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POTENTIAL TECHNIQUES FOR MARKING AND TAGGING SEAHORSES

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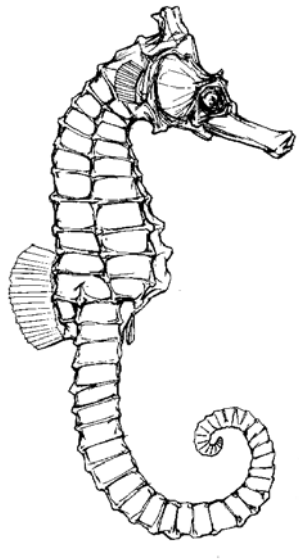

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PROJECT SEAHORSE TECHNICAL REPORT NO. 7: POTENTIAL TECHNIQUES FOR TAGGING AND MARKING SEAHORSES

1. Introduction

Marking seahorses for research and husbandry was first undertaken in the early 1990's. In the last fifteen years there has been notable progress adapting marking and tagging methods for perciform fish to the dissimilar morphology of seahorses, but published data remain scarce. Techniques developed for other taxa such as invertebrates may also be feasible and deserve greater attention (Levin 1990). At present, only one study in the primary literature specifically examines marking and tagging methods for seahorses (Woods & Martin-Smith 2004); other papers mention tagging and marking methods incidentally (Vincent & Sadler 1995, Bell *et al.* 2003, Moreau & Vincent 2004, Curtis & Vincent in press). Two projects have explicitly and incidentally examined the suitability of tagging systems: Le Cheminant (2000) investigated the relative efficacy of three marking systems in the Knysna seahorse, *Hippocampus 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 presently appear in the literature.

Since 1995, members of Project Seahorse and others have worked both *in* and *ex situ*, to develop a suite of marking techniques for seahorses. However, many of the techniques described here were pioneered in other fields and have only recently been applied to seahorses. When writing the original version of this manuscript, the editorial team decided to create two technical reports on marking and tagging. Proven marking techniques would be outlined in condensed form with step-by-step instructions where appropriate; this is Project Seahorse Technical Report 6 (Morgan & Martin-Smith 2004). This second lengthier technical report (this document) was designed to describe techniques that, in addition to those already tried, show promise or remain unexplored, as well as those methods that have proven infeasible or represent a poor investment of time, money and/or animals without significant modification. Contents include information from members of Project Seahorse, as well as colleagues at other institutions. We hope that this information will be of use to those trying to develop new marking methods for syngnathids and will save researchers investing efforts in already disproven techniques. Please also 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 relevant information that has not been addressed in this report (please contact: info@projectseahorse.org). All Project Seahorse Technical Reports can be downloaded as PDF files from our website at: http://seahorse.fisheries.ubc.ca/tech-reports.html

A summary of current techniques for tagging and marking seahorses and their potential utility is given below (Table 1):

Table 1. Report sections by relative certainty of method, from techniques that are known to work at left, to methods that have been disproven at right.

Proven	Promising	Unknown	Unpromising	Unsuitable
2.1. Collar tags 2.2. Visible Implant Fluorescent Elastomer (VIFE) injection 2.3. Passive integrated transponder (PIT) tags	3.1 Decimal coded wire tags (DCWT) 3.2. Elemental otolith marking 3.3. Elemental fingerprinting	4.1. Freeze branding 4.2. Panjet marking 4.3. Fluorescent grit marking 4.4. Other pigments, dyes, stains 4.5. Chemical otolith marking 4.6. Thermal otolith marking 4.7. Genetic markers 4.8. Parasite loads	5.1. Acoustic tags 5.2. Attachment tags 5.3.Satellite tags	6.1. Spine clipping 6.2. Fin clipping 6.3. Frond clipping 6.4. Visible Implant Alphanumeric (VIA) tags 6.5. Silver nitrate branding 6.6. Opalithplattchen (bee tags)

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 between unmarked and marked individuals. In contrast, a tag is a specific form of a mark that physically carries information on either an individual or a batch (Guy *et al.* 1996). 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, we use the verbs tagging and marking 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. Proven Techniques (summary of information from Technical Report No. 6)

2.1. Collar Tags

Collar tags are labeled oval PVC discs (3 mm x 5.5 mm), manufactured by Floy Tag & Manufacturing Inc. (www.floytag.com) that are attached around the neck of a seahorse with cord, to provide individual identification. Collar tags have been used successfully *in situ* in studies of *Hippocampus whitei* (Vincent & Sadler 1995), *H. comes* (Perante *et al.* 2002), *H. capensis* (Bell *et al.* 2003), *H. breviceps* (Moreau & Vincent 2004) and in *ex situ* populations of various species that include at least *H. capensis* (Tops 1999) and an unidentified strain of seahorse (Hughes 1999). Floy tag trials in captive populations of *H. capensis* showed no significant differences in mortality, disease outbreak, feeding rates, growth or behaviour between control and tagged individuals, suggesting that collar tags do not negatively affect seahorse survival (Le Cheminant 2000). No rigorous tests have examined the effects of Floy tags on wild populations. **Floy tags are not recommended in the following circumstances**: (1) In previously unmonitored populations where mobility/site fidelity has yet to be established as tags that are not regularly monitored and resized for growing animals are known to have caused lesions in two populations, (2) When animals are known to move outside regularly monitored areas, (3) If animals are < 40 mm SL as tags are known to impact smaller seahorses' swimming

ability and behaviour (C. Bull, pers. obs.). External marks like collar tags may also act as targets for predators (including fishers). Generally VIFE is recommended over collar tags.

2.2. Visible Implant Fluorescent Elastomer (VIFE) Tags

Visible Implant Fluorescent Elastomer is a marking system available from Northwestern Marine Technology Inc. (NMT) (www.nmt-inc.com) which is an injectable fluorescent polymer that, when mixed with a curing agent, hardens under the skin to leave a permanent, pliable, biocompatible mark. Visible Implant Elastomer is abbreviated to VIE in the case of non-fluorescent colours. Fluorescent elastomer is recommended over non-fluorescent VIE, for long term mark visibility, particularly for heavily pigmented species. Of the fluorescent colours, orange and red are the most easily confused with one another (Curtis in press) but are usually the brightest on dark pigment, followed by yellow and then green. VI(F)E should not be confused with VI (alphanumeric) tags which are described below, nor with microtaggant/visible implant filaments (VIF) which preceded the development of elastomer (Klar & Parker 1986, Buckley *et al.* 1994, Beukers *et al.* 1995, Bailey *et al.* 1998).

