

BIOLOGICAL CONSERVATION

Biological Conservation 123 (2005) 533-545

www.elsevier.com/locate/biocon

# Seahorse declines in the Derwent estuary, Tasmania in the absence of fishing pressure

Keith M. Martin-Smith a,b,\*, Amanda C.J. Vincent a,c

<sup>a</sup> Project Seahorse, Department of Biology, McGill University, 1205 Avenue Dr. Penfield, Montréal, Qué., Canada H3A 1B1
 <sup>b</sup> Project Seahorse, School of Zoology, University of Tasmania, Private Bag 05, Hobart, Tasmania 7001, Australia
 <sup>c</sup> Project Seahorse, Fisheries Centre, University of British Columbia, 2204 Main Mall, Vancouver, BC, Canada V6T 1Z4

Received 31 August 2004

#### Abstract

Seahorses have the potential to be flagship species for estuarine conservation around the world and considerable concern has arisen over their declining abundance in the face of exploitation. All seahorses were recently listed on CITES Appendix II in recognition of threats posed by unsustainable trade. However, the efficacy of this measure and the development of other effective conservation solutions will require an understanding of natural seahorse population dynamics at relevant ecological scales. In this study, we provide quantitative data on seahorse populations over nested spatial and temporal scales up to 10 km and 3.5 years. Unexploited populations of the big-bellied seahorse (*Hippocampus abdominalis*) in the Derwent estuary, Tasmania, Australia consisted largely of mature adults with highly female-biased sex ratios at all sites. While big-bellied seahorses had an aggregated distribution at the spatial scale of our transects (200 m²), most individuals were found alone. When they occurred in groups, the distribution of individuals was random with respect to sex in contrast to the pair bonds found in other seahorse species. All three monitored populations showed significant declines in abundance of 79–98% over the period 2001–2004. Estuarine physicochemical conditions did not change markedly during this period. Possible causes of seahorse declines are interactions with invasive species, disease or reproductive limitation through Allee effects. The magnitude of the declines in the absence of direct exploitation demonstrates that seahorses face multiple threats. Robust fisheries-independent monitoring will be required to demonstrate that conservation actions such as CITES listing are effective.

© 2005 Elsevier Ltd. All rights reserved.

Keywords: Australia; Sex ratio; Size-frequency distribution; Syngnathid; Time series

#### 1. Introduction

Estuaries are important coastal habitats ecologically and commercially. Despite low species richness at any particular location, estuaries support significant marine biodiversity through high levels of species turnover between locations (Price, 2002). Some estuaries have substantial fisheries while others serve as nursery grounds

for large commercial fisheries elsewhere (Blaber et al., 2000; Gillanders et al., 2003). Estuaries are, however, among the most highly modified coastal ecosystems and the most threatened by human activities (Blaber et al., 2000). Continued declines in the condition of estuaries have been reported from around the world because of changes in catchment land-use, coastal development and pollution (e.g., Kiddon et al., 2003; Baird et al., 2004).

Seahorses (genus *Hippocampus*) are potential flagship species for estuarine conservation. Found in estuaries around the world, they are affected by many marine conservation problems including habitat degradation or

 $<sup>^{\</sup>ast}$  Corresponding author. Tel.: +1 61 362 262 522; fax: +1 61 362 262 745.

E-mail address: Keith.MartinSmith@utas.edu.au (K.M. MartinSmith).

loss, direct overexploitation and incidental bycatch in non-selective fishing gear (Vincent, 1996; Baum et al., 2003; Bell et al., 2003). As a resource, seahorses are used in traditional medicine, as curios and as aquarium fishes (Vincent, 1996). In recognition of the threats facing seahorses, all species were listed on Appendix II of the Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES), with implementation in May 2004.

We know little about wild populations of most species of seahorse (Foster and Vincent, 2004). The majority of seahorse species (23 of 33) are listed as Data Deficient on the IUCN Red List (IUCN, 2003). The Knysna seahorse is listed as Endangered on the basis of its limited occurrence in only four South African estuaries (Bell et al., 2003). If we are to measure the effects of our conservation actions we need a thorough understanding of seahorse populations incorporating spatial and temporal structure. There are, however, few long-term data on spatial and temporal patterns for seahorses spanning multiple seasons and years (but see Perante et al., 2002; Curtis and Vincent, in press).

In general seahorses have been found in low abundance in natural ecosystems (Foster and Vincent, 2004). Low abundance may make populations more vulnerable to fishing mortality through Allee effects wherein some component of individual fitness declines as population size declines in a positive density-dependent relationship (Gascoigne and Lipcius, 2004). Mean estimates of density ranged from 0.60 to 110 per 100 m<sup>2</sup> for seven species of seahorse (references in Foster and Vincent, 2004). However, almost all densities >100 per 100 m<sup>2</sup> were found in focal grid behavioural studies where sites were deliberately selected for high numbers of seahorses (Bell et al., 2003; Moreau and Vincent, 2004; Vincent et al., 2004).

The big-bellied seahorse, Hippocampus abdominalis, found in southeast Australia (New South Wales, South Australia, Tasmania and Victoria) and New Zealand, is one of the largest seahorses, growing to over 30 cm height and 30 g wet weight (Lourie et al., 1999). It is listed as Vulnerable on the IUCN Red List (2003) and Data Deficient within Australia (Pogonoski et al., 2002). Whilst not historically known in the traditional medicine trade, it has been the focus of considerable aquaculture efforts as an aquarium species since the early 1990s. Successful development of culturing techniques has led to increasing trade within Australia and internationally (K. Martin-Smith and A. Vincent, unpublished data). The effects of extracting broodstock from the wild are not known nor are there any published assessments of population trends.

