Husbandry Manual
For
White's Seahorse

Hippocampus whitei
Actinopterygii : Syngnathidae

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Disclaimer

HM Statement

These husbandry guidelines were produced by the compiler/author at TAFE NSW – Western Sydney Institute, Richmond College, N.S.W. Australia as part assessment for completion of Certificate III in Captive Animals, Course number 1068, RUV30204. Since the husbandry guidelines are the result of student project work, care should be taken in the interpretation of information therein, - in effect, all care taken but no responsibility is assumed for any loss or damage that may result from the use of these guidelines. It is offered to the ASZK Husbandry Manuals Register for the benefit of animal welfare and care. Husbandry guidelines are utility documents and are „works in progress", so enhancements to these guidelines are invited.

Timeline for Maintenance Activities

	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
BREEDING SEASON												
ENCLOSURE REPAIRS												
ENCLOSURE RENOVATIONS												
FULL CLEANING OF ENCLOSURE												
ROUTINE HEALTH CHECKS Monthly												

Introduction

Description

White's seahorses *Hippocampus whitei* are endemic to Australia, found along the south-eastern coast and south-western coasts of Australia they are common in Sydney harbour and can often be found under jetties. Growing from 13-20cm in length, they're variable from drab greyish brown to yellow, depending on surroundings. Small but distinct knobby crown on head topped by 7 sharp spines. They have 11 body and 32 to 36 tail rings. It has between 16 and 20 dorsal fin rays and between 15 and 18 pectoral fin rays. They have a long, prehensile tail which it will curl around any support such as seaweed to prevent being swept away by currents. The Male will begin to develop a pouch when sexually mature which is around 6 months of age this will also help to identify the males from the female seahorses.

History in Captivity

Australia has five on-shore seahorse breeding setups. The breeding of seahorses in captivity came about from conservation concerns as fishermen around the world take 20 million wild seahorses for the Chinese Medicinal trade. Breeding seahorses in captivity is very difficult as seahorses are picky in finding a mate, seahorses diets are also extremely exacting in their food requirements, and captive reproduction is particularly dependent on diet.

Conservation, education, research and wild population management

The decline of seahorse populations worldwide have encouraged projects such as Project Seahorse (www.projectseahorse.com) founded by Amanda Vincent of Magill University in Montreal which are starting to reverse the decline, and also significantly changing fisheries management at the community regional and national level. Researching wild seahorse populations and educating fisheries on the impact of the overfishing of these animals, and working towards achieving sustainability of wild populations.
Late 2007 Sydney Aquarium released 30 Seahorses bred in captivity into Sydney Harbour as part of an Australian-first study to try boost their numbers, the animals have been tagged and will be closely monitored by the New South Wales Department of Primary Industries (DPI) and a team of divers. (Refer to **Appendix 4)**

Occupational Health and Safety Warnings

INNOCUOUS

Conservation Status

H.whitei is listed as Data Deficient by IUCN. Environment Australia lists the conservation status as Data Deficient, and the Australian populations were moved under the Australian Wildlife Protection Act in 1998 and placed under the Environment Protection and Biodiversity Conservation Act in 2001.
Justification: There are no published data about population trends or total numbers of mature animals for this species. There have been no quantitative analyses examining the probability of extinction of this species. As a result a Data Deficient (DD) listing is consistent with that recommended by an assessment conducted for Environment Australia (Pogonoski et al. 2002)

Taxonomy

2.1 Nomenclature

Class: Actinopterygii
Order: Syngnathiformes
Family: Syngnathidae
Genus: Hippocampus
Species: whitei

2.2 Subspecies

None discovered to date.

2.3 Recent Synonyms

Hippocampus novaehollanidae Steindancher 1866

2.4 Other Common Names

Sydney Seahorse
New Holland Seahorse
Golden Seaponie
Golden Seahorse

3 Natural History

Part of the family Syngnathidae that contains seahorses, pipefish and pipehorses. Seahorses long snout creates suction for feeding on plankton, small shrimp and other crustaceans, they have a prehensile tail used for anchoring themselves onto plants and other stable items allowing them to survive in areas with waves and strong currents. Seahorses and all syngnathidae's have a unique form of reproduction, it is the male seahorse that undergoes a true pregnancy, the female produces the eggs but the male will carry them in a pouch on the underside of the tail.
Little is known about seahorses and further scientific study is required on the family

3.1 Morphometrics

Distinctive features that distinguish fish of the Syngnathidae family from other fish include, body covered in bony plates that are arranged in rings, no **ventral fins**, anal and **caudal fins** small of lacking, some may be only present during larval stages and a long tubular snout with toothless small oblique mouth at end. Great diversity in shapes, pipefish have slender straight bodies and the head in line with the body, and usually with a small to moderate-sized **caudal fin.** Seahorses, pipehorses and seadragons will usually have a head at angle to body and the tail ends at a bony point. In all species the male incubates the eggs.

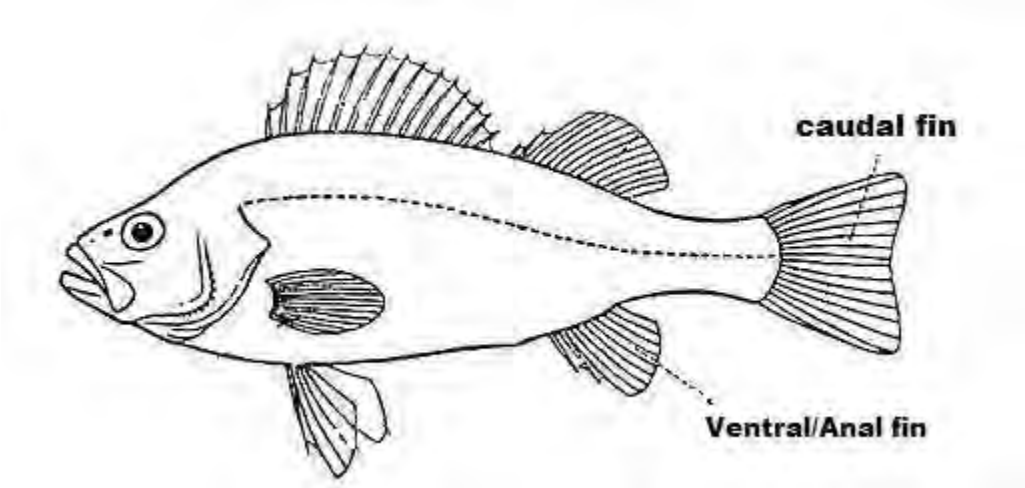

Figure 2.1 Illustration of features of a 'typical' bony fish, the family Syngnathidae lack a caudal fin and Ventral fin.[6]

3.1.1 Mass And Basic Body Measurements

Seahorses range in size from 2.5 cm to 35 cm in length. White's Seahorses can grow from 13-20cm in length from the top of the head to the end of the tail[6].

3.1.2 Sexual Dimorphism

Juveniles and females usually with several pale saddles over back. Male's dark with fine pale spotting and scribbles on face turning to light colour with dark face and chest during courtship[4]. Males are very easy to identify with their pouch, which hangs below the abdomen (refer to figure 2.2)[1].

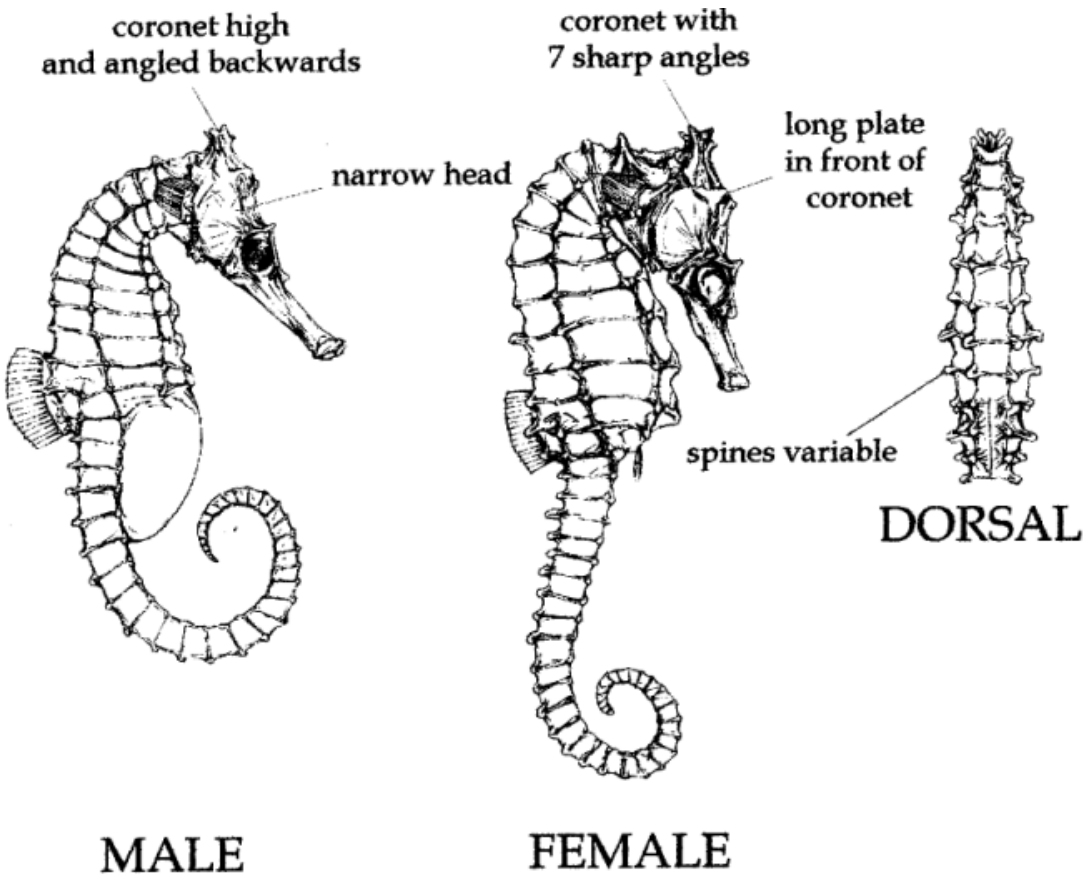

Figure 2.2 Example of male and female White's seahorse.

3.1.3 Distinguishing Features

Variable from drab greyish brown to yellow, depending on surroundings. Small but distinct knobby crown on head topped by 7 sharp spines.
White's seahorse possess 11 body and 32 to 36 tail rings. It has between 16 and 20 dorsal fin rays and between 15 and 18 pectoral fin rays. They have a long snout and their eyes and cheek spines are well developed and sharp. The other spines vary greatly in appearance from low and rounded, moderate, or long and pointed[3].
Usually brown, grey to yellowish, brownish net like markings on spines, almost white spots particularly around head. The seahorse has a long, prehensile tail which it will curl around any support such as seaweed to prevent being swept away by currents[4].

Figure 2.2 Distribution map

3.2 Distribution and Habitat

Fishes of the family Syngnathidae are found in tropical and temperate coastal waters around the world and seahorses occur throughout this range. White's Seahorse however, are **endemic** to Australia. They occur in depths down to about 25 m in temperate marine waters along the south-eastern and south-western coasts of Australia, and common in Sydney Harbour.

Habitat: Various habitats, from shallow seagrass beds in sheltered bays to depths of about 25 m with sponges. Often found under jetties on holdfast of kelp. White's seahorses are non-migratory[6].

3.3 Conservation Status

Red list category & criteria: DD (Data Deficient)
Year assessed: 2003
Justification: There are no published data about population trends or total numbers of mature animals for this species. There have been no quantitative analyses examining the probability of extinction of this species. As a result a Data Deficient (DD) listing is consistent with that recommended by an assessment conducted for Environment Australia (Pogonoski et al. 2002)[7].

3.4 Longevity

3.4.1 In the Wild

Seahorses have an average life span of around 3 years in the wild some species living up to as long as 9 years. Generally the larger species of seahorses live longer than the smaller species.

3.4.2 In Captivity

Seahorses have only been bred in captivity for a few years and their potential lifespan in captivity is not yet known.

3.4.3 Techniques Used to Determine Age in Adults

Juveniles and adult females usually with several pale saddles over back4.
Fry (baby seahorses) are miniature versions of adults. Male will begin to develop a pouch when sexually mature which is around 6 months of age this will also help to identify the males from the female seahorses.

4

4 Housing Requirements

4.1 Exhibit/Enclosure Design

The ideal tank set-up should contain an ample amount of tank furnishings in order for the seahorse to anchor themselves, this is a behaviour that seahorses display in the wild, they will use their prehensile tail in order to hold onto seaweed for support in strong currents. Seahorses do not spend a lot of time swimming and will hold onto seaweed or seagrass for most of the time, feeding on small crustaceans that swim past. Therefore it is important that you provide your seahorse plenty of tank furnishings, not only does it allow the seahorse for somewhere to hold onto but it also gives them a sense of security. Seahorses will hide amoungst seaweed to camouflage themselves from predators, if you dont allow your seahorse for somewhere to hide it will stress the animal and make them more susceptible to disease.

Suitable holdfasts:

- Seaweed or seagrasses (Posidonia australis an Australian seagrass species)
- Coral fans
- Plastic plants (however I recommended using live plants)

Special note for aquarists using live plants, I suggest that you change the seaweed regulary, usually once a week. Once the seaweed becomes crumbly when you rub it in your finger tips, you know its time to change it. Having dead plants in your tank will compromises your water quality.

General principles of a suitable tank set-up:

- Crushed coral substrate
- Live plants
- Coral fans
- Live rocks
- Avoid having the tank positioned in direct sunlight, you want to maintain the water temperature around 22oC-24oC

Refer to **Appendix 1** for an example for a tank design for two mating pairs of seahorses

4.2 Holding Area Design

A smaller separate holding tank that is filtered and has the same water conditions as their enclosure is an ideal holding area for the seahorses while cleaning or maintenance is being done, alternatively it can be used as a quarantine tank for ill or new animals, the tank should be disinfected and all furnishings are thoroughly cleaned after use for quarantine[3]. The holding tank should have the same water temperature as their enclosure so there isn't a sudden change in temperature for the animal which can cause a great deal of stress, some light furnishings will also allow for anchorage while the animal is in the holding area.

4.3 Spatial Requirements

A female seahorse in the wild has a territory size of about 1.4 square metres and the male has a territory size of around 0.5 square metres. It is important that if you are housing male and females together that they have enough room to get away from each other. This allows for hiding areas, as seahorses are very shy animals. The bigger the size of the tank also means a more stable water quality[2].

4.4 Position of Enclosures

Enclosures should be positioned near a window to establish a day/night cycle that coincides with local conditions. However you must ensure that the tank is not in direct sunlight all day as the build up of heat will cause stress to the animal and encourages the growth of algae[3].

4.5 Weather Protection

The tank should be fully enclosed in order to help regulate the temperature, sudden changes in water temperature can cause shock and stress the animal, and can potentially be fatal.

4.6 *Temperature Requirements*

Seahorses are **ectothermic** and are unable to regulate their body temperature therefore changes in water temperature should be made gradually if possible, as a dramatic change in temperature can cause shock. Ideally the changes in water temperature should be in accordance with the seasonal changes that occur in their natural habitat. Bear in mind that the warmer the water the less oxygen it holds than that of cooler water[2].

4.7 Substrate

Types of substrate that can be used:
Crushed coral
Seashells
Dolomite
Coarse sand
Pea gravel
Be careful not use very fine substrates as they can clog the filter[3].

4.8 Nestboxes and/or Bedding Material

Once the male seahorses is pregnant it is a good idea to put him in a separate tank, to minimise him getting stressed. The tank set-up should follow the same principles as a normal tank set-up for seahorses, you still need to supply holdfasts for the seahorse to hold onto. Once the Male gives birth the fry should be moved into another separate tank with no substrate and plastic netting for holdfasts.
Enclosure Furnishings
Seahorses need lots of enclosure furnishings in order to have places to hide and anchor themselves too, as well as for environmental enrichment purposes.

4.9 Enclosure Furnishings

Seahorses need lots of enclosure furnishings in order to have places to hide and anchor themselves too, as well as for environmental enrichment purposes.
Ideal enclosure furnishings include:
Rocks
Live rocks
Live coral, replicas and skeletons
Live plants, replica plants and living marine algae

Coral rock - Helps maintain a safe pH level
Note: Be careful when collecting rocks as some rocks can leach harmful chemicals, lace rock and lava rock are safe choices.