VIFE has been used successfully in both captive and wild populations of seahorses. In *ex situ* studies, VIFE has been used to tag *H .abdominalis* (Woods & Martin-Smith 2004) and *H. capensis* (Le Cheminant 2000). *In situ,* VIFE has been applied to wild populations of *H. abdominalis* (van Dijken 2001. Martin-Smith unpublished data), *H. comes* (A. Diwata & Sian Morgan unpublished data), *H. guttulatus* and *H. hippocampus* (Curtis in press) and *H. whitei* (K. Martin-Smith & J. Clark-Jones unpublished data). In all cases, marked individuals were ≥ 40mm SL, but it appears that VIFE may also be suitable to use in smaller individuals (Frederick 1997).

Seahorses have been marked in a variety of locations including the inside of the tail, the lower jaw and just above the operculum, but lateral body segments provide the greatest number of combinations to create convenient individual marks. All the field studies cited above have marked lateral body segments. Retention rates in captive populations of adult *H. abdominalis* were 100 % over 7 months (Woods & Martin-Smith 2004). Similarly, an *in situ* study of VIFE-marked *H. guttulatus* showed full retention for one year in 150+ individuals, ranging in size from 60-160mm SL (J. Curtis, pers. comm.). In contrast, approximately one third of double-marked *H. comes* individuals lost one tag within six months of tagging; some animals retained marks for one year. It is hypothesized that retention rates may differ due to interspecific differences in bony-plate structure (S. Morgan, unpublished data).

At present, the components can only be mixed in volumes that are large enough to allow several hundred marks, and once mixed, VIFE cures fairly quickly (few hours). However, trials are underway (late 2005) with a new formulation from NMT that can be mixed in much smaller quantities (K. Martin-Smith & D. Harasti pers. comm.). To prolong use, Project Seahorse researchers have successfully kept elastomer-filled syringes in freezers for up to a week (J. Curtis, pers. comm.) and for 24-36 hours on ice in styrofoam containers in the tropics, (S. Morgan, unpublished data).

2.3. Passive Integrated Transponder (PIT) Tags

Passive Integrated Transponder (PIT) tags are glass capsules, each containing a small transponder that has no internal battery, hence the term "passive" available from a variety of sources (e.g. www.allflex.co.nz, www.biomark.com, www.sokymat.com, www.ukid.co.uk). A reader powers (excites) the tag circuitry by radio frequency induction and receives the code back from the tag. Tags are injected or inserted through incisions, either intramuscularly, or into the body cavity. PIT tags have been used in one study to tag seahorses, where they were inserted near the ventral keel, into the intestinal space of *H. abdominalis* (Woods 2005). Intramuscular injection of PIT tags in seahorses seems unlikely because most species lack large muscle blocks. Woods (2005) noted that insertion of PIT tags into male brood pouches was not reliable, and six out of 14 animals in a pilot study expelled tags during courtship displays.

The greatest advantage of PIT tags is that they have the benefits of an internal tag (e.g. no external indication of their presence), while allowing the identification of individual animals without sacrifice (as per coded wire tags for example). Tags also pose no future risk to animals should they leave a study area in contrast to collar tags. In *H. abdominalis*, Allflex ISO FDX-B PIT tags (11.5 mm length, 2.1 mm diameter) were inserted into 24 treatment and 24 control animals. PIT tag retention over three months was 100% with no effect on survival or growth (Woods 2005). Similarly, injection of transponders was rated as simple and retrieval of tag codes was reliable using small hand-held readers.

Size may or may not be problematic, depending on the species and life history stage of interest. The smallest commercially available PIT tags are 8 mm x 2 mm (Woods 2005). Based on the fact that tags risk compromising buoyancy if they exceed 1.5% of the body weight (based however, on external vs. internal tags) (Ross & McCormick 1981) and using the smallest available tags, PIT tagging is a possibility for fish weighing ≥ 4 g. This makes it a realistic option for at least the adults of most seahorse species. Note that survivorship rates may stabilize at some threshold size and rate (Das Mahapatra *et al.* 2001).

Major drawbacks of the technique include applicability to field research and cost. At present, animals with small PIT tags need to be within 5-30 cm of readers (depending on the reader in question) in order for the tag to be read (A Callagher pers. comm.). Most portable readers are made for terrestrial use. This could mean handling and removing animals from the water for identification possibly disrupting behaviours of research interest. Recently, however, portable detectors have been used to detect PIT tagged fishes in small streams (Roussel *et al.* 2000, Barbin Zydlewski *et al.* 2001, Cucherousset *et al.* 2005). Furthermore arrays, large flat plate and annular detectors have all been used in freshwater to study the movements of PIT tagged fish (e.g. Armstrong *et al.* 1996, Lucas *et al.* 1999, Greenberg & Giller 2000, Riley *et al.* 2003, Axel *et al.* 2005). McCormick & Smith (2004) enclosed a PIT tag detector and data logger in a waterproof housing and used it to study visits of damselfish to nest sites on a shallow coral reef in Australia.

These studies indicate that there is considerable potential for using PIT tags in field studies.

Tags cost approximately $9 USD and portable hand held receivers are priced at approximately $300 USD (Woods 2005). These prices may be prohibitive for small research projects and are likely to preclude their use in mass-release experiments. Note however, that costs are significantly less (for small projects) than coded wire tag programs. PIT tags also appear promising for captive studies and managing *ex situ* collections in zoos and aquaria.

3. Promising Techniques

3.1. Decimal Coded Wire Tags (DCWT)

Coded wire tags are small pieces of magnetized stainless steel, that were originally inscribed with an individual binary code (Jefferts *et al.* 1963, Bergman *et al.* 1968). Decimal coded wire tags (DCWTs) are laser-engraved with a unique number code. Tags may be inserted into fish either in areas of cartilage, connective tissue or directly into musculature. DCWTs are 0.25 mm in diameter and the average length is 1.1 mm. For very small animals, 0.5 mm length tags are available, as well as two larger sizes (1.6 mm and 2.2 mm) that increase detectability. Originally developed for use on terrestrial animals, coded wire tags have been used in at least 27 fish families including temperate and tropical reef fishes (Buckley *et al.* 1994, Beukers *et al.* 1995). Coded wire tags are thought to be reliable for fish >11 mm standard length, and possibly smaller (Beukers *et al.* 1995), making them particularly useful for fish that need to be marked when small and recovered at a larger size. DCWTs have not yet been used to tag syngnathids.

The major consideration when assessing the utility of DCWTs for seahorses or other syngnathids will be in finding a site that allows high tag retention, establishing whether individual or batch marks are required and evaluating the costs of the tagging system in relation to other alternatives. In many species tag retention exceeds 90%, application is possible across a wide range of sizes, and tags show little interaction with tissue (Bergman *et al.* 1968, Blankenship 1990, Cook *et al.* 1990). The bony plates of seahorses and may either inhibit insertion or promote retention of DCWTs. For seahorses, suitable sites might be found at the base of the dorsal fin or within tail sections.

