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## Advances in tagging syngnathids, with the effects of dummy tags on behaviour of *Hippocampus guttulatus*

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Artificial marking and tagging techniques have been used to study movement, population dynamics, behaviour, ecology, survival and growth of at least 25 syngnathid species. External necklace-style tags and injection of visible implant elastomer have been the most used techniques, uniquely identifying hundreds of individual syngnathids to study population dynamics, mortality, behaviour, ecology and growth in at least 13 and 12 species, respectively. Only two studies, both on larger syngnathid species, have tested the use of internal or electronic tags. This new case study reveals that dummy tags, weighing up to 6% of individual body mass, have minimal effect on normal *ex situ* behaviour of the long-snouted seahorse *Hippocampus guttulatus*, a smaller syngnathid. In paired aquarium trials, tags did not affect movement, holdfast use or general behavioural state, and only had a short-term effect (1 day) on vertical orientation. Tagged *H. guttulatus* gained more mass during the 5 day trials, a result which warrants further exploration but indicates that tags did not reduce feeding. This study shows promise for using electronic tagging to study *H. guttulatus* and similarly sized syngnathids in the wild.

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Key words: acoustic telemetry; aquarium; movement; seahorse; visible implant elastomer; welfare.

### INTRODUCTION

Studying individual fish provides insight into the biology and management of fish species; insight that would not be gained otherwise. An improved understanding of population structure, movement, mortality or abundance requires re-sampling individual fish, and repeated measures of the same individual can provide growth estimates used in fisheries and population models (Parker *et al.*, 1990; Nielsen, 1992; Pine *et al.*, 2003). Incorporating individual behaviour into population or stock models has helped explain past fisheries collapses and can guide future management and conservation solutions (Jennings, 2001; Rowe & Hutchings, 2003; Palumbi, 2004; Fromentin & Powers, 2005; Botsford *et al.*, 2009).

Studying an individual fish in the wild relies on differentiating that fish from others. When natural markings are unreliable, artificial marking and tagging techniques can be used to identify individuals (Parker *et al.*, 1990; Nielsen, 1992; Nielsen

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*et al.*, 2009). These techniques can be separated into four broad categories: external tags, external (artificial) marks, internal tags and biotelemetric (or electronic) tags (Nielsen, 1992). The most useful techniques should be durable without maintenance (for long-term monitoring and estimation of life-history variables) and should allow for many different combinations (for large sample sizes and population-level estimation), but should also have minimal effects on welfare and behaviour (so that results are representative of unmarked or untagged fishes) (Nielsen, 1992; Jepsen *et al.*, 2002; Bridger & Booth, 2003; Cooke, 2003; Murchie *et al.*, 2004).

Syngnathids are of such biological, evolutionary, ecological, economical and conservation interest that appropriate marking or tagging methods are important. Species within the family Syngnathidae share unusual biological characteristics such as male pregnancy, suction feeding and a distinctive morphology and swimming form (Kuitert, 2000). While these unusual characteristics have been well documented, more basic knowledge is needed for improved biological and ecological understanding and to assess the conservation status of most syngnathid species: only 53 of the 337 syngnathids in the *Catalogue of Fishes* have been assessed in the IUCN Red List and of those 38 are listed as data deficient (Eschmeyer & Fong, 2010; IUCN, 2010). This is troubling as many syngnathids are exploited by humans for use in traditional medicines, as curios and as ornamental aquarium fishes (Foster & Vincent, 2004). Of the syngnathids for which there is sufficient information, all but three are listed in categories that indicate a need for conservation (critically endangered, endangered, vulnerable or near threatened). As in other fishes, marking and tagging studies could help to assess the conservation status of data-deficient syngnathids and guide conservation and management for syngnathids at risk.

The dual aims of this paper were to: (1) review past use of marking and tagging techniques for studying syngnathids and (2) advance knowledge of the effects of tag size on small syngnathids with empirical work. The latter objective was achieved using the long-snouted seahorse *Hippocampus guttulatus* Cuvier 1829 and dummy tags of the same size and mass as the smallest available acoustic tags to test whether similarly sized electronic tags can be used to study small syngnathids without affecting their welfare and behaviour.