Live rocks - Is an important filtration mechanism

Coral skeletons - Are excellent anchorage sites.
Note: Collecting of coral has caused ecological damage and is prohibited in Australia, the United States and other countries. If you choose to use natural coral make sure to only used farmed coral.

Living plants - Provide a natural holdfast and important for the overall health of marine aquariums.

Driftwood - Can be used as anchorage points, an advantage of driftwood is that it is smooth for the seahorses to hold onto3.

5 General Husbandry

5.2 Hygiene and Cleaning

A filtration system will lessen the routine maintenance. Be careful when cleaning enclosure furnishings especially if you use plastic furnishings, as most plastics are porous and will retain harmful chemicals.
Bleach is an ideal disinfectant to use, when used correctly bleach does not leave any harmful residue. Ensure that you always read the label and follow the correct dilution instructions and wear appropriate PPE when using any chemicals.
Be careful to remove all traces of soap and other chemicals from you hands before placing them into the aquarium3.

5.3 Record Keeping

It is important to keep records as it provides archival material for the future, in order to record the numbers and sexes and most importantly it is required by law to keep records. Any notable events such as deaths and births should be noted down, also record keeping helps to communicate with fellow keepers on any changes in diet or behaviour for the animal that needs special attention.
The following is a list of events that should be record:

- Changes in behaviour
- Changes in diet
- Weight loss/ill health
- Death/births
- Numbers and sexes of the animals
- Movements within and between institutions
- Treatments or veterinary examinations

(Refer to **Appendix 2** for a copy of an example of a daily diary record sheet)

5.4 Methods of Identification

Collar tags:

A tag that is physically affixed to the fish, Collar, or fingerling tags were the first methods used to identify seahorses (Vincent & Sadler 1995)

They're a small oval PVC disc with a three digit number on one side, the tags are attached around the neck of the seahorse with cord, to provide individual identification.

VIE – Visible Implant Elastomer or VIFE –Visible Implant fluorescent elastomer:

When conducting research seahorses are commonly tagged with unique identifying marks known as visible implant fluorescent elastomer (VIFE) that has been used worldwide for tagging syngnathids and fish. During the tagging process the animal's size is measured using a ruler and the sex of each individual is recorded. VIFE markers are applied just beneath the skin using a needle and the markers are less than 2mm in size. On subsequent re-sightings in the wild the seahorses are identified and their location recorded[18]

Below are images of examples of VIE tagging in seahorses

(a). *Hippocampus whitei* tagged with three pink tags within body segments viewed under ambient light. © Jonathan Clark-Jones/Project Seahorse.

(b). *Hippocampus abdominalis* tagged with one orange and one green tag within ridges viewed under ambient light. © Keith Martin-Smith/Project Seahorse.

Refer to **Appendix 3** Selected Techniques for Tagging Seahorses

5.5 Routine Data Collection

Daily	Weekly	Monthly
• General Condition of the animal. • Distant Examination should be performed daily • Amount and type of food being fed • Notable events • Water temperature	• Water condition Water should be tested for the following Temperature: 22°C - 24°C, pH 8.2 Salinity sg 1024 Ammonia-Nil Nitrate-Nil Nitrates-<20ppm[14]	• Weight • Measurements

(Refer to **Appendix 2** for a copy of a daily diary record sheet)

6

6 Feeding Requirements

6.2 Diet in the Wild

In the Wild the White's diet consists of small shrimp and other crustaceans, little is really known about the natural diet of seahorses. Seahorses only feed on live food in the wild and they consume large quantities of food which complicates their captive husbandry.[3]

6.3 Captive Diet

Seahorses have a very simple digestive system (*see figure 6.1*) and do not have a true stomach, so food is not retained for any length of time in the body. Therefore seahorses need to feed throughout the day, if you are feeding dead food they will need to be fed three to four times a day. If feeding live food, it should be in the tank with the seahorse all the time.
An Adult seahorse can consume 50 to 100 food items a day, where as growing young may require over 3,000.

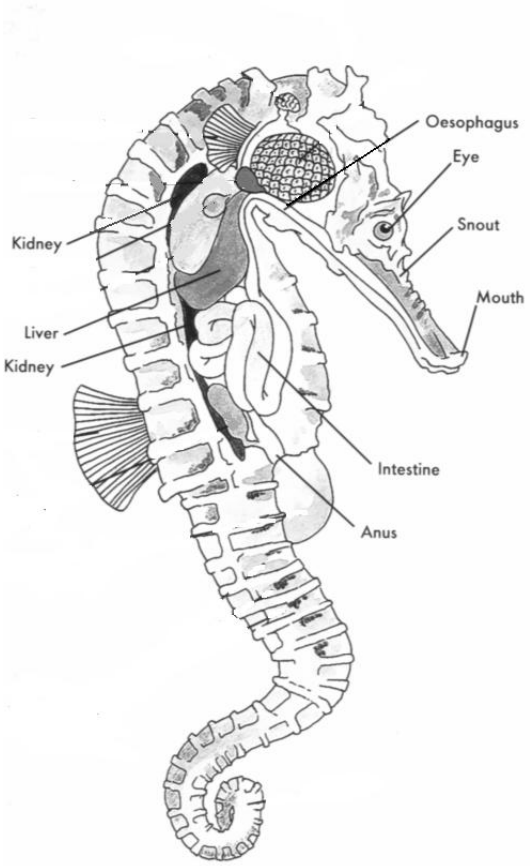

Figure 6.1 Image of anatomy of a seahorse

Small marine shrimps – are among the most nutritious of foods for captive seahorses. Small shrimp should form the bulk of a seahorses diet. Suitably sized individuals of the genera Palaemonetes, Crago, Peneus and Mysidopis which aren't true shrimps can be collected for feeding along shorelines throughout the world and kept in a holding tank to be fed out when needed.

Mysid Shrimps – form the basis of a captive seahorse diets in most public aquariums and are the most nutritionally complete food items. They are commercially available and quite easy to breed.

Freshwater shrimps – are a good food for all species of seahorses however are not suitable as a complete diet. They can be collected and kept in the same way as marine shrimp.

Amphipods (see figure 6.2)[10] – a small, active crustacean, should be kept and bred in a filtered well-lit aquarium and fed tropical fish food flakes and trout chow, various greens and dead fish are also good. A high quality diet fed to food animals translates into healthier seahorses.

Figure 6.2 Image of a amphipod

Copepods, seed shrimps, water fleas and plankton - a cause for concern when collecting saltwater species to feed to your seahorses, is the possibility of introducing fish parasites such as fish lice (Calgus and Argulus spp.) into the aquarium. However the value of nutritional variety, especially for such a sensitive animal as the seahorse, overrides the danger presented by parasites.

Frozen foods – Many of the food items mentioned are available in the pet trade in frozen form. It is a good idea to acclimatise your seahorses to frozen foods. This can be accomplished by mixing bits of frozen food in with the live food and gradually increasing the amount of frozen food offered.
Acclimating seahorses to accept frozen food is a key step to establishing them for the long term, the training process should begin when the seahorses are young, getting the seahorses use to eating frozen foods will then allow you to add such items as shrimps, scallops, mussels, clams, crabs, and fish to their diet. The food should be finely chopped and mixed, then frozen in thin sheets for easy handling.[3]

6.4 Supplements

Feeding Brine shrimps (see figure 6.3)10 although eagerly accepted by nearly all seahorses are inadequate as the sole diet. If being used you should supplement the brine shrimp nutritional quality by allowing the shrimps to feed for two to three days. This is the best accomplished by using products such as Selco, algae pellets, or liquid foods designed for filter feeding invertebrates.
According to Pete Giwojna - Seahorse Nutrition Part V: Feeding Juveniles & Dwarf seahorses the February 1997 issue of Freshwater and Marine Aquarium magazine (FAMA) "I recommend using one of the lipid-rich food concentrates such as Selcon which have recently been developed specifically for use in aquaculture. Selcon Concentrate is rich in highly unsaturated fatty acids (HUFA) and vitamins C and B-12, which makes it an ideal supplement for culturing Artemia."

Figure 6.3 Image of a brine shrimp

6.5 Presentation of Food

Seahorses hunt by sight and are often stimulated into feeding by movement, so the use of live food is imperative, not only from the nutritional aspect but also for the behaviour enrichment of your seahorses. Giving seahorses natural things to do, will encourage them to behave as they would naturally in the wild and, in turn, give them a much better quality of life.

When feeding live food, the food should be kept in the tank with the seahorses, allowing them to feed when they feel like eating. If feeding frozen food or dead food it should be done by placing it in the flow of the filter and allowing it to be powered around the tank; this should stimulate the seahorse into following it thinking that it is alive.[2]

7

7 Handling and Transport

7.2 Timing of Capture and Handling

The capture and restraint handling must be done with care, in order to protect the animals' well-being, and avoid unnecessary discomfort, stress or physical harm.
(as per ARAZPA Accreditation Standards)

Schedule the capture during appropriate climatic conditions. Seahorses are very susceptible to stress, therefore the capture of the animal is best to be done when public is not around; to keep noise levels down and movement outside the tank to a minimum. It is also important that any changes in light should be made gradually.[1]

7.3 Catching Bags

Equipment for animal capture must be in good working order, appropriately stored, maintained and available to authorised, trained personnel at all times.
(as per ARAZPA Accreditation Standards)

During capture the seahorse's **epidermis** can be damaged, creating a site for secondary infection by bacteria or fungus. Therefore it is important that the catching bags used for the capture of seahorses are made of non abrasive materials to minimise damage to the epidermis.
Types of catching bags:

- Mesh Bags
- Strong Plastic (polyethylene) bags

(Syngnathid Husbandry in Public Aquariums, 2005)

7.4 Capture and Restraint Techniques

You need to hold the seahorse just under the surface of the water, making sure you do not cover their gills, allow the seahorse's tail to hold onto one of your fingers this gives it a sense of security and also allows you to hold the tail out of the way whilst performing examinations.[2]

Chemical Restraint

MS-222 is a common and effective form of anesthesia for syngnathids and can be used for chemical restraints a standard fish dose of 50-100ppm works quite well.
(Refer to **Section 8.2 Detailed Physical Examination)**

Below is an image of a seahorse being handled to be injected with a VIE – Visual Implant Elastomer

7.5 Weighing and Examination

Weighing - You will need to learn how to judge a seahorse's weight by sight. Between the ridges of the body, the skin should either bulge outwards or be level; if it sinks inwards the seahorse is seriously underweight and should be offered more or better quality food. The only exception to this is that, when the females are carrying no eggs, the front four or five segments of the belly sink in.[2]

Examination – You will need to take great care when physically handling the animal when performing examinations, you often physically restrain the animal when treating for diseases such as Gas Bubble Disease which is a common problem in seahorses. You need to hold the seahorse just under the surface of the water, making sure you do not cover their gills, allow the seahorse's tail to hold onto one of your fingers this gives it a sense of security and also allows you to hold the tail out of the way whilst performing examinations.[2]

7.6 Release

When releasing seahorses into a new enclosure you need to acclimatise them to the new water. First check that your basic water parameters are within acceptable range which is: Temperature range 20°C – 27°C, optimum temperature 23°C – 25°C, Ammonia 0, Nitrites 0, Nitrates 1-10ppm, pH 8.2 – 8.4, Specific gravity 1.022-1.025.
Acclimate your seahorse slowly, but do not take more than 30 minutes to complete the procedure. Open your box away from bright lights. Check temperature and PH upon arrival in both the shipping water and in the tank. Turn off aquarium lights and follow this procedure[10]:

- Float the bag in your tank for about 10 minutes to equalize temperatures. (see figure 7.1)
- Partially open the bag and add 1 cup of tank water. (Do NOT aerate the shipping bag during acclimation)
- Wait 10 minutes
- Remove 1 cup of water and add another cup of water from the tank
- Wait 10 minutes
- Repeat this procedure again
- Gently use your hands to transfer the seahorse into the tank, discarding the water left in the bag.[10]

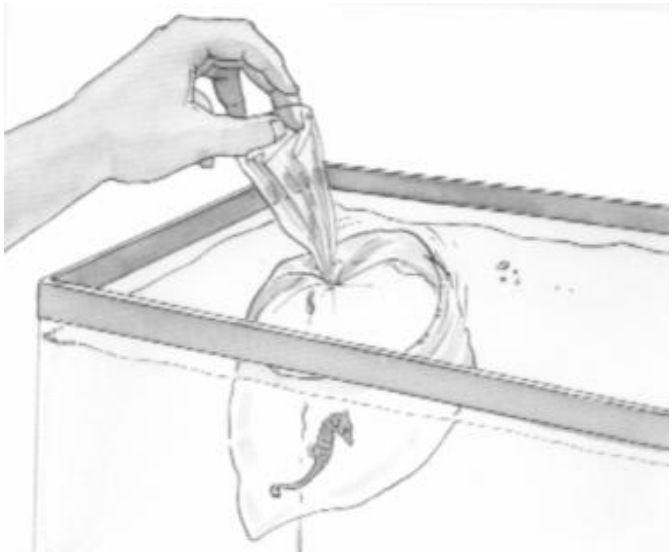

Figure 7.1 Image of a seahorse being acclimatised

7.7 Transport Requirements

Preparations before dispatch
The inner bag must be filled with water to approximately 1/3 of its capacity.
The remaining 2/3 of the container is to be filled with oxygen. Use of ice cubes or chemicals such as methylene blue, volume of water and the amount of fish in the container are the shipper's responsibility. Carriers will not re-oxygenate fish shipments unless by special prearranged agreement. (Refer to **Figure 7.2)**

(as per IATA Live Animals Regulations)

Shippers must pack fish to survive unattended for at least 48 hours from time of acceptance by the airline.
The shipper must clearly mark on the container the local time and the date at which the animals were packed.
The shipper must indicate the acceptable temperature range (in Celsius and Fahrenheit) on the outside of the box in which the animals can be stored.

General care and loading
Animals must be held in areas where the ambient air temperature reduces the heat transfer to the absolute minimum.

No consignment of fish must be accepted if the planned journey exceeds 48 hours. Consignments of live fish must be treated as perishable items and handled accordingly.

For the purpose of providing life support for aquatic species during transport, a cylinder containing oxygen (compressed), UN identification no. 1072, packed in accordance with the IATA Dangerous Goods Regulations, may be carried to oxygenate the water with the approval of the appropriate authority of the States origin, destination and of the operator.

Note: *States may require the physical inspection of the contents of shipments tendered by shippers meeting a specific state mandated criteria as determined by the transporting carrier*

Transportation of Seahorses
The following points should be taken into consideration for the transportation of live seahorses:
1. Transit time must be minimized wherever possible
2. Only healthy individuals should be selected for transportation
3. Packaging must be adequate
4. Seahorses should be packed with compatible animals. Packing seahorses in the same box as corals and live rock is not recommended
(as per Syngnathid Husbandry in Public aquariums 2005 manual)

7.7.1 Box Design

As per IATA Live Animals Regulations
Container construction

Materials
Water-resistant fibreboard, insulating material, plastic or wood, expanded polystyrene or Styrofoam.

Principles of Design
The following principles of design must be met in addition to the General Container Requirements outlined at the beginning of this chapter.

Outer Container
The outer container can be constructed of fibreboard, wood, wood products, or any plastic material of adequate strength. Purpose-built containers made of expanded polystyrene or Styrofoam must be of adequate strength. (see figure 7.2)

Care must be taken to ensure no sharp edges or stapled closing on the outer container punctures the inner plastic bag, which expands from change in altitude.

Inner Container
Strong plastic (polyethylene) bag.

The bag are fastened by twisting the top and folding the twisted part so that it can be sealed with elastic bands. The bags may also be heat sealed. (see figure 7.2)

Warning: *Heat-sealed bags cannot be re-oxygenated in the event of the consignment being delayed.*

It is preferable that each bag is placed in an outer bag of similar size to prevent leakage of water.

Insulation/Cushioning
Expanding polystyrene or expanded polystyrene sheets on all sides including top and bottom is recommended. Alternatively, compressed newspaper wood, wool or approximately 0.6cm (1/4 in) thickness of newspaper or other fibrous material sandwiched between two sheets of craft paper.

