. Zooxanthellae, the unicellular symbiotic algae, are also found within the gastrodermis of zooxanthellate corals (see Section 2.3). See Figure 13 over the page.

These three layers form a tube-shaped polyp that attaches to the skeleton at the base with a mouth surrounded by tentacles at the tip. The tentacles are also tubular in shape and are composed of the

same cell layers as the rest of the polyp. In most species of corals, tentacles have the ability to contract and extend which aids in capturing food. Many species of corals contain specialised stinging cells called **cnidocytes** or **nematocysts** in the epidermis of the tentacles. Nematocysts are used for both defence and for catching food. When chemoreceptors and mechanoreceptors detect prey or competing corals the **cnidocil** is triggered. This results in the release of calcium ions (Ca^{2+}) from the capsule creating a calcium gradient in the cell's plasma membrane resulting in an influx of water into the cell. As the water volume in the cytoplasm increases, pressure causes a coiled tube to eject rapidly. The ability of the tentacles to extend must be taken into consideration with corals displayed in aquariums, as the nematocysts can damage the tissue of surrounding corals. See Figure 14 below.
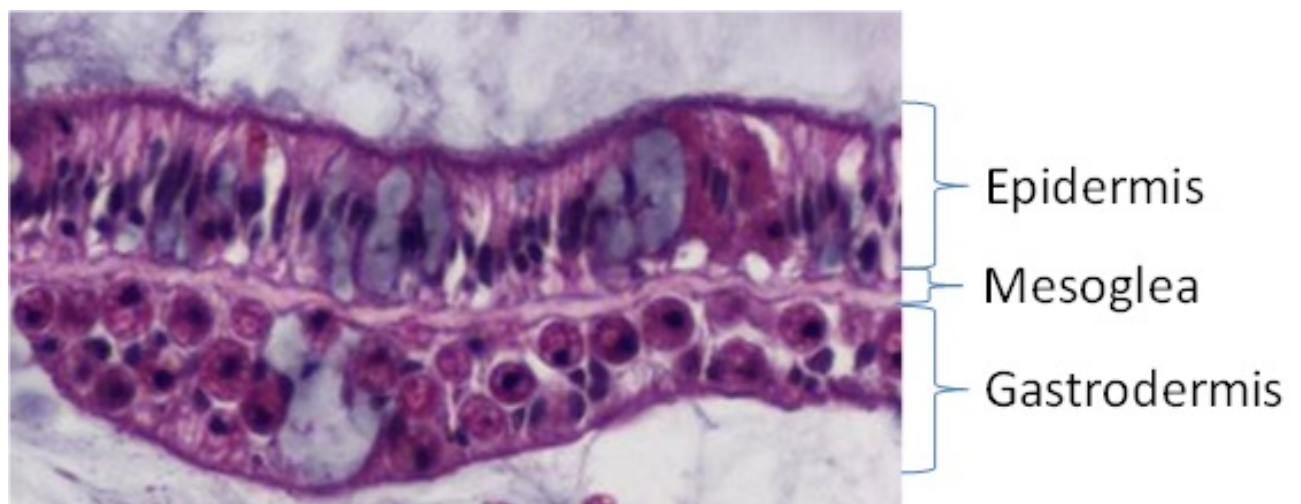

Figure 13- Histology section showing the tissue layers of the living coral. Photo courtesy of Ilze K. Berzins, PhD, DVM

The Aquarium Vet

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Module Ten

Penguins I

Contents

2.   Anatomy and Physiology

As mentioned previously, birds have the following characteristics:

- warm-blooded
- feathered
- winged
- bipedal
- egg-laying

Birds are capable of maintaining a stable internal body temperature, independent of the external environment, just like mammals. This is called **homeothermy** or **endothermy**, and commonly referred to as "warm-blooded". Thermoregulation (particularly in cold climates) will be discussed later in this chapter under the appropriate anatomy sections.

Most birds maintain their body temperature at 40°C (104°F), which is 3 - 4°C (5.4 – 7.2°F) higher than mammals. In diurnal birds their daytime temperature may be a little higher during the day, with the reverse for nocturnal birds.

