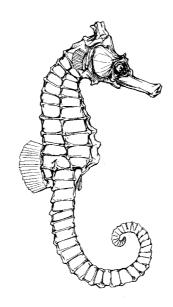


TECHNICAL REPORT SERIES

No. 8

UNDERWATER VISUAL CENSUS FOR SEAHORSE POPULATION ASSESSMENTS

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PROJECT SEAHORSE TECHNICAL REPORT NO. 8: UNDERWATER VISUAL CENSUS FOR SEAHORSE POPULATION ASSESSMENTS

1. Introduction

1.1. Why Is It Important To Assess Populations of Seahorses?

Apart from their intrinsic worth, these fishes are unusual and valuable for a number of reasons including their unusual biology, economic value, medicinal properties and conservation importance.

- **Reproductive Behaviour:** Seahorses (genus *Hippocampus*) exhibit the most highly specialized paternal care of any animal: they incubate young in brood pouches. Some seahorse species also exhibit rare sexual fidelity, called monogamy, to only one partner (e.g. *Hippocampus whitei*, Vincent & Sadler 1995).
- Anatomy: Seahorses are voracious predators that lack scales, teeth and stomachs. They have binocular vision, tube-like snouts, bony plates and unusual grape-like gills. Their fins are reduced in size and most species lack a caudal fin, relying on dorsal and pectoral fins for propulsion. Seahorses grasp holdfasts with prehensile tails and remain stationary during long periods of time.
- *Ecology:* Seahorses contribute significantly to food web dynamics in seagrass communities (Tipton & Bell 1988), and form an important component of their coral, mangrove and estuarine ecosystems.
- *Economics:* Some seahorses provide income for many subsistence fishing families and sell for high prices. Seahorses are also used in the live aquarium fish trade and as dried curiosities or souvenirs (Vincent 1996).
- *Medicines:* Seahorses and pipefishes are used extensively as traditional medicines to treat a range of ailments. The use of these fishes for medicinal purposes is recognized by the World Health Organization.
- *Conservation:* Seahorses are important as attractive species around which we can rally support for a wide range of marine conservation issues, from habitat destruction to overfishing to bycatch. These fishes can therefore be used as an educational tool and seahorses are themselves recognized as threatened, or of conservation concern.

There is still much to learn about seahorse ecology and most seahorse species have yet to be studied in their natural habitat. Basic biological information about seahorse distribution, habitat use, natural densities, population structure, behaviour and life histories is lacking for most species and priorities for research are outlined in Foster & Vincent (in press). Population assessments carried out by divers, local enthusiasts or biologists can provide a valuable contribution to the global understanding of these extraordinary animals. Once the knowledge gained through population assessments has been disseminated, it can then be applied to help protect seahorses and their habitats while allowing a sustainable trade.

1.2. What Is Population Assessment?

A well-managed population assessment is a study that can provide reliable data on the current status of a population. Such data include population abundance, size-frequency distribution and sex ratio. When population assessments are carried out regularly (e.g. bi-monthly, annually), they can be used to track trends (increasing, stable, decreasing) in local population abundance. Carrying out population assessments in many sites provides useful information about species distributions, habitat use and the impacts of large-scale environmental disturbances (English *et al.* 1994). The basic biological information you compile during your seahorse population assessments will be extremely useful to marine resource managers. Your data may become important baseline information available for use by local biologists to evaluate the impacts of a planned or unexpected disturbance event (e.g. illegal fishing, sediment dredging or pollution spill) on the local seahorse community. For example, if there is a pollution spill or a hurricane in the area in the future, your data will give us an idea of seahorse abundance and the

environmental conditions that existed prior to the disturbance. This may allow us to determine what effects the disturbance has had on the seahorse populations. Your assessments may also be the only information available even if the environmental disturbance is planned. If a potentially disruptive human activity is planned in an area (e.g. increased fishing intensity using non-selective gears, dredging out channels, construction of marinas, collection of seahorses, oil drilling), your data will serve to monitor the condition of the seahorse populations in the area before, during and after the activity.

Underwater visual census (UVC) surveys are a non-destructive, fishery-independent method that can be used for estimating abundance, distribution and population structure of seahorse communities. Although the objectives of underwater visual census surveys may differ, most involve a systematic enumeration of animals within a search (census) area coupled with the collection of environmental information. Fishery-independent methods are not based on samples collected from a fishery and therefore do not suffer the biases from such samples, however, they do suffer from their own biases. UVC is subject to observer bias, underestimation of density due to camouflage, and depth constraints (see English *et al.* 1994). Nevertheless, it is widely used because it is practical, non-destructive, relatively quick and easy, and can provide reliable and repeatable relative estimates of population density.

There are many useful resources for learning how to carry out population assessments using underwater visual census techniques (e.g. English *et al.* 1994, King 1995, Sutherland 1996, Samoilys 1997, see also websites in Appendix 9). The more you learn about carrying out population assessments, the more you will come to realize that there is no single best way to assess populations. This technical bulletin is designed as a guide to provide the basic principles for conducting UVC surveys of seahorse populations. We also provide examples to illustrate how this protocol can be adapted to different diving conditions and substrates.

1.3. Population Abundance

Population size is the best predictor of long-term persistence and is one of the main criteria used in evaluating the extinction risk of a population (Meffe & Carroll 1997). Thus, the primary objective of a population assessment is to estimate population abundance. Population abundance or size is estimated by sub-sampling the total population in defined areas. Population density is an abundance measure and is expressed as the number of seahorses per unit of area, often expressed as the number of animals per m², per 1000m² or per km². For example, densities of seahorses range from approximately 0.006 per m² on some coral reefs of the Philippines to 0.95 per m² in estuarine habitats in Europe (see Foster & Vincent in press). By knowing how much area you search and the number of animals you encounter, you will be able to estimate density and compare your findings with other studies. Comparisons are usually based on relative estimates of density, having measured the number of seahorses per census area using the same method in the same habitat. If you want to know the total or absolute population abundance, then you need to know the distribution of the species you are studying in various habitats. These are then sub-sampled and the results are extrapolated to the whole area. For example, if the mean density of a seahorse species on your transects is 0.05 per m² in coralline areas and you've estimated from habitat maps that the coral reefs cover an area of 5 hectares (50 000 m²), then the total estimated population size is 2500 seahorses in that area.

1.4. Population Structure

The density, size distribution, sex ratio, and reproductive activity of a species can give insight into its life history and its responses to disturbance. The structure of a population can be used to estimate life history parameters such as grow, survival, and reproductive rates (See King 1995). Changes in the size distribution, sex ratio or reproductive activity of animals in a population can be used to evaluate the effects of management strategies or disturbance events. For example, when the fishing pressure on a population begins to increase, typically the size distribution of the animals becomes skewed towards smaller sizes and density generally decreases. This is because animals have a lower probability of surviving to an older age and larger size when the probability of being fished increases, and many fishing gears tend to capture larger animals

preferentially (King 1995). By tracking changes in population structure, your data may provide insight into the effects of disturbances on populations.

1.5. Habitat Use And Distribution

Your underwater visual survey may be the first study to document the presence of a seahorse species in your area. Information about habitat use on a number of different spatial and temporal scales is important to help determine the distribution of seahorses in your area as well as assess how management decisions that affect seahorse habitat will impact the populations you study.

Species that occupy many areas are less vulnerable to extinction than species that occupy a few areas. For example, the most threatened seahorse species in the world is the Knysna seahorse, *Hippocampus capensis* that has only been recorded in 4 estuaries in South Africa (Bell *et al.* 2003). By carrying out population assessments, you will help establish the distributional patterns of seahorse species in your area and contribute to the evaluation of the conservation status of these species.

Many seahorse species are found at depths that range from 1- 15 m, which makes them highly amenable to underwater visual census methods. While some seahorses may be found in many different microhabitats (e.g. shells, bryozoans, macro-algae) within a general habitat type (seagrass bed), other species may be strictly associated with a particular feature or microhabitat. The European long-snouted seahorse, *Hippocampus guttulatus* is generally associated with seagrass beds and macro algae, however, within these habitat types, this species uses a variety of microhabitats as holdfasts including seagrass shoots, ascidians, tube-dwelling polychaetes, broken shells and open sand (Curtis & Vincent in review). By contrast, in coral reefs, Bargibant's seahorse, *Hippocampus bargibanti*, is only known to occur on gorgonian corals of the genus *Muricella* (Lourie *et al.* 1999). By carrying out underwater visual surveys in different habitat types, at different depths and at different distances from natural and artificial features of the area, you will be able to identify the kinds of habitats that are most closely associated with seahorses in your area. This information could be valuable in designing marine protected areas for local fish communities.