EXAMPLE:

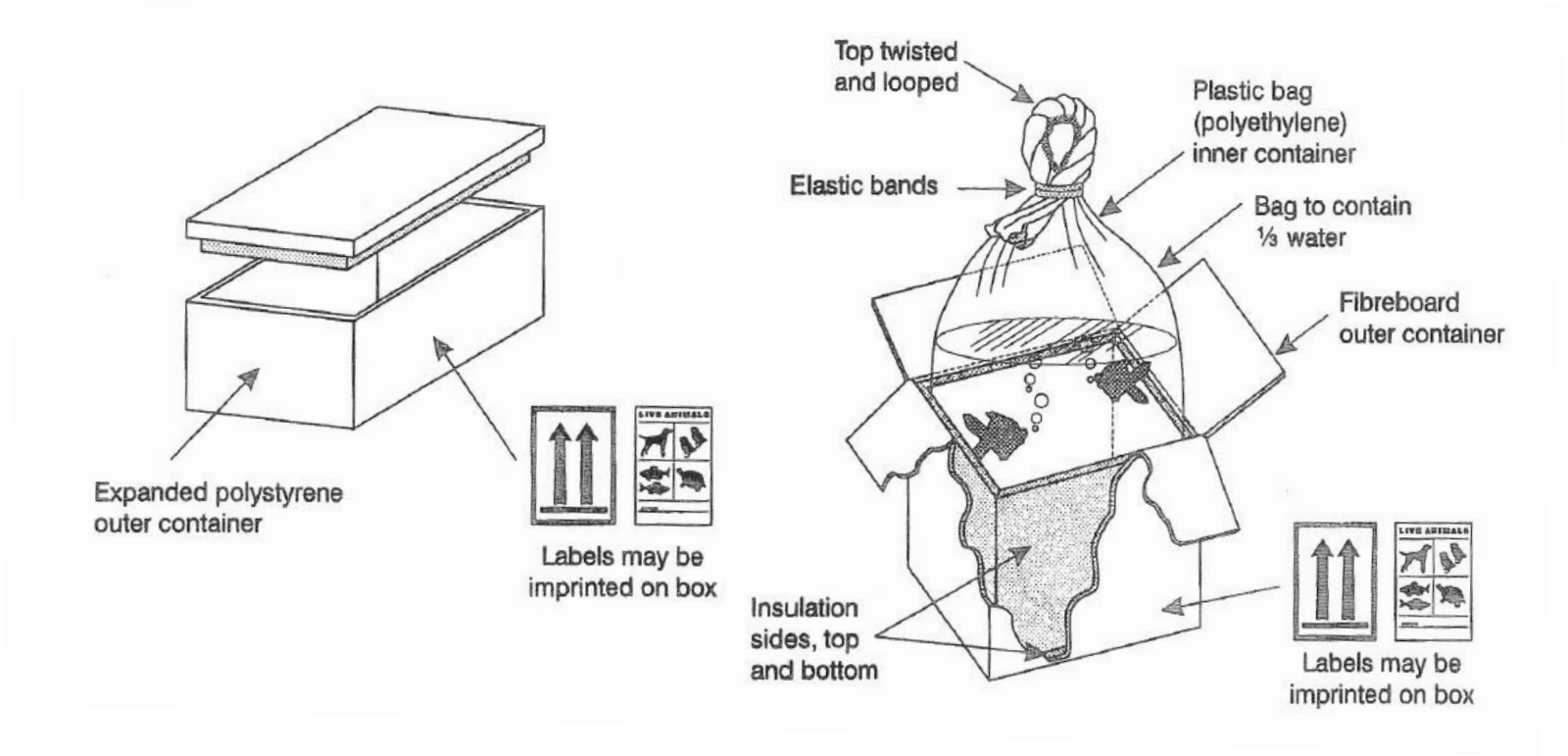

Figure 7.2 IATA Standards Box design

7.7.2 Furnishings

For most species, particularly smaller ones, the use of **holdfasts** in the bag is also recommended which should be held down with a small weight so that they are not floating. It allows these fish that naturally spend a lot of time holding onto something, a place to rest and conserve energy to reduce their metabolism during shipping. Types of holdfasts that have been used include twisted pieces of PVC rod and small plastic plants. (as per Syngnathid Husbandry in Public aquariums 2005 manual)

7.7.3 Water and Food

Water quality and feeding is rarely undertaken during collection and transport, a process that may take several days to a week depending on location. Therefore morality generally occurs either during the transport or within weeks of arrival at the destination as a delayed physiological stress response to collection handling and transport conditions. (as per Syngnathid Husbandry in Public aquariums 2005 manual)

7.7.4 Animals per Box

There must be one species per bag.
(as per IATA Live Animals Regulations)

It is better to have fewer individuals per bag with the use of more bags rather than large bags with a lot of individuals. It is often recommended that seahorses are packed in individual bags. The system is particularly relevant is a seahorse dies in transit.
(as per Syngnathid Husbandry in Public aquariums 2005 manual)

The number of seahorses per container should relate to:
1. Transit time
2. The size of the seahorse
3. The sensitivity of the seahorse
4. Temperature fluctuation (if any) that are expected
5. Changes in transference between transit vehicles
6. Type of transit vehicle
7. Water quality parameters at loading
8. Water quality parameters at destination (transit time can be substantially longer due to acclimation time at destination)
9. Whether individuals are captive-bred, caught directly from the wild, or have been recently transferred through various export/importers

7.7.5 Timing of Transportation

As per DRAFT ARAZPA Guidelines on Animal Transport April 2005:
Ensure that the route and timing of transport is selected in such a away as to:

- Minimise transport time
- Minimise the potential for unplanned delays
- Schedule travel during appropriate climatic conditions.

8 Health Requirements

8.1 Daily Health Checks

A distant examination (DE) of the animal should be done everyday, this can be carried out while cleaning and feeding.

A healthy seahorse should have:

- Strong colour
- Tiny glistening mucus deposits on the head and neck
- Should be of a good weight.

It's good to learn to judge a seahorses' weight by sight. The skin between the ridges of the seahorses' body should either bulge out or be level, if the skin sinks inwards it is a sign that the seahorse is seriously underweight. With the exception of females carrying no eggs then the front four or five segments of the belly sink in[2].

It is important that you note down and understand your seahorses' routine behaviour, deviations from this behaviour can be a sign of ill health.

Things to look out for in your daily routine:

- Constant swimming (this can be a sign of poor water quality or of aggression by a tankmate)
- Increased respiratory rate (this can be due to high water temperatures, the higher the water temperature the less oxygen in the water)
- Lethargy
- Unusual resting posture, like being unattached to an anchorage point
- Reluctance to feed

If your seahorse shows any of the above signs or symptoms, it is then important to identify the cause before treating the problem using medications. If the problem in environmental you can cause more harm than good treating the seahorse with medicines. Check the water quality, temperature, and aquarium inhabitants as soon as you note a potential problem[3].
Because seahorses eat large quantities of food it is important that the tank should be kept clean of excess food and faeces which should be removed. Poor water quality can lead to the ill health of your seahorse[2].

8.2 Detailed Physical Examination

When performing a physical exam, check the seahorses posture and buoyancy, a seahorse bobbing at the water's surface is a positive sign that the animals is abnormally buoyant. An abnormally buoyant animal will find it difficult to maneuver deeper into the water column this can be an indicator that the seahorse has an air entrapment problem such as air in the brood pouch or hyperinflated swim bladder.

Abnormal behaviour to look out for is if your seahorse is lying horizontally for long periods of time at the bottom of the tank, this is an indicator of generalized weakness or could be a sign of negative buoyancy which is associated with swim bladder disease or could be a result of a build up of fluids in the brood pouch or the coelomic cavity.

It is especially important to take note of the seahorses feeding response, seahorses will forage during the day. Consistently refusing live food that is of the appropriate size is an abnormal behaviour that will need immediate attention.

Signs of rapid breathing or irregular respiratory patterns or coughing can be a sign of gill disease. The entire body including fins should be closely examined for any ulcerations, excessive body mucus, lumps or bumps, and any lesions.[11]

Sedation and Anesthesia

MS-222 is a common and effective form of anesthesia for syngnathids, standard fish dose of 50-100ppm works quite well. In the case of a prolonged anesthetic recovery it is advisable to ventilate the animal with freshwater that doesn't contain any **MS-222.** Sea dragons often have a prolonged anesthetic recovery on a dose of 100ppm and it is recommended that dose of 50-75ppm is given for the sea dragon species.
In the situation where assisted ventilation is required the use of a 3.5 to 5.0 French red rubber catheter should be inserted through the snout to the level of the pharynx. A fresh water filled syringe is then attached to the end of the catheter and pumped in a pulsating manner every few seconds until the animal is breathing independently at a normal rate. This technique can also be used to resuscitate an animal in respiratory arrest. When performing any long term anesthetic procedures a flow-through system with oxygen supplementation in the sump or reservoir should be used.[11]

8.3 Routine Treatments

Preventative routine treatments such as worming and vaccinations aren't usually a common practice nor a practical one when it comes to caring for seahorses or any marine fish. Putting medications in the water should be avoided unless your fish are ill, treating seahorses for an illness it doesn't have can be just as bad as the seahorses having a disease.
Instead the following steps should be done routinely, and are better preventative measure than treating your seahorses with medications.

- Routinely cleaning the aquarium
- Cleaning substrate regularly
- Checking filters are working correctly
- Checking water quality, pH, and temperature[3]

8.4 Known Health Problems

The following listed diseases are the most common diseases found to affect seahorses, seahorses are also prone to all other diseases affecting marine fish and should be treated in the same way.

PARASITES

Coral reef disease

Cause –

- Parasite caused by the protozoan Amyloodium ocellatum.
- Highly contagious disease and if left untreated can be fatal.

Signs/symptoms –

- Parasite attaches itself to the fin, gills and body of the seahorse
- Appears as tiny white and yellow spots
- Rapid respiration which is usually carried out at the surface of the water

Treatment –

- Copper based medications; care must be taken when using copper based medications as it is toxic to marine invertebrates. The use of a copper test kit will ensure that copper levels stay at a safe level.
- The parasite will form a cyst during a portion of its life cycle and is immune to medication during this time, treatment should then be continued for at least two weeks to ensure that all parasites have emerged from the cyst and are in the free-swimming stage.

Prevention –

- Chemical treatment
- Flushing
- Filtration[3]

Saltwater ich (white spot disease)

Cause – Parasite caused by the protozoan Cryptocarya irritans. Found in sea water, closely resembles the freshwater parasite Ichthyophthirius.[12]

Signs/symptoms –

- Those affected by the disease will scrape themselves against solid objects in the aquarium
- Are generally very agitated
- Organisms are visible to the naked eye
- Appear as large white spots similar to those of coral reef disease[3]

Treatment – Long durations of copper baths over a considerable period of time. When the parasites can no longer be seen treatment should be continued for an additional five days.[12]

Prevention –

- Chemical treatment
- Flushing
- Filtration
- Avoid sudden changes in water temperature as Saltwater ich usually occurs after times of temperature stress[3]

Figure 8.1 *Cryptocarya irrita* on a seahorses tail

Gas bubble disease

Cause – Water supersaturated with nitrogen or oxygen. Can occur if the aquarium is densely planted or overgrown with algae or is exposed to intense sunlight.

Signs/symptoms –

- Air bubbles appear under the skin and act as a staging area for bacterial infection
- In the male seahorse a gaseous build up in the pouch can occur not allowing the animal to stay submerged and remain head down at the surface of the aquarium.
- External skin ulcerations (seen in extreme cases)

Treatment – A sterilized needle can be used to open the area, this should be performed while the seahorse is held below the water. The air then can be pushed out by massage, and after the area should be treated with a bacterial medication.
In the cases where the males pouch develops a gaseous build up, the interior surface of the pouch can be massaged with pipette expelling the gas (the animal should be kept under water at all times). The pouch should then be flushed out with a marine antifungal medication using a pipette. Treatment should be continued until the symptoms disappear.

Prevention –

- Prevent gas supersaturation by not over planting the aquarium and regularly cleaning to avoid build up of algae
- Avoid having the aquarium set up in direct sunlight.[2]

Figure 8.2 Seahorse with gas bubble disease

Figure 8.3 This diagram shows how to massage gas from the male's brood pouch

Copepods (sea lice)

Cause-

- Copepods are part of the subclass belonging to the subphylum Crustacea. The copepods found on seahorses are ecoparasitic copepods which are also known as "sea lice"
- Poor quality water, water that is already infested with the parasite

Figure 8.4 microscopic images of Copepods

Sign/symptoms-

- The parasite is visible to the naked eye, and can attach itself to anywhere on the seahorses body
- It is easy to spot the female parasite with their long string of eggs that resemble a forked tail.

Treatment-

- Individuals infested with the parasite should be isolated and quarantined as soon as signs or symptoms arise
- Copper based treatments, making sure to use a copper test when using such medications to ensure that copper stays at a safe level.
- Eggs are immune to the medications therefore treatment needs to be for an extended period to ensure that eggs are hatched and the cycle is broken, this can take up to 4-5 weeks

Prevention-

- Any new animals, plants or living organisms introduced to the aquarium are quarantined to ensure they are free from parasites
- Good water quality
- Regular cleaning and water changes
- Good filtration[3]

Internal parasites

Cause-

- Trematodes (Flukes)**,** Cestodes (Tapeworms), Nematodes (Roundworms)
- Attach to the liver or digestive tract and feed off the incoming nutrients, preventing the host from obtaining the nutrients from the food being consumed

Signs/symptoms-

- Weight loss
- Refusal to eat
- Tissue damage
- Lethargy
- Hemorrhaging and eventually death

Treatment-

- Treatment should be given orally in order to be effective
- Metronidazole, safe to use and is difficult to overdose on the medication
- Nicolsamide, effective medication for eliminating nematodes and cestodes however is difficult to find and may need to be obtained from your vet
- Praziquantel, also a very effective medication used against infestation of trematodes, but is also hard to find and may need to be obtained from you vet.

Prevention-

- Isolate any infested animals from the aquarium
- Proper quarantine of new animals to the aquarium should help control the introduction of parasites
- Obtaining food from a reliable source, farming live food as appose to catching it from the wild.[13]

FUNGI AND BACTERIA

Fungal infestations

Cause-

- Decaying organic matter, remains of uneaten food dead eggs encourage the growth of fungi
- Fungal infections are often secondary infections the primary infection often being bacterial[12]

Signs/symptoms-

- Filmy coating on the seahorses body
- Dark areas on the body
- Will affect the skin where there is open wounds or injuries[3]

Treatment-

- There are a variety of fungicides available on the market
- Malachite green
- Methylene blue
- Because fungal infections are usually the secondary infection it is important to identify the primary cause and eliminate it, otherwise treatments such as malachite are unlikely to be successful.[12]

Prevention-

- Remove any uneaten and dead food
- Remove any dead organisms in the aquarium
- Regularly check water quality
- Tank and water should be routinely changed and cleaned
- Avoid overcrowding the tank[3]

“Flesh eating bacteria”

Cause-

- Cause is bacterial infection caused by *Cryptobia sp.* and *Costia sp.* both can be fatal if not caught early

Sign/symptoms-

- Attack the skin eroding the flesh
- Appear as open sores
- Cloudiness of the skin
- Sloughing of the skin
- Cloudy eyes
- Localised swelling
- Lesions, and as the disease worsens the lesions become bloody

Treatment-

- Topical solutions, which great care must be taken when being used avoiding getting the mixture in the fish’s mouth, gills or eyes.
- Sodium Chloride is a topical treatment which can be used in high doses and is safer if ingested, but is not recommended to use if lesions are bloody

Prevention-

- Proper and good husbandry practices
- Quarantine any new animals being introduced into the aquarium
- Monitoring your animals closely and taking note of any signs of ill health and acting as soon as any symptoms of disease are noticed.[13]

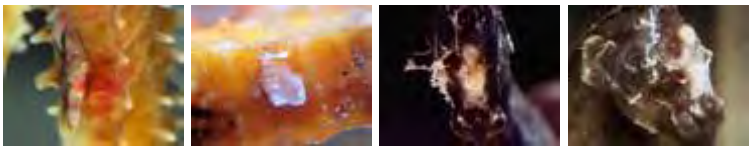

Figure 8.5 Images of seahorses affected with flesh eating bacteria disease

Bacterial infestations (fin rot)

Cause-

- Stress will compromise a seahorses immune system therefore leaving them more susceptible to a bacterial infestation
- Poor water quality
- Another tankmate
- Water temperature

Signs/symptoms-

- Ulcerations of the skin
- Fraying or loss of fin tissue

Treatment-

- Remove stressor
- Can treat with various antibiotics for marine fish
- Check water quality, pH and temperature

Prevention-

- Avoid any stress to the animal by not overcrowding tank
- Regularly check water quality, pH and temperature
- Correct filtration regular cleaning and water changes[3]

DISEASES RELATED TO WATER QUALITY

Heavy metals

Cause-

- Large amounts on copper present in the water is often introduced by tap water
- Heavy metals such as aluminum and lead can be present in rocks or aquarium ornaments
- Plastic plants that contain bendable metal parts

Signs/symptoms-

- Increased respiration
- Lethargy

Treatment-

- Copper test kits, test copper levels in water especially if water is coming from old copper pipes or if treating ill fish with copper-based medications

Prevention-

- Run water for 10-15min if water is coming from a copper pipe system
- Use only farmed lives rocks, as rocks that are collected from the ocean can leach metals
- Make sure that when purchasing plastic plants for your aquarium, that it is suitable for marine aquariums[3]

Ammonia stress

Cause-

- Overfeeding
- Dead tank mate
- Poor filtration system
- Uneaten food or dead organisms in the aquarium
- Overstocked aquarium

Signs/symptoms-

- Rapid breathing
- Become listless
- Occasionally sink to the bottom
- Have problems anchoring themselves to a holdfast
- Can lead to secondary bacteria and fungal infections

Treatment-

- Perform an ammonia test at the first sign of a problem
- Water change

Prevention-

- Regular water changes
- Avoid overstocking aquarium
- High ammonia levels may mean it is overdue for substrate cleaning or a change
- Do not over feed, dead food will increase ammonia levels in the water
- Remove any dead organism as soon as they are discovered.[3]

Exophthalmos / Pop –Eye

Cause-
Can be caused by many factors such as

- Gas supersaturation in the water
- Internal fungal infection (*Ichthyosporidium hoferi)*
- Parasitic infection (usually trematodes)
- Or bacterial infection (*Mycobacterium marinum)*

Signs/symptoms-

- Eyeball protrudes from the socket, which can become damaging to the organ or the tissue surrounding it.