Penguin temperatures are usually a little lower with their body temperature being between 37 and 39 °C (98.6 to 102.2 °F). In a study using wild King penguins (*Aptenodytes patagonicus*), the birds had lower body temperatures (down as low as 36.5 °C or 97.7 °F) when at lower environmental temperatures (-30 °C or -22 °F) (Froget *et. al* 2002).

Birds as a group have the highest metabolic rates of all vertebrates.

2.1 External Anatomy

Skin

The skin of birds is mostly unseen due to the feathers (discussed below), even though as with most animals it is the largest organ in the body. However, there are some bare areas on the bill, parts of the face and feet depending on the bird.

The skin functions to keep out pathogens, by providing a barrier to entry, as well as retaining body fluid and contributing to homeostasis. The skin is also a large sensory organ.

The skin of birds has the same layers as many other animals with an outer layer called the **epidermis** and the inner layer, the **dermis**. Invaginations of the skin form feather follicles but

unlike mammals there are no glands (sweat or sebaceous) associated with these follicles. As such birds cannot sweat or perspire.

Most bird skin is quite thin being only three to five cells thick, but is thicker over the bare parts of the face and legs. This is much thinner than the skin of mammals. The epidermis consists of two layers:

- Stratum corneum
- Stratum germinativium

The outer stratum corneum consists of layers of flattened (squamous) keratinized cells (called keratinocytes), which are combined with lipids almost in a bricks and mortar arrangement (lipids are the mortar) providing an impermeable barrier.
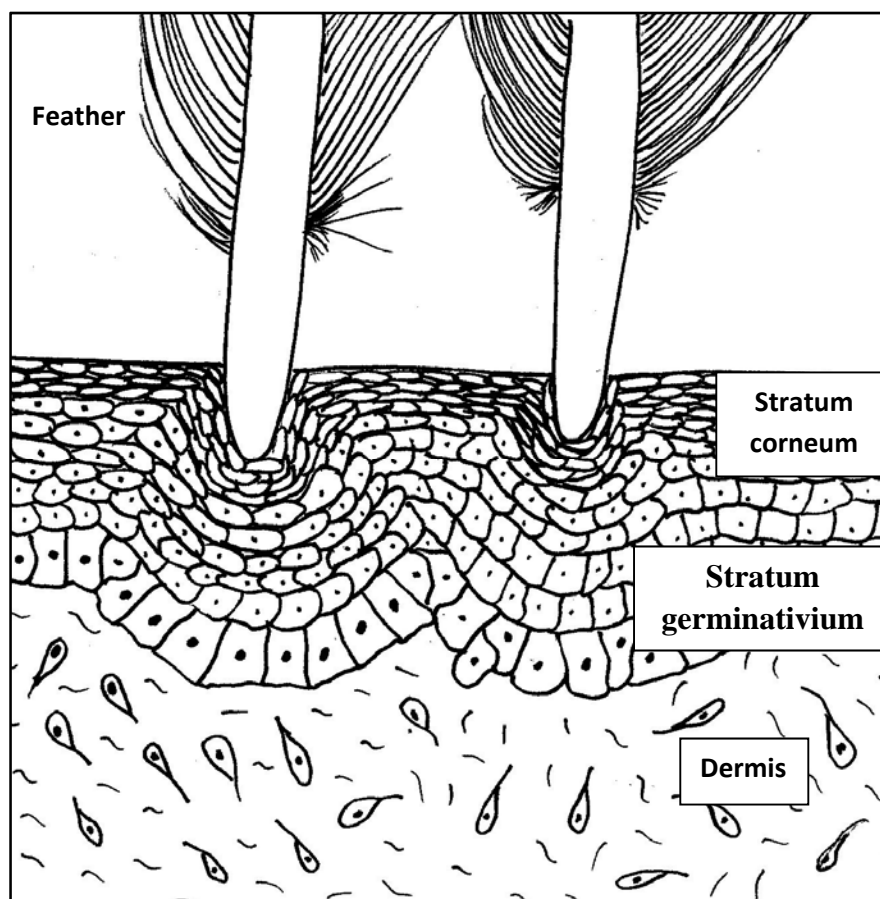
Figure 15 - Diagram showing the various skin layers.