1.6. What Is Involved In The Assessment?

There are six steps in a population assessment:

- 1 Deciding on a study area.
- 2 Deciding on population parameters to be recorded.
- 3 Selecting a sampling design.
- 4 Carrying out the underwater visual surveys.
- 5 Analyzing data.
- 6 Reporting or disseminating data and results.

This technical report is designed to guide you through the first 3 steps, although we do provide some hints and suggested reading for steps 4 and 5 below. Because no two sampling areas are alike, and different population assessment teams will have access to different resources, we present a basic underwater visual survey protocol and discuss modifications or considerations for different kinds of SCUBA diving conditions, marine environments and habitat types using examples.

1.7. What Kind of Data Will Be Collected During The Assessment, And Why?

In the protocol, we describe methods for collecting two main types of data. The first relates to the fishes themselves:

| Parameters | Community and Population-level Parameters |
|-----------------------|---|
| Species presence | -distribution |
| | -density |
| | -associations among species |
| | -fish community structure |
| Location in transect | -inter- and intra-specific interactions among individuals |
| | -spacing (e.g. aggregated, uniform, random) |
| | -group size |
| Size | -size distribution |
| | -growth rates |
| | -survival rates |
| Sex | -sex ratio |
| | -insight into mating pattern |
| Reproductive activity | -proportion of reproductively mature and/or active adults |
| | -duration of reproductive season |
| Behaviour | -activity patterns |
| | -inter- and intra-specific interactions among individuals |
| Condition | -environmental stresses |

Parameters highlighted in bold italics are considered essential for a population assessment, while the others are optional.

The importance of these data is obvious: we need information about the fishes themselves to be able to determine population parameters including (1) the population density, (2) the size-structure, (3) the sex ratio, (4) the reproductive status of the fishes, and (5) behavioural interactions among fishes of different species, sex and size. Information about the condition (e.g. presence of lesions, injuries) of the fishes can also help identify environmental stresses affecting the populations.

The second data set relates to the fishes' environment:

| Environmental Data | Purpose |
|--|---|
| Habitat type (e.g. seagrass bed, coral reef, mangrove, macroalgae, | -general habitat associations |
| muddy bottom) | |
| Percent cover of habitat types | -correlations between seahorse density and availability of different habitat types. |
| Microhabitat (e.g. holdfast) | -specific habitat requirement. |
| Salinity | -habitat characteristics often related to a species' |
| Temperature | distribution, and to diving conditions which may affect |
| Horizontal visibility | ability to detect seahorses. |
| Tidal data | Tidal state may relate to fish behaviour |
| Wind conditions | · |
| Sea surface conditions | |
| Cloud cover | |

The second set of data is important to allow us to understand the type of environment the seahorses inhabit and the types of stresses they might experience. Correlations between environmental variables and population-level parameters will help predict the habitat requirements of seahorse populations and the responses of populations to disturbance events that affect their habitat.

These population-level and environmental variables are data outputs of a seahorse population assessment. Remember that the primary goal of a population assessment is to estimate population abundance, therefore not all parameters listed are essential; those in bold italics

indicate the critical parameters. You may choose to record additional variables that are important in your area (e.g. seagrass shoot density, coral height, habitat rugosity, presence / absence of other fish species). Consult other sources (see below) for detailed descriptions of habitat quantification and survey methods in the different habitat types you may encounter in your area.

1.8. What Happens To The Data I Collect?

Your data can be analyzed and summarized using a range of simple statistical analyses, many of which can be carried out in an Excel worksheet using the data analysis tools. Consult statistical references (e.g. Zar 1999), local biologists, non-profit organizations, or Project Seahorse members for assistance with your data analysis. If you are unfamiliar with statistical analysis, you may choose to simply collect the data and have a colleague or collaborator analyze them.

Once the data are analyzed, it's important to make the data and results available. There are a number of ways to disseminate what you've learned to the local scientific community and the public:

Send a copy of your data and results to Project Seahorse. There it will be added to our seahorse reference library and made available to researchers interested in wild seahorse populations. Your data will also be added to life-tables that summarize the studies that have been carried out on those species and the information that we know about them.

Bring copies of your results to your local marine conservation organizations as well as government and university associated marine biologists and fisheries scientists. They may incorporate your data into existing biodiversity databases.

Publish your results in a scientific peer-reviewed journal.

Generate public interest in your work by writing an article for your local newspaper, or SCUBA club newsletter. Contact your local radio station or television station and offer to do an interview about your work and findings.

1.9. How Often Should I Do The Assessment?

Although a one-off survey will provide useful results, a number of surveys at different times provides a temporal perspective on the population which is much more complete. Temporal sampling is conducted to see whether population abundance changes in response to natural or anthropogenic factors. Natural changes may be driven by factors such as tide, lunar phase and/or season. If you are able to do more than one survey then you need to decide on the temporal scale of the questions you are asking. For example if you are interested in seasonal changes then surveys should be conducted in each season. If you want to monitor population abundance in response to anthropogenic factors such as establishing a marine protected area, then surveys should be conducted annually at the same time each year. It is very important to think of temporal patterns in abundance when designing your surveys. For example, if you survey only once a year, then the timing of the surveys should be standardized to the same season and lunar phase each year.

Surveys may also be replicated within a time period to measure small-scale variability in your populations. For example 2-3 surveys within a season will give you a better estimate of abundance for that season and will greatly increase your confidence in the results.

A single population assessment acts as a snapshot of the population and therefore consecutive dives should be done as close together as possible in time, within a few days.

1.10. Before You Start...

Get to know the seahorses in your area.

Even trained seahorse biologists occasionally confuse similar species. Take the time to carefully study the morphological characteristics of the species you intend to monitor. Cross reference photographs, line drawings and descriptions of local species from several fish identification books in order to select key characteristics for identification. Visit your local museum, aquarium and university to view and compare specimens of the species you will be assessing. Contact Project Seahorse for an updated list and description of species known to occur in your area.

Meet your local marine conservation allies.

Project Seahorse maintains a network of scientists who study seahorses and may be able to put you in contact with someone in your area. Local biologists, SCUBA divers, amateur underwater photographers, the coast guard, fishers, and parks officials will also have knowledge about the habitat, SCUBA diving conditions and previous studies carried out on seahorses in your area. Fishers, local divers and biologists will be able to tell you where they find seahorses, how often they are encountered and what species are present. You can obtain navigation and habitat maps from local governments, libraries, universities and SCUBA diving clubs. This information will be useful in designing an effective underwater visual survey and may lead to some productive collaborations.

Prepare a one-page description of your population assessments.

A brief outline of your goals, methods, dive schedule and outputs is useful when making contacts, requesting information or applying for research permits and funding.

Obtain the necessary dive permits and research permits if required in your area.

Seahorses are associated with shallow marine environments (Lourie et al. 1999) and when populations are located near urban areas, diving in those areas may require special permits or additional safety considerations. Contact your local coast guard, maritime police, department of fisheries or coastal resources, university or park authorities for information about diving and research regulations in your area. Even though you won't be collecting specimens, you may still need a permit to carry out an underwater survey.

Seek community support.

Carrying out underwater visual surveys is time intensive and can be expensive if carried out over the long term. There are grants available for marine conservation initiatives, many of which can be applied for over the Internet. Founding a local seahorse population assessment team and securing some funding to support your work will greatly facilitate your logistics and empower you and your collaborators.

2. Basic Underwater Visual Survey Protocol

2.1. Deciding On A Study Area And Describing Its Habitat

It's important to know your study area before you begin assessing the seahorse populations that exist there. Try to obtain a map of substrate types for your proposed sample area. If habitat maps are not available, carry out some dives to roughly map out the distribution of major habitat types in your area. The kinds of habitat or substrate you may encounter include seagrass beds, macro-algae, open sandy or muddy bottoms, kelp beds, coarse sand or shell fragments, sea urchin aggregations, rocky reefs, coral reefs and mangroves. Some artificial habitats you may encounter include pilings, concrete walls, large predator nets, and abandoned fishing gears. Consult other resources (e.g. English *et al.* 1994. Kingsford & Battershill 1998) for outlines of underwater mapping techniques and underwater visual survey techniques specific to the habitat types you will be surveying.