High capital costs are required for the equipment used to insert and detect DCWTs. The system only really starts to become affordable when mass marking is undertaken, reducing the cost of each tag individually. Specific project needs would require detailed assessment prior to trials, as various application and detection systems are available. Individual tags are inexpensive, costing only $120 USD/1000 tags. However the least-expensive detectors and multi-tag injectors cost $6200 USD and $7000 USD respectively. Rental of a hand-held injector and tag detector is possible through companies such as Northwest Marine Technologies at a rate of approximately $1200 USD per month.

A last important consideration is that full recovery of tag codes requires removal of the tag through sacrifice. However, metal-detecting equipment can determine the presence of tags in the field in live or dead specimens if a presence/absence batch mark is sufficient. Tag detection may be an issue in some species, particularly older fish that were tagged when small (Bumguardner *et al.* 1990).

3.2. Elemental Otolith Marking

Teleost otoliths are effectively small, metabolically synthesized rocks, found in the cranial cavity, that function as sound receptors, for balance and orientation. There are three pairs of otoliths in every fish; the largest pair being the sagittae and the two smaller pairs being the lapillae and asterisci respectively. Otoliths marking takes advantage of the fact that otoliths form through concentric additions of alternating protein and carbonate bands, laid down around a central nucleus; this process can be manipulated to incorporate a mark. In elemental marking, rare elements are introduced into the fish's environment and incorporated into the crystal matrix of developing otoliths.

To be useful as a mark, an element must be: (1) Rare in the environment and in fish tissue, (2) Taken up and retained in fish tissue, (3) Nontoxic and (4) Measurable by standard analytical techniques. Elements such as strontium, rubidium and manganese are commonly used for marking fish. The rare earth elements lutitium, europium and samarium have also been used because they have low background concentrations in the plankton and low instrument detection limits (Levin *et al.* 1993). Like chemical marking, elemental marking can be used to mark tissue, scales and bones as well as otoliths.

Elemental marking might be suitable for batch identification within wild syngnathid populations for tracking movement or connectivity. Strontium in particular is inexpensive, simple to apply, and non-toxic if consumed. Like chemical marks, elemental marking may be applied to very young animals via immersion and recent research has showed that young can be marked embryonically by injecting gravid females (S. Thorrold unpublished data.). The major advantages of elemental marks over chemical marks are that: (1) Naturally occurring elements can be used to mark fished populations if toxicity effects are a concern for particular chemical marks, (2) It appears possible to mark successive broods from the residual element-load in injected parent's tissue (S. Thorrold unpublished data.), (3) Marked individuals can be unambiguously distinguished from unmarked individuals (S. Thorrold unpublished data.), (4) Marks allow a simple way of mass-marking large numbers of embryonic young via injection of gravid parents; this approach is possible with chemical agents, but is difficult to optimize, as is adult immersion (chemical) for embryonic marking.

3.3. Elemental Fingerprinting

Elemental fingerprinting uses trace elements present in water that are incorporated into bones, scales or otoliths, to distinguish among groups of fish. This is in contrast to elemental marking where rare elements are intentionally introduced to act as a mark. Here we discuss elemental signatures in otoliths, since syngnathids do not have scales, and because as a destructive method, otoliths are usually more informative than bone.

Since otoliths are metabolically inert and are not reabsorbed, fish populations from different water masses should have otoliths with different elemental compositions; also called fingerprints or signals. Other body parts such as bone, scales and various soft tissues also have compositions that reflect ambient water masses, but in some body parts signals may be distorted by metabolic reworking after initial deposition.

A variety of elemental fingerprinting methods exist, using different equipment and appropriate for asking different questions. Common approaches include wavelength-dispersive electron microprobe (WD-EM), energy-dispersive electron microprobe (ED-EM), proton-induced X-ray emission (PIXE), (laser ablation/isotope dilution) inductively coupled plasma mass spectrometry (LA/ID)(-ICPMS) and energy dispersive X-ray microanalysis (EDX) (Mulligan *et al.* 1987, Gunn *et al.* 1992, Campana *et al.* 1997, Sawhney & Johal 1999). Elemental signatures from whole otoliths can be used in to assign individuals from wide-ranging or aggregating (schooling and spawning) species, to discrete stock populations (Edmonds *et al.* 1991, Campana *et al.* 1995, Begg *et al.* 1998, Campana *et al.* 2000). The utility of whole otolith elemental signatures for seahorses is at present unknown although perhaps of limited utility given that most species are thought to spend the most of their lives in fully marine waters with relatively homogenous water chemistry. However, in coastal and estuarine species that are exposed to anthropogenic pollution such as heavy metals (e.g. *H. abdominalis, H. capensis, H. guttulatus, H. kuda*), whole otolith elemental signatures could be used to distinguish between populations.

Probes can also be used to examine the chemistry of particular sections or bands of the otolith, corresponding to different portions of the life history. This is useful for species where juveniles occupy shallow inshore waters with variable water chemistry, allowing discrimination of nursery grounds. This method may therefore have the potential to assign individuals back to their source populations (e.g. Swearer *et al.* 1999). For syngnathids, the potential may exist to confirm reported trade routes and to understand patterns of dispersal. Preliminary examination of probe-based otolith microchemistry will be attempted in 2005 at the University of British Columbia, using *H. guttulatus* from estuarine populations along the coast of Portugal (J. Marcus, pers. comm.).

4. Techniques With Unknown Potential

4.1. Freeze Branding

Freeze branding is generally accepted as the most effective branding method for fish (Bryant 1990). Agents used to cool brands for freeze branding include liquid nitrogen (Smith 1973, Herbinger *et al.* 1990), compressed CO_2 (Bryant 1990), dry ice (solid CO_2) (Fujihara & Nakatani 1967), freon and laser beams (Brock & Farrell 1977). Of these approaches, compressed CO_2 branding may be a feasible approach for seahorses; both because it has been used on fish as small as 32mm SL (Smith 1973) and because the equipment is lightweight, easy to use and reliable under field conditions (Everest & Edmundson 1967, Bryant 1990). This is in contrast to most freeze branding methods where transporting and storing coolants at remote field bases without refrigeration can be

a major obstacle. The major drawback of the technique – distortion of the marks through growth – may be minimal in seahorses given the relatively small difference between the size of fish at branding and adult size. This technique would need to be thoroughly evaluated under laboratory conditions before use in field studies because of the known susceptibility of seahorses to skin necrosis following injury.