## SYNGNATHID MARKING AND TAGGING

Identifying individuals using natural markings is ideal as it does not require interference with the animal. There has been enough variation in body morphology to allow for identification and study of individuals of at least five syngnathid species without the need for artificial tagging or marking (Table I). While three of these species were studied using either small sample sizes (nine to 20 individuals) or short time periods (<1 year), the diverse facial markings of worm pipefish *Nerophis lumbri-ciformis* (Jenyns 1835) and abdominal markings of weedy seadragons *Phyllopteryx taeniolatus* (Lacépède 1804) allowed for reliable identification of 133 and 43 individuals over 18 and 19 month periods, respectively (Monteiro *et al.*, 2005; Martin-Smith, 2011). While other morphological characters such as appendages have been used to confirm identifications (Martin-Smith, 2011), they are less reliable than facial or abdominal patterns and can change over time making them unsuitable for long-term studies or even for identifying among species (Connolly *et al.*, 2002a; Curtis,

TABLE I. Published syngnathid studies using tagging and marking techniques, with maximum standard length ( $L_S$ ) and wet mass ( $M_w$ ) for each species

Syngnathid species	Technique used	Maximum Size		Focus of study	References
		$L_S$ (cm)†	$M_w$ (g)		
<i>Anarchopterus criniger</i>	EM	10.0	—	Population dynamics	Masonjones <i>et al.</i> (2010)
<i>Corythoichthys haematopterus</i>	EM	19.8	—	Behaviour	Matsumoto & Yanagisawa (2001); Sogabe & Yanagisawa (2007a, b, 2008); Sogabe <i>et al.</i> (2007)
<i>Entelurus aequoreus</i>	ET	60.0	47.5§	Reproductive ecology	Vincent <i>et al.</i> (1995); Bauchot & Bauchot (1978)§
<i>Hippocampus abdominalis</i>	ET, EM*, IT*	35.0	44.4‡	Growth, ecology, population dynamics, effect of tags	Woods & Martin-Smith (2004)*; Martin-Smith & Vincent (2005); Woods (2005a*, b**‡)
<i>Hippocampus breviceps</i>	ET	15.0	2.08‡	Behaviour, ecology	Moreau & Vincent (2004)‡
<i>Hippocampus capensis</i>	ET	12.1	2.58‡	Ecology, population dynamics, effect of tags	Le Cheminant (2000)*‡; Bell <i>et al.</i> (2003)
<i>Hippocampus comes</i>	ET	18.7	10.7‡	Behaviour, ecology	Perante <i>et al.</i> (2002); J. Meeuwig & P. LaFrance (unpubl. data)‡
<i>Hippocampus erectus</i>	EM	19.0	14.9‡	Population distribution	Masonjones <i>et al.</i> (2010); Zhang <i>et al.</i> (2010)*‡
<i>Hippocampus guttulatus</i>	ET, EM	21.5	22.5‡	Growth, mortality, behaviour	Curtis & Vincent (2006)‡; Palma <i>et al.</i> (2008)*; Garrick-Maidment <i>et al.</i> (2010)
<i>Hippocampus kuda</i>	ET	30.0	14.9‡	Growth	Lipton & Thangaraj (2007); Lin <i>et al.</i> (2009)‡
<i>Hippocampus reidi</i>	NM, ET	17.5	14.9§	Behaviour, population distribution, ecology	Felicio <i>et al.</i> (2006); Rosa <i>et al.</i> (2007); Castro <i>et al.</i> (2008); Freret-Meurer & Andreata (2008); Hora & Joyeux (2009)**§
<i>Hippocampus subelongatus</i>	ET	20.0	—	Behaviour	Kvarnemo <i>et al.</i> (2000, 2007)
<i>Hippocampus whitei</i>	ET, EM	13.0	9.70‡	Behaviour, reproduction, habitat use	Vincent & Sadler (1995); Vincent & Giles (2003)‡; Vincent <i>et al.</i> (2004, 2005); Harasti <i>et al.</i> (2010)

TABLE I. Continued

Syngnathid species	Technique used	Maximum Size		Focus of study	References
		$L_S$ (cm)†	$M_w$ (g)		
<i>Hippocampus zosterae</i>	EM	5.00	0.234‡	Population dynamics	Masonjones (2001)§; Masonjones <i>et al.</i> (2010)
<i>Nerophis lumbriciformis</i>	NM	17.0	1.95§	Behaviour	Lyons & Dunne (2003)§; Monteiro <i>et al.</i> (2005)
<i>Nerophis ophidion</i>	ET	30.0	5.93§	Ecology	Vincent <i>et al.</i> (1995); Gurkan & Taşkavak (2007)§
<i>Phycodurus eques</i>	NM, BT	35.0	—	Behaviour, ecology, population abundance	Connolly <i>et al.</i> (2002a, b)
<i>Phyllopteryx taeniolatus</i>	NM, EM	46.0	18.2‡	Behaviour, growth, reproduction, population dynamics, ecology	Kvarnemo & Simmons (2004)‡; Sanchez-Camara & Booth (2004); Sanchez-Camara <i>et al.</i> (2005, 2006); Martin-Smith (2011)
<i>Syngnathus abaster</i>	NM	21.0	4.97§	Behaviour	Verdiell-Cubedo <i>et al.</i> (2006)§; Silva <i>et al.</i> (2010)*
<i>Syngnathus acus</i>	ET	50.0	57.0§	Reproductive ecology	Vincent <i>et al.</i> (1995); Valle <i>et al.</i> (2003)§
<i>Syngnathus floridae</i>	EM	25.0	3.96*‡	Population dynamics	Ripley & Foran (2008)*‡; Masonjones <i>et al.</i> (2010)
<i>Syngnathus fuscus</i>	ET	33.0	4.81*‡	Behaviour, ecology	Roelke & Sogard (1993); Ripley & Foran (2008)*‡
<i>Syngnathus louisianae</i>	EM	38.0	—	Population dynamics	Masonjones <i>et al.</i> (2010)
<i>Syngnathus rostellatus</i>	ET	18.7§	2.83‡	Reproductive ecology	Vincent <i>et al.</i> (1995); Gokoglu <i>et al.</i> (2004)‡
<i>Syngnathus scovelli</i>	EM	18.3	—	Population dynamics	Masonjones <i>et al.</i> (2010)
<i>Syngnathus springeri</i>	EM	38.0	—	Population dynamics	Masonjones <i>et al.</i> (2010)
<i>Syngnathus typhle</i>	ET	35.0	19.0‡	Behaviour	Vincent <i>et al.</i> (1994, 1995); Valle <i>et al.</i> (2003)§