Treatment-
Treatment should be done in the following order.

1. **Treat for gas supersaturation,** eliminate the cause and remove the fish from aquarium into water that is not supersaturated.
2. **Treat for a parasitic infection,** check to see if the eyes are cloudy is this is the case use medications for external parasites, if eyes are not cloudy treat seahorses for internal parasites.
3. **Treat for bacterial infection,** *Mycobacterium marinum* or also known as "fish tuberculosis" is very difficult to treat, treatment for this type of infection is rarely successful and the medications used are very harsh.
4. **Treat for fungal infection,** antifungal treatments are generally very harsh and can cause other complications such as loss of appetite.

Prevention-

- Avoid gas supersaturation by not over planting aquarium or overcrowding
- Regularly clean water and check water quality and temperature
- Remove any dead or uneaten food
- Remove sick animals straight away and isolate them in a separate quarantine tank[13]

Figure 8.6 A seahorse infected with Exophthalmos

DISEASE RELATED TO DIET

Nutritional Deficiencies

Cause-

- A common problem in marine fish species that is brought about due to lack of knowledge about the nutritional requirements in the diet.
- Poor diet and quality of food
- Nutritional deficient diet

Signs/symptoms-

- Skin between the ridges of the body are sunken in
- Animal is not feeding
- Colour changes, becomes pale

Treatments-

- In terms of nutritional deficiencies prevention is always better than cute
- Humanly euthanizing the animal if extremely malnourished

Prevention-

- Research the animals wild diet
- Seahorses hunt by sight and are often stimulated into feeding by the movement of their prey, therefore feeding live food is often the best way to get seahorses to feed.
- Avoid feeding brine shrimp as the staple of the seahorse's diet. Brine shrimp is nutritional deficient
- Training seahorses from a young age to eat frozen food, a combination of frozen food and live food will allow for a nutritionally balanced diet
- If feeding frozen food place the food in the current of the filter so it appears to the seahorse that the food is living.
- Trial and error, individual seahorses will have different preferences of food and it is a matter of trial and error to find a diet that suits your seahorse.[3]

8.5 Quarantine Requirements

When adding new animals to the aquarium, they should first be housed in a quarantine tank for the first month or so. This allows you to closely monitor the animal for any signs of illness.
A quarantine tank should include the following:

- Separate tank (Quarantine tank, for sick or new animals)
- Separate filter system, this prevents any disease causing organisms spreading to other tanks thru the filtration system and infecting other animals
- Clean substrate
- Correct water conditions, temperature, oxygen levels and pH
- Furniture, use fake or plastic furniture and plants as holdfasts as an alternative to live plants, to control the introduction of bacteria or parasites. Plastic furniture can also be re used after being sterilised after use.[3]

9 Behaviour

9.1Activity

White's seahorses like most seahorses are active during the day time. However there are some species of seahorse like the Tiger Seahorse *H.comes* and the Pacific Seahorse *H.ingens* that are to believed to be nocturnal.[3]

9.2Social Behaviour

Seahorses are monogamous and are often found in mating pairs instead of large groups. Individuals will have their own territory, the female has a territory size of about 1.4 square metres and the male has a territory size of around 0.5 square metres. When housing pairs or groups of seahorses it is important that the aquarium is of adequate size to allow them to get away from each other and have places to hide. Seahorses are very shy animals and overcrowding of the tank will stress the animal.
Mating pairs will perform a daily greeting ritual which reinforces their bond, after this courtship display is finished the pairs will go back to their own territory and feed separately.[1]

9.3Reproductive Behaviour

Partners reinforce their bond everyday with a courtship dance. This display can last from anywhere from 30 minutes to an hour sometimes even more. The courtship dance often takes place every morning, the dance centres around an object and the male will circle the female with a stiffened body posture and with his chin on his chest. The male seahorse changes to a lighter colour, and the female changes her colour to match his in response and she too circles him and copies his body posture.[1]
Time of year: breeding and courtship displays occur between the months of October through to April

9.4Water conditions

Temperature: 22°C - 24°C,
pH 8.2
Salinity sg 1024
Ammonia-Nil
Nitrate-Nil
Nitrates-<20ppm[14]

9.5Behavioural Problems

Stereotypic behaviour: None such behaviour has been observed in this animal. Little is known about the wild behaviour which makes it difficult to identify signs stereotypic behaviour in captivity.

Aggression: According to the study *Faithful pair bonds in wild seahorses, Hippocampus Whitei, by Amanda C.J. Vincent & Laila M. Sadler* Male aggression was only seen on once occasion in 1993. When a neighbour tried to greet the female of a pair that had just finished greeting. The partner of the Female snapped at the intruding male with his snout. Seahorses are classified as innocuous and pose little threat to keepers or the public. Displays of aggression has rarely been observed in Seahorses, however little is known about aggressive or territorial behaviour of seahorses.[18]

Behaviour created by public feeding animals:
The aquarium should be set up so that only keepers can access the animals for feeding or cleaning, this is to prevent any member of the public attempting to feed the animal something that it shouldn't be eating. Food stuffs can throw off the water conditions and cause stress or the spread of disease.

9.6 Behavioural Enrichment

Tank Furniture: Plenty of vegetation, sea grasses and coral fans/skeletons offer anchorage points for the Animal, this is important as in the Wild seahorses anchor themselves to an object to hide or camouflage themselves from prey, and to prevent them from being swept up in currents. Seahorses are slow moving and are opportunistic feeders and will stay attached to a holdfast and will just eat whatever is swimming past. Plenty of holdfast allows for your seahorse to hide and feel safe and secure, thus minimising stress.[3]

Live Food: Seahorses have independent moving eyes like a chameleon and hunt by sight and are often stimulated into feeding by movement, so the use of live food is imperative, not only from the nutritional aspect but also for the behaviour enrichment of your seahorses. If feeding frozen food or dead food it should be done by placing it in the flow of the filter and allowing it to be powered around the tank; this should stimulate the seahorse into following it thinking that it is alive.[3]

Pair Bonds: Seahorses are monogamous and will form a pair bonds for life. Try to house seahorses in mating pairs, in the wild it is not very often you find single seahorses unless the partner has passed and usually a seahorse will try find another mate. Housing in pairs is a one of the greatest forms of enrichment for these animals as it means they can display their daily courtship ritual which is an important part of reinforcing their pair bonds.[3]

9.7 Introductions and Removals

Quarantine requirements: All new animals should be quarantined, especially for saltwater fish where often specimens are caught from the wild where the animals are not use to being kept in captivity. The animals can become stressed and come down with a disease that you want to prevent being spread to your already existing collection. For quarantine tank requirements and setup refer to the Known Health Problems section of this Husbandry manual. [3]

Aggression problems associated with introductions: Seahorses aren't known to display aggressive behaviour to other animals, however the introduction of new animals to the aquarium should be done slowly in order to prevent any stress to the animals.
Ensure you acclimatise you new tank mate to the water, as a quick change in water temperature is stressful to the animal.

9.8Intraspecific Compatibility

Aggressive behaviour between males: Housing two males together shouldn't be too much of a problem, however you try to house seahorses in pairs; one male and one female. As long as there is sufficient space in the aquarium for the seahorses to get away from each other there shouldn't be a problem with aggressive behaviour towards each other.

9.8.1 Interspecific Compatibility

Spread of disease: Seahorses can catch any disease or parasite that any other marine fish can contract. Introducing any new specimens to the aquarium either it be another seahorse or other marine animal, you should also under go the proper quarantine measure to prevent the spread or outbreak of diseases or parasites to your already existing collection.

Territory: Although seahorses maintain their own territory, territorial behaviour hasn't really been observed in seahorses. However aggressive behaviour from another tank mate can occur, removal of such stressors will ensure that you maintain a safe and secure home for your seahorses and increase its longevity in captivity.

Cannibalism: Seahorses aren't known for eating the offspring or eggs of other species. However once the male gives birth, the fry should be removed and reared in a separate tank, this is because seahorses hunt by sight and their fry can be mistaken for a food item.

9.8.2Suitability to Captivity

Little is known about seahorses and because of this its husbandry needs are not always understood. One of the most difficult aspects of seahorse husbandry is seahorse nutrition. Getting your seahorses to eat in captivity and having enough good quality nutritional food is difficult as seahorse can consume 4,000 brine shrimp a day.

Changes in water temperature, and quality can cause an outbreak of disease and even death of your seahorse. Good water quality needs to be maintained, along with a good nutritionally balanced diet. These factors are what make it difficult in keeping seahorses in captivity.[3]

10 Breeding

10.1 Mating System

Seahorses have one of the most unique and unusual breeding behaviours of any animal. It is the male that gets pregnant, seahorses are the only creature where the male has a true reversed pregnancy. Seahorses are monogamous and will pair for life, however they have separate territories and only come together to perform their courtship dance.[2]

Figure 10.1 ***Hippocampus whitei*** Displaying their courtship dance

Courtship dance

Once a seahorse finds a partner they reinforce their bond everyday with a courtship dance. This display can last from anywhere from 30 minutes to an hour sometimes even more. The courtship dance often takes place every morning, the dance centres around an object and the male will circle the female with a stiffened body posture and with his chin on his chest. The male seahorse changes to a lighter colour, and the female changes her colour to match his in response and she too circles him and copies his body posture.[1]

Mating

The courtship dance takes on a different intensity when the pair is ready to beginning mating. The male takes the lead in the courtship display and the pair mimic each other and will repeatedly rise together in the water column[1]. The male contracts his pouch in order to send hormonal signals out into the water this also allows to flush out the pouch ensuring it is clean and ready to receive the females hydrated eggs.[2]

This courtship display will last for about half an hour, when the male and female will face each other at the top of their rise in the water then the female will then put her ovipositor into the male's pouch. She can deposit between 90-250 eggs at a time which only takes a matter of seconds.[1] Timing is important when is come to seahorse reproduction. This is because the female must add water to her eggs shortly before depositing it into the males pouch, and she can only hold these hydrated eggs for 24 hours. If the male doesn't accept them in this time frame then the eggs are released into the water and fail to hatch.[3]

Figure 10.2 A female depositing her eggs into the male's pouch

After depositing her eggs the female doesn't have much more to do with the pregnancy and the pair will only have contact in the mornings for their reinforcement courtship display[2]

10.2 Ease of Breeding

Once your seahorses have paired, and they're in good health and the water conditions are right then most seahorses will then breed.
However getting a compatible breeding pair to mate isn't the only challenge in breeding seahorses, the challenge is in feeding all the seahorse fry. White's seahorses can give birth to about 10-250 fry which are a lot of mouths to feed.[1]

Triggers for breeding

- Space - In the wild the female has a territory of around 1.5 sq m. and you will need to provide sufficient space in order to encourage breeding.
- Temperature and water pH changes are often triggers for various species to reproduce.
- Changes in temperature and day length are triggers for Temperate-zone seahorses
- Tropical species are triggered into breeding by significant changes in pH and salinity levels.
- A breeding pair - seahorses are monogamous and you will need to have an established breeding pair before breeding can occur.
- Both animals will need to be in fairly good condition, and be both fed a diet rich in nutrients

Manipulating water temperature and day lengths can be done with the use of thermostats and light timers. Gradual water changes are the best way to achieve changes in salinity and pH levels.[3]

10.3 Techniques Used to Control Breeding

Wild populations of seahorses are under threat as seahorses are quite often captured and used for traditional Chinese medicines and for the souvenir trade.[2] Breeding seahorses in captivity will mean that the pressure on the wild population will diminish.
Breeding seahorses is one of the most challenging parts of keeping them (next to feeding) this is because seahorses can give birth from anywhere from 10 to 1,500 fry.[2] Housing and feeding this many fish is difficult and many institution may not be able to facilitate such large numbers of fry.

Culling

It is pointless and difficult to try and keep all the fry, due to the difficulties in feeding. Instead select 20 of the strongest looking fry and concentrating on caring for them and the remaining fry can be left in the tank with the adults for nature to take its course.[1]

10.4 Occurrence of Hybrids

Identifying a seahorse by species can often be difficult and it is not uncommon for aquarists to unknowingly pair up two seahorses of different species, while we may not be able to tell the difference the seahorses definitely can. Crossbreeding from such a mismatched pair like this usually does not occur.[15]

Physical and biological barriers usually prevent the hybridisation of wild populations of seahorses and syngnathid species are generally reproductively isolated from each other. Hybridisation of seahorses should be avoided as such matings may compromise the captive breeding programs in place and poses a risk to the wild populations if they are released into the wild.[17]

10.5 Timing of Breeding

Breeding season begins when the water temperature becomes warmer, most mating occurs in the days just before a full moon. Mating occurs between the months of October-January.[14]

Figure 10.3 A pregnant male *Hippocampus abdominalis*

10.6 Age at First Breeding and Last Breeding

Is sexually active from 6 months old.[14]

10.7 Ability to Breed Every Year

Paired seahorses, that are in reasonable good health will breed every year and can have up to 3 separate births in the season.[14]

10.8 Ability to Breed More than Once Per Year

Can have up to 3 births a year.[14]

10.9 Nesting, Hollow or Other Requirements

Rearing Tank

- Once the male becomes pregnant he should be moved into a bare rearing tank, with the proper water condition and correct temperature already set up and ready.[15]
- Avoid moving the male when he is close to giving birth as they can tend to sometimes abort the fetuses if they become stressed, these premature young may look normal but rarely survive.[15]
- Once the fry are born they should be moved into a tank with no substrate or furnishing, this makes cleaning a lot easier and allows the young fish to find their prey more easily
- Transport the fry into the rearing tank with the water they were born in, so the change isn't too stressful for the animal
- Be sure the filter isn't too strong or they will get sucked into it, good circulation is also need so the use of several air stones should be spread out in the tank.
- For the first week or so you will not need any tank furnishings, after this time you should place in some artificial plants (these are easier to clean)[2]

10.10 Breeding Diet

Seahorse nutrition is extremely important and complex. A diet rich in nutrients will equal a healthy seahorse and healthy offspring. Seahorses are exacting in their food and are opportunistic animals, catching any prey swimming past that is small enough for them to eat.[2] The most common form of food fed to captive seahorses is Brine shrimp, they're easy to breed and readily available but unfortunately a poor quality source of food. When breeding seahorses avoid feeding Brine shrimp as the staple of their diet, instead mysis shrimp should form the basis of a captive seahorse's diet they are the most nutritionally complete food items and are commercially available and quite easy to breed.[3]

Fry Foods

Feeding seahorse fry are one of the most difficult parts of breeding seahorses. The young require large amounts of food, up to 3,000 food items a day. Finding food small enough for them to eat is also a challenge. In the wild seahorse fry will spend the first two weeks in the plankton layer of the ocean. Wild-collected zooplankton is the best food to feed to newborn fry however collection is time consuming.[2] Enriched baby brine shrimp (naupulii) is often a common food type to feed newborn fry, however a diet made up of strictly brine shrimp will result in nearly 100 percent mortality before they reach the age of 8 weeks.[15]