4.2. Panjet Marking

A panjet marker uses a spring-loaded trigger system to force a solution from a nozzle at high pressure through cell membranes. Its standard application (injecting anaesthetic for medical treatments) has been adapted for use as a fish-marking tool. Panjets are normally used to mark fish skin or fins with Alcian blue (e.g. Herbinger *et al.* 1990) but tattoo artists' pigments have also been applied successfully (Laufle *et al.* 1990). The hand-held injector is portable and lends itself to field studies for both individual and batch marks. Panjet marking is useful, but marks vary in size and longevity, where retention rate is dependent upon mark location, water temperature and growth rate. Care should be taken when marking small fish where tissue damage may be incurred by the high pressure injection.

Experimental application of panjet marks on dead seahorses represent the only attempts to use this method on syngnathids. Alcian blue was mixed with distilled water and injected on three thawed seahorses (two *H. reidi* and one *H. kuda*). Pigment was sprayed through a template with a 2 cm diameter hole to contain the dye and concentrate the mark. Marking tried at various distances and angles to the template to showed best results when the panjet was held 2 cm away from the body and lined up directly with the template hole. Occasionally small (1 cm. diameter) marks were created with high concentrations of pigment. The stain successfully penetrated the skin to produce a semi-permanent mark. Marks were often irregular due to the use of a panjet marker that fired irregular volumes from the nozzle (C. Bull unpublished data).

Panjet marking is known to work well on fin rays, and may have the potential to produce batch marks on the base of dorsal and pectoral fins in seahorses; the operculum and the base of the coronet are also possible marking locations.

The major drawback was that many marks were difficult to see against heavily pigmented skin. Occasional puncture wounds < 1 cm diameter were created by high pressure on soft tissue, but did not puncture bony plates or appear to cause excessive tissue disturbance. It may be possible to mix a quantity of fluorescent pigment granules with Alcian blue stain to allow identification under UV light. Retention times of Alcian blue, injected by panjet in seahorses, remain unknown. This technique is believed to be worth further investigation in live animals (C. Bull pers. comm.), but may not be suitable for *in situ* work where populations are harvested for trade (aesthetic and toxicity issues).

4.3. Fluorescent Grit Marking

Fluorescent grit marking is essentially a variation on panjet marking, that uses modified sand-blasting equipment to implant dye under the skin in mass-marking programs.

Pigment is sprayed across the whole body surface and retained at various locations in the epidermal layer. Grits range in size from < 20 μm to 350 μm (Phinney *et al.* 1967) and are available in at least red, yellow and green fluorescent colours (Nielson 1990). Several thousand individuals can be marked per hour using this technique. Successful spray marking has been reported in juvenile Pacific salmon species, brown trout, and Atlantic salmon, among others (Phinney *et al.* 1967, Strange & Kennedy 1982). Retention over 12 years has been reported in cutthroat trout (Nielson 1990).

Fluorescent grit marking may be worth attempting in seahorses. One drawback is that grit is usually applied to the whole body. Since seahorses rely on crypsis to avoid natural predators and fishers, individuals marked with fluorescent grit may stand a greater chance of predation/capture. Furthermore, one of the major advantages of the technique is the ability to mark a large number of individuals at once. For most syngnathids, it is seldom that a large group of individuals is collected for simultaneous marking. Complications from grit marking may arise in seahorses because there is only a thin layer of skin covering bony plates, which may be severely damaged if grit is arrested by the presence of bone, allowing pathogenic infection. This approach should first be tried on recently dead specimens to predict its potential applicability.

4.4. Other Pigments, Dyes and Stains

In this report, we have specifically addressed VIFE and panjet/fluorescent grit marking as two forms of pigments, dyes and stains, but other approaches may deserve consideration. The major advantage of dyes and pigments is that they are easy to obtain, inexpensive, and simple to apply making them particularly well suited for mass-marking programs. However, since much syngnathid research requires methods that can mark individuals, we choose here to simply list pigments that other researcher may wish to consider: paints, inks, liquid latex, plastics, metallic compounds, fluoresceins, and radioactive isotopes. These may be administered through injection, tattooing, blasting or immersion.

A number of injected dyes have been used successfully on small intertidal fish which share with seahorses non-traditional body morphology that precludes the use of external tags in confined and spatially complex habitats (e.g. Pholidae, Stichaeidae, Bleniidae, Gobiescodiae). Injected dyes that have been successfully used on small species include: neutral red (Davis 1971), lead acetate (Ichikawa & Hiyama 1954), lead versenate (Fry et al. 1960), National Fast Blue 8GXM and hydrated chromium oxide (Kelly 1967), India ink and procion yellow (Thresher & Gronell, 1978). Bismarck brown and chemicals such as those listed in section 4.5. have been administered through immersion (Davis 1971). Each of these dyes has different retention times that are likely to vary with species, administration and behaviour. Major disadvantages of stains include the fact that some may be lethal or affect swimming behaviour, may be too watery to use easily, or may be toxic if consumed. None of these stains has been used on syngnathids. In most cases VIFE is likely to be a more suitable option given its proven retention and tissue biocompatibility.

4.5. Chemical Otolith Marking

Chemical marking relies upon the introduction of chemicals or elements into the body, which are later detected in otoliths to identify a particular set of animals within a larger group. Chemicals are normally introduced either through feeding, injection or immersion. One group of chemicals particularly well suited to marking calcified structures such as otoliths are fluoresceins (note that it is also possible to mark bones, fin rays, scales and tissue with fluoresceins). Fluoresceins are chemicals that bind with calcium as they are deposited in the growing matrix of otoliths or bone and fluoresce under UV light. Three fluoresceins commonly used to mark fish otoliths include (oxy)tetracycline (Hettler 1984, Muncy *et al.* 1990, Thomas *et al.* 1995), calcein (Wilson *et al.* 1987, Muncy *et al.* 1990, Mohler 1997) and alizarine complexone (Adkins 1965, Thomas *et al.* 1995). Under UV light, these chemicals fluoresce yellow-green, yellow-orange and orange-red respectively. Successive immersion events can create multiple banding patterns useful for distinguishing between different batches of fish.

The advantages of chemical marks are that they might be applicable to all life history stages, including eggs, and that many animals can be marked simultaneously, cheaply and rapidly. Marks are long lasting and are applied non-intrusively. The disadvantages include the fact that chemical retention in most tissues is short and otolith examination requires sacrifice. Chemical marking cannot generate individual marks and detailed testing is required for each taxon to determine suitability.