Our aim in this study was to quantify the spatial and temporal distribution of wild populations of the big-bellied seahorse. We established study sites in southeastern Tasmania and conducted underwater visual censuses at intervals of one to two months over more than three years. Life-spans for larger species of seahorse have been estimated at three to five years (Foster and Vincent, 2004). Thus, while the life-span for the big-bellied seahorse is not known in the wild, sampling over three years is likely to be ecologically relevant with respect to growth, mortality and recruitment. One problem in detecting long-term trends in abundance in this kind of sampling programme is estimating the contribution of short-term stochastic variability (Krebs, 1999). Therefore, we also sampled populations at temporal scales of days and weeks. These data allowed us to produce quantitative evaluation of population abundance, size structure and sex ratio, and changes in these parameters over time.

## 2. Materials and methods

#### 2.1. Study sites

This study was conducted in the estuary of the River Derwent in Hobart, southeastern Tasmania (42°S 147°E). The estuary covers approximately 200 km<sup>2</sup>, is relatively deep (20-44 m maximum depth) and highly stratified in the narrow upper reaches with freshwater input chiefly over the austral winter (June-August). It has been heavily affected by human activities with high levels of heavy metals and nutrients, 70 introduced marine species and historical losses of macroalgae and seagrass (Green and Coughanowr, 2003). The estuary has significant levels of nutrient enrichment with nitrite, ammonia and phosphate regularly exceeding Australia and New Zealand Environment and Conservation Council guidelines (Green and Coughanowr, 2003). Commercial fishing in the estuary is restricted to the lower reaches below the sampling sites used in this study (Green and Coughanowr, 2003). Similarly, recreational fishing in the area of the Derwent estuary shown in Fig. 1 is restricted to hook-and-line only.

Six sites were established in the Derwent estuary: a single site was used for pilot work in 1988 and five further sites were selected for work in 2000–2004 (Fig. 1). Initial work was conducted around two similar piers of a marina where there were anecdotal reports of high numbers of seahorses. Each pier covered approximately 1100 m<sup>2</sup> of seabed with a water depth of 6-8 m. We chose later sites to be representative of conditions in the Derwent estuary and these were located 0.5-3 km apart. The substratum at all sites was predominantly soft sediment with varying amounts of fine sand, shell debris, boulders, rocky reef and anthropogenic debris (Table 1). We consider these latter features to be microhabitats, as their spatial extent (<3 m) was considerably smaller than that of our sampling unit (50 m length). We have presented all of our data by site from north to

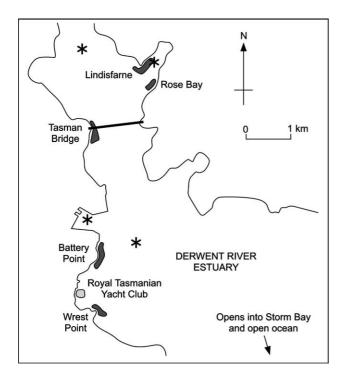


Fig. 1. Map of lower Derwent estuary, Tasmania showing locations of our study sites. 1988 site is shown in grey; 2000–2004 sites are shown in black. Asterisks indicate sampling stations for water quality parameters collected by the Derwent Estuary Program.

south, representing progressively more oceanic conditions.

Water quality parameters for four stations close to our sampling sites (Fig. 1) were collected by the Derwent Estuary Program, Department of Primary Industries Water and Environment as part of estuary-wide environmental monitoring. Data for selected parameters (temperature, dissolved oxygen, total nitrogen and total phosphorus) for the period December 2000–March 2004 were abstracted to provide a description of physicochemical changes during the period of our study.

## 2.2. Underwater visual censuses

We quantified seahorse populations using underwater visual censuses in December 1988 and from December 2000 to March 2004. In December 1988 we conducted a one-month long intensive study wherein seahorse populations were quantified around two piers at time intervals ranging from 3 h to 7 days (for a total of 32 samples; Table 1). On three occasions, the seahorses were tagged with uniquely numbered plastic 'necklace' tags (Vincent and Sadler, 1995). We also conducted three night dives starting at 2130 or 2200. On each dive the whole area around one pier and the pilings of the pier itself was searched. We recorded the seabed area covered by each pier but it should be noted that abundance estimates could not be directly compared with later censuses because of the additional three-dimensional structure provided by the piers. We noted the location of each individual. its sex and size. We defined seahorses as adults if their sex could be determined visually: males with a developing or developed brood pouch and females with a pronounced rounded abdomen (Woods, 2000). This does not necessarily equate to reproductive capability. Size is difficult to measure in seahorses because of their unusual body morphology; our measurements refer to height (see Lourie et al., 1999; Foster and Vincent, 2004 for details).

