The Aquarium Vet

E-quarist Course®

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 of the Fish and Aquatic Invertebrate Modules to give you an insight into the course.

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The initial course consisted of ten modules with a focus on fish and aquatic invertebrates. From 2018 onwards, modules on Penguins, Sea Turtles and Chelonians, Amphibians and Alligators / Crocodiles will be available. The current modules available are:

Module 1. Fish Anatomy and Physiology

Module 2. Disease Concepts and Diagnostics in Fish

Module 3. Water Chemistry and Quality

Module 4. Aquatic Life Support Systems

Module 5. Fish Diseases and Treatments

Module 6. Nutrition and Reproduction

Module 7. Elasmobranchs

Module 8. Invertebrates I – Cephalopods and Crustaceans

Module 9. Invertebrates II – Sea Jellies and Echinoderms

Module 10. Invertebrates III – Coral Husbandry

Module 21. Penguins I

Module 22. Penguins II

Module 23. Penguins III - under development and should be available early in 2022.

Module 31. Chelonians I – under development and should be available early in 2022.

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Module One

Anatomy and Physiology

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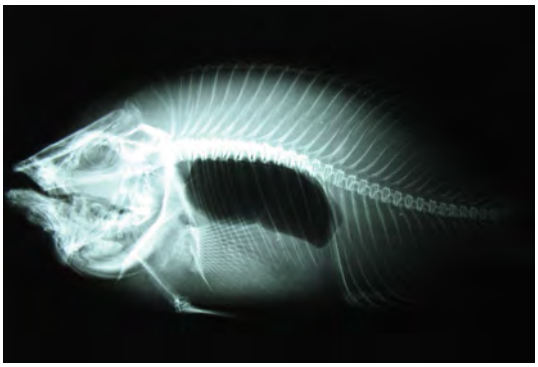

Contents

Section of Chapter 3 - Cardio-vascular and respiratory systems

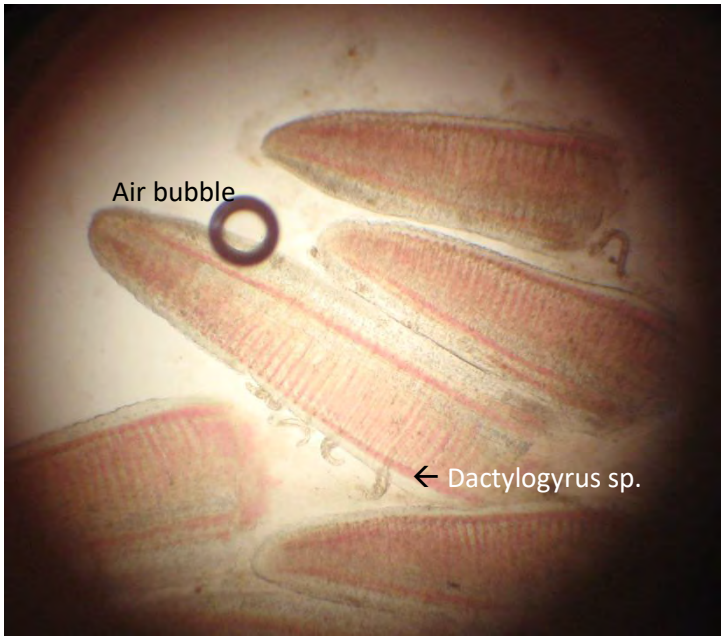

Figure 59 - Discus (*Symphysodon spp.)* – gill monogeneans (*Dactylogyrus sp*.) a very common discus problem (40 times magnification). Monogeneans were previously called flukes. Note air bubble with black circular outline. Air bubbles are often seen and are not pathological.

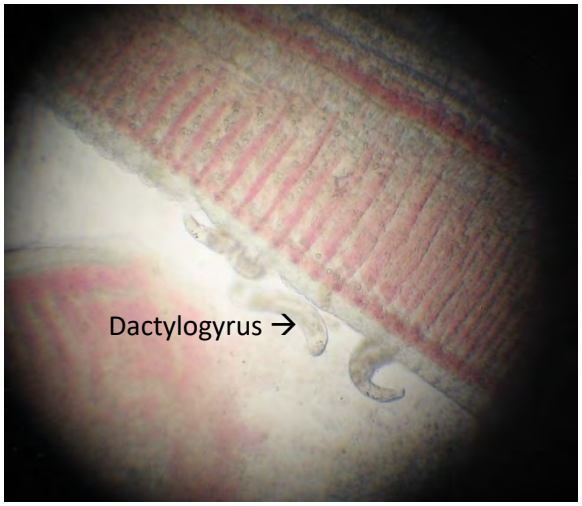

Figure 60 - same case as figure 59 Discus (*Symphysodon spp.)* (100 times magnification).

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.

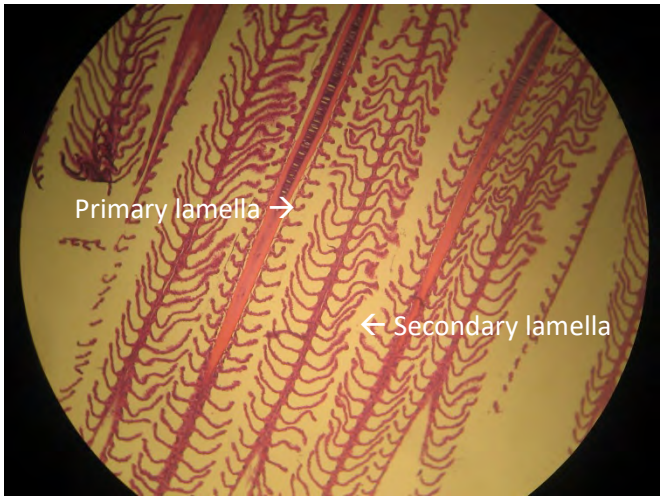

Figure 61 - Barber Perch (*Caesiperca razor)* normal gills (100 times magnification)