Chemical otolith marking has been attempted in seahorses at the Shedd Aquarium in *H. erectus* ("Ocean Rider" strain). Three marking chemicals – oxytetracycline hydrochloride (250 mg/L for 24 hours), calcein (250mg/L for 24 hours) and alizarin complexone (100mg/L for 24 hours) – were used to immerse two pregnant males. Young from treated males were then systematically harvested at one, three and six months after marking to examine differences in retention as a function of time. There was no difference in the fluorescence of otoliths between marked and control individuals (S. Morgan, unpublished data). Any number of factors may be responsible for the absence of fluorescent bands. These include: marking young too early in development, insufficient immersion time, insufficient chemical solutes in marking solution, or human error. Chemical otolith marking may still be worth pursuing, but the effects of the above factors need to be examined under carefully controlled conditions to identify the factors that influence marking success.

4.6. Thermal Otolith Marking

Thermal marking is a method of creating distinct, non-random patterns on otoliths by exposing fish to temperature changes. This marking technique has been used largely for temperate fish (mainly salmonids), and usually for marking early life history stages (e.g. Negus 1999). Marking usually consists of exposing fry to rapid temperature pulses (e.g. an increase of 10°C), sustained for a few hours, and interspersed with periods of normal temperature to create banding patterns on otoliths. Different patterns can be used to distinguish different batches or cohorts of fish, and detection usually requires only a compound microscope with transmitted light. Thermal marking is presently used in both

Washington and Alaska on a production scale for identification and management of commercial fish stocks (Volk *et al.* 1990, Hagen *et al.* 1995).

Thermal marking has been attempted in relatively few fish, particularly tropical species. It is unknown whether this method would be feasible for marking syngnathid otoliths. Many researchers remain skeptical of applying thermal marking to tropical species, which are more sensitive to rapid or drastic temperature fluctuations than temperate species. However, thermal marking is a new approach and deserves investigation. Candidate species might include tropical seahorses such as *H. barbouri*, H. *comes* or *H. kuda* that inhabit shallow waters and regularly tolerate rapid temperature changes with tidal ebb and flow (e.g. 5°C change over 30 minutes S. Morgan, pers. obs.). It would also be necessary to investigate the incidence of "natural" thermal banding to determine how/whether artificially induced marks could be detected.

The major drawback of thermal marking is the need to strictly control temperature in marking aquaria. This is not always possible under field conditions, particularly in developing countries or where electricity is not readily available. It is debatable whether marks would be easily distinguished under transmitted light without sectioning, given the hemi-spherical morphology of seahorse otoliths.

4.7. Genetic Markers

To create genetic marks, naturally occurring, but very rare, genes are intentionally introduced into wild populations to "mark" some segment of a population. The advantages of using genetic markers are that: (1) Identification is achieved using natural methods, (2) Marks last throughout the life of the individual, (3) Information is passed between generations. Disadvantages include: (1) Need for tissue samples from fish, occasionally necessitating sacrifice, (2) Introduction of individuals who carry a rare allele. The latter usually has unknown effects on fitness, particularly if the allele in question is under selection. The techniques are complex and expensive and information is usually only obtained about groups and not individuals. The earliest application of biochemical genetics to aid fish identification was carried out in Pacific salmon in the 1960s and since then, techniques and applications have rapidly expanded to be used in over 200 fish species.

The potential for using genetic marks in seahorses is unknown. This is a relatively sophisticated and expensive technique, and is probably more advanced than necessary to elucidate basic biological parameters for most syngnathids. The fact that genetic markers introduce rare alleles into populations that may already be vulnerable through over-exploitation and habitat loss, would require serious consideration.

4.8 Parasite Markers

Parasites have been used successfully to differentiate between populations of fish, particularly those that live in freshwater, or spend some portion of their life in freshwater. Compared to standard marking techniques, parasites are inexpensive, tend to be stable in

populations from year to year, do not require mark-recapture, and can be analyzed with smaller sample sizes than most marking techniques (Moles *et al.* 1998)

The potential for using parasite markers in seahorse remains completely unexplored and may be worth pursing for the purposes of stock identification, particularly applicable in tracing trade routes. However, it will require significant work to first determine whether suitable parasites exist in marine seahorse populations, then characterize these organisms, and investigate whether such parasites could be extracted from dried specimens.

5. Unpromising Techniques

5.1. Acoustic Tags

Biotelemetry is a means of tracking the movement of animals both on land and in water via the transmission of radio and acoustic waves. Transmitters may be attached intragastrically, within musculature, or externally. In water, a battery-powered tag transmitter emits signals, which are received and converted by hydrophones, then detected and recorded by receivers. Radio waves are used to track terrestrial animals and freshwater fish, while acoustic tags are used in marine environments. The simplest acoustic transmitters are called pingers. These emit pulses at a fixed rate and are used to simply locate and identify an animal. Individuals tracked on the same frequency are distinguished by slightly different pulse periods that vary between 900 and 1200 milliseconds. More complex tags with sensors are also available and can track such variables as pressure (depth), temperature, velocity, muscle contractions, and heart rate.

At present, it does not appear that biotelemetry via acoustic tagging is a practical option for seahorses due to the size and placement of tags. The smallest available telemetry tags are sold by Biotrack (www.biotrack.co.uk) whose transmitters weigh 0.35 g, but are designed for birds. The smallest acoustic tags for fish are sold by VEMCO (www.vemco.com) and measure 9 mm x 20 mm, weigh 2 g and last anywhere from 16-229 days depending on pinger frequency (D. Webber pers. comm.). Transmitter diameter controls the transmission frequency of tags and sound propagation is proportional to frequency. Therefore, tag diameter is traded-off against detection range, making it unlikely that the present form of acoustic tags will decrease significantly in size in the near future without the development of better detectors.

Given their large size and weight, acoustic tags are not appropriate for seahorses. A general rule of thumb in telemetry is that tags should exceed no more than 1-2% of an animal's body weight (Ross & McCormick 1981) although some studies have implanted transmitters representing up to 10% of body weight in juvenile fish (Adams *et al.* 1998). However, even small/light telemetry tags may affect physiology and behaviour (Bridger & Booth 2003). In the case of seahorses, the adults of most species weigh between 0.5 g and 15 g so even the smallest current acoustic tag would represent 13% of the largest seahorse species adult weight. Furthermore, tag insertion in seahorses may be difficult because a high proportion of the body is covered with bony plates.

Acoustic tags can also be attached externally where weight is offset through the attachment of positively buoyant foam. For external transmitters increasing the size of the tag is likely to increase swimming drag and the chances of entanglement. The body cavity of seahorses – assumed to be the best site for tag insertion – could not support the present size of tags particularly those enlarged by the attachment of positively buoyant foam.

However, telemetry technology is evolving rapidly, and researchers interested in tagging seahorses should remain in contact with companies such as VEMCO and Lotek (www.lotek.com) for regular updates (see Appendix 1). Almost all telemetry companies design their tags for specific projects, and there is reason to hope that acoustic telemetry will soon be a possibility for the largest species of syngnathid.