BT, biotelemetric—electronic tags; EM, external marks; ET, external tags; IT, internal tags; NM, natural markings.

\**Ex situ* study.

†From Froese & Pauly (2010) unless otherwise indicated.

‡Maximum size reported in reference.

§Mass calculated from maximum  $L_S$  using reported length and mass relationship.

2006a). Unfortunately, many syngnathid individuals are too similar for reliable identification using natural markings. Estimating population-level characteristics or life-history variables in these species will require artificial marking and tagging techniques.

Artificial marking and tagging (both external and internal) have contributed to understanding the biology of at least 25 syngnathid species (Table I), but the syngnathids' small body size [pipefishes average maximum standard length ( $L_S$ ) = 246 mm, seahorses average maximum  $L_S$  = 143 mm: Wilson, 2009] and distinctive morphology pose challenges (Vincent & Sadler, 1995; Le Cheminant, 2000; Woods, 2005a). There has been a general, albeit disputed, guideline that tags should be less than 2% of body mass to minimize negative effects on welfare and behaviour of fin fishes (Jepsen *et al.*, 2005). Maximum masses of syngnathid species marked or tagged in the past range from 0.05 to 57.00 g (Table 1; Masonjones *et al.*, 2010). Under the 2% guideline, tags weighing <0.001 to 1.100 g would be needed for those species. The rigid body and reduced fins of syngnathids make it difficult to inject or attach tags that are normally either placed in the body cavity or on the dorsal fins of larger fishes (Nielsen, 1992). The upright swimming posture of seahorses can make tagging particularly difficult as placement on the dorsal fin could alter balance and make swimming difficult (Moreau & Vincent, 2004).

External tagging methods (*i.e.* attaching externally visible foreign material for means of identification) have been used to study reproductive ecology, behaviour, movement, activity patterns, growth and social structure in at least 15 syngnathid species (Table I). For many fish species, external tags need to be secured through a fin or other part of the body (Nielsen, 1992). The presence of bony scutes in syngnathids, and the horse-like shape of *Hippocampus* species in particular, has meant that tags could instead be tied around the neck or tail without slipping off the body, a less invasive method of attachment. Coloured and patterned thread has been tied around the bodies of syngnathids to identify groups (*i.e.* those which have been measured or from a given location) (Felício *et al.*, 2006; Rosa *et al.*, 2007). The addition of small, individually numbered discs to such necklaces has made it possible to follow hundreds of syngnathids in the wild. Lipton & Thangaraj (2007) used numbered necklace-style tags to identify and study the growth of 452 spotted seahorses *Hippocampus kuda* Bleeker 1852 with minimal effect on short-term behaviour. Although tied necklaces have minimal short-term effect on behaviour, they must eventually be loosened, replaced or removed to prevent injury as the fish grow (Vincent & Sadler, 1995; Perante *et al.*, 2002; Bell *et al.*, 2003). This technique is therefore suitable only for closely monitored populations and is less suitable for the measurement of life-history variables such as mortality that require long-term data. Vincent *et al.* (1994) used shrinking plastic to secure ends of the necklace, which may allow the loop to expand and eventually fall off if broad-nosed pipefish *Syngnathus typhle* L. 1758 were not recaptured. External tags could be attached to bony appendages (Connolly *et al.*, 2002b), but such appendages are not found in all syngnathid species or even on all individuals within a species (Curtis, 2006a). Alternative methods for attaching external tags to syngnathids, such as gluing tags to the body, have proven unsuccessful (Le Cheminant, 2000).