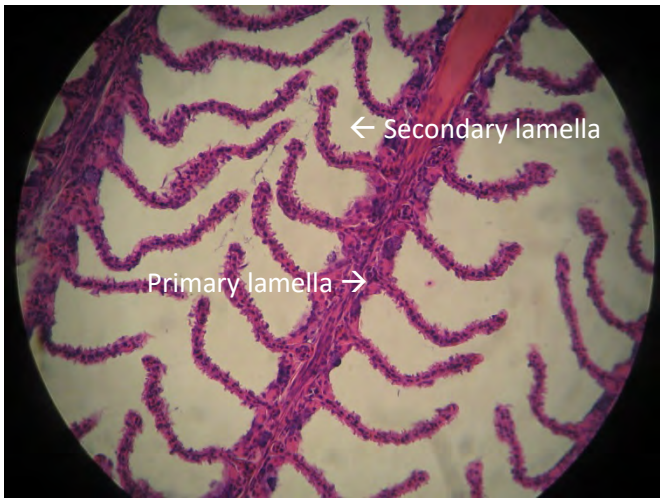

Figure 62 - Barber Perch (*Caesiperca razor*) normal gills (400 times magnification)

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.

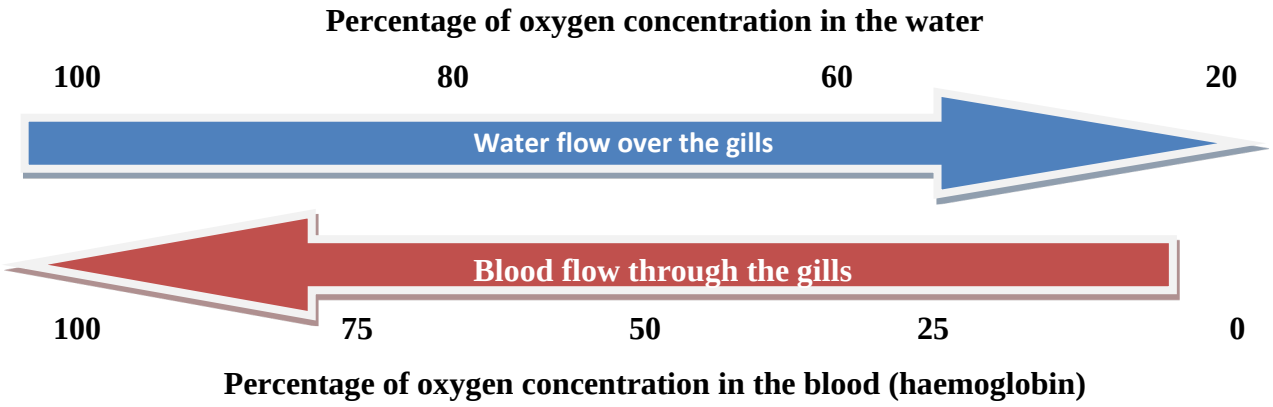

Figure 63 – Diagram of the counter-current mechanism in the gills

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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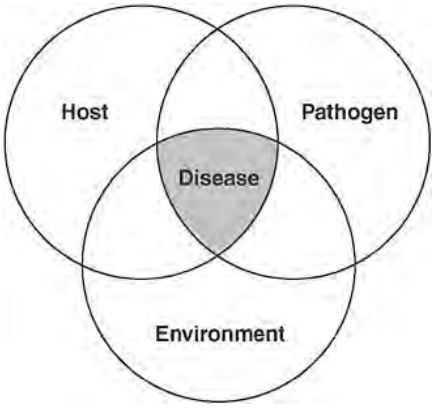


Contents

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.

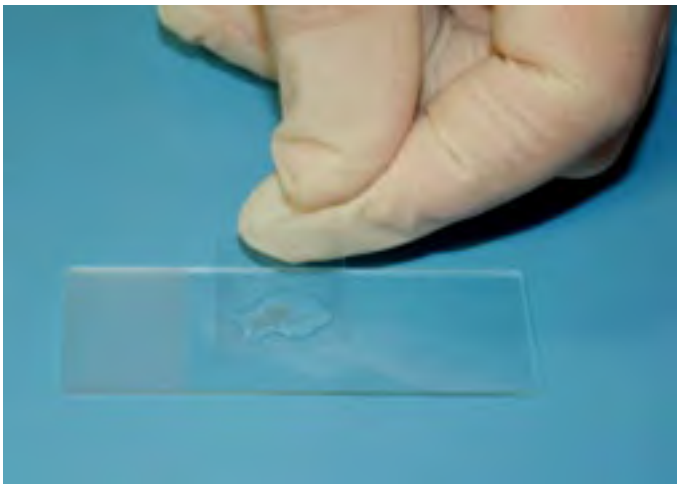
Usually a small area on the side of the body is targeted, although the area at the base of the fins is recommended by some. Using the back of a scalpel blade or a scissor blade, a **gentle** scrape is taken and the resulting sample – mainly mucous - is placed on a microscope slide, add a few drops of 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



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Module Three

Water Chemistry and Quality

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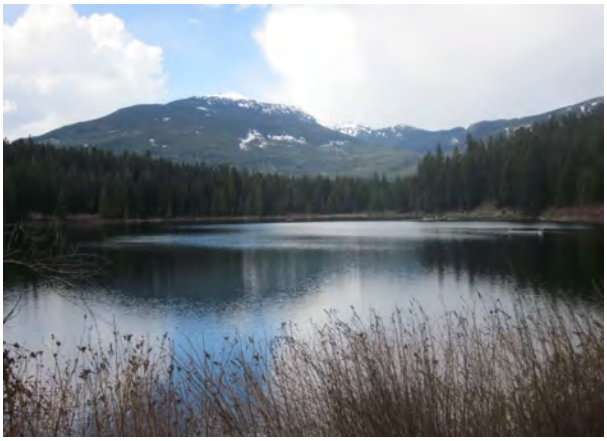

Contents

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

$$\mathbf{NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-}$$

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^-).