5.2. Attachment Tags

Attachment tags are physically affixed to fish for identification and are by far the most common means of identifying fish. The most common tags include dangler tags, Petersen discs and spaghetti T-bars. However, long term studies have shown that external attachment tags can affect growth and mortality making it dangerous to treat tagged fish as representative of untagged individuals (McFarlane & Beamish 1990).

Attachment tags, with the exception of collar tags (see section 2.1), are likely to be inappropriate for use in seahorses. This is because of their unusual body shape and small size relative to most commercially valuable perciform fishes. Most attachment tags are affixed through the dorsal musculature of perciform fish, near or under the dorsal fin, and dangle freely. Seahorses are unusual in having musculature that is covered by bony plates, covered in a thin layer of skin without scales. There are therefore no suitable attachment points for standard fish tags such as Petersen discs, spaghetti tags, dart tags, fingerling tags, anchor tags, hydrostatic tags or archer tags (see Floy Tag Inc., Appendix 1). Furthermore, the fact that many syngnathids rely on camouflage to avoid predation and move through complex habitat, mean that dangling tags are likely to pose a threat in terms of both predation and entanglement. A common site for non-dangling attachment tags is through the operculum (e.g. bachelor buttons or opercular straps). However, a closed operculum and suction feeding in syngnathids precludes these options.

5.3. Satellite Tags

Satellite tags are presently too large for use on any but very large fish, and are impractical for seahorses. Satellite tags are now being used to track marine animals ranging in size from elephant seals to Adelie penguins (www.wildlifecomputers.com/Default.htm). The major advantage of satellite tags is their ability to transmit position information via satellite to computers without the need to track animals by hydrophone as per acoustic methods. The smallest satellite tags that now exist for marine tracking are SPOT4 tags that weigh on the order of 30 g and are suitable for seals, turtles, large and small cetaceans, penguins, and albatross. Not surprisingly these remain much too large to use on syngnathids, comprising nearly twice the total body weight of the largest seahorse species.

6. Unsuitable Techniques

6.1. Fin Clipping

Fin clipping is not recommended as a marking technique either for seahorses or other syngnathids although there may remain unexplored potential in the latter. Reasons for this include: (1) Mutilation of dorsal or pectoral fins via fin-clipping may negatively affect swimming performance, (2) Tissue re-growth may provide only short term marks, (3) Non-invasive marking methods that pose less risk of infection already exist. Seahorses have few fins compared to most teleosts and rely almost exclusively on their dorsal fin for propulsion while pectoral fins also provide a small amount of power (Blake 1976, 1980, Consi *et al.* 2001). Agility rather than swimming speed is probably more important for seahorses in their often complex benthic habitats (Blake 1980). Seahorse fins are known to work together, and even portions of a single fin may work at different angles from others (Blake 1976). Therefore, clipping either dorsal or caudal fins may compromise either propulsion and/or orientation.

Two small studies have assessed the effects of fin-clipping seahorses and both were designed for genetic sampling, as opposed to marking (Lourie 2003). In 2000, S. Lourie and D. Melchinsky removed tissue from the dorsal fins of three adult seahorses (*H. erectus* and unknown species) at the Birch Aquarium, Scripps Institute of Oceanography, San Diego, USA. The researchers recorded neither behavioural effects nor mortality. In 2001, a more extensive experiment involving 100 seahorses (*H. kuda*) was undertaken at the Balai Buddaya Laut, Lampung, Sumatra Indonesia by S. Lourie and A. Hafiz al Qodri. Unfortunately, the results were inconclusive due to strong tank effects, but did indicate rapid regeneration of fin tissue within two weeks of clipping (S. Lourie, unpublished data).

For research that has identified fin clipping as a priority, the anal fin may be worth investigation as it does not appear to play a role in propulsion. This fin may be important during display or reproductive behaviour. A full investigation into its function and ability to regenerate would be required prior to consideration.

6.2. Frond Clipping

Frond clipping is the only form of mutilation that has been used for the intended purposes of identifying seahorses. Like fin clipping, frond clipping is a temporary marking technique, but is limited to species that reliably grow fronds. These include (but may not be limited to): *H. abdominalis, H. breviceps, H. guttulatus, H. sindonis, H. whitei* and *H. zosterae.* Shortcomings of this marking method include regrowth of tissue and the possibility of infection.

For small studies, fronds could be clipped in targeted locations to create individual marks. However, the only field study to use frond clipping found that skin filaments started to re-grow within two days of clipping (J. Curtis, pers. comm.). This may be

beneficial for some studies and undesirable in others. In most cases, frond clipping is more appropriate for batch marking, and might be considered as a short-term double mark in elastomer studies, particularly for highly mobile species where collar tags are not appropriate. Frond clipping is the most favourable of the three mutilation methods listed for obtaining genetic samples where it is an option. In all cases, controlled trials examining potential for infection and regeneration are recommended.

6.3. Tail Clipping

Tail clipping, like fin clipping, has been used to collect genetic samples from seahorses. This method is not recommended for marking because of risk of infection and it may compromise reproductive success and ability to grasp holdfasts. Tail clipping was developed subsequent to fin clipping for collecting greater volumes of tissue while being (theoretically) less detrimental to swimming performance. Animals with reduced tails have been observed in the wild in good condition, maintaining pair bonds and breeding successfully. This would appear to indicate indicating that tail clips, if healed successfully, may not impede basic functioning such as feeding and reproduction. (A. Vincent, pers. comm.).

Nonetheless, it is recognized that trials would be needed to evaluate the effects of tail clipping. Unlike fin clipping, tail clipping is a permanent marking technique and there is no indication that seahorses are able to regenerate tail tips, although this too remains to be tested.

6.4. Visible Implant Alphanumeric (VI Alpha) Tags

VI Alpha tags were invented in response to the limitations of external and coded wire fish tags for projects that: (1) Needed individual identification, (2) Wanted to read tags without sacrifice (Haw *et al.* 1990, Bergman *et al.* 1992). VI Alpha tags are made of elastomer, alphanumerically labeled, available in two sizes (1.0 x 2.5 mm or 1.5 x 3.5 mm) and three fluorescent colors (red, orange and yellow) and designed for internal application under clear tissue. Tags are inserted using a specially adapted, flat, large-gauge needle and plunger. The VI Alpha tagging system is available from Northwest Marine Technologies (www.nmt.us/products/via/via.htm).

Pilot trials undertaken by NMT staff in 1999 using preserved adult *H. comes* (>70 mm SL), indicated that there were no suitable locations to insert a VI Alpha tag because of both pigmentation and skin tears associated with injecting tags into a thin epidermis over body plates (D. Thompson pers comm.). It was suggested that larger, more lightly coloured seahorses might be suitable, where puncture trauma should be less and there may be larger areas suitable for insertion.