The stratum germinativium is the layer where the keratinocytes originate. As they multiply they migrate upwards, lose their nucleus, become keratinized and form the stratum corneum. It is in this layer that the lipids mentioned in the previous paragraph are produced. These lipids are hydrophobic and contribute to waterproofing in combination with the secretion of

the uropygial gland (see later in this section). They also assist by keeping the feather keratin from becoming brittle and possess some antibacterial and antifungal properties.

The dermis layer is thicker than the epidermis. It contains fat deposits, nerves and nerve endings, blood vessels, lymphatics and smooth muscle. The smooth muscle controls feather movement. The base of the feather follicles are in the dermis layer, often closely associated with sensory nerve endings.

The sub-cutaneous tissue (mainly collagen and fat) lies below the dermis separated by a thin layer of elastic fibers.

Temperate penguins (genus *Sphensicus*) have bare skin between the face and eyes. This helps with evaporative cooling and increasing heat loss in warmer climates.

Penguins have a thick layer of fat below the skin which assists with insulation (analogous to blubber in marine mammals). Sadly in the period from the 1860's to the 1890's there was commercial penguin harvesting in the Falkland Islands and on Macquarie Island with the production of many gallons of penguin oil. See Figure 16.

Figure 16 - Necropsy of a Gentoo penguin (*Pygoscelis papua*) showing the fat layer under the skin (arrow).
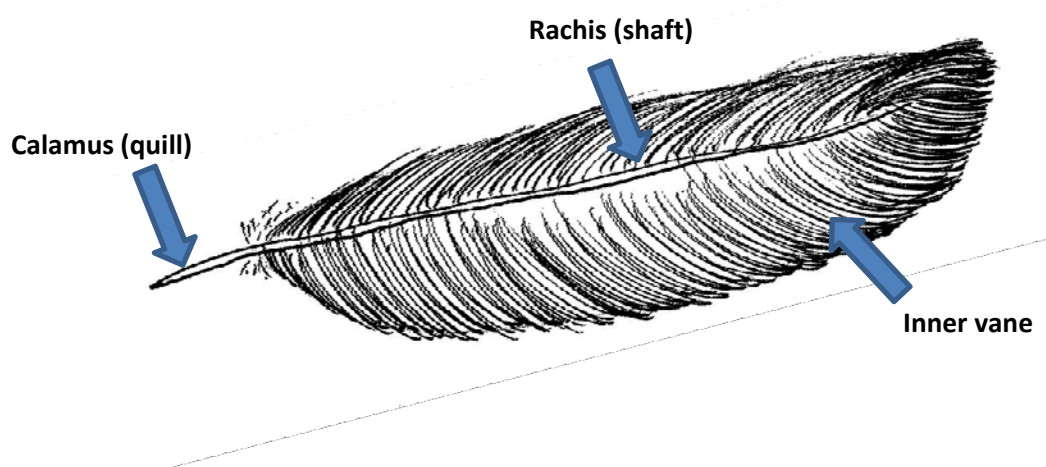
Feathers

Feathers are the main characteristic that differentiates birds from all other animals. Feathers are composed of a unique form of keratin only present in birds. Keratin is a fibrous structural protein that composes human hair and fingernails. In birds keratin is also present in scales that may cover the feet and lower legs.

Feathers make up 4 to 12 % of the birds bodyweight, depending on the species. There are two main types of feathers:

- Vaned feathers
- Down feathers

Vaned feathers are the outer layer of feathers, the typical quilled feather. The stiff central shaft is called the rachis. Flat, blade-like vanes are present on either side of the rachis. The vanes are composed of a series of barbs, extending obliquely from the rachis. Each barb in turn has finer branches called barbules. The barbules are held together by barbicels (small hooks that run the length of the barbule and hook together to form a smooth surface. See Figures 17 and 18 below and over the page.

Figure 17 - Diagram of a vaned feather.

In growing feathers, the central core or pulp (inside the quill) consists of vascular (blood vessels) connective tissue. This pulp regresses as the feather grows and is completely absent in mature feathers.