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).

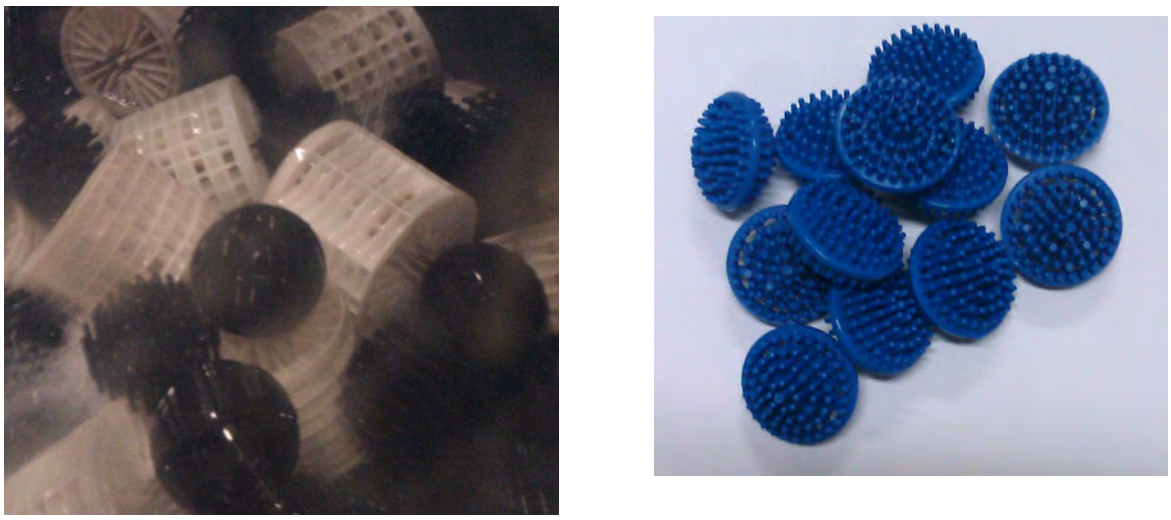

Figure 26 – Common biofilter media (large surface areas)

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^{o}C. 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 nitr**ate** 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).

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Module Four

Life Support Systems

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Contents

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.

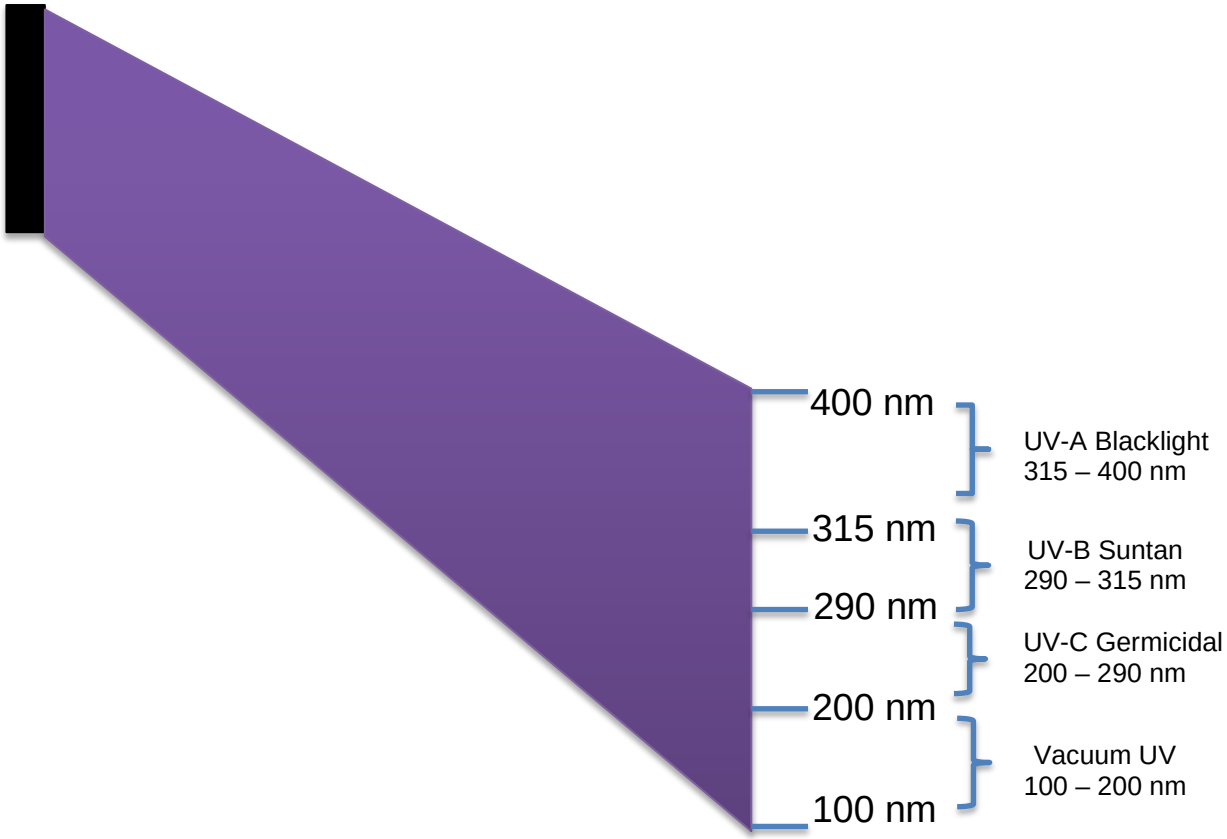

Figure 61 - Various UV subtypes

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).