In an ideal world, a population assessment would be carried out over the entire area in which the populations of interest live. In practice, however, this may not be as easy as it sounds:

Some seahorse populations may extend over a much larger space than you could hope to survey.

Seahorses may also live in areas that are too difficult or dangerous for you to assess. These factors may include strong tides and currents, heavy boat traffic, abandoned fishing gears, low visibility, extremely dense vegetation and poor accessibility.

You may not have the use of a boat, in which case your surveys will be restricted to sites that are accessible from shore.

Generally speaking, you should try to make the study area as large as possible, but not so large that it becomes impractical. You will need to work around your limitations and exclude areas that are not possible to survey. Use a marine chart and information about the area that you gathered from local fishers, biologists, divers and personal experience to help you delineate a safe and accessible study area, within which you can randomly sample your populations (see Figure 1 below).

Once you have a map of general habitat types in your study area, you may choose to stratify your population assessment by surveying transects in different habitat types (e.g. sandy bottom, seagrass bed, coral reef). This way you can compare population-level parameters (e.g. density, sex ratio) among habitat types. If your study area is dominated by one habitat type (e.g. seagrass bed), then you won't need to stratify your sampling.

If you're assessing populations on artificial structures (e.g. wall, predator net, pilings), your study area will be largely limited to the structure that you are assessing and the surrounding area. To ease the selection of transects, you should either obtain or draw a plan of the structure before continuing with the protocols.

2.2. Diving Considerations

Depending on the type of habitat you are examining, the procedures for assessing the populations will vary. Some population assessments may be carried out without SCUBA equipment, but when depths are greater than approximately 0.75m in a dense seagrass bed for example, the use of SCUBA equipment will greatly facilitate your surveying. Practical experience from our own research activities have shown us that in good diving or snorkeling conditions, the search area that can be surveyed within a few hours by two observers varies according to SCUBA diving conditions (e.g. visibility, current strength, sea surface conditions, depth), diver skills, habitat complexity and the density of seahorses. In the Ria Formosa coastal lagoon in southern Portugal, with low visibility (<1m), strong tidal currents, patchy vegetation, moderate seahorse density (approximately 0.005 - 1 per m²) and moderate depths (1-7m), two experienced divers can search an area of approximately 180-360 m² within 1-3 hours. A few pilot dives will be useful for practicing underwater writing, handling equipment, communicating underwater and measuring fish as well as developing a search image for seahorses. Practice dives will also allow you to estimate the total search area you can manage in your area during each dive.

2.3. Underwater Visual Census Transects

Transects are straight lines that are laid down on the substrate to mark the census area. Plastic measuring tape, rope marked with numbered tags, or a weighted line of known length may all be used to mark a transect. The search area of your transect is given by the length of the transect line multiplied by its width, usually the sum of a predetermined distance on either side of the transect line. This type of transect is called a belt transect. Transects used in underwater visual surveys of seahorses are typically 20-50m long and 2-5m wide depending on water clarity (visibility), substrate type, diving conditions and the density and mobility of the species being surveyed. For example, Project Seahorse members working in Portugal, use three randomly placed 30m transects and depending on the visibility, habitat complexity and density of

seahorses, the transect width is either 2 or 4m. In Portugal, the probability of encountering seahorses on each transect is quite high (>95%). In the Philippines, however, Project Seahorse carries out underwater visual surveys with six to eight randomly placed 50m x 5m transects because the probability of encountering seahorses on each transect is lower.

Once you've decided on your transect length and width, carry out a few practice census dives to fine-tune your underwater and data-collection skills. During these practice dives, have your buddy search through an area you've just searched to check your seahorse search skills. If you miss some, keep developing your search image until you feel confident about your ability to find seahorses in your study area. Practice measuring seahorses as well and have your buddy measure the same ones you measure to ensure that you are both measuring consistently and correctly (see Project Seahorse Technical Report No. 4).

Have a look at the data you collected during your practice dives. If there is a high degree of variance in your data among your first few transects you may need to carry out more transects per population assessment, that is increase your sample size. Variance measures how much spread there is among samples and is used to make inferences about how much confidence one should have in the estimated mean of a parameter. If variance in a population-level parameter or habitat variable is high among your practice transects, then increase the number of replicate transects.

If one of your end goals is to use the size distribution of seahorses to estimate growth and survival rates, you should aim to sample at least 200 animals per survey. This is likely to involve searching many more transects than you might need for your abundance estimate. Keep in mind that there is a trade-off between cost and precision. The more you sample, the more precise your parameter estimates will be, but you may need to secure additional funding to carry out your assessments.

2.4. Transect Locations

Once you've selected your study area and familiarized yourself with the major habitat types within it, randomly select starting points for your transects. Random selection is important to make the study accurate and valid. There are many subtle environmental factors that determine how many fish will be present in an area, and in which patches of the area they will choose to live (e.g., tide and current patterns, habitat type, food availability, light exposure, proximity to shore, salinity). If you were to choose your transect locations without randomizing, these factors might bias your choice of transect locations, with the consequence that your study would not cover a representative portion of the study area. Avoid haphazardly selecting starting points for transects: haphazard sampling often results in under-sampling marginal habitats and will invalidate any inferences you make about the population based on your survey results. Systematic sampling - sampling in a strictly regular fashion - is a good method of determining the distribution of a species, however the usual formula for sample variance does not apply when samples are systematically collected (King 1995). By randomly locating transects you will hopefully account for natural and unpredictable variations in these environmental factors while also eliminating bias. If you decide to stratify your sampling according to habitat type, randomly select at least three transect starting points for each habitat type you will survey.

Even when your selected study area is a regular artificial structure, the starting point and compass direction of your transects should be randomly selected. Examples of random selection are outlined in two appendices: Appendix 3 describes the selection of transects on a vertical structure where you will examine the fishes associated with the structure at various depths and Appendix 4 describes the selection of transects on the sea floor near a structure such as a dock.

Figure 1 below shows an example of transect selection in a small bay. You can randomly select starting points for your transects by following these steps:

- Obtaining maps of your study area that are marked with latitudinal and longitudinal units. Use a random number generator (available in Excel or any statistics textbook) to randomly select coordinates as transect starting points and compass degrees for determining the direction in which the transect will be laid. If you don't have access to a random number generator, draw equally sized and numbered pieces of paper to randomly select the transect coordinates and orientation (consult Zar 1999 for more details).
- 2 Enter the coordinates and orientation into a global positioning system (GPS) and mark them on your map. If you don't have access to a GPS unit, see Appendix 7 for suggestions on land-based navigation.
- 3 Ensure that the transects fall entirely within the subtidal zone (water line at low tide) and that none of the transects overlap. Your transects should be at least 15-20m away from each other, or far enough away from each other that they will be independent samples (Consult Zar 1999 for more details about sample independence). You want to minimize the probability that individuals from one transect will swim onto other transects during your assessment, or that individuals on one transect will be disturbed by your activities on nearby transects. Keep selecting new coordinates until you have the desired number of transects plotted onto your map.

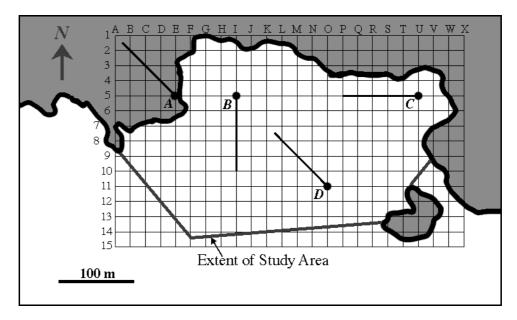


Figure 1. Map of study area and demonstration of transect selection.

2.5. Pre-Dive Preparation

Gather your sampling equipment together and go over communication, SCUBA diving plan and survey protocols with all team members.

Ensure all observers are comfortable with survey and measurement protocols.

Ensure SCUBA safety equipment is working and in place (e.g. cell phone, dive flags).

Take note of the location, the transect number, the GPS co-ordinates, team members and tide, wind, sea and cloud conditions (see Appendix 7 for estimating cloud cover). Remember to take compass bearings before descending to facilitate underwater navigation.