10.11 Gestation period

One the eggs are fertilised they are embedded in the pouch wall, the pouch acts like a womb it is complete with placental fluids that provides the nutrients and oxygen to the developing embryos[17]

Gestation varies depending on the size of the species, smaller species have a gestation period of about 14 days, whereas larger species such as the Big Bellied Seahorse can have a gestation period of up to 4 weeks.[1]

The gestation period for White's seahorses' is approximately 21-25days (3 weeks) the gestation period is shorter in warmer temperatures and longer at cooler temperatures.[14]

Birth

The birth itself can be a long process, the contractions can last up to 12 hours, and the male pumps and pushes to empty his pouch. The birth usually happens at night. Nearing the time the eggs are ready to hatch the fluid inside the pouch becomes like seawater, this is so the developing seahorses become acclimatised to the world they are about to enter.[16] Soon after giving birth the male can usually be seen lying exhausted on the bottom of the tank, and despite the stressful and exhausting ordeal of giving birth the male will become pregnant again within a couple of days.[1]

Figure 10.4 Male *Hippocampus whitei* giving birth

10.12 Brood size

The brood size is dependant on the age and size of the parents, the brood size can be between 10-250 individuals, the average brood size for adults is 100.[14]
When born seahorse fry are miniature replicas of an adult seahorse,

10.13 Age at Weaning

Seahorses do no cannibalize but the young should be removed from their parents and reared in a separate tank so that feeding and cleaning is a lot easier to perform.
In the wild less than one in a thousand will survive to maturity this is usually due to predation, seahorse fry will usually fall prey to other fish.[2]
After giving birth the male is able to fall pregnant again within a couple of days.[1]

10.14 Age of Removal from Parents

Newborn fry are precocial (independent) once they are born. The Male seahorse has little to do with the young once he has given birth to them. It is best to separate the newborn fry from the adult so that feeding and water quality is more easily controlled.
The fry should be moved into a bare tank, they should be transported in the water they were born in so that the change in water is less stressful to the animal.
In the wild seahorse fry often fall prey to other fish, having the fry in a separate tank will prevent them being eaten by other tank mates.[1]

Figure 10.5 Image of Seahorse fry

10.15 Growth and Development

Newborn fry are miniature replicas of adult seahorses. They are only a few millimeters in size at birth but already look like an adult seahorse. In the first dew weeks of their life growth is quite slow. In larger species of seahorse (*H. reidi*) they reach the size of about 2.5 inches long in four months, after 5 months their size is approximately 3 inches and after 8 months 5inches in size, the adult size of *H. reidi* is about 6 to 8 inches. http://www.seahorses.de/seahorses.htm

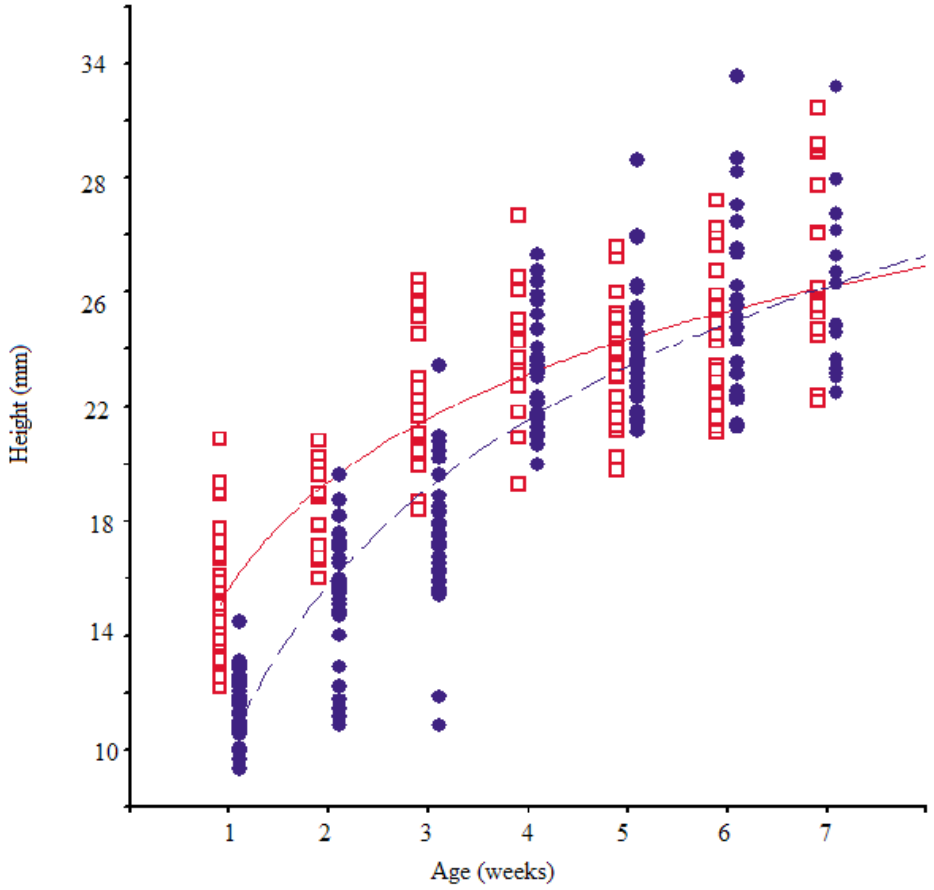

Figure 10.6 Example of growth curves between weeks 1 and 7for the offspring from and older couple (red squares) and a young couple (blue circles)

11 Artificial Rearing

11.1 Incubator Type

Once the male becomes pregnant he should be moved into a bare tank, with the correct water conditions and temperature already set up and ready. Try to avoid moving the male when he is close to giving birth, stress caused to the animal can cause him to abort the fetuses. When the fry are born they should be moved into a tank with no substrate this makes it easier to clean and for the dry to find there food more easily.

11.2 Incubation Temperature and Humidity

You should maintain your tank at a constant temperature between 22oC-24oC sudden changes in temperature will stress the animals and can eventually lead to their demise. The gestation period for White's seahorses' is approximately 21-25days (3 weeks) however depending on the water temperature the gestation period will be shorter in warmer temperatures and longer at cooler temperatures.[14]

11.3 Desired % Egg Mass Loss

N/A Seahorse give birth to live young

11.4 Hatching Temperature and Humidity

N/A Should be maintained at the suggested water temperature

11.5 Normal Pip to Hatch Interval

N/A

11.6 Diet and Feeding Routine

Diet

- Amphipods
- Brine shrimp are an important component for the diet of seahorse fry
- *Artemia* nauplii
- Mysids

Temperature of food

If possible food should be fed out live. If using frozen food, defrost in cold water before tipping the bucket of food and water together into the tank.

Methods used to feed

Pour the bucket of cold water and defrosted food into the tank, uneaten food should be siphoned out. Same method applies to live food

Amount of food

Considering that seahorses can give birth up to 250 individual fry its a good idea just to pick 10 of the strongest and healthiest looking fry to keep. Baby seahorses can eat up to 3,000 individual food items per seahorse per day.

Frequency of feeds

Like most fish, seahorse fry are born with a small yolk sac. This will provide nutrition for about the first 6-12 hours of life. Food can be offered within the first few hours but is no necessary, It can take up to several days for them to start eating at the full rate.
Food should be added to the tank at least three times a day, you'll need to add enough food for the seahorses to eat for about 15-20 minutes. Only feed during daylight hours and the lights should be switched off at night.[14]

11.7 Specific Requirements

Fry should be housed in a bare tank with no substrate for easy cleaning and so the fry can catch their food easily.
Its also a good idea to cover the overflow with a mesh grill so that the seahorses don't get sucked into the overflow
A plastic netting can be used as a holdfast instead of using live plants this is so its easier to see the animals

Figure 11.1 Image of *H.whitei* fry at 3-4months of age in a nursery tank set-up

11.8 Data Recording

Good idea to keep a diary or a record sheet so you can note down when you fed out how much and take notes on any observations made. Recording when siphoning was done (Refer to **Appendix 2** for an example of a daily diary sheet)

11.9 Identification Methods

Forms of identification such as VIFE tagging is impractical in a captive situation and the fry are far to small to tag, tagging seahorses are often used more for research purposes of Seahorse wild populations. Once seahorses get older they're easier to identify as they often develop individual markings and colourings.

11.10 Hygiene

Routine siphoning of the tank, each time this is done it should be recorded on the daily diary (Refer to **Appendix 2)**, try to minimise the amount of disturbance made siphoning the tank as it can cause stress to the animals. Siphon 2-3 times a week or when needed. Uneaten food should be removed. Water quality should be tested on a weekly basis. Refer to section 7.5 Release, for specifics on nitrate and pH levels suitable for seahorses

11.11 Behavioural Considerations

Not much is known about human imprinting affecting seahorses. However you need to provide an environment that is like that of there wild environment in order to allow them to perform there natural behaviours.

11.12Weaning

N/A seahorses are born **precocial**

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Figure 8.1,2,4,5,6 -
http://www.syngnathid.org/articles/diseaseGuide.html

Figure 8.3 -
http://www.saseahorse.com/stable.htm#Raising%20Fry

Figure 10.1 -
http://images.jupiterimages.com/common/detail/32/40/22564032.jpg

Figure 10.2 -
http://www.spiracanada.com/spiralbound/2004/beltaine/seahorse.jpg

Figure 8.3 -
http://www.saseahorse.com/stable.htm#Raising%20Fry

Figure 10.3 -
http://www.divegallery.com/seahorse_15.jpg

Figure 10.4 -
http://www.amonline.net.au/FISHES/fishfacts/fish/hwhitei3.htm

Figure 10.5 -
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14 Glossary

Caudal fins ~ The tail fin on a fish

Ventral fins ~ Paired fins behind or below the pectoral fins

Pectoral fins ~ The anterior or uppermost of the paired fins, which correspond to the anterior limbs of the higher vertebrates

Endemic ~ Belonging to once place

Ectothermic ~ Cold blooded

Epidermis ~ The outer layer of the skin

Holdfasts ~ An anchorage point eg: Seaweed

Precocial ~ Able to move about freely when hatched

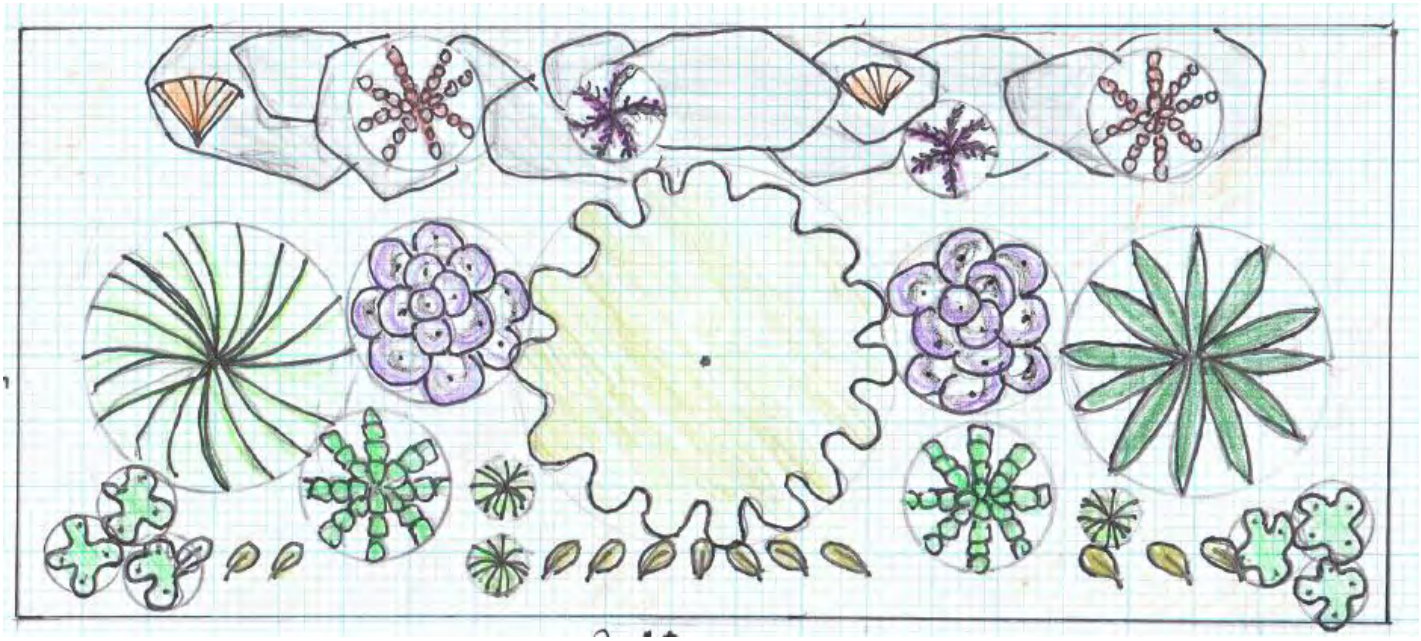
Appendix 1

Enclosure design

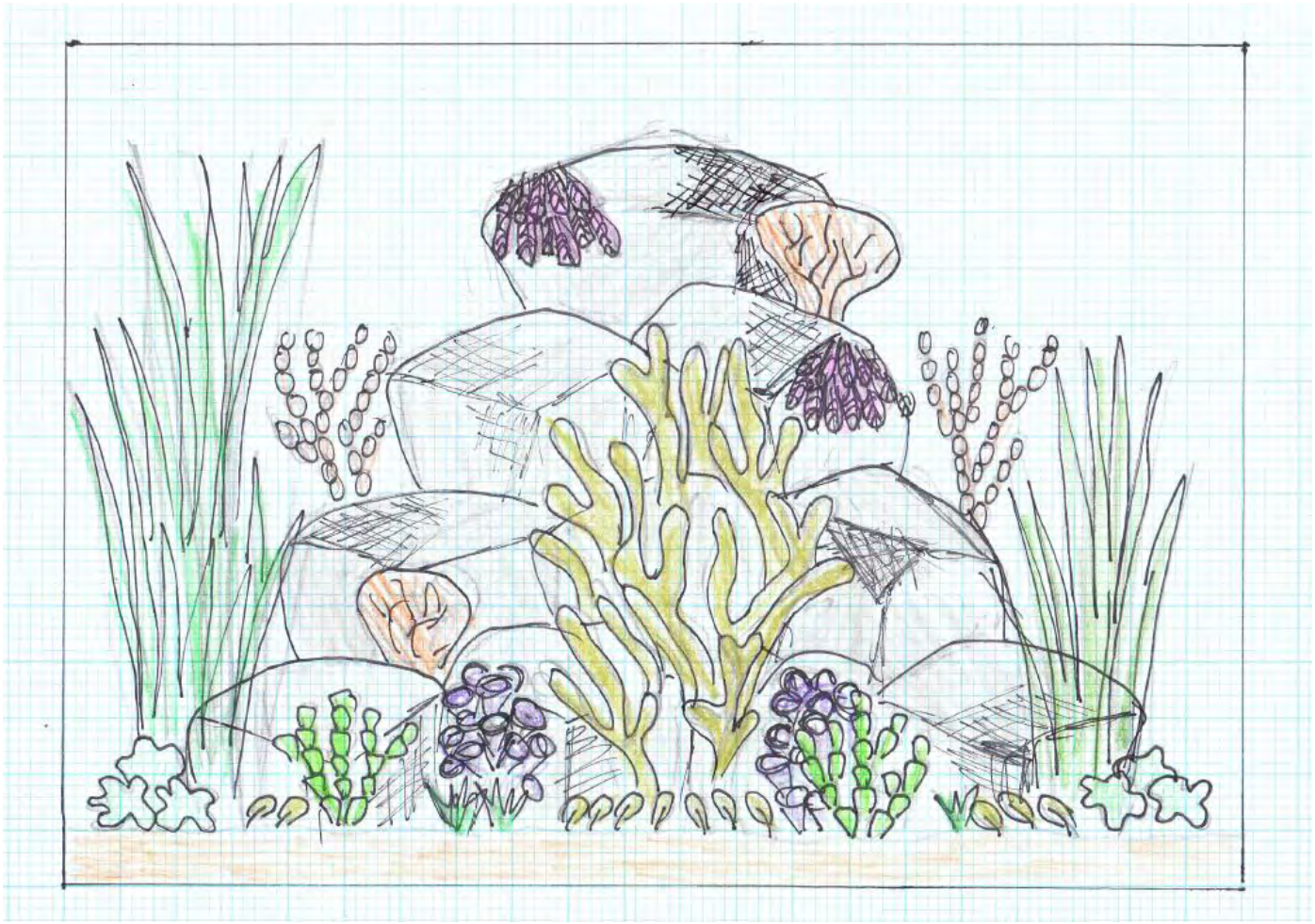
Photo view from all positions around the exhibit