Vaned feathers are of various types. The smaller feathers which cover the body are called **contour feathers**. Flying birds possess two rows of long flight feathers, called **remiges**, being primary and secondary remiges. Above and below the remiges, are rows of **covert feathers**. Tail feathers are called **retrices** and similarly are associated with covert feathers.
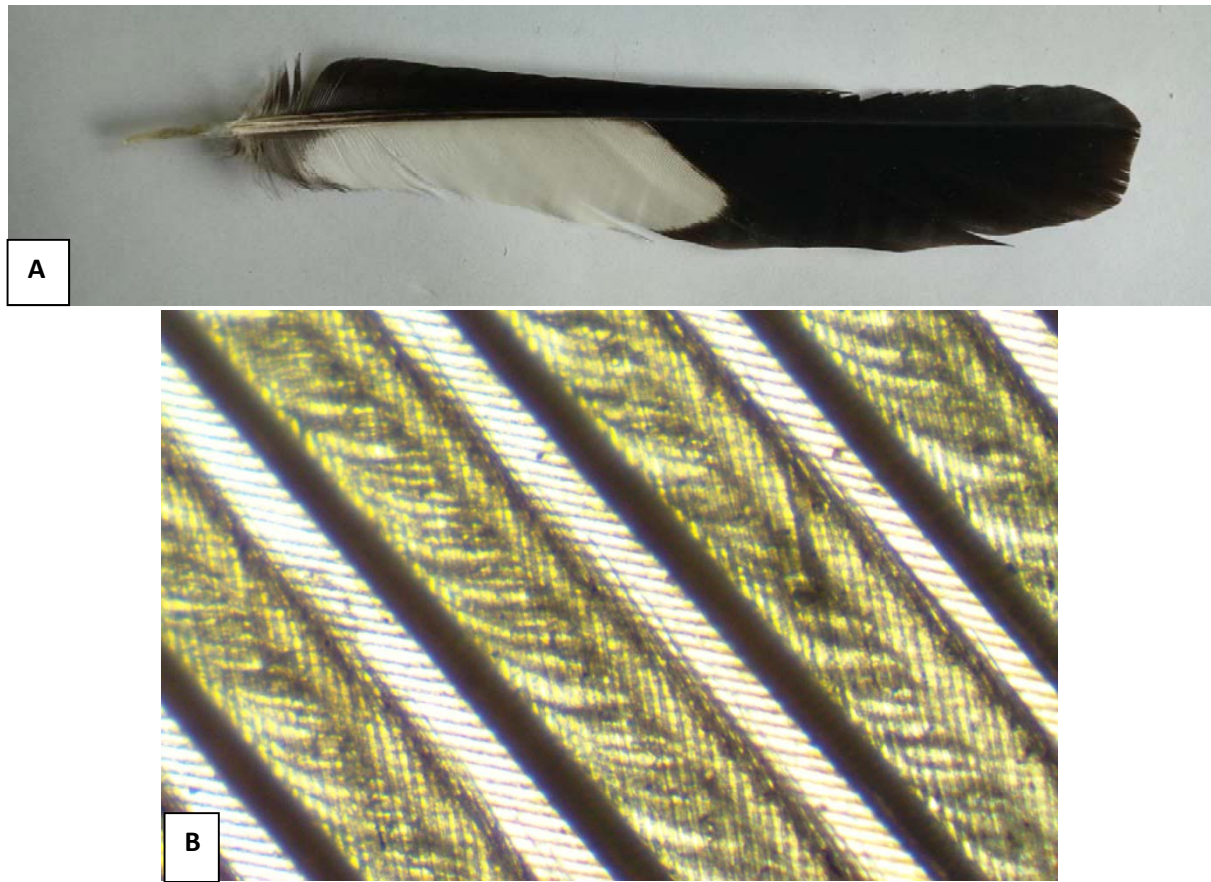
Figure 18 - A = Australian Magpie (*Cracticus tibicen*) feather; B = same feather x 40.

Penguins do not possess long fight feathers. However, they do possess long and bristle like tail feathers (retrices). See Figure 19 over the page.

Down feathers form a layer next to the skin and provide warmth. They lack a rachis. The barbules do not possess barbicels. Hence they are not smooth like vaned feathers, but rather are fluffy. This allows them to fill with air pockets, which assists with maintaining body temperature. In cold weather, birds will "fluff up" increasing the volume occupied by the feathers, which conserves heat by acting as insulation.