Externally visible marks, created by injecting visible implant fluorescent elastomer (VIFE) beneath the skin, have been used to identify hundreds of syngnathids

uniquely (up to 637 *H. guttulatus* in one study), have minimal effect on behaviour or mortality and remain visible for years (Woods & Martin-Smith, 2004; Curtis, 2006b; Curtis & Vincent, 2006). VIFE has been used to study reproductive behaviour, population dynamics, growth, habitat use, movement, home range and survival of at least 12 syngnathid species (Table I). VIFE had minimal effect on behaviour, growth and mortality in all syngnathids studied and marks remained visible for up to 2.5 years in the wild (Le Cheminant, 2000; Matsumoto & Yanagisawa, 2001; Woods & Martin-Smith, 2004; Sogabe *et al.*, 2007). Batch marking and assessment of population abundance have been achieved with single colours of elastomer while multiple colours and injection sites have allowed the unique marking of hundreds of individuals. Other external marking techniques such as uprooting dorsal spines and injecting acrylic paint caused severe skin irritation and should not be used (Matsumoto & Yanagisawa, 2001). A disadvantage of both external marking and external tagging is that they require recapture or re-observation of marked fishes. With such techniques, many individuals must be marked to obtain sufficiently high recapture rates.

Internal VI-alpha tags are numbered to allow for even more fish to be uniquely identified than VIFE but they have only been tested on one syngnathid species. As with VIFE, these small numbered tags are injected just beneath the skin so their numbers can still be seen through the skin. VI-alpha tags did not affect the growth or mortality of the big-bellied seahorse *Hippocampus abdominalis* Lesson 1827 (Woods, 2005a). VI-alpha tags, however, need to be larger (1 mm × 2.5 mm) than VIFE marks for the numbers to remain visible. Although they did not affect the welfare of *H. abdominalis*, they may be less suitable for smaller syngnathid species (Table I; Woods, 2005a). As with VIFE, reading VI-alpha tags requires repeated capture or observation.

Electronic tags can be used to remotely track individual fish and collect environmental data but have hitherto seldom been used with syngnathids, partly because of these tags' larger size. The newest of these tags can store and remotely transmit information about water temperature, depth, time and location of multiple fishes without a need to recapture or observe the fishes after tagging (Nielsen, 1992; Nielsen *et al.*, 2009). Ultrasonic (acoustic) tags have been used successfully to study the movement and habitat use of the leafy seadragon *Phycodurus eques* (Günther 1865) (Connolly *et al.*, 2002b), and passive integrated transponder (PIT) tags had only minimal effects on the growth of *H. abdominalis* (Woods, 2005a). These are two of the larger syngnathid species (Table I), however, making larger tags less of a burden. Their suitability for smaller syngnathids has not been tested but the emergence of smaller electronic tags may make this technique more generally applicable (Nielsen *et al.*, 2009). Acoustic tags can be attached to smaller syngnathids using necklaces or around the appendages of *P. eques* and should be attached so that they will fall off after their battery life has expired (Connolly *et al.*, 2002b). The battery life of acoustic tags has traditionally limited their usefulness to short-term studies, although battery life can be extended by programming tags to shutdown and start up periodically. This emerging technology provides an opportunity to learn more about the movement and environment of these fish on much finer time scales than was previously possible. This is an exciting new opportunity, but before these tags enter broad usage more must be known about their effects on fish welfare and behaviour.

## MATERIALS AND METHODS

### SPECIES DESCRIPTION

*Hippocampus guttulatus* is a small-bodied fish living in shallow, macrophyte-dominated environments of the north-eastern Atlantic Ocean, Mediterranean Sea and Black Sea (Lourie *et al.*, 2004). In a wild population in southern Portugal, this species ranged in size from 65 to 215 mm  $L_S$  and 0.57 to 22.5 g wet mass ( $M_w$ ) (Curtis & Vincent, 2006). After settling, *H. guttulatus* maintain relatively small home ranges of 1.4 to 400 m<sup>2</sup> (Curtis & Vincent, 2006; Garrick-Maidment *et al.*, 2010). Further information is needed to assess the global conservation status of *H. guttulatus* but it is protected regionally in the U.K. under the 1981 Wildlife and Countryside Acts and in Slovenia under the 1993 Protection of Threatened Animals Act and is listed in the Red Books of France and Portugal (Project Seahorse, 2003). The fish used in this study were originally collected from Portugal's Ria Formosa lagoon (36° 59' N; 7° 51' W) with permission from national authorities at the Parque Natural da Ria Formosa. Research protocols were approved by the University of British Columbia Animal Care Committee (approval A07-0077).

### AQUARIUM TRIALS

Individual fish were marked with dummy tags modelled after the smallest commercially available transmitters at the time of the experiments (October to December 2008), those produced by Lotek Wireless Inc. ([www.lotek.com](http://www.lotek.com)) and VEMCO ([www.vemco.com](http://www.vemco.com)). Lotek produces a cylindrical acoustic transmitter measuring 6.2 mm in diameter and 13 mm in length, weighing 0.9 g in air and 0.6 g in water (model MAP6\_1). Dummy tags (*i.e.* tags without the electronics of acoustic tags meant only for testing) were built with the same dimensions and mass as Lotek's MAP6\_1 transmitter.