From December 2000 to February 2001, we conducted intensive seahorse population surveys at five sites using stratified random belt transects, each one  $50 \times 4$  m. We surveyed between six and twelve transects at each site on each sampling occasion at intervals of 2–7 days (Table 1). We stratified our sampling by time of day (early morning, late morning, early afternoon, late afternoon), tide height and phase of moon (new, first quarter, full, third quarter). For safety reasons, we started each survey with the deepest transect and moved progressively shallower. The start

Table 1
Details of sampling locations and frequency for underwater visual censuses of seahorses in the Derwent estuary, Tasmania, arranged from North to South

Site	Depth (m below	Habitat description		of sampling (transects)
	chart datum)		Intensive	Long-term
Lindisfarne	1.8-6.1	Extensive silt with small patch of rock reef. Little anthropogenic data.	6(54)	_
Rose Bay	1.7-8.3	Low rocky reef surrounded by extensive silt. Little anthropogenic debris.	4(26)	_
Tasman Bridge	2.3-8.2	Large expanse of silt with extensive large anthropogenic structures close to base of bridge.	8(84)	16 (154)
Battery Point	0.9–9.0	Mainly silt with areas of shell debris. Some areas of low rock and extensive rock/ oyster reef in shallow depths (1–2 m). Large amounts of anthropogenic debris.	10 (92)	21 (186)
Royal Tasmanian Yacht Club <sup>a</sup>	1.0-7.5	Mooring piers for recreational vessels. Wooden pilings and extensive anthropogenic debris. Silt substrate.	32	-
Wrest Point	1.1-8.1	Large boulders (1-3 m) emergent from silt and fine sand. Some anthropogenic debris.	9(80)	11 (98)

a Pilot site in 1988.

point of each new transect was a randomly assigned horizontal distance between 1 and 25 m from the previous transect. We sampled transects at depths between 1 and 9 m below mean chart datum on each occasion except at one site (Lindisfarne) where strong currents precluded depths greater than 6 m. Each transect was run at approximately the same depth throughout, i.e., parallel to depth contours. Two observers swam side by side whilst one unreeled a 50 m fibreglass tape. Each observer searched a belt 2 m wide on one side of the tape only. Initial training dives allowed us to estimate this distance visually. This sampling procedure was designed to detect all seahorses, adult or juvenile, within the sampling area.

Whenever we encountered seahorses within the area of the belt transect, the distance along the transect was recorded. We recorded the sex of each individual and whether there were other seahorses within visual range (approximately 2 m). We did not measure height directly but recorded the size in one of six size classes by holding the seahorse against scale drawings of known size. The smallest size class (0) consisted of all individuals smaller than 160 mm height while all subsequent size classes corresponded to size class intervals of approximately 25 mm. We also recorded the microhabitat where the seahorse was found in the following categories: anthropogenic debris, bare sediment, macroalgae (Codium spp., Ulva spp., unidentified red algae), other organic material (ascidians, holothurians, branches, etc.) or rock (including seahorses on a rock or within 50 cm). For each site overall microhabitat availability was assessed qualitatively on a six point scale: l = very rare (<1%)available area); 2 = rare (1-5%); 3 = moderately rare(5-10%); 4 = common (10-25%); 5 = abundant (25-10%)50%); 6 = dominant (50-100%).

From December 2001 to March 2004, we surveyed seahorses at a subset of three sites (Tasman Bridge, Battery Point and Wrest Point) at one to two-monthly intervals (Table 1). We also surveyed Lindisfarne and Rose Bay at irregular intervals of 6-9 months (January 2002, August 2002, April 2003 and January 2004). We used the same belt transect method as described above but measured all seahorses directly. Size data were grouped into 10 mm height classes. From November 2002 to April 2003 size-frequency distributions were supplemented with additional data from a separate mark-recapture study that we conducted at Tasman Bridge and Battery Point. Our sample design allowed the possibility that an individual seahorse could move among transects and be recorded more than once during a dive. However, we released seahorses (which invariably swam away) in the opposite direction to our next transect. Marked individuals were also rarely encountered more than once in a dive and thus we are confident that the majority of seahorses that we measured were unique individuals.

# 2.3. Data analyses

We used data from our intensive 1988 sampling to provide bootstrap error estimates for our long-term monitoring. We constructed matrices of abundance differences between all possible pairwise combinations of samples and converted these to percentage of the pairwise mean. As there was no relationship between time and the magnitude of the abundance difference, we calculated the overall mean as a bootstrap stochastic error.

As we had unequal replication between sites during our intensive sampling, we used a general linear model to investigate differences in seahorse abundance between sites. We used a hierarchical model with sampling occasions nested within our main factor, sites. We used Yates-corrected  $\chi^2$  tests to detect significant deviations from 1:1 sex ratios at each site except for Rose Bay where we encountered too few seahorses for meaningful analysis. We investigated the influence of time of day, tide height and phase of moon on abundance (density), size-frequency and sex ratio of seahorses using our combined intensive data from December 2000 and January 2001. We calculated mean abundance and mean size for each sampling occasion and sex ratio for sampling occasions with more than 10 seahorses. We then used periodic regression (also known as linear-circular regression) with mean abundance, mean size or sex ratio as the response variable and time of day, tide or phase of moon as the continuous predictor variable (deBruyn and Meeuwig, 2001). We repeated these analyses using linear regression. We investigated the relationship of seahorse abundance and size with depth in a similar manner except that individual transects were used as replicates.

To investigate the spatial patterns of seahorses among transects we used the standardized Morisita's index of dispersion  $(I_p)$  (except for Rose Bay where there were too few data). This index is considered to be one of the best measures of dispersion as it has been found to be independent of population density and sample size (Krebs, 1999). The index, ranging from +1.0 (clumped) through 0 (random) to -1.0 (uniform) is associated with a known sampling distribution and 95% confidence limits at +0.5 and -0.5 (Krebs, 1999). To determine whether seahorses preferentially associated with others of the same sex or the opposite sex we compared observed and expected frequencies using  $\chi^2$  tests under the null hypothesis that groups were random associations between individuals. Spearman rank correlation tests were used to compare microhabitat availability and seahorse microhabitat use.