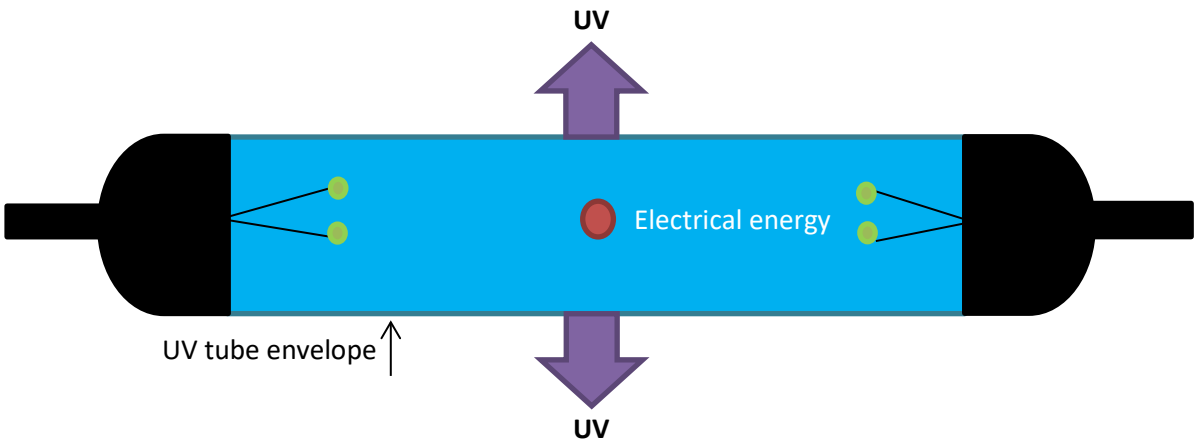

Figure 62 – Diagram showing the production of UV radiation

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^{o}C (104^{o}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.

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Module Five

Diseases and Treatments

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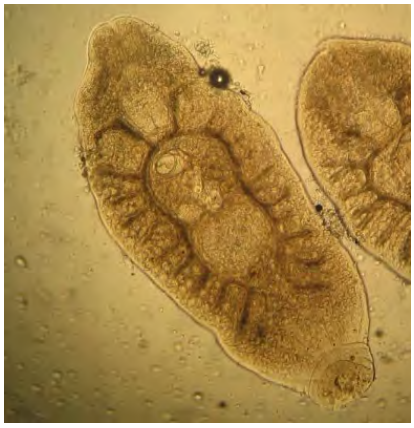

Table of Contents

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.

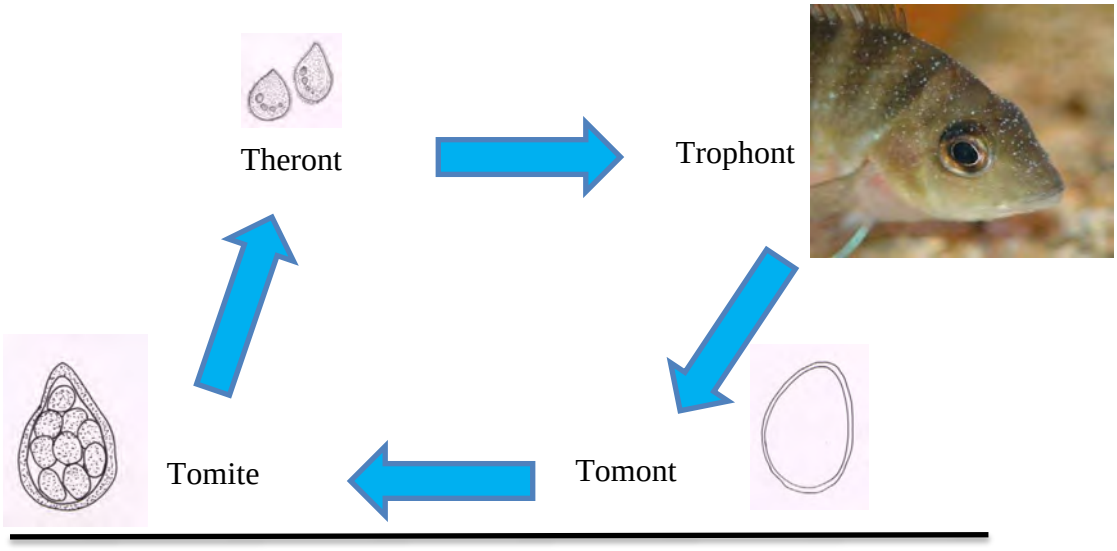

Figure 48 - Life cycle for the protozoan *Ichthyophthirius multifilis*. The dark line at the bottom represents the substrate of the tank.