Aquarium trials were used to assess how dummy tags affect individual *H. guttulatus* behaviour and mass. Recorded behaviours included movement, orientation, holdfast use and behavioural state. Holdfast use was recorded because *H. guttulatus* curl their prehensile tails around marine plants and animals to remain sedentary and upright (Curtis & Vincent, 2005); differences in how often a holdfast is used might suggest distress. Differences in the general behavioural state may also indicate stress induced by tagging. Finally, food intake has been used to assess welfare in a variety of fish species (Huntingford *et al.*, 2006; Faleiro *et al.*, 2008), and mass change was used as a proxy for food intake.

Trials were conducted in four 80 l cubic tanks housed at the Ramalhete field station of the University of Algarve in Faro, Portugal. Filtered sea water was fed into the tanks using a flow-through system. Temperature of the tanks was maintained at 18° C, range  $\pm 0.1^\circ$  C. An air hose and stone provided moderate aeration. Tanks were illuminated from above using two 36 W fluorescent tubes at a photoperiod of 12L:12D (0800–2000 hours) controlled by a timer. Before and during experiments, food (frozen shrimp, mysids and adult *Artemia* sp.) was provided daily *ad libitum*. A plastic mesh grid was placed on the bottom of the tank to allow for measurement of movement. The mesh was separated into a 5 × 5 cell grid, each cell measuring 100 mm × 100 mm. Two different materials were attached vertically to the grid to mimic the variety of holdfasts available in the wild: (1) a 150 mm length of wire coated with plastic to simulate rigid holdfasts (*e.g.* tube worms and sea squirts) and (2) a 150 mm length of balloon ribbon to simulate less rigid holdfasts (*e.g.* seagrass and algae). Holdfasts were attached in the centre of each grid cell, with each type alternating between cells.

Paired aquarium trials were conducted with one tagged and one untagged *H. guttulatus* per tank. Fish were originally collected from the Ria Formosa lagoon in 2007 for use in a feeding study (Palma *et al.*, 2008). Except for the 12 weeks of those feeding experiments, the individuals used in the tagging trials had been housed in two 500 l circular tanks for 2 years. Thirty-two *H. guttulatus* (16 males and 16 females) were measured, weighed and sexed, then each was marked with a unique colour combination using VIFE. The fish were paired with matching size and sex, resulting in eight female and eight male pairs. One of the individuals in a pair was randomly assigned to have the tag and each pair was randomly assigned to one of the four tanks and one of the four trials. Individual VIFE marks were used to ensure

fish were used in the tank and trial to which they were assigned and each fish was used for only one trial. The dummy tags were tied around each fish's neck using cotton thread. Each untagged fish was handled similarly, but without actually attaching a necklace or tag. After each 5 day trial, tags were removed and fish were re-weighed before releasing them back into a 500 l tank with the remaining fish.

Each pair of *H. guttulatus* was observed for 30 min each day over the 5 day trial period. The following were recorded once per minute during the focal period for each fish: grid location within the tank, orientation (vertical or not), holdfast and behavioural state. If a fish changed locations from 1 min to the next, the straight-line distance between grid locations was calculated for movement. As a holdfast, fish could choose one of the types provided (wire or balloon ribbon), other materials in the tank (e.g. air hose, mesh base and water outflow), the other fish or none. Behavioural states were categorized *sensu* Faleiro *et al.* (2008) as stationary (St), head movement (Mh), slow body movement (Ms), fast body movement (Mf) and swimming (Sw), with the addition of tail movement (Mt) and clicking (Cl). Only the initial behavioural state was recorded each minute (*i.e.* behaviours were treated as mutually exclusive). Tail movement was assigned when the fish was curling or uncurling its tail while keeping the rest of its body stationary. Clicking was assigned when the fish moved its jaw, creating a sound, but otherwise remained stationary. Clicking has been associated with feeding as well as negative responses to environmental stimuli and aggressive interactions (Fish, 1953; Colson *et al.*, 1998).