We used non-linear regression to examine trends in long-term patterns of abundance, fitting negative exponential curves to abundance data from December 2001 onwards. Instantaneous mortality rates were derived from the slopes of these curves and converted to finite yearly survival rates (Krebs, 1999). As above, we used

Yates-corrected  $\chi^2$  tests to detect significant deviations from 1:1 sex ratios at each site. All statistical tests were performed using SPSS 11.0 with a significance level of  $\alpha = 0.05$  and all means are reported  $\pm$  SE unless otherwise stated.

## 3. Results

## 3.1. Physicochemical patterns

Temperature in the Derwent estuary ranged from approximately 11 to 18 °C seasonally over the course of the study (Fig. 2(a)). Dissolved oxygen levels were generally above 5 mg l<sup>-1</sup> and fluctuated unpredictably spatially and temporally with two records of very low oxygen levels below 3 mg l<sup>-1</sup> (Fig. 2(b)). Total nitrogen and total phosphorus levels remained reasonably constant over time with some spatial and temporal variation (Fig. 2(c) and (d)).

# 3.2. Short-term patterns

Seahorses were abundant on and around both piers during our initial sampling in 1988 and 1989 with mean abundance of  $3.57 \pm 0.30$  and  $2.56 \pm 0.21$  per  $100 \,\mathrm{m}^2$  (Fig. 3(a)). There appeared to be no systematic changes in abundance over the brief sampling period (30 days) although we found higher numbers of seahorses on two of three night dives (Fig. 3(a)). Bootstrap estimates of variation in abundance were 30% and 35% of the mean for the two piers. All seahorses that we observed

were adults ranging from 164 to 207 mm for females and 167–214 mm height for males. There was no evidence of multiple cohorts in the size-frequency distribution (Fig. 3(b)). Sex ratio at one of the two piers was highly female-biased (approximately three females for every one male) and remained consistent over time while there was a systematic change from male-biased to female-biased sex ratios at the second pier (Fig. 3(c)).

During our sampling in 2000 and 2001 seahorse abundance differed significantly among sites (F = 1.57, p = 0.026). We found highest densities at Battery Point (mean  $1.11 \pm 0.12$  per  $100 \text{ m}^2$ ), similar densities at Lindisfarne, Tasman Bridge and Wrest Point (means  $0.56 \pm 0.10$ , 0.65 + 0.10 and  $0.75 \pm 0.08$  per  $100 \text{ m}^2$ , respectively) and low density at Rose Bay (mean  $0.12 \pm 0.04$  per 100 m<sup>2</sup>). Again, all seahorses that we observed were adults. Size-frequencies were broadly similar unimodal distributions at Tasman Bridge, Battery Point and Wrest Point sites while Lindisfarne had a greater proportion of smaller seahorses (Fig. 4). Female seahorses at all sites were significantly larger than males by approximately half a size class (13 mm). Sex ratios remained consistent over the period of sampling so we combined all data for subsequent analyses. We found significantly female-biased populations at all sites (Yates corrected  $\chi^2 > 5.24$ , p < 0.022 in all cases).

We found no relationships between seahorse density, mean size or sex ratio and time of day or tide height (Table 2). There was a very weak negative relationship between seahorse density and sin (2 \* moon) with slightly higher abundance at days 11 and 25 of the lunar cycle but this relationship was not apparent with linear

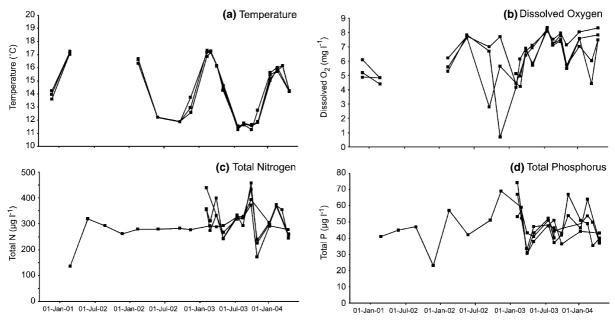


Fig. 2. Physicochemical parameters for water sampled at the stations shown in Fig. 1 for the period December 2000–March 2004. Data courtesy of the Derwent Estuary Program, DPIWE.

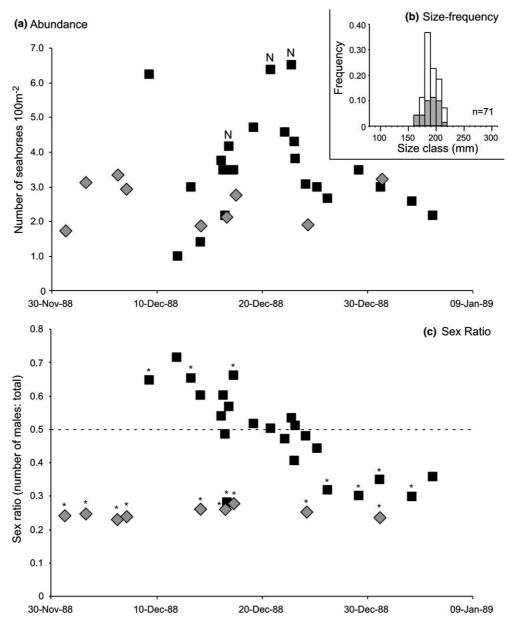


Fig. 3. (a) Abundance trends for seahorses, (b) size-frequency distribution, and (c) trends in seahorse sex ratio from sampling at the Royal Tasmanian Yacht Club from 1 December 1988 to 5 January 1989. The two replicate piers are shown with different symbols and shading. (a) N indicates night dives, (b) females are hatched and males white, (c) asterisks indicate significant departures from a 1:1 sex ratio using  $\chi^2$  tests. The size-frequency distribution was constructed from uniquely identified individuals only.

regression. Similarly, there was a weak negative relationship between seahorse size and cos (2 \* moon) suggesting slightly smaller seahorses were found on full and new moons (Table 2). We found a positive linear relationship between seahorse abundance and depth but this was strongly influenced by two outliers of deep transects with high numbers. With either of these transects removed the relationship was no longer significant and the proportion of total variance explained by depth was very low (Table 2).