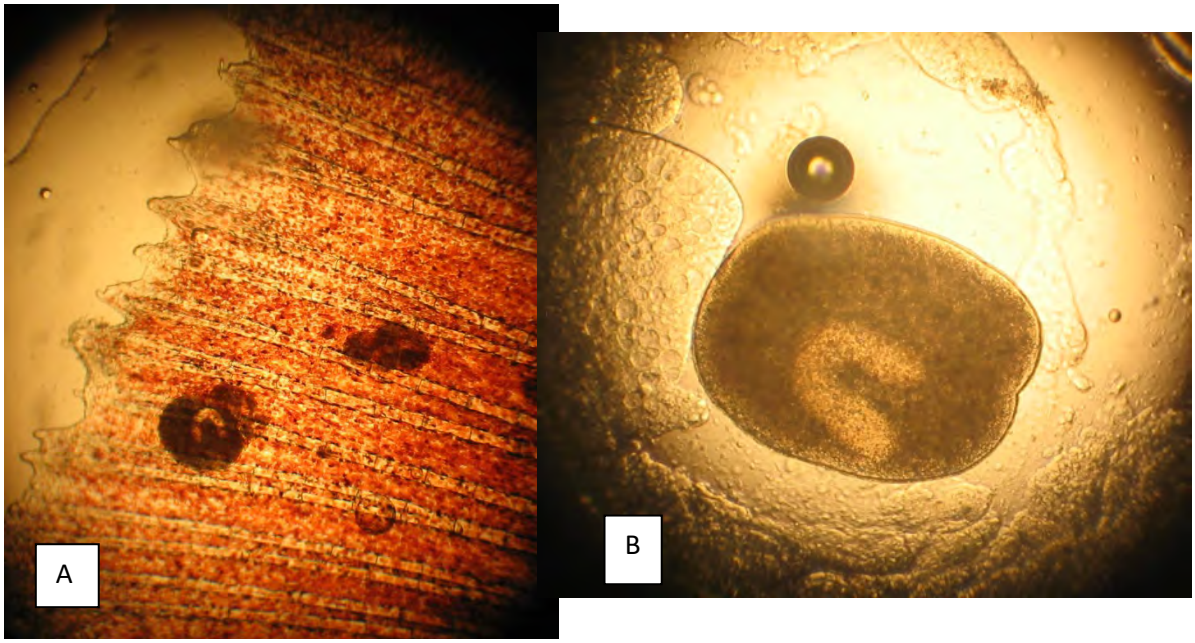

Figure 49 - Ichthyophthirius multifilis trophonts from an infection in a Murray Cod (Maccullochella peelii) A = Fin clip (40 X magnification); B = Skin scrape (400 X magnification). Note the "smiley face"nucleus.

- **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.

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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.

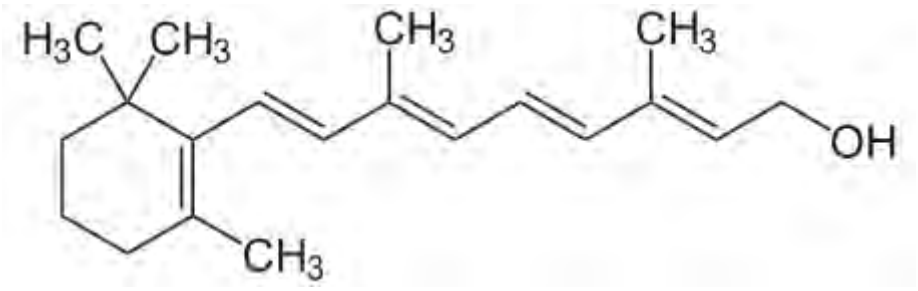

Figure 13 - Vitamin A (retinol) (from https://commons.wikimedia.org/wiki).

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.

REPRODUCTION SECTION

Contents

The Aquarium Vet

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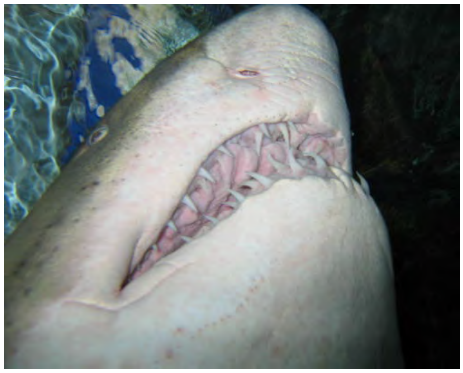
Module Seven

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.

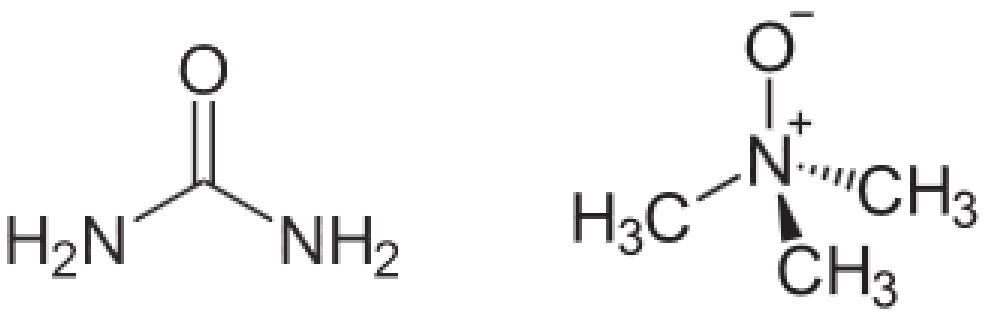


Figure 58 - Chemical structure of urea (left) and tri-methylamine oxide (right) (courtesy of Wiki Commons).

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.

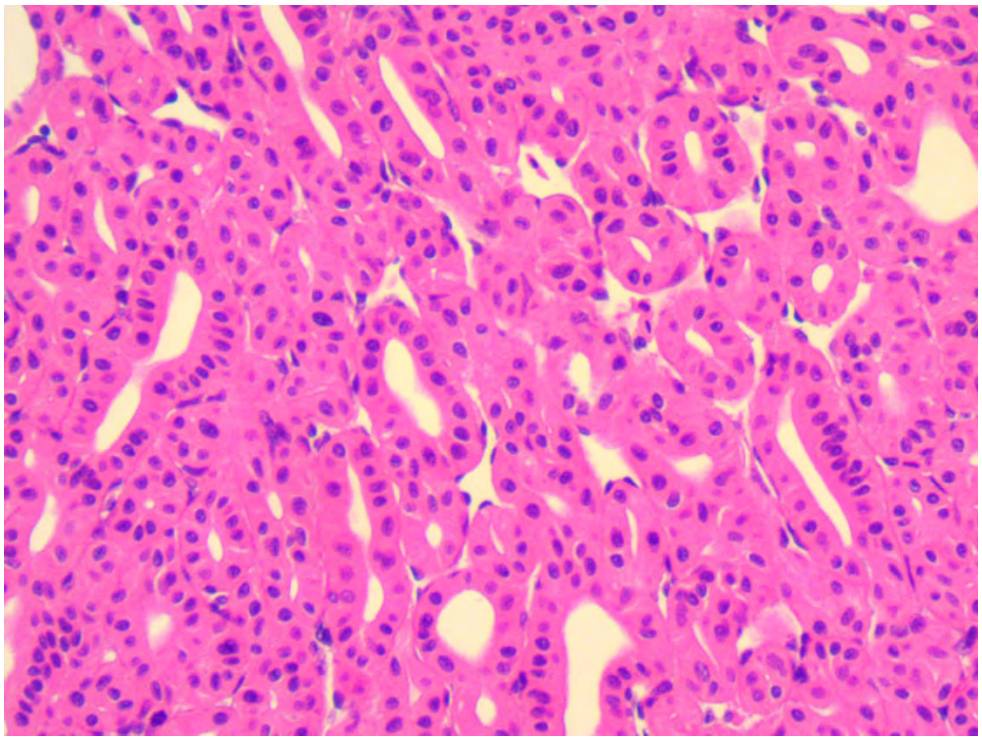


Figure 59 – Histology of rectal gland from an Australian swell shark (*Cephaloscyllium laticeps*) x 100

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

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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.