The effects of tagging were analysed using paired comparisons (paired *H. guttulatus*), while the effect of time (*i.e.* day since first tagged) was analysed using repeated measures of individual fish and comparisons between sexes were unpaired. Before each comparison, the data were first tested for normality and constant variance. Parametric comparisons were used unless the assumptions of normality and constant variance were violated. Initial wet mass of *H. guttulatus* was compared between treatment groups (*i.e.* tagged and untagged) to determine whether pairs were matched appropriately and between sexes. The effect of tagging on wet mass before and after trials was compared using a two-way repeated measures ANOVA with treatment (tagged and untagged) and time (before and after) as the two factors. Bonferroni post-tests were used to identify whether there were differences in mass within each treatment group. A one-sample *t*-test, comparing mass change (final – initial mass) against a mean of zero, was used to test whether there was significant mass gain or loss in either treatment group. Mass change was further compared between tagged and untagged fish (paired comparisons) and between sexes (unpaired comparisons). To identify any tagging effects on behaviour (*i.e.* distance moved, frequency of vertical orientation, frequency of using a holdfast and frequency of observing each behavioural state), each day was treated as a replicate (*i.e.* the sum of the behaviours over the 30 min of observation per day). Before analysing the effects of tagging, each behaviour was first compared between days using repeated measures to determine whether there were changes in *H. guttulatus* behaviour over the trial period. If there were no differences between days, the total for each fish over the trial period was calculated and this total was used in the paired analysis of tagging effects. If there were differences between days, paired analysis of tagging was done for each day. In addition to comparing the distance moved between the two sexes (unpaired comparisons), the relationship between movement and *H. guttulatus* size (wet mass) was analysed using regression.

## RESULTS

### MASS

Tagged *H. guttulatus* gained significantly more mass than untagged *H. guttulatus* over the 5 day trial period (Fig. 1). Initial masses were between 15.6 and 35.4 g (median 20.9 g) with males significantly heavier than females (Mann–Whitney *U*-test,  $n = 16$ ,  $P < 0.001$ ) but no significant mass difference was detected between treatment groups (Wilcoxon matched pairs test,  $n = 16$ ,  $P > 0.05$ ). There was a significant difference in initial and final mass for tagged *H. guttulatus* (two-way repeated

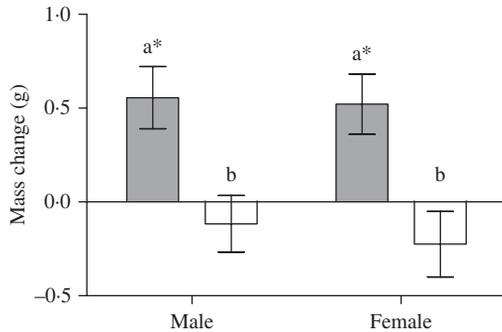


FIG. 1. Mean  $\pm$  s.e. mass change for male and female *Hippocampus guttulatus* tagged with dummy tags (■;  $n = 8$  in each case) compared to those that were not tagged (□;  $n = 8$  in each case) over a 5 day trial period. Bars labelled with the same lower case letter are not significantly different ( $P > 0.05$ ). \*, mass change is significantly different from zero ( $P < 0.05$ ).

measures ANOVA with Bonferroni post-tests,  $P < 0.001$ ) but not for untagged fish ( $P > 0.05$ ). There was a significant difference between the change in mass of tagged and untagged *H. guttulatus* (paired  $t$ -test,  $n = 16$ ,  $P < 0.001$ ) with tagged *H. guttulatus* gaining significant mass overall (one-sample  $t$ -test,  $n = 16$ ,  $P < 0.001$ ) while untagged *H. guttulatus* neither gained nor lost mass throughout the trial period (one-sample  $t$ -test,  $n = 16$ ,  $P > 0.05$ ). There was no significant difference detected in the change in mass between sexes (unpaired  $t$ -test,  $n = 16$ ,  $P > 0.05$ ).

## MOVEMENT

*Hippocampus guttulatus* moved distances of between 0 and 238 cm (median 13.4 cm) during the 150 min of total observation. Distance moved by individual fish did not differ significantly from one day to another (paired Friedman test,  $n = 32$ ,  $P > 0.05$ ) so daily movement data were combined. Total distance moved did not differ significantly between tagged and untagged seahorses (Wilcoxon matched pairs test,  $n = 16$ ,  $P > 0.05$ ), but male *H. guttulatus* moved significantly greater distances than females (median distance moved: males = 33.1 cm *v.* females = 8.74 cm; Mann–Whitney  $U$ -test,  $n = 16$ ,  $P < 0.05$ ) (Fig. 2). There was no correlation between the distance moved and wet mass of each *H. guttulatus* (Spearman rank correlation,  $n = 32$ ,  $P > 0.05$ ).

## ORIENTATION

*Hippocampus guttulatus* were oriented vertically the majority of the time (84% of observations). There was a significant difference in frequency of vertical orientation between days (Friedman test,  $n = 32$ ,  $P < 0.01$ ), so each day was analysed separately. On the first day, untagged fish were oriented vertically more often than tagged fish (median frequency of vertical orientation day 1: untagged fish = 30 *v.* tagged fish = 21; Wilcoxon signed rank test,  $n = 16$ ,  $P < 0.01$ ), even after applying a Bonferroni correction to adjust  $P$ -values for multiple tests ( $P = 0.05/5 = 0.01$ ). On days 2 to 5, however, there was no significant difference between tagged and untagged fish after Bonferroni correction (Wilcoxon signed rank tests,  $n = 16$ ,  $P > 0.01$ ).