2.6. Laying Down Transects

Transects can be laid out using a plastic measuring tape, held in place temporarily with tent pegs and rolled up after the area is searched and the habitat has been described. Alternatively, you can use a weighted and line measured out at intervals (e.g. Bell *et al.* 2003) – although this may be heavier to handle both below and above water. Peg the beginning of the transect down, and as the rope is laid out, each diver should scan their side of the transect line looking for seahorses. When a seahorse is located, record all the relevant information and continue. Once the outbound swim is complete peg down the other end of the transect. The two divers can then swim back along the fixed transect noting habitat features (e.g. type, % cover, vegetation/habitat height, sediment composition, slope, depth, topographical features and associated species). After all the data have been recorded, be sure to collect all equipment (e.g. pegs, transects) before surfacing.

2.7. Carrying Out The Observations

Your methods will be very similar whether you carry out your survey on natural substrates or on artificial structures associated with the sea bottom (e.g. base of pilings, lost fishing gears). When laying down transects, do so as calmly as possible remaining as high above the bottom as possible (>0.5m). One person can slowly roll out the transect line while the second person gently pegs the line in place with tent pegs.

At the beginning of the transect, record the depth, visibility and temperature and take a sample of water (in a clean, pre-labeled plastic bottle) for salinity measurements if you have a salinity meter. Record the time when you are ready to begin searching the transect.

Record the substrate type (e.g. silt/mud, fine sand, coarse sand, shell fragments, rock, coral). If it's an artificial substrate, record what the structure is made of (e.g., wood, concrete, nylon netting). Also record the dimensions of the structure (e.g., diameter of pilings, bar size and/or stretched-mesh size of netting (see Appendix 9), size of rope in netting) and describe any regular or prominent attachments to the structure (e.g., anchor lines, nails, spikes, floats).

Note animal and plant communities (e.g. seagrass bed, sandy bottom dominated by sea urchins).

Record any other information that you think might be important (e.g. presence of debris, interactions between seahorses and other animals, unusual diving conditions, description and reasons for deviations from sampling protocol).

The two divers should swim together slowly, one on each side of the transect, and scan for fishes within the predetermined distance from the transect line (usually 1-2.5m). By swimming together, you will be less likely to record the same individual more than once. If the substrate is vertical (e.g. concrete wall, predator net), the two divers should swim together slowly and scan for fishes with diver A on the top half of the transect and diver B on the bottom half (see Appendix 6).

Seahorses camouflage themselves by changing colours to match their backgrounds. They also remain quite still for extended periods of time, and are therefore difficult to spot. You will have to look quite closely and carefully at the substrate, and you should gently brush aside any seaweed or other covering that may obscure your view. You should be especially on the lookout for tails that are wrapped around small objects—they are much easier to spot than heads, which can be hidden. Look for slow movements among seagrass shoots or macro algae and take the time to look ahead for any seahorses that may be swimming off the transect as you approach. If you find these, gently catch them, record the information below and the location where you first saw them.

When a seahorse is located, fill in the information for each column. To save time underwater, you can use abbreviations as long as you clearly explain their meaning

somewhere in your data and write out the full results so that you will remember what they are and others will be able to understand them.

Note the fish's behaviour. Typical behaviours include stationary, feeding (sucking motion), foraging, swimming, aggressive interaction (chasing, snapping, tail wrestling) courtship (tail wrapping, quivering, brightening, promenading) and mating. Record anything unusual about the fish's behaviour (e.g. trapped in a net, unresponsive, dead).

After habitat use, depth, time and location (along and away from transect) have been recorded, gently remove the fish from its holdfast or microhabitat. Measure the standard length of pipefishes (tip of snout to tip of tail) and the height and trunk length of seahorses to the nearest mm (Measuring Seahorses Technical Bulletin). Next note species, sex, reproductive status and condition. Note that sometimes juveniles and adults look somewhat different. For example, inexperienced divers often mistake juvenile *Hippocampus guttulatus* for adult *Hippocampus hippocampus* because neither of these have the long skin filaments that are characteristic of adult *Hippocampus guttulatus*. Be sure to use as many characters as possible to distinguish between species.

To measure seahorses, gently press the seahorse against the ruler and clipboard with one hand, holding the head perpendicular to the trunk with your thumb. Use the other hand to gently uncurl the tail to measure height and trunk length. Seahorse height is measured from the top of the animal's coronet (the crown-like structure at the top of the head) to the tip of its tail. Seahorse trunk length is measured from the last trunk ring (just above first tail ring) to the intersection between the first trunk ring and the cleithral ring. Refer to the Project Seahorse Technical Report No. 4 for line drawings and details. Be patient with seahorses and often they will settle down long enough to measure. If not, try to obtain at least the trunk length: height can be estimated from a regression of height against trunk length obtained from the rest of your data.

Sex is difficult to determine in juvenile seahorses. In seahorses, mature males will have fully developed brood pouches and can easily be distinguished from the females. Juvenile male seahorses on the threshold of maturity can be distinguished by small oval skin flaps beneath their abdomens that gradually form a narrow slit. These are the early stages of pouch development and are difficult to spot without practice. Some male pipefish brood embryos beneath fleshy folds of skin and others have their eggs glued ventrally. When male pipefish are not brooding young, sex determination may be more difficult. Consult your identification guidebooks for useful hints on sex determination in the species of interest. Foster and Vincent (in press) summarize size at 50% maturity, which will give you an idea of the size at which your species becomes a mature adult. If you cannot determine the sex, indicate it as unknown.

Identifying the reproductive status of male seahorses is relatively straightforward: if a male seahorse is brooding young (swollen pouch in seahorses), then he is pregnant, otherwise he is non-pregnant (empty pouch in seahorses). Gently feel the pouch to ensure that it is indeed full because sometimes non-pregnant males inflate their pouches with water. The reproductive status of females is difficult to assess using external characteristics and is not generally recorded during population assessments.

Look for injuries (clipped tail, missing fin), parasites, lesions or other signs that might indicate environmental stresses and record them on your data sheets.

In most cases, when you return an animal to its holdfast or microhabitat, it will remain there. But in cases when the fish is determined to swim away, gently encourage it to swim away from the area you or your partner are about to search. This will lower the probability of recording the same animal more than once during a survey.

It is tempting to include seahorses in your survey data that fall outside your search area, however, doing this will inflate estimates of density and bias your data. If you encounter

seahorses outside your predetermined search area, you may record all the information about them (species, sex, size, reproductive status, condition, habitat use etc...), but make sure to note that this fish was *outside* the search area and exclude the animal from your estimates of density. The information you collect from that fish will still be valuable for quantifying the structure of that community (species composition, sex ratio, size structure, reproductive activity).

Once you reach the end of the transect record the time and depth again.

You may choose to quantify habitat more rigorously than described above using a number of methods (consult English *et al.* 1994). Line intercept methods and quadrats are two good ways of quantifying the percent cover of different habitat types in your search area. In order to quantify habitat cover using the line intercept method, record the proportion of your transect that crosses over different habitat types. Typically this is done to the nearest 10cm, but your precision can be as coarse as you need it to be. In order to use the quadrat method, randomly select the location of 3-5 quadrats per transect. When you arrive at the location, mark out the corners of your quadrat using the ruler on your clipboard. Then estimate the percent cover of benthic organisms or substrate within your quadrat. Manageable quadrat sizes are $0.25\text{m}^2 - 1\text{m}^2$.

Conduct the same procedure for each transect, doing one or more transects per dive, depending on how much time it takes you to assess each transect.

2.8. Diving Hints

Plan your dive and dive your plan. Discuss and agree upon your dive protocol before getting into the water. This will make your sampling more efficient and less likely to deviate from the UVC protocol. During the dive, maintain visual contact and communication with your buddy.

During the entire survey, maintain neutral buoyancy at a minimum of 0.5 - 1m above the sediments. Skull with your fins side to side rather than using scissor kicks: this will prevent the bottom being disturbed by your finning motion.

Plan your dives according to depth: do the deepest dive first and respect your time limits at various depths.

If there is a current, orient yourself so that you are facing into the current. If the current is cutting across your transect at an angle, make sure that the diver (diver A) who's body is crossing onto the second diver's (diver B) side of the transect is down-current of diver B so as not to obstruct diver B's search area.

Clip all of your equipment to yourself with the exception of your transects. This will prevent loss of equipment and data. You can clip clipboards or underwater notebooks to each diver. Diver A can carry a bag with tent pegs, while diver B can carry a bag with plastic measuring tape(s), sample bottles, etc).