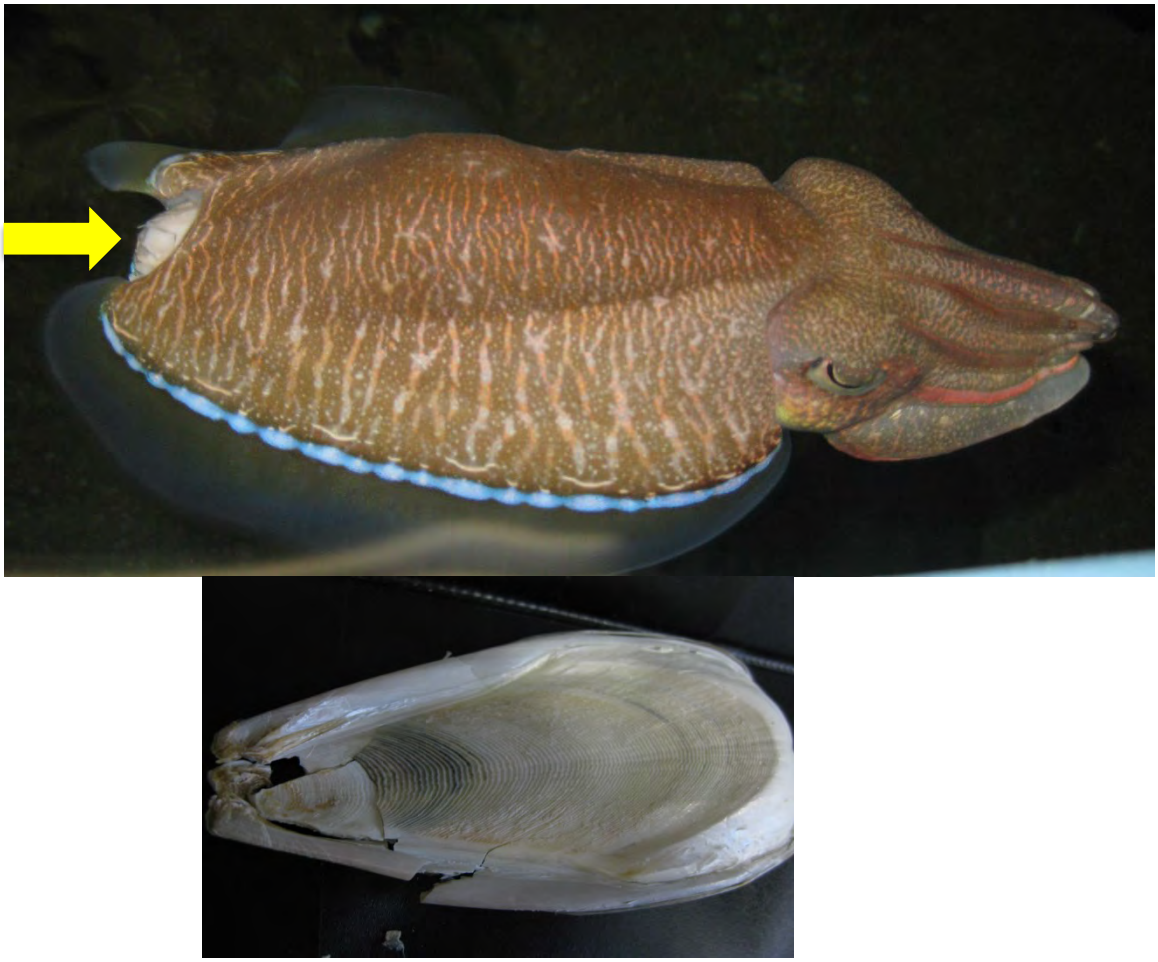

Figure 38 - Top: Mantle laceration on pharaoh cuttlefish (*Sepia pharaonis*) (arrow); Bottom: Fractured cuttlebone from impact against hard surface. Photos by Bret Grasse, Monterey Bay Aquarium.

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.