At the spatial scale of our belt transects seahorses were significantly clumped at all sites with all  $I_p$  values

greater than +0.5 (Table 3). However, at each site 60–75% of individuals were found alone with no other individual within visual range (Table 3). When seahorses were found in association with others they were found in same-sex pairs, male-female pairs, same-sex groups or mixed-sex groups, the largest group size being 10 individuals at Battery Point (Table 3). There were very strong positive relationships between abundance of seahorses and the number of groups or the numbers of individuals found in groups (linear regression: adjusted  $r^2 = 0.865$ , p = 0.046, n = 4 and adjusted  $r^2 = 0.998$ , p = 0.001, n = 4, respectively). The distribution of sea-

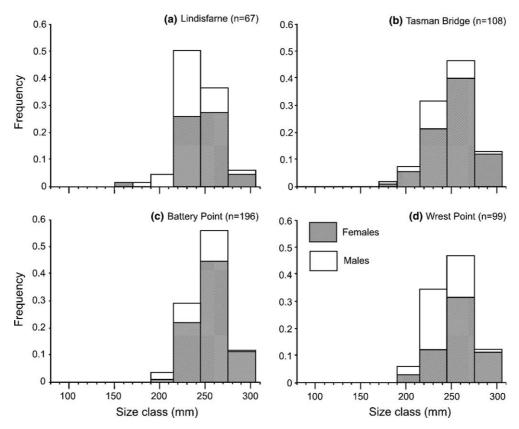


Fig. 4. Size-frequency distributions for seahorses for four sites in the Derwent estuary for January 2001. No data are presented for Rose Bay as only six seahorses were found. Size class intervals are approximately 25 mm except for the smallest size class (<160 mm), represented by a single individual at Lindisfarne.

Table 2
Summary of results from periodic regression of seahorse variables against physical predictors for data collected during sampling period December 2000–January 2001 irrespective of site

Seahorse variable	N	Time of day	Tide height	Phase of moon	n	Depth
Mean density	37	NS	NS	Weak –ve relationship with sin (2 * moon) Spearman rank correlation $r_s = -0.108$ , $p = 0.048$	336	Weak +ve relationship Spearman rank correlation $r_s = 0.080; p = 0.143$
				Linear regression $r^2 = 0.001$ , $p = 0.672$		Linear regression $r^2 = 0.005$ , $p = 0.018$
Mean size	37	NS	NS	Weak –ve relationship with cos (2 * moon) Spearman rank correlation $r_s = -0.058$ ; $p = 0.216$ Linear regression $r^2 = 0.011$ , $p = 0.023$	199	NS
Sex ratio	24	NA	NA	NS	_	NA

For each sampling occasion mean density and size were calculated and regressed against time of day, tide height or phase of moon. Sex ratio data were only used if more than 10 individuals were found and only analyzed with respect to moon phase.

To investigate the relationship of seahorse density with depth, individual transect data were used.

Similarly, individual transect data with seahorses present were used to investigate the relationship of seahorse size with depth.

NS = no significant relationship, NA = not analyzed.

horses in same-sex or mixed-sex groups was not significantly different from random association based on overall sex ratios (p > 0.22 for all  $\chi^2$  tests).

Seahorses used a variety of microhabitats as holdfasts but were also found resting on the substrate without grasping on to anything with their tails. Seahorses did not use microhabitats in proportion to their availability at all sites (Spearman rank correlations  $R_s < 0.667$ , p > 0.219) (Table 4). However, there was no consistent

choice of one type of microhabitat across all sites with greater use of anthropogenic debris at Lindisfarne and Battery Point, macroalgae and other organic material at Tasman Bridge and rock at Wrest Point (Table 4).

# 3.3. Long-term patterns

Seahorses showed significant declines in abundance at all three long-term monitoring sites (Fig. 5). Using

Table 3
Spatial patterns of seahorses at four sites in the Derwent estuary, Tasmania in January 2001

Site	Number of transects	Numbe	er of seah	orses i	n speci	fied group	size				
		$I_{\rm p}$	Alone	2F	2M	1F1M	>2F (# groups)	>2M (# groups)	>2 mixed (# groups)	Total F	Total M
Lindisfarne	54	0.696	51	6	0	0	0	0	10(2)	39	28
Tasman Bridge	84	0.784	71	14	0	8	9(3)	0	6(1)	86	22
Battery Point	92	0.880	118	18	2	10	12(4)	0	36(6)	154	42
Wrest Point	80	0.811	65	6	0	20	0	4(1)	4(1)	57	42

No data are presented for Rose Bay as too few seahorses were found for meaningful analysis.

Seahorses were significantly clumped at the spatial scale of the transect; all standardized Morisita's index of dispersion values were greater than +0.5. Within transects, the number of seahorses found alone or in association with conspecifics of the same or opposite sex are given.

mean abundance in January 2001 as a baseline value in comparison with the mean abundance of the last four sampling occasions declines were 98% at Tasman Bridge, 79% at Battery Point, and 80% at Wrest Point. At Battery Point seahorses appeared to have been declining from December 2000 as better fits were achieved using the whole data set  $(r^2 = 0.742)$  rather than data from December 2001 onwards ( $r^2 = 0.350$ ; Fig. 5(b)). For the other two sites declines seemed to have begun later (early 2002) with good fits to exponential decay curves ( $r^2 = 0.859$  for Tasman Bridge, Fig. 5(a);  $r^2 = 0.531$  for Wrest Point, Fig. 5(c)). Instantaneous mortality rates were -2.625 for Tasman Bridge, -0.621 for Battery Point and -1.749 for Wrest Point representing yearly survival rates ( $\lambda$ ) of 7.2%, 17.4% and 53.7%, respectively.