Exhibit name:	White's Seahorse tank
Address, Suburb State, Postcode:	West Esplanade Manly NSW 2095
Phone number:	02 8251 7877

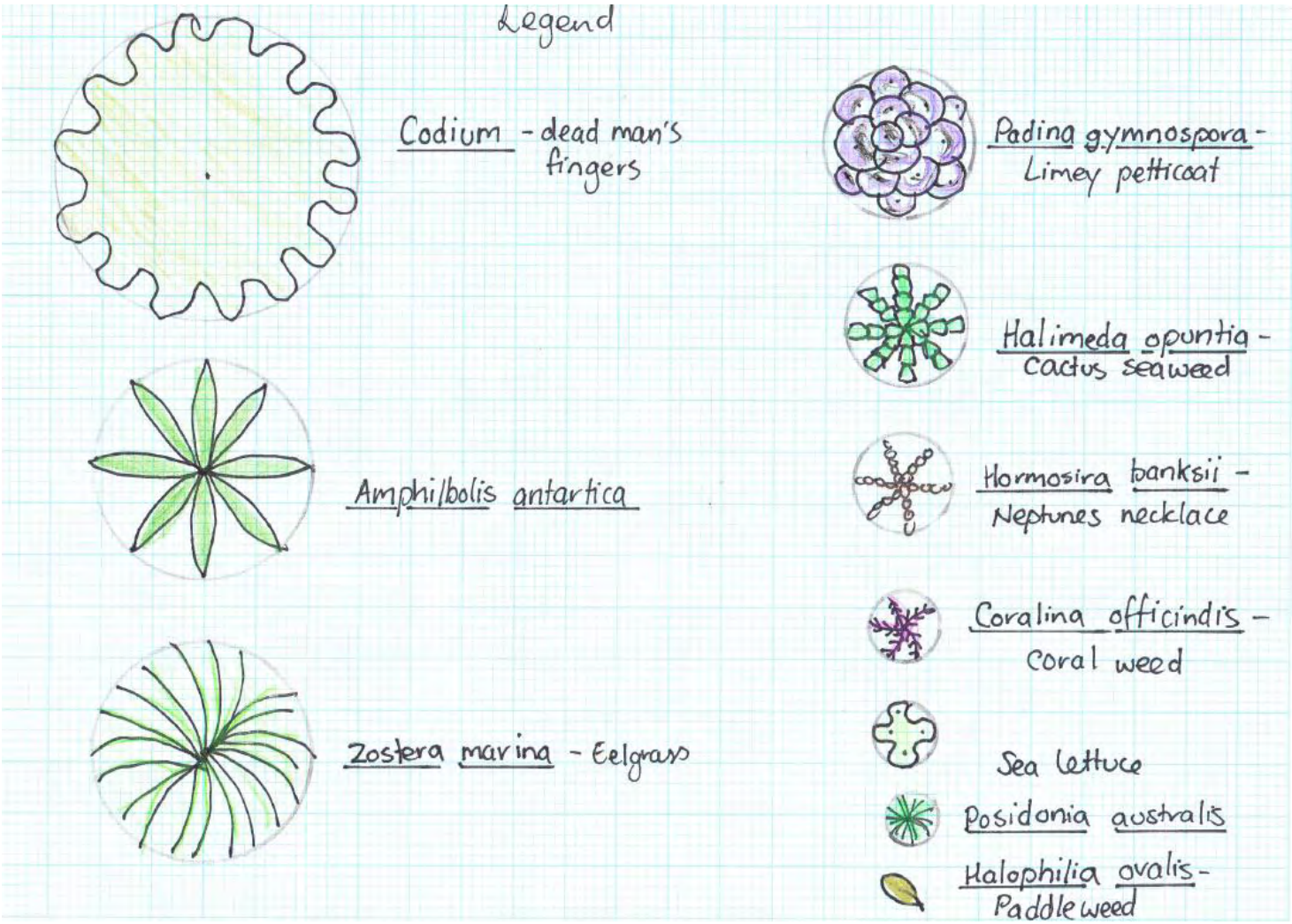
Species to be housed	Sex	Age	Comments
Hippocampus whitei	*2:2:0*	*Unknown*	



Top



Front



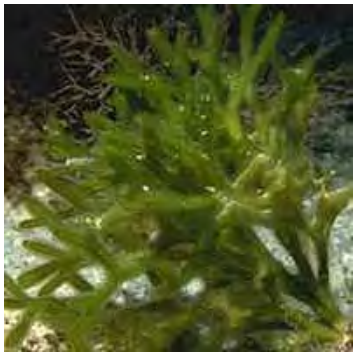
Plant species key

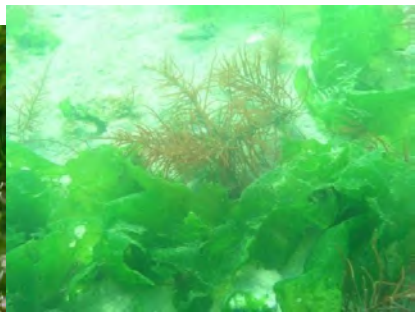
Plant species list

Limey Petticoat ~ *Padina gymnospora*


Neptunes Necklace ~ *Hormosira banksii*


Coral weed ~ *Coralina officindis*


Dead man's fingers ~ *Codium*



Sea lettuce ~ *Ulva lactuca*


Eelgrass ~ *Zostera*


Paddleweed – *Halophilia ovalis*


Amphilbolis


Posidonia australis


Cactus seaweed ~ *Halimeda opuntia*

Appendix 2

Daily diary sheet

Tank number: 3	Species: Seahorses	
Date	Notes	Initials
1	Mysids, Siphon, scrub, change weed	KP
2		
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Appendix 3

Selected Techniques for
Tagging Seahorses

Project Seahorse
ADVANCING MARINE CONSERVATION

TECHNICAL REPORT SERIES

No. 6

SELECTED TECHNIQUES FOR TAGGING SEAHORSES

Sian Morgan and Keith Martin-Smith

Version 1.0. March 2004

Suggested reference
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Table of Contents

PROJECT SEAHORSE TECHNICAL REPORT NO. 6: SELECTED TECHNIQUES FOR TAGGING SEAHORSES

1. Introduction

Techniques for identifying animals are important tools for research, husbandry and management. Tagging techniques originally developed for mammals and birds have been successfully adapted for use in fish and the evolution of techniques has accelerated in recent years in response to greater information needs over a range of species and life history stages. There now exist a wealth of marks and tags that have been developed for fish (see McFarlane *et al*. 1990 for review): here we focus on two selected techniques that have been used successfully on seahorses (*Hippocampus* spp.). This technical report is intended: a) to provide step-wise guidelines for these established seahorse tagging/marking methods and b) to discuss the relative advantages and disadvantages of each.

At present, only one study exists in the primary scientific literature that specifically examines marking and tagging methods in seahorses (Woods & Martin-Smith 2004); other papers mention tagging and marking methods incidentally (Vincent & Sadler 1995, Bell *et al*. 2003, Moreau & Vincent in press). Non peer-reviewed work has also explicitly and incidentally examined the suitability of tagging systems. Work by Le Cheminant (2000) investigated the relative efficacy of three marking systems in the Knysna seahorse, *H. capensis*, while Van Dijken (2001) monitored 43 tagged big-bellied seahorses (*H. abdominalis*) in the wild in New Zealand to understand their general ecology. However, many more techniques have been tried than are presently reported in the literature.

Given the extensive range of potential tagging and marking techniques available, Project Seahorse has produced two Tagging and Marking Technical Reports. The present document (Project Seahorse Technical Report No. 6) only examines necklace tags and visible implant elastomer tagging: methods that have been used extensively and for which the most information exists. A longer document (Project Seahorse Technical Report No. 7) discusses general marking and tagging methods and their success or potential use in seahorses. For researchers interested in *ex situ* tank studies or *in situ* focal studies at the individual-level, we recommend reading this document and consulting Technical Report No. 7 for further information. For scientists working with other syngnathids, or conducting population level studies, we recommend perusal of Technical Report No. 7 which will give a sound overview of seahorse tagging and may stimulate the development of new methods or the improvement of old ones. Bear in mind that techniques are modified regularly, technology is improving rapidly, and hybrid methods are often the answer. Project Seahorse would appreciate receiving any information that may be unreported in these documents (please contact: info@projectseahorse.org). Both versions can be downloaded as PDF files from our website at: www.projectseahorse.org.

1.1. Definitions of Marking and Tagging

Both for technical correctness and clarity, it is necessary to understand that tagging and marking are not synonymous terms. A mark is the most general term for any means of identifying an organism: marks do not carry information and simply act to distinguish unmarked animals from marked animals. In contrast, a tag is a specific form of a mark that physically carries information on either an individual or a batch. It is important to recognize that the two terms are not mutually exclusive in that a mark can be placed such that, although it does not actually carry information, its location may confer some meaning. In this document, the verbs tagging and marking are used as they refer to tags and marks in the technical sense. The verb "to label" is used as a general term to encompass marking and tagging where we do not wish to infer the technical connotation of either word.

2. Collar Tags

Collar tags are a form of attachment tag, physically affixed to fish for means of identification (McFarlane *et al*. 1990). Collar, or fingerling tags, were the first method used to identify seahorses (Vincent & Sadler 1995) and are small oval PVC discs with a three-digit number on one side (3 mm x 5.5 mm, manufactured by Floy Tags – see Appendix 4). Tags are attached around the neck of a seahorse with cord, to provide individual identification. This method and has been used with success in many focal studies: *H. capensis, ex situ* (Tops 1999) and *in situ* (Bell *et al*. 2003); *H. comes, in situ (*S. Morgan unpublished data, Perante *et al*. 2002;); *H. breviceps, in situ* (Moreau & Vincent in press), *H. erectus, ex situ* (Hughes 1999), *H. whitei, in situ* (Vincent & Sadler 1995). Fingerling tag trials in captive populations of *H. capensis* have shown no significant differences in mortality, disease outbreak, feeding rates, growth or behaviour between groups of control and tagged individuals, suggesting that Floy tags do not negatively affect seahorse survival (Le Cheminant 2000). No rigorous tests have examined the effects of fingerling tags on wild populations. Fingerling tags are not recommended for use on seahorses <4cm SL, where tags impact smaller seahorses' swimming ability and behaviour (C. Bull pers. comm.). External marks like collar tags can also act as targets for predators (including fishers), and researchers have seen seahorses dragged by their necklace tags by nearby perciform fish, apparently attempting to feed directly on tags, or on epiphytic growth on tags (J. Curtis pers. comm., S. Morgan pers. obs). *It is recommended that prior to tagging, animals should be monitored for at least a month without being tagged in order to assure fidelity, as this method should not be used for mobile species that will leave the study area.*

Below is a modified version of the original protocol used for marking a wild population of *H. whitei* (Vincent & Sadler 1995). Further modifications arose from studies of both *H. comes* (S. Morgan pers. obs.) and *H. guttulatus* (J. Curtis pers. comm.), where lesions were discovered on regularly monitored animals. In the case of *H. comes*, a modified collar design was used successfully without any recurrence of lesions (section 2.3 & Appendix I). In *H. guttulatus*, the technique was abandoned in favour of elastomer marking.

2.1. Cord Material

Seahorses have been successfully collar-tagged using cotton and soft nylon such as that used in nylon clothing. Note that hard transparent nylon such as fishing line is not an appropriate cord material and causes lesions. The principal problem with firm cord materials is that sharp points at the ends of the line aggravate neck tissue. Even if knots are tied so that ends face away from the neck, the weight of knots often causes them to hang under the chin/snout area where they are largely surrounded by tissue and in a zone of high mobility during feeding. It has been suggested that elastomeric threads and resorbable suture materials may be useful cord material for collar tags. Resorbable sutures such as Vicryl, Monocryl and Chromic Gut (www.ethicon.com) are biodegradable and could be useful at the beginning of tagging studies where researchers need to begin data collection prior to establishing site fidelity. Use of biodegradable materials would require more frequent retagging, and higher tag loss, but would insure that collar tags pose not danger to seahorses that leave study sites. Resorbable sutures have not yet been used as a cord material for tagging seahorses.

2.2. Ordering Tags

Fingerling tags can be obtained from Floy Tags, and should be ordered with a triple digit numeric code. This will allow any tag to be changed or replaced quickly and yet still retain the same number, which is essential for record keeping. For example, an animal originally tagged (0)21 can later be retagged (1)21, (2)21 etc. without changing its designated identity of M21. Note that tags are available in the following opaque colours: green, yellow, orange, blue, and white. Tags made from transparent plastic are also available and are recommended as they pose minimal threat to camouflage. For studies with <100 animals, Project Seahorse recommends ordering a few hundred-1000 tags; where the number of replacements should correspond to the duration of the study. A rough guideline is to estimate the need for one tag per animal for every three months of monitoring. Even for short studies, at least 1-2 replacement tags are recommended for each study animal.

2.3. Tagging Preparation

- On one side, tags have a three digit numeric code, while on the opposite side, each tag should be marked with a simple symbol, specific to the tag (e.g. filled circle / open circle / letter A / right slash etc). These symbols serve to cross-validate tag numbers, which can be difficult to read reliably in low light. Alternately, tags may be ordered with the same number on both sides, although this is not recommended for transparent tags. Tags should be read left to right, with the hole to left of the tag.
- It is useful to tag in ascending order, with the first tagged animal given the lowest number; one sex should be given odd numbers and the other sex even numbers. Project Seahorse convention to date has been to label males with odd numbers and females with even numbers.
- **Design A:** Make collars by putting a 15cm length of lightweight nylon gut through the hole in the tag. Lay a 15 cm length of heavy cotton thread alongside it, but not through the hole. Take nylon and thread together as one strand and tie reef/square knot (L over R, R over L) around tag. Cut ends of nylon close to knot,

leaving the tag attached to the collar by gut (suture material), but leaving only thread to tie around the seahorse. This is important as nylon abrades the seahorse's skin much more rapidly. Note that this method has resulted in neck lesions in a population of *H. guttulatus*.

- **Design B** (see **Appendix 1**)**:** An alternate type of collar tag, recommended for long term focal studies (where elastomer is not available/not practical) uses soft nylon cord. Start with 15 cm of nylon then fold this length in half. Obtain a short (<10cm) piece of narrow diameter telephone cable or electrical cable. Remove the metal wiring from the cable, leaving only the plastic casing. Cut the plastic tube of casing into 1mm disc cross-sections using a scalpel. Using a needle-threader, thread the two ends of the soft nylon through one plastic disc; the paired cord should *just* fit through the casing and must not be loose – this won't be possible without a needle threader. Next thread on a fingerling tag, followed by a second plastic disc. Estimate the maximum possible neck diameter during the period of monitoring; slide the array of disc/tag/disc up to this point. Tie the two ends of the string in a knot approximately 0.5 cm below the bottom of the disc/tag/disc array. The advantage of this method is that it allows for adjustment of the collar diameter as seahorses grow.

2.4. Tagging Procedure (see Appendix 1)

The following protocol assumes that you are conducting a study where is it possible to collect seahorses from their habitat, remove them to a boat or the shore and then return them to their collection location. If this is not possible (because of decompression risk to diver or seahorse for example) then seahorses can be tagged underwater; however, this will be more difficult and should ideally be practiced on aquarium or dead specimens before attempting field experiments.