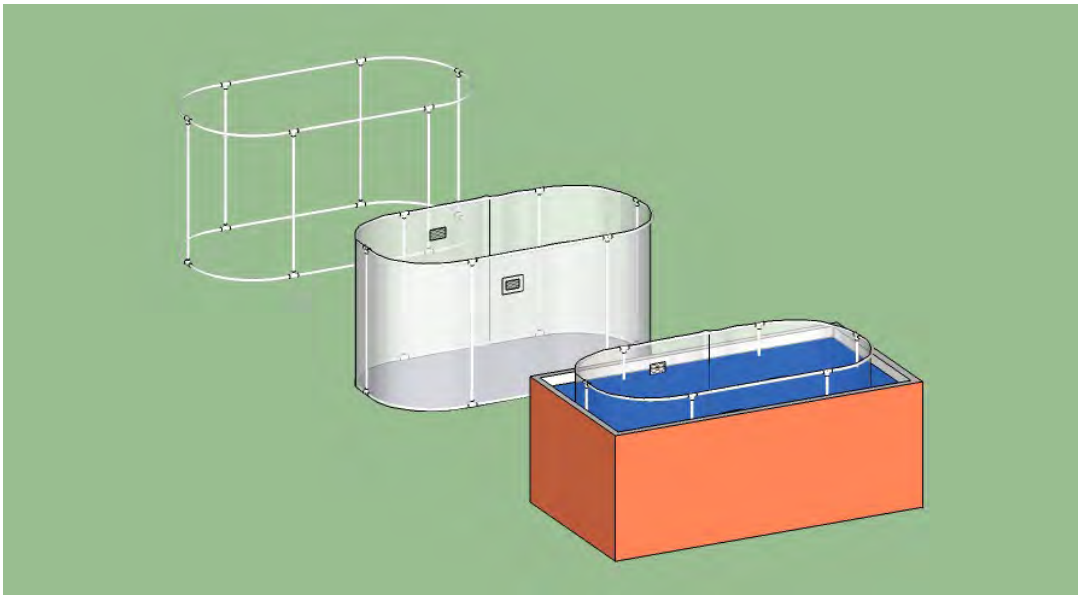

Figure 39 - Soft-sided tank prototype, Sketchup model by Bret Grasse, Monterey Bay Aquarium.

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.

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.

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Module Nine

Sea Jellies and Echinoderms

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.

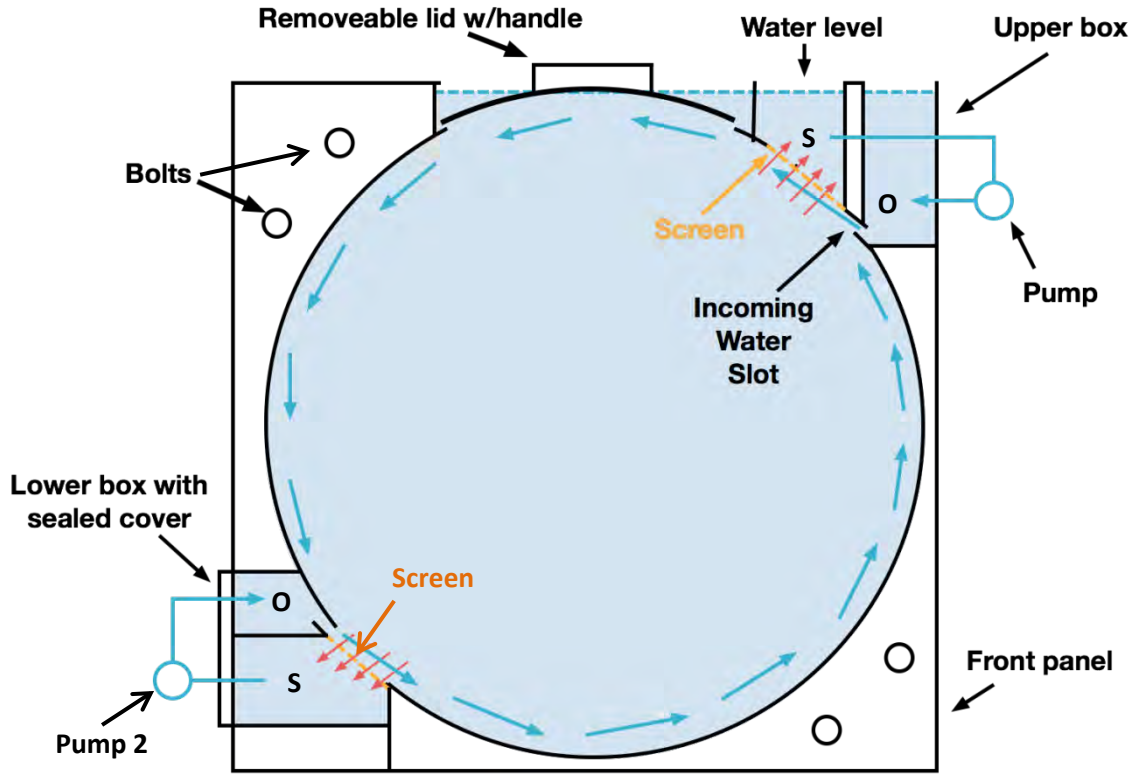

Figure 19 - The standard kreisel tank designed at the Monterey Bay Aquarium, a modified version of Hamner 1990. (Arrows indicate water flow; O = outlet; S = suction). Figure by Wyatt Patry.

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).

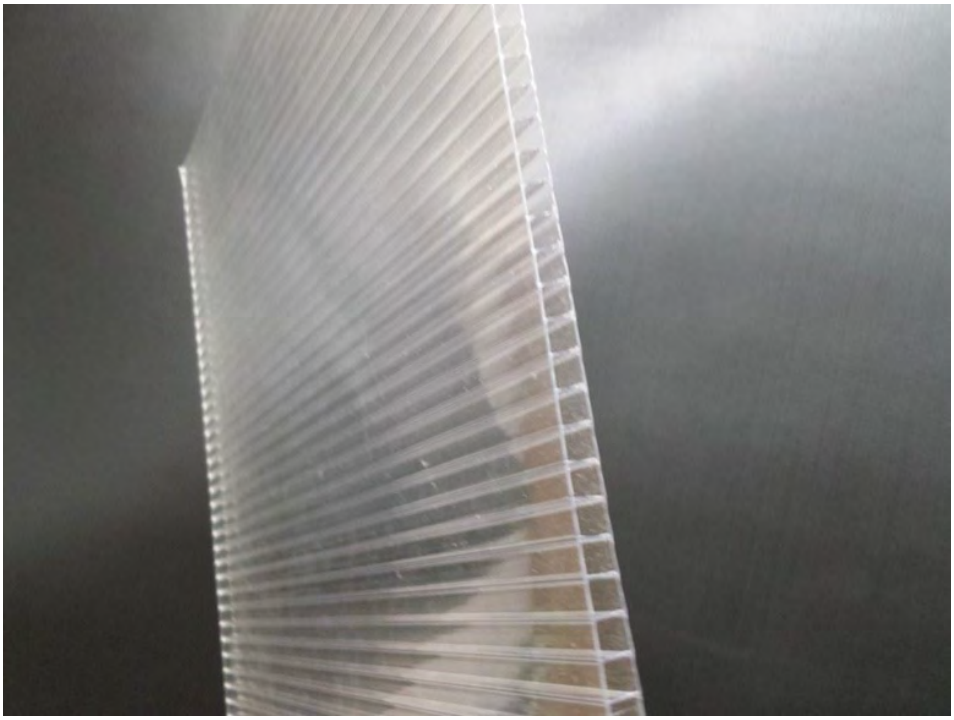

Figure 20 - Corrugated plastic material, commonly used in greenhouses, fills the slots of the kreisel box.