2.9. Key Points To Remember

It is important to carry out population surveys in the same manner each time so that population parameters can be compared and trends extracted from the data. If possible, always dive with the same buddy to prevent differences in search skills from biasing your data. If you can't always dive with the same person, then ensure that your dive buddy's search skills are similar to yours by each carrying out several practice surveys within the same area and comparing notes.

Transect locations must be selected randomly within your search area, otherwise your data may be biased.

It is important to not make assumptions about seahorse distribution and population structure prior to your surveys. For example, although most studies suggest that seahorses are associated

with seagrasses in temperature ecosystems (Foster & Vincent in press), *Hippocampus abdominalis* is frequently observed on bare sandy substrate in Tasmania and *H. hippocampus* appears to prefer open sand flats to densely covered vegetation in Portugal.

In order to estimate density, the total search area and the number of animals encountered within that search area must be known. Do not include animals encountered outside your search area in your density estimate.

After the assessment, enter your data into a worksheet (e.g. Excel) or a database (e.g. Access database). Make sure that if you use abbreviations in your worksheets that these are clearly explained at the top. For more information about creating worksheets and databases as well as analyzing data see English *et al.* (1994) and Samoilys (1997).

Organize your data sheets according to date and location and store them in a secure place. Photocopy your data sheets and leave the photocopies with a colleague or friend. Similarly, back up your entered data and analysis results to prevent loss of work.

Disseminate your findings. If you publish or share your data and/or results with colleagues, biologists, fisheries scientists, environmental non-government organizations, Project Seahorse, politicians and the general public, your study will make a lasting contribution to the conservation of this unique group of fishes.

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Appendix 1: Underwater Survey Equipment Checklist.

This list is meant to be a guide and may not be exhaustive for your diving conditions.

| Equipment | Purpose and Hints |
|---|--|
| Compass | Taking bearings for underwater and land based |
| - | navigation. |
| Data sheets | Pre-print data collection tables onto waterproof |
| | paper. "Aqualaser" paper is available from |
| | www.aquascribe.com and it can be |
| | photocopied or printed onto directly. Using |
| | data sheets is better than a bound notebooks |
| | because if you lose your notebook, you may |
| | lose all of your data. If you lose a sheet, at |
| | least you still have all the other data you |
| | collected to date! |
| Clipboard with ruler. | Look for transparent clipboards with rulers |
| 1 | built into the side of them. Otherwise, glue |
| | rulers on to one full length of the clipboard to |
| | facilitate measuring fish and the distance away |
| | from the transect. |
| Pencils | Tie them on with rope to clipboards, bring a |
| | spare pencil or dive knife for sharpening. You |
| | can purchase graphite pencils that have a larger |
| | diameter without wood: these are less likely to |
| | break underwater. |
| Flagging tape | Useful for marking permanent transects and |
| | tying to dark equipment under low visibility |
| | conditions. |
| Floats | Useful for marking permanent transects |
| Hammer | Useful for driving pegs / posts into substrate |
| | for permanent transects. |
| Pegs | Use light plastic pegs for laying down |
| | temporary transects in soft bottoms. Heavier |
| | pegs will be needed to keep permanent rope |
| | |
| | transects in place. |
| 30 – 50m transects | For laying out temporary transects and |
| 30 – 50m transects | • |
| 30 – 50m transects | For laying out temporary transects and measuring habitat dimensions (height of shark net, distance between pilings, length of a |
| 30 – 50m transects | For laying out temporary transects and measuring habitat dimensions (height of shark net, distance between pilings, length of a seagrass bed). Plastic measuring tapes (20–50 |
| 30 – 50m transects | For laying out temporary transects and measuring habitat dimensions (height of shark net, distance between pilings, length of a seagrass bed). Plastic measuring tapes (20–50 m in length) are easily purchased at hardware |
| 30 – 50m transects | For laying out temporary transects and measuring habitat dimensions (height of shark net, distance between pilings, length of a seagrass bed). Plastic measuring tapes (20–50 m in length) are easily purchased at hardware or outdoor stores and their plastic handles |
| 30 – 50m transects | For laying out temporary transects and measuring habitat dimensions (height of shark net, distance between pilings, length of a seagrass bed). Plastic measuring tapes (20–50 m in length) are easily purchased at hardware or outdoor stores and their plastic handles makes working with them convenient |
| 30 – 50m transects | For laying out temporary transects and measuring habitat dimensions (height of shark net, distance between pilings, length of a seagrass bed). Plastic measuring tapes (20–50 m in length) are easily purchased at hardware or outdoor stores and their plastic handles makes working with them convenient underwater. Measuring tapes can also be used |
| | For laying out temporary transects and measuring habitat dimensions (height of shark net, distance between pilings, length of a seagrass bed). Plastic measuring tapes (20–50 m in length) are easily purchased at hardware or outdoor stores and their plastic handles makes working with them convenient underwater. Measuring tapes can also be used to estimate horizontal visibility. |
| 30 – 50m transects Rope | For laying out temporary transects and measuring habitat dimensions (height of shark net, distance between pilings, length of a seagrass bed). Plastic measuring tapes (20–50 m in length) are easily purchased at hardware or outdoor stores and their plastic handles makes working with them convenient underwater. Measuring tapes can also be used to estimate horizontal visibility. Durable rope marked out at 1m intervals for |
| | For laying out temporary transects and measuring habitat dimensions (height of shark net, distance between pilings, length of a seagrass bed). Plastic measuring tapes (20–50 m in length) are easily purchased at hardware or outdoor stores and their plastic handles makes working with them convenient underwater. Measuring tapes can also be used to estimate horizontal visibility. Durable rope marked out at 1m intervals for permanent transects. Mark rope such that |
| | For laying out temporary transects and measuring habitat dimensions (height of shark net, distance between pilings, length of a seagrass bed). Plastic measuring tapes (20–50 m in length) are easily purchased at hardware or outdoor stores and their plastic handles makes working with them convenient underwater. Measuring tapes can also be used to estimate horizontal visibility. Durable rope marked out at 1m intervals for permanent transects. Mark rope such that location along transect will be easy to |
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| | For laying out temporary transects and measuring habitat dimensions (height of shark net, distance between pilings, length of a seagrass bed). Plastic measuring tapes (20–50 m in length) are easily purchased at hardware or outdoor stores and their plastic handles makes working with them convenient underwater. Measuring tapes can also be used to estimate horizontal visibility. Durable rope marked out at 1m intervals for permanent transects. Mark rope such that location along transect will be easy to determine. Global positioning system, if available for |
| Rope GPS unit | For laying out temporary transects and measuring habitat dimensions (height of shark net, distance between pilings, length of a seagrass bed). Plastic measuring tapes (20–50 m in length) are easily purchased at hardware or outdoor stores and their plastic handles makes working with them convenient underwater. Measuring tapes can also be used to estimate horizontal visibility. Durable rope marked out at 1m intervals for permanent transects. Mark rope such that location along transect will be easy to determine. Global positioning system, if available for marking transect locations. |
| Rope | For laying out temporary transects and measuring habitat dimensions (height of shark net, distance between pilings, length of a seagrass bed). Plastic measuring tapes (20–50 m in length) are easily purchased at hardware or outdoor stores and their plastic handles makes working with them convenient underwater. Measuring tapes can also be used to estimate horizontal visibility. Durable rope marked out at 1m intervals for permanent transects. Mark rope such that location along transect will be easy to determine. Global positioning system, if available for marking transect locations. For determining study area and transect |
| Rope GPS unit Navigation charts, habitat maps | For laying out temporary transects and measuring habitat dimensions (height of shark net, distance between pilings, length of a seagrass bed). Plastic measuring tapes (20–50 m in length) are easily purchased at hardware or outdoor stores and their plastic handles makes working with them convenient underwater. Measuring tapes can also be used to estimate horizontal visibility. Durable rope marked out at 1m intervals for permanent transects. Mark rope such that location along transect will be easy to determine. Global positioning system, if available for marking transect locations. For determining study area and transect locations. |
| Rope GPS unit | For laying out temporary transects and measuring habitat dimensions (height of shark net, distance between pilings, length of a seagrass bed). Plastic measuring tapes (20–50 m in length) are easily purchased at hardware or outdoor stores and their plastic handles makes working with them convenient underwater. Measuring tapes can also be used to estimate horizontal visibility. Durable rope marked out at 1m intervals for permanent transects. Mark rope such that location along transect will be easy to determine. Global positioning system, if available for marking transect locations. For determining study area and transect locations. Useful for dive planning where currents can be |
| Rope GPS unit Navigation charts, habitat maps | For laying out temporary transects and measuring habitat dimensions (height of shark net, distance between pilings, length of a seagrass bed). Plastic measuring tapes (20–50 m in length) are easily purchased at hardware or outdoor stores and their plastic handles makes working with them convenient underwater. Measuring tapes can also be used to estimate horizontal visibility. Durable rope marked out at 1m intervals for permanent transects. Mark rope such that location along transect will be easy to determine. Global positioning system, if available for marking transect locations. For determining study area and transect locations. |