Recently, VI Alpha tags were used to mark one of the largest seahorse species, *H. abdominalis* (Woods 2005). Smaller-sized tags (1.0 x 2.5 mm) were applied to the lateral surface of the first two anterior tail segments, where they showed 100% retention and had no significant effects on the length, weight or specific growth rate of treatment versus control animals after three months. All individuals survived tagging. However, tag

readability was low (see also Mourning *et al.* 1994) and secondary incisions often had to be made to avoid skin tears. Skin tears may be avoided through creating an incision with a scalpel before inserting the tagging needle. It was concluded that VI Alpha tags are not a reliable individual tagging method for seahorses (Woods 2005), although they may be suitable for batch-marking. Similar results were found in a separate trial with *H. abdominalis* (K. Martin-Smith, unpublished data). Other significant disadvantages include: (1) Anaesthesia is required, (2) The technique is slow (~120 seconds per seahorse), (3) It cannot be performed *in situ*, (4) Tags may affect susceptibility to predation. Previous researchers have noted that VI Alpha tags did not affect the mortality of small temperate reef fishes (Malone *et al.* 1999) but they have shown poor longterm retention (0-9% over 245 days) in juvenile temperate reef fishes (Buckley *et al.* 1994).

6.5. Silver Nitrate Branding

Branding is a marking process that uses heat, ice or chemicals that either displace or concentrate pigment to leave a mark. Brands tend to be simple to apply and heal rapidly with little risk of infection. The major drawback of branding is that it leaves only temporary marks and is therefore of limited use in long-term studies.

Silver nitrate branding is a technique that uses a paste of silver nitrate and has been tried only once in seahorses. It was concluded that the technique was not an appropriate method for the genus (C. Bull, pers. comm.). However, the approach was assayed once, on one individual, that was not feeding prior to marking and may have already been in unusually poor condition when marked.

The individual in question (*H. reidi*) was marked twice on the ventral surface of the tail between tail rings. Silver nitrate was dissolved in water and applied once using a hypodermic syringe that resulted in a 3 mm diameter mark (spreading occurred because the silver nitrate paste was too thin), while a second mark was applied with a "match-like" applicator and produced a larger mark. Both marks appeared initially white, and some sloughing of tissue occurred. Within 24 hours the mark made by the match-like applicator had turned black, surrounded by white necrotic tissue, encircled by red tissue. A similar but less severe response was noted in the mark made by the syringe which was not black at the centre. At this time, the area was treated with antibiotic ointment, but necrosis continued and the seahorse died five days later. The excessive tissue damage was unexpected both by the researcher and collaborating veterinary staff (John G. Shedd Aquarium). It was hypothesized that the rapid necrosis may have been due to the presence of bony plates immediately beneath the skin that caused the silver nitrate to spread over the thin skin layers horizontally, rather than remaining concentrated in a small area.

6.6. Opalithplattchen (Bee Tags)

Bee tags are 2.5 mm plastic numbered discs, available in five colours and most commonly used for marking queen bees and other insects (Freilich 1989, Seely 1995). Opalithplattchen have been used to tag marine mollusks, but few other marine species

(Le Cheminant 2000). One study tagged seahorses with bee tags (n=5 total), where tags were attached with two types of glue, Shellac (Chr. Graze, Endersbach, Germany) and Vetbond (3M Animal Care Products). Tags were applied on the area between the 7th and 8th body trunk rings, which was identified as the largest flat area away from sensitive locations (fins, head or areas of constant movement such as the tail). The area was dried as much as possible, a single drop of glue was applied, and the tag positioned with forceps. Tags affixed with Shellac were retained for only one hour, while tags affixed with Vetbond were retained for a maximum of 4.5 hours. Five to six days after tagging, white patches, which suggested the loss of skin pigmentation, appeared at tag sites. Four days later the patches were smaller and were covered by what appeared to be new skin. Eleven days after application, the marks had disappeared with no mortalities. One hundred percent tag loss within 24 hours of labeling and unacceptable skin irritation indicates that the method is inappropriate for even short-term seahorse tagging.

7. Tagging Considerations and Conclusions

7.1. Experimental Design Considerations

Many marking experiments use improper design, thereby jeopardizing the validity of their findings. When undertaking a marking study, the following issues deserve consideration. Basic experiments should be comprised of three groups:

Treatment group
A group of mixed-parentage/same parent animals that should have the actual tag or mark applied.

Blank treatment group
This group should have the same level of handling and treatment as group A, but do not actually have the mark or tag applied. This allows investigation of handling effects and the tagging technique on survival and growth.

Control group
This group should remain undisturbed and provide a "benchmark" group from which to estimate the normal (or expected) growth, behavior and mortality within the study population.

In populations where tagged individuals will be interacting with untagged individuals, it is necessary to double-tag treated fish, so that should the experimental marks of interest be lost, the individual will be recognized as a fish with a lost tag, as opposed to an unlabelled individual. Be aware that two tags may have synergistic effects, and where possible, the least invasive second mark should be used. For short-term studies, metrics such as standard length or body markings may be sufficient "double marks".

Most tags are lost soon after insertion, and monitoring programs should be designed accordingly. Therefore, populations should be monitored most intensively immediately after marking and less frequently as time goes on.

One constraint associated with syngnathid research is that large numbers of animals may not be available for experiments. Similarly, it can be difficult to obtain size- or age-matched young from different fathers to combine simultaneously in treatments, because pairs mate on different individual reproductive cycles. Where the availability of size- or age-matched young is a critical, two alternative approaches are possible. One option is to simply increasing the sample size and repeat the manipulation on broods from many different males as they become available (unpaired design – where any male only contributes one brood to either a control, treated or blank group). With sufficient broods, the variation due to parentage should be minimal relative to the variation due to treatment effects. If the logistics of matching of young *and* obtaining broods from sufficient males is problematic, it may be necessary to use successive broods from the same fathers. This decreases the overall number of animals needed, and may be the most feasible approach for small-budget work. As long as the same number of broods from each parent are used in each manipulation (control, treatment, blank) the results will be comparable and treatment effects, if any, valid: this is a paired design.

7.2. Standardizing Experimental Species

As seahorse species share the same overall anatomy, it might be practical to concentrate preliminary *ex situ* tagging and marking efforts on one species or strain that has proven to be easy to hold in a captive environment. Appropriate strains may be *Hippocampus* species (putative *H. fuscus*) maintained successfully at the Chester Zoo, England, *H. erectus* from Birch Aquarium at Scripps, USA or with *H. capensis* (a species that has proved less difficult to culture in captivity).