Our infrequent visits to Lindisfarne and Rose Bay confirmed that seahorses were also largely absent from these sites; we found a total of two seahorses over four visits.

Throughout the entire sampling period seahorse populations consisted almost entirely of adult animals. From December 2001 onwards we found only nine juveniles out of a total of 418 seahorses, all but two in the winter months of June, July and August. Where there were sufficient data, size-frequency distributions of adults remained broadly similar with some evidence of smaller seahorses entering the population in June–August 2002 and December 2002–January 2003 (Fig. 6). Females showed size-frequency distributions skewed further to the right than males with larger size classes (270 mm height and greater) almost exclusively female (Fig. 6).

Sex ratios did not appear to show any systematic changes over time at any of the three sites although there were some month-to-month fluctuations (Fig. 7). There were insufficient data (<10 seahorses) to calculate sex ratios on two occasions at Tasman Bridge and five occasions at Wrest Point. At Tasman Bridge and Battery Point seahorse populations were highly female-biased on almost all sampling occasions including three occasions where no males were found at all (Fig. 7(a) and (b)). Although the sex ratio at Wrest Point was slightly

female-biased during our initial intensive sampling, subsequently it was never significantly different from equality except in February 2002 when there were more males than females (Fig. 7(c)).

#### 4. Discussion

Our data represent the first regular population monitoring of seahorses over relevant ecological time scales. We also provide the first field estimates of abundance, sex ratio and size structure for the big-bellied seahorse. This work documents significant declines in populations of big-bellied seahorses over different sites in the Derwent estuary, unrelated to direct fishing pressure. Abundance of big-bellied seahorses in our study during 2000 and 2001 were similar to those found for H. hippocampus in Portugal (Curtis and Vincent, in press) and H. reidi in Brazil (Dias and Rosa, 2003), but then diminished greatly. We also found that big-bellied seahorse populations have very different spatial structure to other studied seahorse species, with highly skewed sex ratios at some sites and the majority of individuals not found in association with opposite-sex partners.

We are confident that population declines in our long-term sampling reflects real changes over time. We used sampling at nested temporal scales to partition variance due to stochastic variability, periodic physical changes and long-term directional changes (Krebs, 1999). Our short-term intensive sampling in 1988 provided a bootstrap estimate of stochastic variability. Similar intensive sampling in 2000–2001 showed that there were few effects attributable to periodic variations across time of day, state of tide or phase of moon. The significant effects that we did detect were small in magnitude (explaining less than 2% of variation in all cases) compared with stochastic variation and probably not biologically relevant.

Seahorse population declines were not related to direct fishing or incidental capture as bycatch. Seahorses are protected species in Tasmanian state waters requiring permits for collection (*Living Marine Resources Management Act* 1995). No collection of broodstock

Microhabitat availability and microhabitat choice for seahorses at four sites in the Derwent estuary, Tasmania in January 200

Site	Number of seahorses	Anthropogenic debris	genic	Bare substrate	strate	Macroalgae	ae	Other organic material	ganic	Rock	
		Av	MC	Av	MC	Av	MC	Av	MC	Av	MC
Lindisfarne	54	-	33	9	28	1	2	2	22	2	15
Tasman Bridge	101	3	12	9	47	2	15	2	17	3	10
Battery Point	172	2	24	9	47	3	8	2	2	3	19
Wrest Point	85	1	5	9	17	3	27	1	9	4	46
No de de consequence	No date one measured for D on Don to the form of the f	the form the second	d for mooning	ful one lucie							

point qualitative scale (see text for details) while microhabitat choice (MC) is given as a percentage of all seahorses found. Bay as too few seahorses were found for meaningful analysis. Availability (Av) is given on six data are presented for Rose

for aquaculture took place in the Derwent during the study and there was no evidence of illegal collection of seahorses. Similarly, non-selective fishing gear (trawls and dredges) was prohibited in the area of our study. We are also confident that our presence did not have adverse effects on seahorse populations. Mark-recapture work on the same species elsewhere in Tasmania and in New South Wales over two years with similar levels of handling did not produce population declines (K. Martin-Smith and J. Clark-Jones, unpublished data).

Possible stressors of seahorse populations include water quality, physical disturbance invasive species or disease. It seems unlikely that water quality or physical disturbance were responsible for the observed declines. Water quality has generally improved in the Derwent estuary over the sampling period through measures to reduce heavy metal and sewage pollution (Green and Coughanowr, 2003). We saw no systematic changes in levels of nutrients or dissolved oxygen. While levels of heavy metals in sediments remain very high but this has been the case for at least 40 years (Green and Coughanowr, 2003). Physical disturbance during the study was minimal.

Both invasive species and disease remain possibilities for the seahorse declines. Whilst apparently not direct competitors with seahorses, two invasive species, the northern Pacific starfish *Asterias amurensis* and the European green crab *Carcinus maenas*, are highly abundant in the Derwent estuary (Ross et al., 2004). As a voracious generalist predator, the northern Pacific starfish in particular can have significant effects on benthic ecosystems through physical or trophic modification (Ross et al., 2003). Very little is known about disease in wild seahorse populations but seahorses suffer from a variety of pathogens in captivity (e.g., Alcaide et al., 2001).