| ENOX clips | For clipping equipment bags and clipboards to BCD. |
|------------------------------|--|
| Light nylon bags | Transporting pegs, measuring tapes, sample bottles, etc. |
| Spare parts kit | Have extra data sheets, pencils, dive straps etc., on hand just in case! |
| Plastic bottles | For collecting water during your transect survey |
| Thermometer | Many dive computers or SCUBA gauges record temperature. |
| Salinity Meter | For suggestions on types of salinity meter, consult English et al. (1994). |
| Dive Safety Equipment | Dive flags, emergency plan, spare parts kit. |
| Species identification cards | Laminate line drawings or photographs of the local fauna and flora to help with identification underwater. |
| Underwater Camera | Photographing seahorses for future identification and presentations. Documenting habitat use and changes in habitat over time. |
| Waterproof flashlight | For searching in dense vegetation, among corals and in crevices. |
| Watch | Recording time |
| Appropriate SCUBA gear | You'll need a regulator (including depth and pressure gauges), BCD, tank, mask, fins, snorkel, weight belt and dive knife. The exposure suit you wear will depend on water temperature. We use drysuits in 10-14 C water, 7mm two-piece wetsuits in 15-22 C water and a simple skin suit (to prevent abrasions) even in 28-32 C. |

Appendix 2: Transect Selection And Marking For Vertical Artificial Structures

This appendix explains the procedure for selecting transects when assessing seahorse populations on an artificial structure, such as a predator net. The two diagrams below, Figure 2, show an example of a set of transects on a section of a predator net.

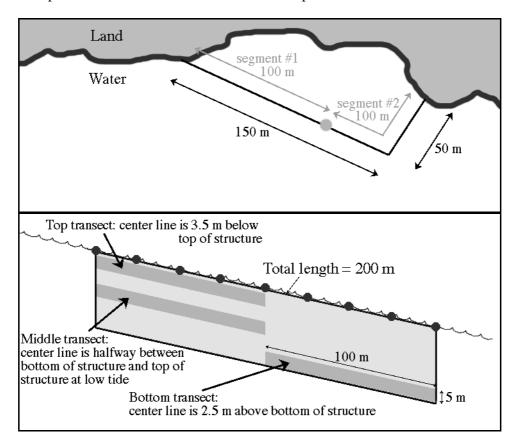


Figure 2. Demonstration of transect layout on a predator net. Top: a "map" view of the net from above. This diagram shows the division of the net into 100-m segments. Bottom: a side view of the net, shown as a single straight net for simplicity. The darker gray regions are the transects. Here in this example, the three transects are 100m long and 5m wide.

A2.1. Choosing The Transects Within The Study Area

The length and width of your of your transects will depend on diving conditions (depth, visibility, diver experience) and seahorse density. You will need to randomly select the starting points of your transects as well as orientation and starting depths, keeping in mind the following:

- the top of the shallowest transect should be at least 1 m below the surface at low tide: if your transect width is 4m, then the centre line of your transect should be at least 3m below the surface of the water at the lowest low tide.
- the bottom of the deepest transect can be as deep as the artificial structure, while respecting dive table constraints.
- When surveying your transects on a vertical structure, remember to survey the deepest transect first, and the shallowest last.

To help with the selection of transects, you should draw a plan of the structure as shown in Figure 2, eliminating parts of the structure that are not searchable for safety or other reasons. As you randomly select starting points, orientations and depths, ensure that all transects will fit onto the structure and that they are all at least 15-20m from each other. If this is not possible, (i.e. your structure is too small to fit three transects that are spaced at least 15-20m apart), you

will need to consider whether placing transects closer together will cause them to be non-independent samples. Draw your transects onto your map.

A2.2. Marking The Transects

If you decide to use permanent transects on the artificial structure, you will need to mark their locations at least 3 days before carrying out your first population assessment. Transects on artificial structures can be marked with flagging tape, brightly coloured clips, pegs or rope. Try to mark our your transects with numbered tags to facilitate the location of fish during your surveys (see Fig. 3).

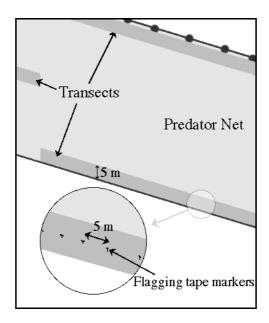


Figure 3. Diagrams of marked transects on a predator net. The transects are shown in dark gray. In the inset, the small black marks represent flagging tape used to mark the center of the transect every 4-5 m.

Appendix 3: Transect Selection And Marking For The Sea Floor Near Artificial Structures

This appendix explains the procedure for selecting transects when assessing seahorse populations on the sea floor near an artificial structure, such as a dock.

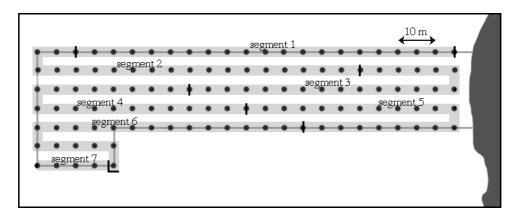


Figure 4. A plan of the pilings of a dock, demonstrating the selection of transects along the sea floor at the bottom of the pilings. In this example the light gray area is a 5-m-wide strip that will be used as the basis for the transects. This strip is divided into 100-m-long segments, three of which are selected for assessment of seahorses.

A3.1. Choosing Transects Within The Study Area

The structure around which you will be assessing fish populations will help immensely in the placement of your transects.

- 1. Draw a plan of the structure as shown in Figure 4. This diagram should show the distance between the parts of the structure.
- 2. Draw a continuous line that passes through all portions of the structure. In Figure 4, this is shown as a light gray strip that passes through all the pilings. Randomly select starting points, orientations and depths while ensuring that all transects will fit onto the structure and that they are all at least 15-20m from each other. If this is not possible, (i.e. your structure is too small to fit three transects that are spaced at least 15-20m apart), you will need to consider whether placing transects closer together will cause them to be non-independent samples. Draw your transects onto your map.
- 3. Note that if the pilings have a wide diameter (e.g. >20cm), this may greatly bias your estimate of density (i.e. your actual search area will actually be less than your transect area). In this case, you may choose to run your transects between the pilings, or calculate the total area taken up by the pilings and substract it from the transect area.

A3.2. Marking The Transects

1. Find the pilings at either end of each segment that you've selected. Mark these at the top of the piling or structure so that you can find them before descending. To mark the structure, tie a piece of flagging tape around it. Descend to the bottom of the structure and mark several pilings along each transect. We suggest that you mark the transect at least every 1-2 m.

Appendix 4: Data Sheets

The following are examples of data sheets that you may want to use for data collection:

- 1. The first sheet is an example of a "Transect Record Sheet." This sheet has space to record most of the general information about the transects. It has 6 sections:
- The first section contains very general information on the dive, and places to record temperature, salinity and time.
- The second section has space to record the start and end times and depths of each transect.
- The third section is for recording a description of the substrate.
- The fourth section is the bulk of the sheet; it has space to record data on the fishes.
- The fifth section is an open space to record any other information as you see fit.
- The sixth section contains a list of abbreviations that you may find useful for recording data.

Each diver should carry several copies of this sheet. For example, in Portugal where seahorse densities are high, we carry enough sheets to record observations for a total of 50 fish each.