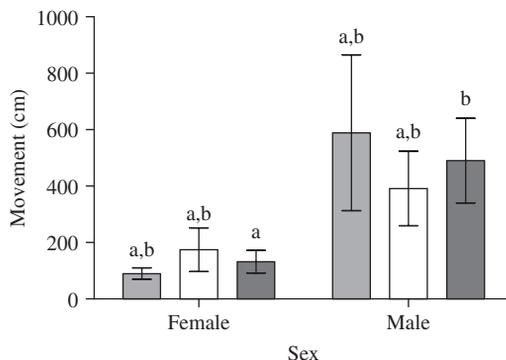


FIG. 2. Mean  $\pm$  S.E. distance moved over 5 days by female and male *Hippocampus guttulatus* tagged with dummy tags (■;  $n = 8$  in each case), untagged (□;  $n = 8$  in each case) and both combined (■;  $n = 16$  in each case) when observed for 30 min each day. Bars labelled with the same lower case letter are not significantly different ( $P > 0.05$ ).

## HOLDFAST USE

*Hippocampus guttulatus* used holdfasts the majority of the time (94% of observations) and used the rigid wire holdfasts more than the balloon ribbon (31 *v.* 0.02% of observations). Other holdfasts used included the filter, the grid, the air stone, the heater, the air hose and their paired fish (36, 14, 9, 3, 0.4 and 0.1% of observations, respectively). The frequency with which fish used holdfasts in general (as opposed to using none) did not differ from day to day (Friedman test,  $n = 32$ ,  $P > 0.05$ ) so observations were combined for each fish. When summed observations were compared, there was no significant difference between how frequently tagged and untagged *H. guttulatus* used a holdfast (Wilcoxon matched pairs test,  $n = 16$ ,  $P > 0.05$ ).

## BEHAVIOURAL STATES

All *H. guttulatus* remained stationary most of the time (93% of all observations) and tagging had no effect on behavioural state (Fig. 3). Behavioural states of individual seahorses did not differ from one day to the next (Friedman tests,  $n = 32$ ,  $P > 0.05$  in all cases), so observations were combined. None of the seven behavioural states differed significantly between tagged and untagged fish (Fig. 3; Wilcoxon matched pairs tests,  $n = 16$ ,  $P > 0.05$  in all cases).

## DISCUSSION

### SYNGNATHID MARKING AND TAGGING

External tagging with necklaces and external marking with VIFE have been the most utilized marking or tagging techniques in studies of syngnathid biology. Any long-term use of necklaces would require attachment using shrinking plastic (Vincent *et al.*, 1994), and regular monitoring to ensure necklaces do not cause injury as fishes grow, whereas VIFE injected beneath the skin remains visible for years in the wild

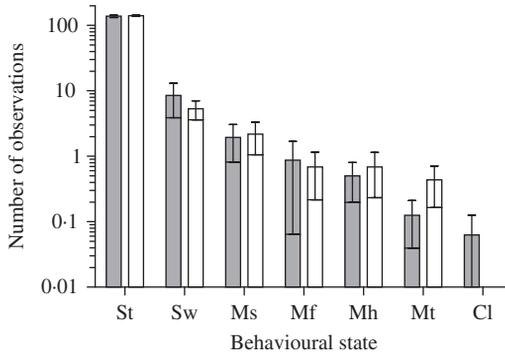


FIG. 3. Mean  $\pm$  s.e. number of times, on a logarithmic scale, tagged ( $\blacksquare$ ;  $n = 16$  in each case) and untagged ( $\square$ ;  $n = 16$  in each case) *Hippocampus guttulatus* were observed in each of seven behavioural states (St, stationary; Sw, swimming; Ms, slow body movement; Mf, fast body movement; Mh, head movement; Mt, tail movement; Cl, clicking) during 30 min focal periods per day for 5 days.

without the need for maintenance. Both techniques could be used to verify growth rates and estimate life-history variables (Matsumoto & Yanagisawa, 2001; Curtis, 2006b). Using many colours and injection sites with VIFE or individually numbered plastic discs on necklaces, hundreds of individual syngnathids can be differentiated to measure population-level characteristics such as total mortality, immigration, emigration and abundance. Marked animals, however, must be reliably recaptured for these techniques to be useful. This may be less difficult for the many syngnathids that are sedentary as their small home ranges may make it easier to find marked individuals (Kuitert, 2000; Foster & Vincent, 2004). A major limitation of external marking and tagging is that one cannot monitor individual movement continuously and archive the environmental conditions experienced by fishes (Nielsen *et al.*, 2009). This problem can be overcome using electronic tags.