- Collect seahorse and put in numbered/coloured nylon bag, marking source location with numbered/coloured clip. If multiple seahorses are being collected, pairs of number/coloured clips in each bag should be used; the clip remaining in the bag allows you to ensure the seahorse is returned to the correct location. Attaching a 10-20 cm streamer of coloured material (flagging tape or similar) to the clip makes it much easier to relocate if you are working in a complex or low visibility environment. Numbered Ziploc bags may also be useful for temporary transport as they provide a water cushion around animals, and do not catch on spines like many mesh or nylon bags.
- Take seahorse to boat or shore then place in beaker/tray of seawater. Weigh and measure, keeping in water as much as possible, then turn to tagging.
- Cup seahorse in the palm of the hand and allow it to hold your little finger. With larger or more mobile species, like *H. abdominalis*, or smaller species, like *H. breviceps*, you may need somebody else to hold the animal for you.
- **Design A:** Tie thread around neck very loosely, just tightly enough so it doesn't slip off the head, using a reinforced reef knot / square knot. This is very important as poor knots will slip. Tie L over R, R over L, then reinforce with L over R, and even one more R over L if you like. Cut ends of thread 10 mm from knot. Check tightness again.
- **Design B**: In order to fit each collar properly, only knot nylon when the tag is around a seahorse's neck. Estimate the total potential neck diameter of any individual over the study period, and knot the nylon here. Then slide discs and tag

back up the paired cord to obtain the appropriate fit. *Like Design A, this method is not recommended for use in any populations where animals are not monitored regularly or where escapement is expected.*

2.5. Monitoring

- Allow animal to recover for 5-10 minutes (optional).
- Return to original location and gently guide it to grasp a holdfast.
- Watch for about 5 minutes to ensure that it is maintaining a good grasp and behaving normally. Seahorses usually resume usual social interactions within minutes of being returned to the water.
- *Monitor seahorse closely thereafter, relocating it at least once a week.* This is critical, as one must monitor the tightness of the tag regularly. *It is inappropriate to use collar tags if you cannot maintain a regime of regular, preferably daily, observations.*
- Tags usually become covered with algae, but can be scraped if their identity is in question. It is advisable to develop the capacity to recognize individual animals (without depending on location) to minimize the need to examine the collar number. Checking the collar tag definitely disrupts the animal and should only be done occasionally to verify presumed identity. It should not be necessary to check the tag on each sighting. Beside each observational record, note whether tag was examined or if identity was inferred from individual recognition.
- **Design A:** tags should be replaced as soon as they begin to get tight, and long before abrasion begins. Remove the animal from the sea as above, and cut off old collar. Retag with new collar with same last two digits and symbol. Return to sea as above. Cut thread and cut off old tag and remake for next retagging episode of same animal. Thus, it may be retagged from (3)21 to (4)21, then back to 3(21) next time. It may be necessary to retag every 4-8 weeks.

2.6. Checking for Lesions

- When seahorses are handled, they often pull their snouts tight against the ventral surface of their torsos and can be difficult to manipulate. Note that lesions usually develop under the chin/neck area which, for the above reason, can be difficult to examine. When checking for lesions, it is best to work quickly (but still carefully) with the animals; rapidly "unwrapping" them from their holdfasts and simultaneously inserting a finger under their chin. If they have taken up a defensive posture, you can release them in midwater so that they swim, again allowing you to insert your finger under the chin for examination. *Forcing the head up via pressure on the snout is strongly discouraged.*
- If a lesion is detected, the collar should be removed immediately. Morphometric measurements and notes of individual markings should be made prior to release. In low mobility, highly site faithful species, it should not be difficult to wait the 3-4 weeks necessary for lesions to heal. Modify the method as necessary prior to re-tagging. Note that the lesion itself and lack of tag should act to identify any healing individual in an otherwise tagged population.

3. Injected Marks - Visible Implant Elastomer Marking

Visible Implant Elastomer (VIE) is a marking system available from Northwestern Marine Technology Inc. (NMT) (Appendix 4) (see references in Woods & Martin-Smith 2004 and at http://www.nmt-inc.com/References/VIE.htm). VIE consists of an injectable plastic polymer that, when mixed with a curing agent, hardens under the skin to leave a permanent, pliable, biocompatible mark. VIE is available in four fluorescent colours (yellow, green, orange and red) and three non-fluorescent colours (blue, purple and black). Fluorescent elastomer may be referred to as VIFE.

Seahorses are particularly well suited to elastomer marking because of the segmented nature of their bodies, which provide natural, easily identifiable sites for marking that can be used in different combinations to create a large number of individual tags. In *ex situ* studies, VIFE has been used on *H. abdominalis* (Woods & Martin-Smith 2004) and *H. capensis* (Le Cheminant 2000). *In situ*, elastomer has been used as a marking technique on wild populations of *H. abdominalis* (Van Dijken 2001, K. Martin-Smith unpublished data), *H. comes* (A. Diwata unpublished data, S. Morgan, unpublished data), *H. guttulatus* (J. Curtis unpublished data), *H. hippocampus* (J. Curtis unpublished data) and *H. whitei* (K. Martin-Smith unpublished data). In most cases, marked individuals were >70mm SL, but if results from perciform fish are applicable to seahorses, elastomer may also be suitable for marking much smaller individuals (Frederick 1997, Buckley *et al.* 1994). In addition to seahorses, weedy seadragons *Phyllopteryx taeniolatus* (Sánchez-Cámara 2002, K. Martin-Smith & M. Davey unpublished data) and spiny pipehorses *Solegnathus spinosissimus* (K. Martin-Smith & M. Davey unpublished data) have been successfully tagged with VIFE.

3.1. Mark Locations (see Appendix 2)

Seahorses have been elastomer marked in a variety of body locations, where the number of sites available is largely governed by size. Researchers at NMT at who injected dead, ethanol-preserved adult *H. comes* (140-170 mm SL), suggested the inside of the tail, the lower jaw and an area just above the operculum as marking sites (D. Thompson pers. comm.). These areas are appropriate for batch marking, but are unlikely to be suitable for individual marks in anything but very small studies. Marks on the inside of tails may be lost if skin from this area is shed or rubbed while seahorses cling to holdfasts. In *H. capensis* (65 mm SL), the inside of the tail was too small to allow application of a reasonably sized mark (Le Cheminant 2000). However, *H. abdominalis* have been successfully tagged with VIE on both lateral sides of the upper tail, below the end of the dorsal fin (Van Dijken 2001). In smaller seahorses, lateral body segments appear to provide the largest and most suitable injection sites. At least four field studies have marked lateral body segments to create individual marks (*H. comes* A. Diwata unpublished data; *H. comes* S. Morgan unpublished data; *H. abdominalis* K. Martin-Smith unpublished data; *H. guttulatus* J. Curtis unpublished data). Where possible, it is strongly recommended to choose injection sites that are separated by segments that are never used as marking locations. This way, one can have marking sites that are simply designated as, for example, high, middle or low, or that are can be distinguished without counting segments (Appendix 2,). In some species, injection along the ridges of body segments may

work best, particularly if these areas are less pigmented than concave body segments (Appendix 3, Figure b).

3.2. Fluorescing Marks

Fluorescent elastomer is recommended over non-fluorescent VIE, for long term mark visibility. While most elastomer marks are visible under the skin immediately after injection, marks can quickly become difficult to see under white light as successive pigmented layers overgrow the mark (*H. abdominalis* Woods & Martin-Smith 2004; *H. comes* S. Morgan unpublished data). The ability to fluoresce VIFE elastomer with a UV torch is very useful for long term marking studies because fluorescent colours are usually visible, even deep under heavily pigmented skin (*H. abdominalis* Woods & Martin-Smith 2004; *H. comes*, S. Morgan, unpublished data). Of the fluorescent colours, orange and red are usually the brightest on heavily pigmented animals, followed by yellow and then green. If using a two-colour marking system, it is recommended to use either orange or red, and yellow or green, to avoid any confusion between similar colours - particularly when fluoresced.

3.3. Retention

VIFE mark retention has been examined in three seahorse species and shown interspecific differences in retention. In *ex situ* work, *H. abdominalis* showed 100% mark-retention over seven months (Woods & Martin-Smith 2004). Similarly, an *in situ* study of elastomer marked *H. guttulatus* showed 99% retention for one year in 150+ individuals, ranging in size from 60-160mm SL. (J. Curtis unpublished data). In contrast, approximately one third of double-marked *H. comes* lost one tag in the first 6 months post-marking; some animals retained marks for up to one year (S. Morgan unpublished data). This may have been related to the particularly bony structure of *H. comes* as opposed to softer-bodied species such as *H. abdominalis* and *H. guttulatus*. Observation of VIE-marked *H. whitei* also suggests tag-loss. Like *H. comes*, and unlike its sympatric congener *H. abdominalis*, *H. whitei* also has a bony, inflexible body structure (K. Martin-Smith pers. obs.).

Tag loss is probabilistic and will depend on biocompatibility between the tag and the study species (a fixed effect for any species/marking technique pair), and how many times each individual is marked (not fixed). For example, if the tag retention rate is 95% for a given species, each tag will have a 0.05 probability of loss (5%), or a 1 in 20 chance of being lost. If individuals are marked twice to create a single individual mark, then the probability of tag loss increases multiplicatively, so that the chance of losing a mark on a double-injected individual is 0.95 x 0.95 = 0.9025, or closer to a 1 in 10 chance of losing a mark. Note that this is only true where both marks are required to identify the individual. If either of the two marks is sufficient to identify the individual and each mark is actually insurance against the loss of the other, the chance of tag loss remains 1 in 20 for each mark, and 1 in 40 that you will be able to identify the individual.

3.4. Effects on Growth

Woods & Martin-Smith (2004) measured the growth of VIFE-tagged and control *H. abdominalis* over a period of 7 months. Although the growth rate of VIFE-tagged

seahorses was slightly lower than control animals (approximately 6% for length growth and 10% for weight growth) this was considered to biologically insignificant. However, experimental confirmation of growth effects should be undertaken if life-history parameters are to be estimated from tagging data.

3.5. Infection

Due consideration should be given to the relative merits of using aseptic technique when making consecutive VIE injections on different animals. This is particularly true in populations where there is no information on pathogen communities. However, note that use of aseptic technique also poses potential health risks to the animals involved through capture and handling. Animals should be monitored for signs of secondary infection at the injection site. This has been documented in 0.5% of marked animals in a large population of *H. guttulatus* (J. Curtis unpublished data).

3.6. Readability

One other factor to consider is tag readability in conjunction with observer training. In a study of *H. guttulatus*, only 1% tag loss was recorded, but triple-marked seahorses were misidentified in 7% of cases through tag-misreading (J. Curtis pers. comm.).

3.7. Storage

Once mixed VIE cures quickly. NMT advises that VIE may be used for up to 24 hours if retained at low temperatures (e.g. in a freezer), however Project Seahorse researchers have successfully kept VIE-filled syringes for up to a week in freezers for repeated use (J. Curtis pers. comm.). In developing countries, VIE-filled syringes have been kept under tropical conditions in small Styrofoam coolers with blocks of ice without curing for 24-36 hours (S. Morgan pers. obs.). Small perciform fish have successfully retained polymer without curing agent (K. Martin-Smith pers. comm.). Should this method be applicable to seahorses, it would significantly reduce the waste and cost of using VIE. After mixing a batch of VIE it is advisable to load small amounts into a number of injecting syringes rather than into only 1 or 2 syringes. If the injecting needle is bent or becomes blunt, it is much easier to swap syringes than attempt to transfer VIE from one syringe to another. Furthermore, unused syringes can be retained on ice to prolong their working life.

3.8. Cost

The major drawback of the VIE system is its expense. At present, the component latex and curing agent can only be mixed in volumes sufficient for several hundred marks. Commonly, fewer marks are required and the material is wasted. The minimum recommended mixing volumes (1cc latex + 0.1cc curing agent) correspond to a cost of approximately $5.63 Can/$4.45 USD. Half volumes (0.5cc latex + 0.05cc curing agent) have been mixed with success (K. Martin-Smith & S. Morgan pers. obs.), although proper hardening is not as reliable given the potential for error in the latex: curing agent ratio at small volumes.

3.9. Study Planning

- Estimate the number of animals that you will be marking, or could have to mark in this population. This will affect how many positions you will need to mark in, and the number of colours to use. Note that some proportion of the population should be allocated as control subjects and these individuals can be subtracted from the total number of marks required for the study population. Ideally it is best to perform double controls, with sets of both handled and unhandled individuals.
- Survey your population to identify the smallest individuals for marking. Use these individuals to establish which body positions it will be possible to inject. For example, in average sized adult seahorse of *H. comes* (14-17cm SL), body segments 1-4 are often too small to easily insert a marking needle into, therefore only body rows 5-10 are available for injection.
- Ascertain whether it is possible to make injections in the segments of all rows. Depending on size and species, some individuals may have particular segments that are too concave to inject. This may hold true for whole columns (superior, dorsal, inferior) of body segments.
- Note that there is a trade-off between minimizing tag loss and maximizing tag readability. Maximizing tag readability generally means leaving some segments blank and only injecting in others. This reduces the number of unique tags available and increases the likelihood of needing to tag with two colours; double-marking increases the chance of tag loss
- Having established a) the body segments available for injection, b) those that you will use as sites c) the number of marks per animal and d) the number of colours that will be used, the number of possible marks can now be generated according to the following formulae:

  # of Unique Codes with 1 Tag/Individual = L
  # of Unique Codes with 2 Tags/Individual = $L * (L-1) * C^2/2$
  # of Unique Codes with 3 Tags/Individual = $L * (L-1) * (L-2) * C^3/6$
  where L = # of body locations, and C= # of colours used
  (http://www.nmt-inc.com/Products/VIE/vie.htm)
- If this number is insufficient for the projected study population, you have the option to a) increase either number of sites for injection per animal, b) increase the number of marks per animal or c) increase the number of colours used in the study.

3.10. Tagging Procedure (see Appendix 3)

As in section 2.4, the following procedure assumes that you are removing the seahorse to the shore or a boat for marking. However, *H. abdominalis*, *H. guttulatus* and *H. hippocampus* (and the spiny pipehorse *Solegnathus spinosissimus* and the weedy seadragon *Phyllopteryx taeniolatus*) have all been successfully marked underwater with VIFE (J. Curtis, K. Martin-Smith & M. Davey unpublished data). In these cases, it is advised that injection syringes (and caps if possible) be attached with string to a scribe or buoyancy jacket. Both the syringe and syringe cap are positively buoyant and very easy to lose when marking underwater.

- Remove the individual to be marked, leaving a colour-/number-coordinated clip on holdfast. Transfer seahorse into a colour/number coded nylon bag or Ziploc baggie – see section 2.4.

- Photograph seahorse prior to marking to aid in future identification and where possible perform morphometric measurements.
- Hold seahorse in your left hand (if right-handed), letting the tail curl around your little finger and stretching the trunk by grasping the neck firmly and gently between thumb and first two fingers, to expose and stabilize the torso for injection.
- Remove VIE-filled syringe (see instructions from NMT for mixing) from cooler. While the syringe can be used directly to tag seahorses, the hand injector available from NMT makes the procedure considerably easier and more controllable.
- Researchers should have clean, wet hands when holding animals. If possible the whole animal should be held underwater while making the injection; the gills at least should remain submersed. In some cases (species with stiff bodies that are difficult to inject) this may not be possible and it may be easiest to hold the seahorse against a flat surface which should be covered with a soft, wet covering such as a seawater-dampened towel or sponge. Be alert to the possibility of dessication in hot, windy environments.
- Insert the needle under the epidermis at one side of the body segment. This may require exerting more force than you would expect and the needle will probably have to be inserted at shallow angle relative to body. Be careful not to insert the syringe too deeply as it is possible to puncture the body cavity. Marks inserted too deeply are also difficult to see. Once inserted, the needle should be pushed gently but firmly under the skin. A good indication that you are not too deep is that you should see the syringe pushing the epidermis upwards as it is moves across the segment.
- Alternatively, the skin immediately adjacent to body segment ridges, or the base of body segment ridges may be marked.
- When the needle reaches the opposite side of the segment, slowly and firmly depress the plunger of your syringe with your thumb (or with your palm if you are using the hand injector) and simultaneously withdraw the needle a fraction. When you see elastomer coming out of the needle, draw the syringe smoothly backwards. Stop pushing on the plunger just before you exit the skin to minimize seepage from the injection site.
- Wipe off any excess elastomer with a wet finger.
- Transfer the seahorse to a bucket for 5 minutes of observation. Check marking site again before replacing the seahorse, and again remove any excess elastomer.