An alternative hypothesis is that seahorse populations undergo large natural fluctuations and that our initial sampling coincided with high recruitment success or subsequent declines were due to recruitment failure. We consider this hypothesis to be unlikely. Although recent analysis has suggested that a European species, *H. guttulatus*, has some life history parameters that conform to opportunist or 'r-selected' species (rapid growth, early age at maturity and short generation times; J. Curtis & A. Vincent, unpublished data), seahorses do not attain most opportunists' high abundance and biomass over large geographic areas (references in Foster and Vincent, 2004). Moreover, quite unlike most opportunists, seahorses exhibit obligatory parental care, structured mating patterns, and restricted home ranges.

Potentially, big-bellied seahorses could show large recruitment pulses as they may have brood sizes of >1000 offspring and be reproductively mature in less than a year (Woods, 2000; K. Martin-Smith, unpublished data). Big-bellied seahorses are born at

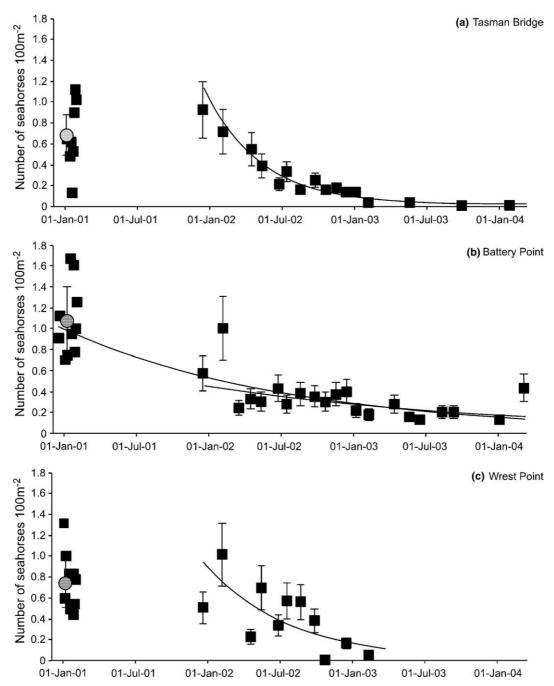


Fig. 5. Abundance trends for seahorses from (a) Tasman Bridge, (b) Battery Point, (c) Wrest Point. Mean of short-term samples from December 2000–January 2001 shown in grey. Errors bars represent bootstrap estimate of 35% (see text for details).

15–20 mm height (Woods, 2000) and presumed to spend some time in a planktonic dispersal phase (Kuiter, 2000). The smallest seahorses observed in our study were 80–90 mm and probably at least four months old (Woods, 2000). Thus increased mortality in juvenile habitats, which are unknown for any species of seahorse (Foster and Vincent, 2004), could have contributed to population declines. However, the population mortality rate at two of our three sites (Tasman Bridge, Wrest Point) was considerably greater than would be expected

for simple natural mortality of a species with a life span of approximately five years, even if little or no recruitment occurred during the study.

A third proximate hypothesis for the declines could be some form of reproductive limitation or Allee effect relating to the dearth of males in the population, especially if males limit the rate of reproduction in *H. abdominalis* as they do in *H. fuscus* (Vincent, 1994). Some support for this hypothesis is provided through the observed lower rate of decline at the site with highest

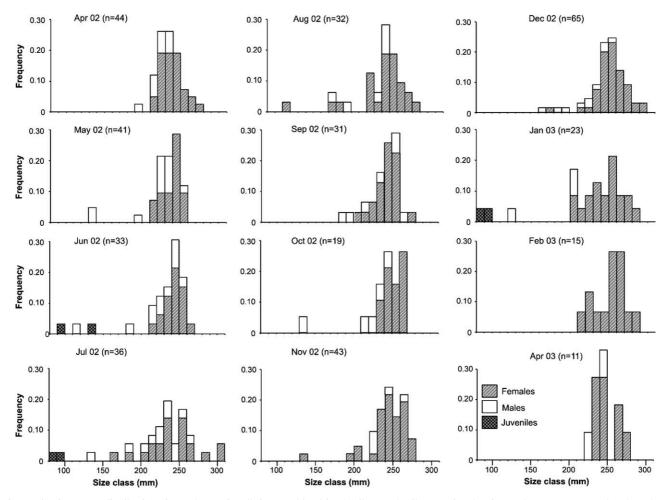


Fig. 6. Size-frequency distributions for seahorses for all sites combined for April 2002–April 2003. Size class intervals are 10 mm. No data have been given from May 2003 onwards as sample sizes were fewer than 10. Data represent all seahorses observed during censuses.

seahorse abundance (Battery Point), presumably with greater encounter rates between individuals. Preliminary microsatellite DNA data reveals sexual monogamy in wild big-bellied seahorses, at least within one reproductive cycle (T. Wilson and K. Martin-Smith, unpublished data). However no evidence of social monogamy i.e. male-females pairs was observed, as is generally found in other seahorses (Foster and Vincent, 2004). Instead, we found most seahorses alone or associating in groups, apparently at random. We also observed numerous incidents of courtship behaviour at Battery Point between groups of females and single males (K. Martin-Smith, unpublished data), with courtship between groups of males and sexually receptive females observed ex situ (Woods, 2000; Kuiter, 2000). More information about operational sex ratios (relative reproductive rates of the two sexes) or about population declines at different sex ratios would help evaluate this hypothesis.