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.

ECHINODERM SECTION

Contents

The Aquarium Vet

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Module Ten

Coral Husbandry

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.

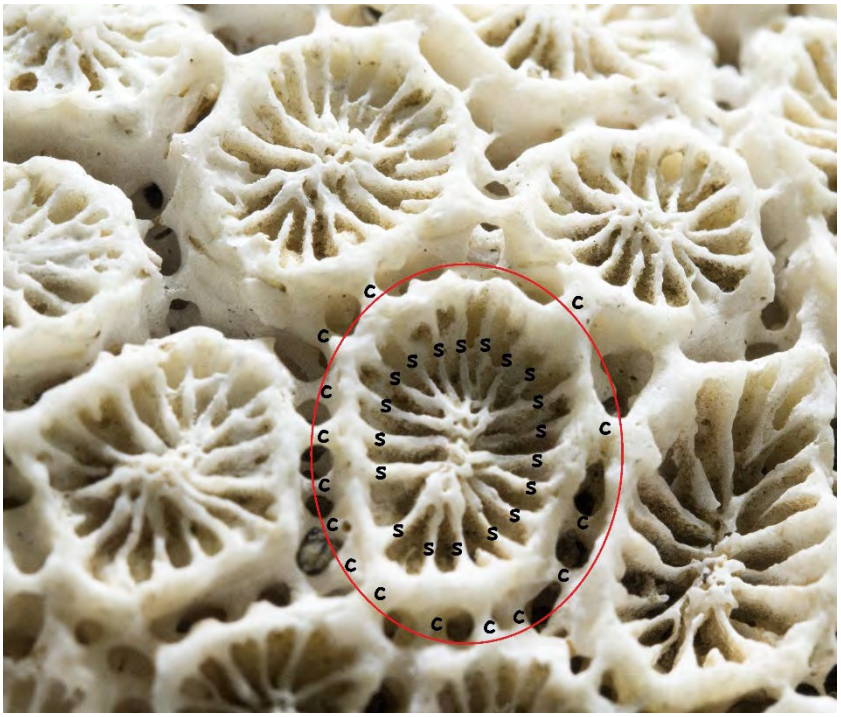

Figure 9 - Coral Skeleton which has distinct septae walls. The basic skeletal structure the corallites is outlined in the red circle. The septae are labelled with "s" and the costae are labelled "c" (courtesy of Shutterstock).

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**.

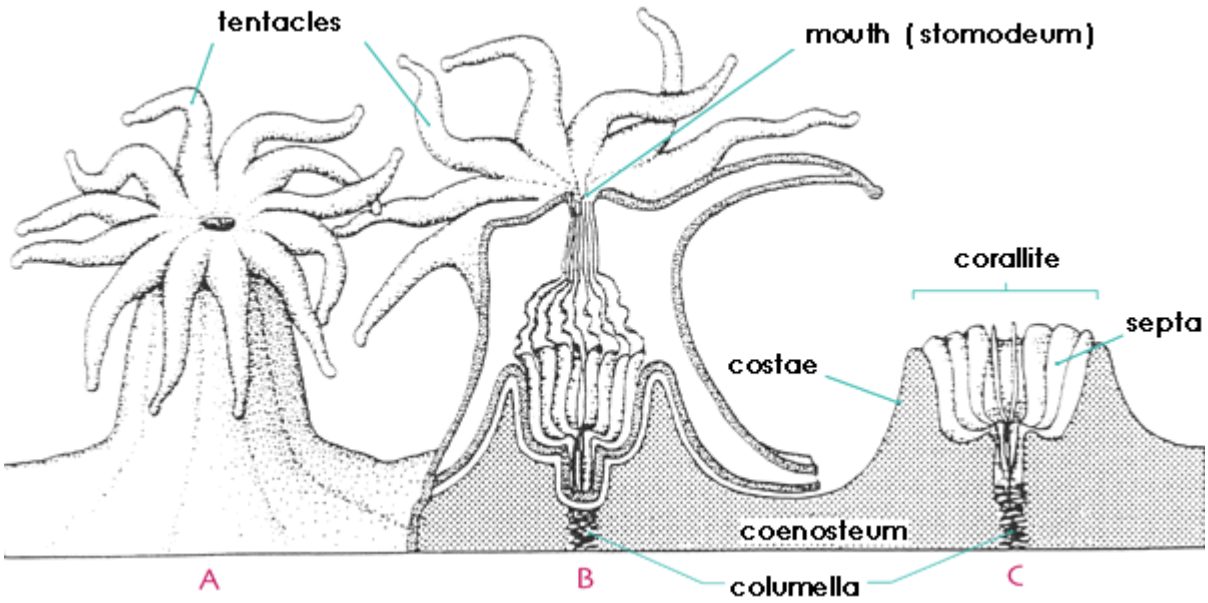

Figure 11 - Coral skeletal structure in association with the living tissue of an individual polyp. A = Polyp; B = Cross section of a polyp and of the skeleton; C = Skeleton (Corallite) only. Source: A Coral Reef Handbook: A Guide to the Geology, Flora, and Fauna of the Great Barrier Reef, edited by Patricia Mather and Isobel Bennett 1993.