Figure 19 - Tail feathers (retrices) on a king penguin (*Aptenodytes patagonicus*).

In the Antarctic, Emperor penguins (*Aptenodytes forsteri*) and King penguins (*Aptenodytes patagonicus*) huddle together to conserve body heat. Chicks will do the same in the formation of crèches in some species.

Down feathers are very well developed in aquatic birds. Many penguin species have evolved to survive sub-zero Antarctic winters. A large component of this is the fluffy layer of down feathers. The original eiderdown for bedding was harvested from the nests of eider ducks in Scandinavian countries and archeological digs indicate that the Vikings possessed eiderdown on their voyages.

The overlapping feathers of penguins create a surface practically impenetrable to wind or water. The down feathers trap air, which provides about 80% of the thermal insulation for penguins. This air dissipates during diving and a stream of bubbles is often seen when they dive.

Heat loss also occurs via the bill and any bare facial areas. Birds tuck their head into or under their wing to maintain body heat.

Natal down refers to the feathers on baby birds. These down feathers arise from the same feather follicles that the contour feathers will develop from at **fledging**. Down feathers in adults arise from a separate group of feather follicles specifically for down feathers.
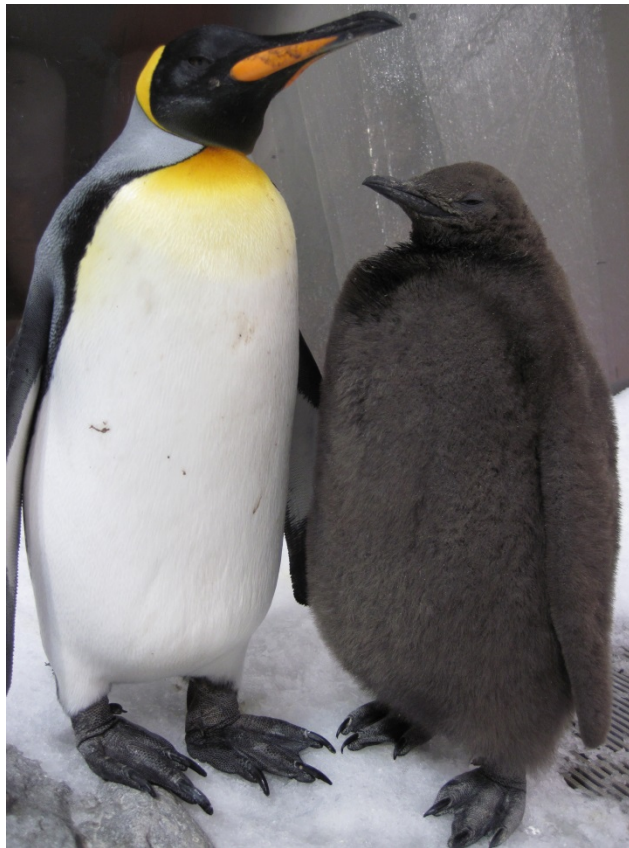
Figure 20 - King penguins (*Aptenodytes patagonicus*) at SEA LIFE Melbourne Aquarium. On the right is a chick displaying natal down.

The colour of skin and feathers is usually produced by three pigments:

- **Melanin** – produced by melanocyte cells in the epidermis
- **Carotenoids** (carotenes and xanthophylls) – synthesized by plants and taken up in the diet
- **Porphyrins** – red and green pigments synthesized in the bird cells

The crests of Macaroni penguins (*Eudyptes chrysolophus*) and other Eudyptes members fluoresce under a UV light a yellow-green colour. Investigation has revealed a new class of pigments called **pterins**, dissimilar to the carotenoid and melanin pigments which form most bird plumage. In Snares penguins (*Eudyptes robustus*), there has been a documented correlation between the intensity of this yellow pigment and body condition (heavier and healthier birds).

Feathers are held in the feather follicle by feather muscles, keratin bridges between the feather and follicular epidermis, as well the opposing concave and convex surfaces of the feather shaft and feather follicle.