7.3. Pilot Studies on Dead Animals

Preliminary assessment of possible marking locations can be made on dead animals. The preservation method should be known prior to experimentation, as this may confound or misrepresent results by changing the strength and consistency of tissue layers in both a relative and absolute sense.

7.4. Conclusions and Recommendations.

As the dried, aquarium and curiosity trades continue to put pressure on wild populations, viable means of marking and tagging syngnathids will provide us with one set of tools to obtain information critical to managing both wild and captive populations. At present we lack many of these methods. To date, only VIFE, collar tags, PIT tags and VI Alpha tags have been rigorously tested as marking techniques for seahorses; and these on relatively few species within the genus *Hippocampus* and fewer still, within the family Syngnathidae.

Given the potential threats to wild populations, there is a need to proceed with marking and tagging trials, with a focus on well-planned research under controlled conditions. Laboratory rearing should help to minimize variation and clearly identify tagging effects prior to their application in the field. Progress and results, particularly from unpublished

pilot work, should be reported widely amongst the research, conservation and management communities with interests in syngnathids

It would appear that a range of tags and marks may be useful for seahorse and other syngnathid research, fisheries management, trade monitoring and captive population maintenance. Appropriate techniques (focused on seahorses), by application, are displayed in Table 2.

Table 2. Examples of syngnathid research projects that may require tagging or marking and factors relevant in the determination of the most appropriate technique.

Study topic	Juvenile Disperal	Adult Movements	Fishery Exploitation	Stock Origin	Stock Discrimination	Effects of Introductions	Identification of Captive Animals	Population Management in Aquaria	Life History & Physiology in Aquaria
Location of Study	***In situ***	***In situ***	***In situ***	***In situ***	***In situ***	***In situ***	***Ex situ***	***Ex situ***	***Ex situ***
Timescale of Retention	**Years**	**Months**	**Years**	**Years**	**Years**	**Years**	**Years**	**Years**	**Months**
External recognition	✗	✓	✓	✓	✗	✓	✗	✗	✓
Internal Recognition	✓	✗	✓	✓	✓	✓	✓	✓	✗
Batch Recognition	✓	✗	✓	✓	✓	✓	✓	✗	✓
Individual Recognition	✗	✓	✗	✗	✗	✗	✗	✓	✓
Life Stages to be Identified	**All**	**Adult**	**Sub-adult, Adult**	**Sub-adult, Adult**	**All**	**All**	**Sub-adult, Adult**	**Sub-adult, Adult**	**All**
Life Stages to be Tagged	**Fry**	**Adult**	**All**	**–**	**Fry**	**All**	**Fry**	**Sub-adult**	**All**
Retrieval from Culled Animal	✓	✗	✓	✓	✓	✓	✓	✗	✗
Retrieval from Live Animal	✗	✓	✗	✓	✓	✓	✗	✓	✓
Repeated Retrieval from Live Animal	✗	✓	✗	✗	✗	✓	✗	✓	✓
Consumption by People Possible?	✓	✓	✓	✓	✓	✓	✓	✗	✗
Tagging Options	Chemical marks Thermal marks Elemental marks	VIFE Dye marks PIT tags DCWTs Genetic	Genetic VIFE CWTs	Elemental fingerprinting Parasite load	Elemental fingerprinting Chemical marks Thermal marks DCWTs	DCWTs PIT tags VIFE	Chemical marks Thermal marks Elemental marks	PIT tags VIFE DCWTs Collar tags	VIFE Dye marks Collar tags

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Appendix 1. 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 Amick
VIFE/VI Alpha/DCWT			
Northwest Marine Technologies	(USA) 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 (Europe) Northwest Marine Technology, Foundry Farm, Kiln Lane, Redlynch, Salisbury, Wilts, SP5 23HT Great Britain (Asia) Northwest Marine Technology 5007 Chambers Creek LP, SE Olympia, WA 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 Tel: +44-1725-512523, Fax: +44-1725-512964 Tel: (360) 455-4731 Fax: (360) 455-4814	Jan Sanburg office@nmt.us David Solomon david.solomon@nmt.us Yong Huang yong.huang@nmt.us
Bee Tags	Christian Graze KG Postfach 2707 7056 Weinstadt-Endersbach FRG		
PIT Tags			
Allflex	(Canada) Allflex Canada 3555 Boul. Choquette St-Hyacinthe, QC J2S 7Z8 CANADA (USA, Small Animals) Allflex Small Animal Division 2820 Wilderness Plaza, Suite A Boulder, Colorado 80301 USA (Europe) Allflex Europe ZI DE Plague Route des Eaux Vitre, France 35502 (Australia) Allflex Australia 33 Neumann Rd. Capalaba, 4157 Brisbane, Australia (New Zealand) Allflex New Zealand Private Bag 11003 Palmerston North New Zealand	Tel: (866) 505-TAGS [8247] Tel/Fax: (877) 456-3639/FAX Tel: (303) 449-4509 Tel/Fax: (303) 449-4529 Web: www.Allflex-Boulder.com Phone: 33 299 75 7764 Fax: 33 299 75 7700 www.allflex.co.uk Web : www.allflex-europe.com Tel : 61(07) 32459100 Fax : 61(07) 32459110 Tel : 0011 646 356 7199 Fax : 61(07) 32459110 Web: www.allflex.co.nz	Bob Stewart (Boulder, Colorado): r001stew@cris.com Bob Stewart (Boulder, Colorado): r001stew@cris.com Phillipe Dubouix (Vitre, France): phdubouix@compuserve.com John Boyd (Australia): john.boyd@allflex.com.au Marketing Manager: ray@allflex.co.nz

Biomark	Biomark, Inc. 7615 W. Riverside Dr. Boise, Idaho 83714	Tel: (208) 275-0011 Fax: (208) 275-0031 Cell: (208) 859-3208 E-mail : biomark@micron.net Web: www.biomark.com	Audrey Callagher : audreyc@biomark.com
Sokymat	Company merging with Metget	www.sokymat.com	
Acoustic Tags			
Biomark (see above)			
Lotek Marine Technologies	115 Pony Drive Newmarket Ontario Canada L3Y 7B5 114 Cabot Street St. John's Newfoundland Canada A1C 1Z8	Tel: (905) 836-6680 Fax: (905) 836-6455 E-mail: telemetry@lotek.com Web: www.lotek.com Tel: (709) 726 3899 Fax: (709) 726 – 5324 E-mail: marine.telemetry@lotek.com	Padraic O'Flaherty poflaherty@lotek.com
VEMCO Limited	100 Osprey Drive Shad Bay Nova Scotia Canada B3T 2C1	Tel: (902) 852-3047 Fax: (902) 852-4000 Web: www.vemco.com	Fred Voegeli sales@vemco.com