- 2. The second sheet is the "Surface Data Sheet" which should be used in conjunction with the first sheet. Someone on the surface (e.g. boat skip) should use this sheet to record environmental observations, or the divers prior to diving should collect the data.
- 3. The third sheet combines the Transect Record Sheet and the Surface Data Sheet into one sheet and includes a space to record line intercept data for habitat. A separate sheet is used for each transect, and sometimes This sheet is filled in with examples of data collected.
- 4. The fourth sheet is similar to the third sheet, but it contains a space for recording the percent cover of benthic organisms using a quadrat method. Again, examples of data are included in this sheet.

You should feel free to customize your data collection sheets as long as they contain sufficient space to record the essential data. Make sure you fill in all the information while you are on site; it is very easy to forget details even within a few hours! Enter your data into a spreadsheet as soon as you can and file your data sheets into a binder for future reference. Make copies of all your work (paper and electronic) to back up your data.

1. Transect Record Sheet (to be used in conjunction with a Surface Data Sheet)

| Diver's Name: JC | Area: Hudson Bay | Dive start time: 3:45pm | Dive end time: 5:56pm | Maximum Depth: 8.1 m |
|-------------------|--------------------|-------------------------|-------------------------------|----------------------|
| Date: 9 July 2002 | Salinity: 36.7 ppt | Temperature: 4 °C | Notes: Very cold, but good vi | sibility >5m. |

| Transect | Start time | Start depth | End time | End depth |
|-----------|------------|-------------|----------|-----------|
| 1/Deep | 3:56 pm | 23 ft | 4:28 pm | 19 ft |
| 2/Middle | 4:33 pm | 20 ft | 5:14 pm | 22 ft |
| 3/Shallow | 5:27 pm | 19 ft | 5:48 pm | 16 ft |

Substrate description:
Sandy bottom dominated by ascidians, anemones and macroalgae, mostly *Ulva* spp., but also *Sargassum* and some unidentified algae.

| Species | Transect name/# | Depth (m) | Dist. along transect (m) | Dist. away (m) | Trunk (cm) | Height (cm) | Sex | Reprod. State | Habitat | Behaviour | Other Comments |
|---------|-----------------|-----------|--------------------------|-------------------|------------|-------------|-----|------------------|---------|-----------|-------------------|
| HG | 1 | 6.7 | 20.4 | 1.8 | 4.0 | 13.8 | M | NP | SG | For | Feeding from sand |
| | | | | | | | | | | | |
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| Miscellaneous notes: | <u>Habitat</u> | <u>Species</u> | Height = top of | Sex | Reprod State | Behaviour |
|--|----------------|----------------------|----------------------------|--------------|--------------|------------------|
| have taken photographs for | SG = | Hg = Hippocampus | coronet to tail tip | M = Male | N = Not | Sed = Sedentary |
| identification of algae and | seagrass | guttulatus | <u>Trunk</u> = midpoint | F = Female | pregnant | Crt = Courting |
| fish. | SD = | Hh = Hippocampus | of cleithral ring to | J = Juvenile | P = Pregnant | For = Foraging |
| | Sand | hippocampus | last trunk ring | ? = Unknown | N/A for | Sw = Swimming |
| | Ul = Ulva | Sa = Syngnathus acus | Standard = $tip of$ | | Females | Oth = Other |
| | | | snout to tip of tail | | | |
| | | | 1 | | | |

| • | CC- | D | (1) | 4 |
|----|---------|--------|---------|---|
| Z. | Surface | · Keco | ra Snee | 1 |

| Date: | Location: |
|------------------------------------|-----------|
| Site: | |
| Area and Transect Number(s): | |
| GPS Co-ordinates: | |
| Diving team: | |
| Surface Supporter / Boat operator: | |

Tide (please circle)

Low 1 hour either side of low water 1 hour either side of high water High

The period between high and low water The period between low and high water Falling Rising

Wind (please circle one)

| , , (5 _ 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 | | | | |
|--|-------|-------|--|--|
| Beaufort | Wind | speed | | |
| Scale | knots | km/hr | | |
| 0 | <1 | <1 | | |
| 1 | 1-3 | 1-6 | | |
| 2 | 4-6 | 7-12 | | |
| 3 | 7-10 | 13-19 | | |
| 4 | 11-16 | 20-30 | | |
| 5 | 17-21 | 31-39 | | |
| 6 | 22-27 | 40-50 | | |
| | | | | |

Sea (please circle one)

Mirror-like to small Calm

ripples

Slight Large wavelets, crests

breaking

Many white caps forming Moderate

Large waves, 2-3m, Rough

white caps

Cloud Cover: Number of sectors with cloud cover: _____

3. Transect Record Sheet with Line Intercept Method for Habitat Quantification

| Date | 2 Oct 2002 | TRANSECT # | 1 | Depth Start | 2.3m | Current Strength | Slow |
|----------------|------------|-----------------------|------------|--------------|-----------|----------------------------|----------|
| Time Start | 7:49 am | Area | Barra | Depth Finish | 2.1m | Current Direction (from) | NW |
| Time Stop | 8:34 am | GPS North | 37.58.220 | Salinity | 37 ppt | Wind Strength | Mod. |
| Names(s) | JC & VS | GPS West | 007.53.320 | Water Temp | 78 F | Wind Direction (from) | W |
| Search area | 30m x 2m | Orientation (towards) | 275 ° | Other notes | fish with | "*" were found outside sea | rch area |
| Substrate type | Sand | Visibility | 2.5m | | | | |

Hg = Hippocampus guttulatus, Hh = Hippocampus hippocampus, Sa = Syngnathus acus, St = Syngnathus typhle.

X is the distance along the transect line from the starting point of the survey, Y is the perpendicular distance from the transect line (0-2.5m)

| Depth | X (m) | Y (m) | Trunk (cm) | Height or Total (cm) | Sex | Species | Reproductive Status | Habitat | Behaviour | Other (e.g. description of injuries, color pattern, interactions, etc) |
|-------|----------|----------|---------------|----------------------|-----|---------|---------------------|-------------------|---------------------|--|
| 2.1 | 1.9 | 0.4 | 3.5 | 12.8 | M | Hg | Not pregnant | Seagrass | Greeting | Brightening, tail wrapping, promenading |
| 2.1 | 1.9 | 0.4 | 3.6 | 13.0 | F | Hg | N/A | Seagrass | Greeting | Brightening, tail wrapping, promenading |
| 1.9 | 2.3 | 0.8 | 2.1 | 6.5 | ? | Hg | juvenile | Seagrass blade | Stationary | No skin filaments |
| 1.7 | 6.6 | 0.2 | N/A | 26.3 | F | Sa | N/A | sand | Swimming | |
| 1.8 | 18.4 | 1.1 | 3.4 | 12.6 | F | Hh | N/A | Codium spp. | Feeding, stationary | * |
| 2.0 | 21.4 | .04 | 4.2 | 14.2 | M | Hg | Pregant | Tunicate | Stationary | Very thin, missing tip of tail |

Line Intercept Habitat Data:

| From | То | | From | То | | From | То | General Notes |
|------|------|----------------------|------|------|----------------------|------|----|------------------------------|
| 0 | 2.4 | Seagrass (C. nodosa) | 27.8 | 29.2 | Seagrass (Z. noltii) | | | there were many juvenile |
| 2.4 | 6.7 | Sand | 29.2 | 30.0 | Macro algae (Ulva) | | | Diplodus spp., an octopus, |
| 6.7 | 21.0 | Seagrass(C. nodosa) | | | | | | and a discarded refrigerator |
| 21.0 | 27.8 | Sand | | | | | | |

4. Transect Record Sheet with Quadrat Method for Habitat Quantification

| Date | 2 Oct 2002 | TRANSECT # | 3 | Depth Start | 2.3m | Current Strength | Strong |
|----------------|------------|-----------------------|------------|--------------|--------|--------------------------|--------|
| Time Start | 7:49 am | Area | Mar Menor | Depth Finish | 2.1m | Current Direction (from) | SSE |
| Time Stop | 8:34 am | GPS North | 38.57.610 | Salinity | 38 ppt | Wind Strength | Calm |
| Names(s) | JN & CML | GPS West | 014.13.821 | Water Temp | 85 F | Wind Direction (from) | N |
| Search area | 50m x 5m | Orientation (towards) | 15 ° | Other notes | | | |
| Substrate type | Sandy | Visibility | 6.5m | | | | |

Hg = Hippocampus guttulatus, Hh = Hippocampus hippocampus, Sa = Syngnathus acus, St = Syngnathus typhle.