3.11. Monitoring

- It is highly recommended that you monitor retention following marking. There is preliminary evidence that different species may have different rates of elastomer retention and it remains unknown whether retention varies between sites or populations within species.
- In past Project Seahorse studies, marked animals have been monitored at the following intervals following injection: 0.5 hours, 2 hours, 4 hours, 8 hours, and 24 hours. Animals are then monitored daily for the first week, once a week for the next month, and once monthly after that (M. Samoilys pers. comm.). Take note of any changes in the size of marks or signs of secondary infection at the injection site.
- One concern with elastomer is that although marks show very high retention after the first week, visibility may decrease with time as marks are obscured by

pigmentation. It is unknown how visibility effects affect mark-recapture probabilities and hence population estimates. Visibility also varies between white light and UV light (for VIFE) and data on mark visibility should be recorded under both types of light. In order to monitor changes in visibility, it is pragmatic to create a standard against which to compare animals' marks. Standards have been prepared by covering multiple patches of VIE with different numbers of layers of translucent paper. These patches can then be assigned visibility values that range from complete visibility, to complete invisibility. This standard can be compared to marks on animals to maintain a constant baseline for visibility values.

- Marked animals should be measured for growth to see whether control animals are growing at different rates from marked animals.
- Noting the condition at the site of injection or any associated indications of disease are also recommended.

4. Conclusions

Both collar tags and VIE have been used to tag seahorses successfully. The exact technique chosen for any particular study will depend on the objectives, the species of seahorse involved and logistic considerations. It is strongly recommended that pilot studies are undertaken before any large-scale tagging is commenced. For any study where seahorses are not monitored regularly, or are highly mobile, collar tags should not be used as there is an unacceptable risk of mortality from neck constriction as the animal grows.

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Appendix 1. Design B Collar Tag

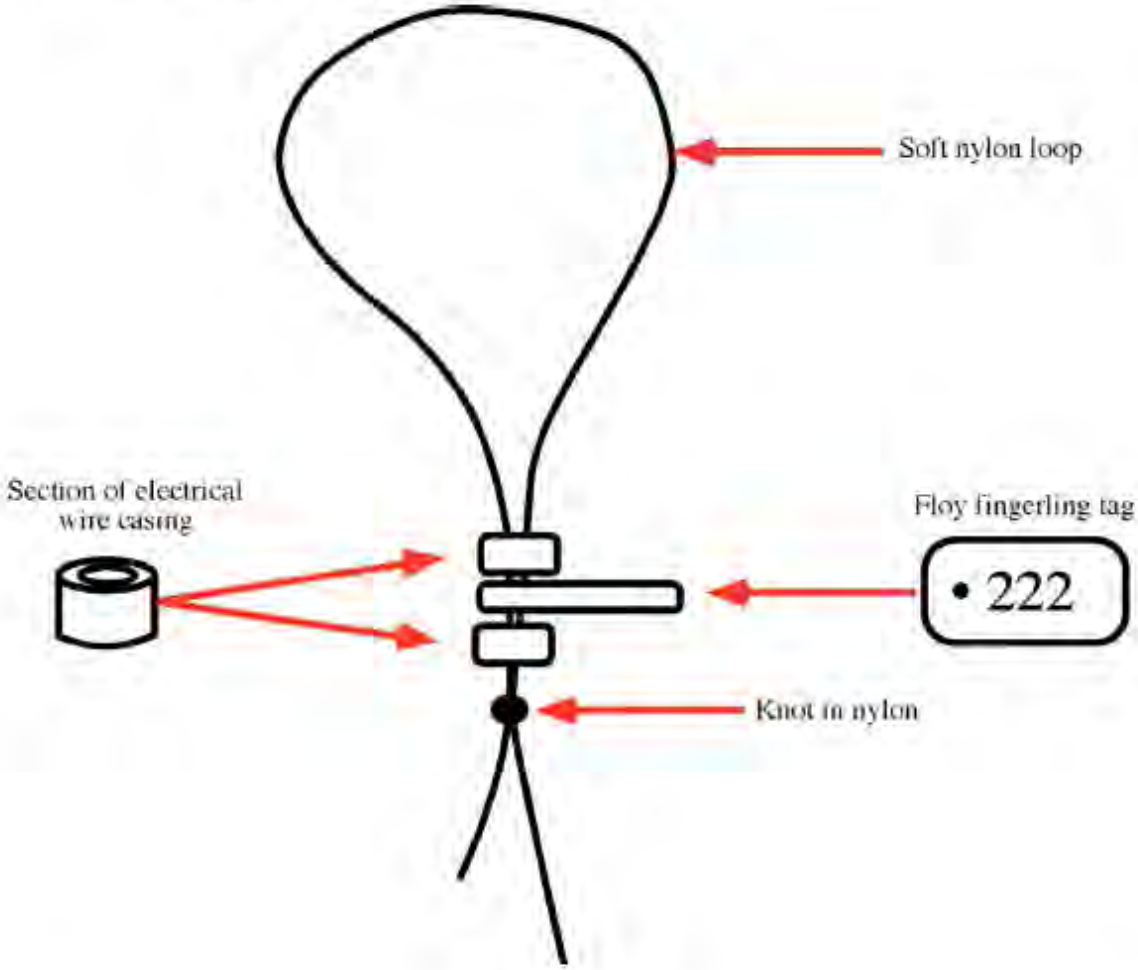


Figure 1 – Design B, modified collar tag design. Note that in this diagram electrical wire casing discs and Floy tags are separated for the purposes of illustration. These should be abutting one another when the tag is in use.

Appendix 2. Example of a Coding System for VIE Tagging

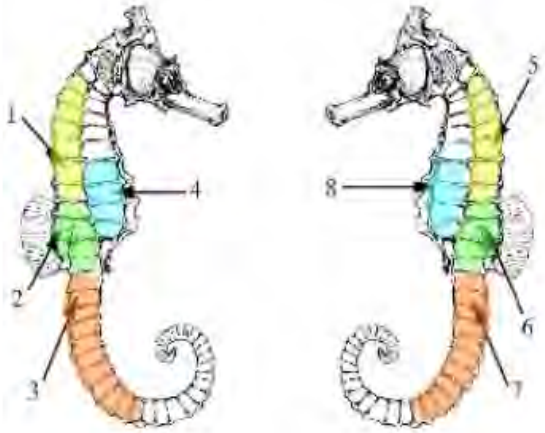
The following pages show one example of a coding system used for seahorses. Body locations are not individual segments but areas so that individual tags can be separated and thus identified unambiguously. With three tags per animal, four VIE colours and eight body locations over 700 unique marks can be created. In this example, seahorses were only marked on one side of the animal.

Master Tagging Sheet

Tag Positions (see diagram)

1 & 5	2 & 6		
Bounded by mid-lateral trunk ridge: located above insertion of dorsal fin	Bounded by mid-lateral trunk ridge: located within insertion of dorsal fin	3 & 7 On tail below lower insertion of dorsal fin	4 & 8 Located on ventral side of mid-lateral ridge

Colours	Code
Orange	O
Yellow	Y
Pink	P
Green	G



Note: tags placed on one side of seahorse only
Formula for number of unique codes:
$L! \times C!/n!$ where L is no. locations, C is no. colours

Extracts from master code sheet for tags 1-40 (using only one colour) and 161-205 (using two colours).

Single Colour: Orange

Position	Tag #1	2	3	4	5	6	7	8	9	10
1	OOO				OO	OO	OO	O	O	O
2		OOO			O			OO		
3			OOO			O			OO	
4				OOO			O			OO
	11	12	13	14	15	16	17	18	19	20
1	O	O	O							
2	O	O		OO	OO	O	O	O		
3	O		O	O		OO		O	OO	O
4		O	O		O		OO	O	O	OO
	21	22	23	24	25	26	27	28	29	30
5	OOO				OO	OO	OO	O	O	O
6		OOO			O			OO		
7			OOO			O			OO	
8				OOO			O			OO
	31	32	33	34	35	36	37	38	39	40
5	O	O	O							
6	O	O		OO	OO	O	O	O		
7	O		O	O		OO		O	OO	O
8		O	O		O		OO	O	O	OO

Two Colour: Orange & Green

Position	161	162	163	164	165	166	167	168	169	170
1	OGG				OOG				O	O
2		OGG				OOG			O	
3			OGO				OOG		G	O
4				OOG				OOG		G
	171	172	173	174	175	176	177	178	179	180
1	O	G	O	O					G	G
2	G	OG			O	O	O		O	
3			OG		O	OG		O	O	O
4	G			GG	G		GG	GG		O
	181	182	183	184	185	186	187	188	189	190
1	O	O	O	O				O	O	O
2	O	OG			O	O	O	O	O	
3			OO		O	OO		O		O
4	O			OO	O		OO		O	O
	191	192	193	194	195	196	197	198	199	200
1	O	O	O	OO	OO	OO				OO
2	O	O		O			OO	GG		O
3	O		O		O		O		GG	
4		G	G			O		O	O	
	201	202	203	204	205					
1	OO	OO								
2			OO	OO						
3	O		O		OO					
4		G		O	O					

Appendix 3. Elastomer Tagging

(a). *Hippocampus whitei* tagged with three pink tags within body segments viewed under ambient light. © Jonathan Clark-Jones/Project Seahorse.

(b). *Hippocampus abdominalis* tagged with one orange and one green tag within ridges viewed under ambient light. © Keith Martin-Smith/Project Seahorse.

(c). *Hippocampus abdominalis* tagged with one orange tag within segment viewed under blue light. © Chris Woods.

Figure 3. Examples of VIE tagging in seahorses.

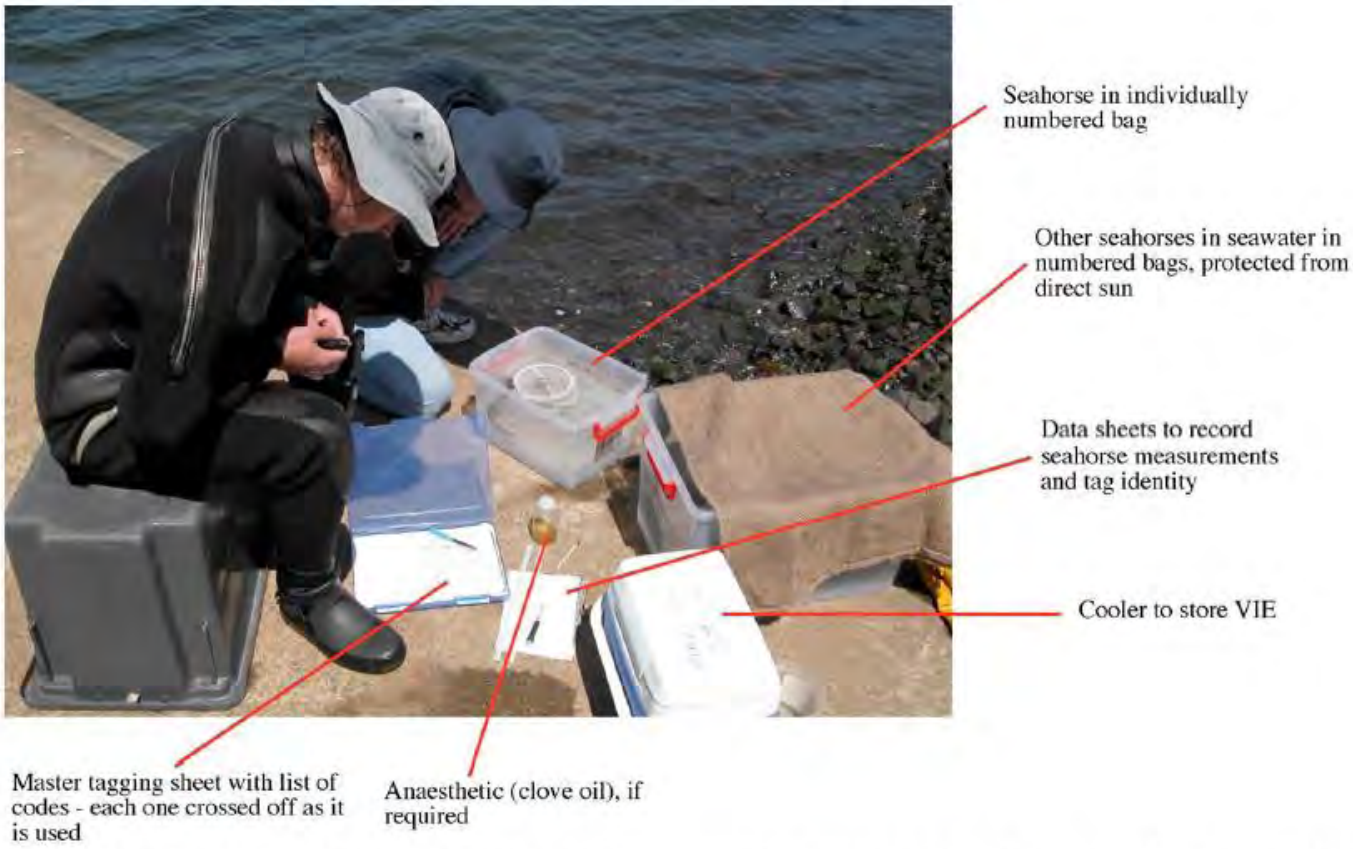


Figure 4. Field setup for shore tagging of *H. abdominalis* during population dynamic studies. © Keith Martin-Smith/Project Seahorse

(a) Preparation for tagging

(b) Needle of syringe inserted at shallow angle under skin, parallel to body ridge in this case and plunger of syringe depressed using palm of hand (hand injector)

(c) Tag is completed, pressure on plunger released and the needle is withdrawn.

Figure 5. Series of photographs illustrating VIE tagging of *H. abdominalis* in the laboratory. © Keith Martin-Smith/Project Seahorse

Appendix 4. Tagging and Marking Contact List

Tagging Method/ Company	Mailing Address	Tel/Fax/E-mail/Website	Contact person
Collar tagging			
Floy	Floy Tag Inc. 4616 Union Bay Place NE Seattle, WA USA 98105	Tel: 1-800-843-1172 Tel: (206) 524-2700 E-mail: floytag@halycon.com Web:www.halcyon.com/floytag	Betsy A. Conrad Fish and Crustacean Tags (Sales & Operations Manager)
Elastomer Marking			
Northwest Marine Technologies	Corporate Office P.O.Box 427 Ben Nevis Loop Road Shaw Island, WA USA 98286 Biological Services 955 Malin Lane SW Tumwater WA USA 98501	Tel: (360) 468-3375 Fax: (360) 468-3844 E-mail: office@nmt.us E-mail: biology @nmt.us E-mail: techsupport@nmt.us Web: www.nmt-inc.com	Jan Sanburg <office@nmt.us> Geraldine Vander Haegen <geraldine.vanderhaegen@nmt.us>

Appendix 4

ABC News story
Seahorses released into Sydney Harbour

Seahorses released into Sydney Harbour

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Swimming free: Seahorses are prepared for release in Sydney Harbour (ABC News: Rebecca Hyam)

The Sydney Aquarium has released 30 seahorses bred in captivity into Sydney Harbour as part of an Australian-first study to try to boost their numbers, but it is concerned poachers may intervene.

The tiny black animals were carefully transported from a tank into the netted area at Manly Cove, in northern Sydney, this morning.

The five-centimetre-long white's seahorses have been tagged and will be closely monitored by the New South Wales Department of Primary Industries (DPI) and a team of divers.

There is already a colony of about 300 of the animals, only known to exist in NSW waters, near the sea net at Manly Cove but some have been poached from there in the past.

It is hoped the research will lead to the release of captive-bred seahorses along the NSW coast and in areas overseas where seahorse populations are in danger of disappearing.

Claudette Rechtorik, from the Sydney Aquarium Conservation Fund, says the animals should not be too hard to keep track of, even though they are small.

"Each of these 30 babies have been tagged," she said. "The tag actually expands as they grow so we'll be able to watch for these tagged animals in amongst these wild populations."

Poaching fear

Seahorses are protected in NSW but there is a market for seahorses both within the aquaria trade and also within alternative therapies, where they are used for various medicinal purposes.

The animals are vulnerable to poaching and can fetch up to $120 each on the black market.

Ms Rechtorik also says seahorses are in decline because they live in vulnerable habitats.

"Sea grasses are quite sensitive to pollution, so our sewage outfalls and nutrient enrichment of the local waters have quite an impact on sea grass," she said.

"Without sea grass, without habitat, the seahorses don't actually have anywhere to live."

'Clinging onto trolleys'

But she says man-made structures, like the sea net, often provide good environments for seahorses.

"You will find little seahorses clinging onto trolleys at the bottom of Sydney Harbour," she said.

"As much as you'd like to be able to remove these bits of human remnants of trash out, you have to be quite careful because sometimes it is actually quite a rich home for many different creatures, including the seahorse."

The researchers, led by DPI marine scientist David Harasti, will come back in three days to check how the seahorses are faring

http://www.abc.net.au/news/stories/2007/11/13/2089359.htm