## AQUARIUM EXPERIMENTS

The aquarium experiments presented here suggest that acoustic tagging technology has become small enough to be a viable technique for monitoring *H. guttulatus* and similarly sized syngnathids. Although dummy tags were used in the experiment, they were the size and mass of acoustic tags and this size seems to have had minimal effect on behaviour when attached as necklaces. Tags did not seem to affect movement, holdfast use or general behavioural state. An initial difference in vertical orientation was a short-lived response and, by itself, small differences in orientation may not matter given that wild *H. guttulatus* have been observed oriented both vertically and horizontally (I. R. Caldwell, pers. obs.).

The finding that tagged *H. guttulatus* gained more mass than untagged clearly needs more probing, did they somehow eat more or expend less energy? In a search of other tagging studies, no others could be found that resulted in mass gain. There is no evidence that tagged *H. guttulatus* moved shorter distances (Fig. 2) or were less active (Fig. 3). On the other hand, tagged fish are the only ones that clicked (Fig. 3), and clicking has been associated with feeding in other seahorse species (Colson *et al.*, 1998). Although further trials would be needed to determine the true cause,

this limited evidence does hint that the mass difference might have been due to food intake rather than energy expenditure. In any case, mass was measured in this study to determine whether tagging reduced feeding and that at least did not seem to happen.

For short-term studies, these syngnathids seem to be able to cope with tags exceeding the 2% guideline normally used for fishes. The tags in the present study, as heavy as 5.77% of body mass, had minimal effect on fish movement or behaviour over a 5 day period. The sedentary nature of *H. guttulatus* and many other syngnathids may allow them to carry larger tags than more active fishes without affecting their behaviour. *Hippocampus guttulatus* in the present study rarely moved, using rigid holdfasts to remain stationary. This sedentary behaviour, in combination with their unusual body morphology, allows seahorses and many other syngnathids to ambush prey and escape predation using camouflage (Foster & Vincent, 2004). If such syngnathids can remain hidden and feed with a large tag attached, they may be better suited to carry heavy tags than fishes that need to swim to find prey or escape predation. The utility of the 2% guideline, however, has also been questioned for fishes that need to swim greater distances, with tags weighing up to 10% of body mass found to have no significant effect on swimming speeds of juvenile Chinook salmon *Oncorhynchus tshawytscha* (Walbaum 1792) (Jepsen *et al.*, 2005; Brown *et al.*, 2006). Indeed, the threshold in tag mass to body mass for syngnathids may not have been reached in this aquarium study, given that the *H. guttulatus* used were larger than average in the wild (Curtis & Vincent, 2006). While the present study suggests these large tags are suitable for short-term studies, this may or may not be the case for longer periods.

Caution should be taken before assuming that acoustic tags will have no effect on these fish in the wild. Despite the positive results from the present *ex situ* experiment, *in situ* experiments should follow. These should use a larger sample size to increase the power to detect any small effects of tagging that may not have been detected in the present study and could prove to be important in long-term studies in the wild. Many of the natural threats to the welfare of wild fish were not present in aquaria and results may only apply to a specific size range of *H. guttulatus*. In the wild, fish are exposed to threats from predators, variable food resources, parasites, disease and variable environmental conditions, none of which was present in aquaria (Huntingford *et al.*, 2006). Tags might interfere with a wild syngnathid's camouflage making them more vulnerable to predation or making it harder to ambush prey. The additional stress of carrying a tag might also impair the ability to fight parasites or disease. The movement constraints in the aquaria may have produced results that do not predict the effects of tagging on long-range movement *in situ*. Before any large-scale deployment of transmitters on *H. guttulatus* or similarly sized syngnathids, experiments should be conducted to test whether tagging effects are influenced by the natural threats to which wild fish are exposed.

If acoustic tagging technology can be used to study wild syngnathids, it would help establish conservation status of data-deficient species and protect threatened species. Sedentary syngnathids may be less capable of escaping negative environmental change than more mobile species. Acoustic tags, however, could be used to follow these more sedentary fishes when exposed to change to determine their true capability for long-distance movement. Following syngnathids with acoustic tags after displacement could help identify habitats associated with settlement and those of greatest importance. Information on habitat use, movement and occurrence can

be integrated into IUCN assessments of data-deficient species such as *H. guttulatus* and used to appropriately site and manage marine reserves.

Advancements in marking and tagging techniques for syngnathids have provided life history and population information that is necessary for conservation assessment and action. There are now a variety of marking and tagging techniques, which can be used to track small fishes such as syngnathids and provide information on movement, population dynamics and behaviour. The most advanced of these is the use of electronic tags, which allow fishes to be tracked remotely and continuously with stationary receivers, allowing for precise measurement of their movement and environmental conditions. This method has been limited to large fishes in the past due to large tag sizes but here is shown to be increasingly suitable for smaller fishes. Other marking and tagging methods, such as VIFE and external tagging, cannot be used for such precise measurements but have proven equally useful in studies where fine temporal scales are not needed (*e.g.* estimating population abundance, verifying growth estimates or understanding population structure).

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