X is the distance along the transect line from the starting point of the survey, Y is the perpendicular distance from the transect line (0-2.5m)

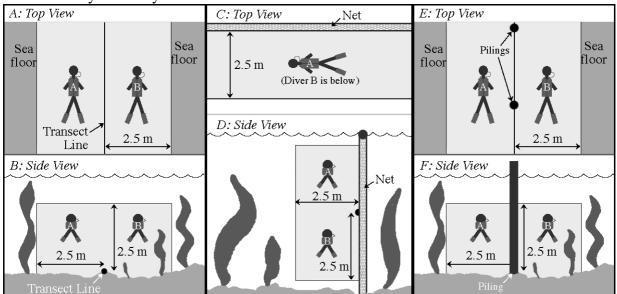
| Depth (m) | X (m) | Y (m) | Trunk (cm) | Height or Total (cm) | Sex | Species | Reproductive Status | Habitat | Behaviour | Other (e.g. description of injuries, color pattern, interactions, etc) |
|-----------|----------|----------|------------|----------------------|-----|---------|---------------------|----------|------------|--|
| 3.6 | 12.4 | 0.2 | 2.6 | 6.8 | M | Hg | juvenile | seagrass | Stationary | Has oval skin fold developing |
| | | | | | | | | | | |
| | | | | | | | | | | |
| | | | | | | | | | | |

| Locatio | n of Quadrat 1: 16-17 m, right side | Location | of Quadrat 2: | Location of Quadrat 3: | | |
|---------|-------------------------------------|----------|---------------|------------------------|-------------|--|
| % | Description | % | Description | % | Description | |
| 60 | Seagrass (Cymodocea nodosa) | | | | | |
| 10 | Shell fragments | | | | | |
| 20 | Macro-algae (Ulva lactuca) | | | | | |
| 10 | Various ascidians | | | | | |
| | | | | | | |
| | | | | | | |

Appendix 5. Extent Of Transects.

The following are diagrams of the volume that is to be examined for the presence of seahorses for each type of substrate. Some miscellaneous points to note on all three diagrams:

- The actual area that is to be examined (i.e., the transect) is shaded in light gray in all diagrams.
- The vertical distance in diagrams B, D and F has been greatly under-represented. We've shown the water surface, sea floor and seaweed to give you a sense of perspective, but you may survey some of your transects in much deeper water.
- The divers are included in the drawings to give you a sense of where you will swim, but you will of course need to move around to get a closer look at some fishes. The divers in diagrams B, D and F are swimming away from your view point ("into" the paper).
- Note the similarity between diagrams A and E, and between B and F. This reflects the similarity in the methods, and that the only difference in the two assessments is that, around a dock, the pilings are used in place of a rope to determine the center of the transect.
- Note that in these figures, 2.5m is given as an example of the distance to search from the transect line. If the total transect width is 2m, then you will search 1m on one side while your buddy searches 1m on the other side. Similarly, if the total transect width is 4m, you and your buddy will each search 2m on either side of the transect line.



- A shows the extent of the transect to either side of the transect rope from a top view.
- **B** shows the same extent from the divers' perspective as they move along the transect line.
- C looks down on a predator net from above and shows the extent of the transect.
- **D** looks at the same transect from the divers' perspective as they move along the transect line. A solid structure, such as a wall, would be assessed in an identical manner to a predator net.

E shows the extent of the transect when examining a dock piling. This diagram shows a view of the transect area from above. Keep in mind that the deep transect will "rest" on the bottom, so the 2.5-m circle around the bottom of the piling should be examined for attached organisms.

F shows the same transect from an end view.

Appendix 6. Navigating From Land-Based Features

This section explains how you can find the site that you've selected for the start point of your transect. You will need three pieces of equipment: a marine chart of your study area, a compass and a protractor.

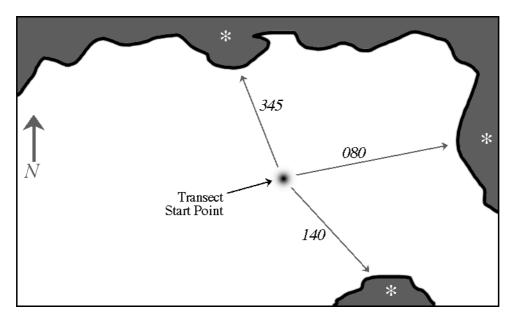


Figure 5. A marine chart showing how to find a point in mid-water by navigating from land-based features.

- 1. Once you've chosen the start point of your transect, mark it on the marine chart.
- 2. If they are not shown already on the chart, draw the positions of prominent land features (e.g., large rocks, islands, buildings, etc.) as accurately as possible. It is very important that these features be as close as possible to, or even within, the study area. In Figure 5, the features are shown as white asterisks.
- 3. Draw a straight line between the transect start point and the land-based features.
- 4. The chart should have some indication of compass bearings on it. If nothing else, it will indicate north with an arrow. Compass bearings are often represented in degrees, where north is 000, east is 090, south is 180 and west is 270. Extend the lines you've drawn as necessary and measure the angle that your line makes with the north line. This should give you a bearing from your transect start point to each land-based feature. These bearings are shown in italics in Figure 5.
- 5. Bring this chart with you on the boat. Move the boat to the general area where the transect will start.
- 6. Use the compass to read the bearings to each land-based feature.
- 7. Move the boat as necessary until the bearings that you read match those that you calculated on the chart. You are now at the transect start point!
 - This procedure can be done with as few as 2 land-based points, but 3 or 4 will make your navigation more accurate.

Appendix 7. Measuring Environmental Characteristics

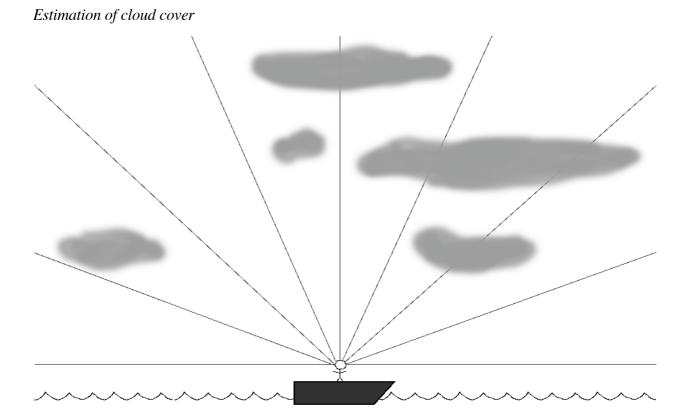


Figure 6. Estimation of cloud cover from a boat.

Here is a quick and easy method of estimating cloud cover prior to a popuation assessment. Pick any axis (e.g., north-south) and divide the sky into eight approximately equal sectors. Then count the number of sectors that have <u>any</u> degree of cloud cover, <u>no matter how thin</u>. This count is recorded as the cloud cover score on the data sheet. In the above example, the cloud cover score would be 5.

Appendix 8. Dimensions To Measure On Netting

If you are assessing seahorse populations associated with a net (e.g., a predator net), a variety of dimensions will be useful to measure, as listed on page. Among these dimensions are the bar size and stretched mesh size of the netting. These are described in Figure 7.

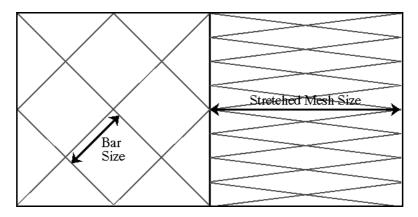


Figure 7. Demonstration of dimensions to be measured on netting. Left: the bar size is the length of the mesh from one junction to the next. Right: the stretched mesh size is measured across a mesh square while the mesh is being stretched as far as possible.

Appendix 9. Website Resources:

Australian Institute for Marine Science (AIMS): http://www.aims.gov.au/

FAO-ICLARM Online users Manual for the FAO-ICLARM Stock Assessment Tools, Fisat II, a statistical software package designed to help fisheries managers analyze data collected during population assessments and use results to manage fish populations. http://www.fao.org/fi/statist/fisoft/fisat/WebHelp/FiSAT2.htm

Random number generator: www.randomizer.org

The Secretariat of the Pacific Community (SPC) http://www.spc.org.nc/artreact.htm

The World Conservation Union: www.iucn.org

UNEP-World Conservation Monitoring Centre http://www.unep-wcmc.org

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