Species-based conservation instruments will only be effective if they address the causative factors of population declines. The CITES listing for seahorses was based

on work demonstrating the threat of international trade. Monitoring the effects of a CITES listing often relies solely on trade records (Wijnstekers, 2003). In this study we have demonstrated substantial declines, at a local scale, unrelated to fishing or trade. This illustrates the necessity of robust monitoring which is fisheries- or trade-independent in order to avoid drawing false conclusions about the efficacy of particular conservation measures. In the case of seahorses, we advocate monitoring 'sentinel' populations around the world in locations where seahorses are exploited and where they are protected or unexploited.

We believe that the charismatic nature of seahorses may provide a powerful means of mobilizing political will and public support to develop appropriate conservation solutions to be broadly applied across estuarine and other marine systems. Seahorses have already led the way in new approaches to marine conservation, e.g., becoming the first commercial marine fish to be listed on CITES. There is certainly an urgent need to alert policymakers, resource users and the general

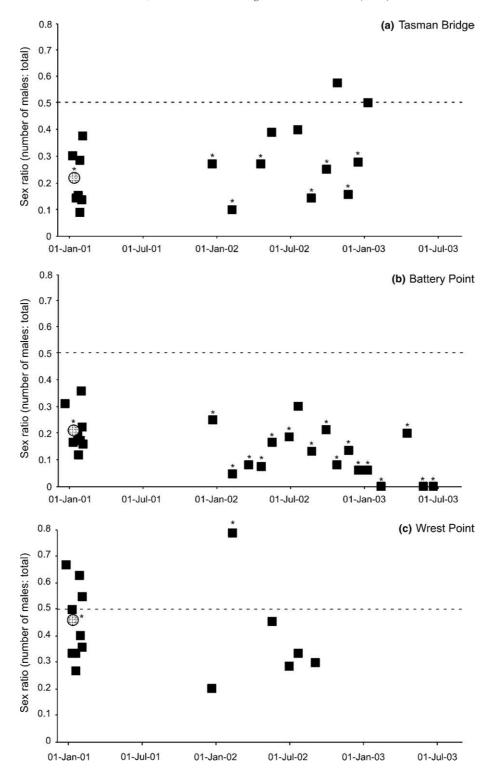


Fig. 7. Sex ratio trends for seahorses from (a) Tasman Bridge, (b) Battery Point, (c) Wrest Point. Mean of short-term samples from December 2000—January 2001 shown in grey. Dotted line represents 1:1 sex ratio and points marked with asterisks are significantly different from this. No data have been given where total number of individuals was fewer than 10.

public to the increasing anthropogenic pressures threatening estuaries and their many ecosystem linkages (Blaber et al., 2000; Gillanders et al., 2003). If not, then we face further losses of resources, biodiversity and ecosystem function.

# Acknowledgements

This is a contribution of Project Seahorse. We are very grateful to our field assistants and volunteers, Emma Cronin, Marlene Davey, Matt Edmunds, Narelle Hall, Scott

Ling, Emma Morgan, Adam Stephens, Dave Stevenson, Simon Talbot and Joe Valentine, and the constructive comments of Jean Marcus. We thank the Derwent Estuary Program, DPIWE for water quality data. We heartily thank our partners at the John G. Shedd Aquarium (USA) and Guylian Chocolates (Belgium) for their particularly generous financial support. AV's initial 1988 study was funded by the British Federation of University Women, a Corpus Christi College (Cambridge) Research Scholarship, the Canadian Centennial Scholarship Fund (Canadian Women's Club), and a Lundgren Studentship (University of Cambridge Board of Graduate Studies). This study was carried out under University of Tasmania Animal Ethic Committee Approval Number A6433.

#### References

- Alcaide, E., Gil-Sanz, C., Sanjuán, E., Esteve, D., Amaro, C., Silveira, L., 2001. Vibrio harveyi causes disease in seahorse, Hippocampus sp. Journal of Fish Diseases 24, 311–313.
- Baird, D., Christian, R.R., Peterson, C.H., Johnson, G.A., 2004. Consequences of hypoxia on estuarine ecosystem function: energy diversion from consumers to microbes. Ecological Applications 14, 805–822
- Baum, J.K., Meeuwig, J.J., Vincent, A.C.J., 2003. Bycatch of lined seahorses (*Hippocampus erectus*) in a Gulf of Mexico shrimp trawl fishery. Fishery Bulletin 101, 721–731.
- Bell, E.M., Lockyear, J.F., McPherson, J.M., Marsden, A.D., Vincent, A.C.J., 2003. First field studies of an Endangered South African seahorse, *Hippocampus capensis*. Environmental Biology of Fishes 67, 35–46.
- Blaber, S.J.M., Cyrus, D.P., Albaret, J.-J., Ching, C.V., Day, J.W., Elliott, M., Fonseca, M.S., Hoss, D.E., Orensanz, J., Potter, I.C., Silvert, W., 2000. Effects of fishing on the structure and functioning of estuarine and nearshore ecosystems. ICES Journal of Marine Science 57, 590–602.
- Curtis, J.M., Vincent, A.C.J., in press. Habitat use of two Red-Listed seahorses, *Hippocampus guttulatus* (Cuvier 1829) and *H. hippocampus* (Linnaeus 1758) in a coastal lagoon. Marine Ecology – Progress Series.
- deBruyn, A.M.H., Meeuwig, J.J., 2001. Detecting lunar cycles in marine ecology: periodic regression versus categorical ANOVA. Marine Ecology – Progress Series 214, 307–310.
- Dias, T.L.P., Rosa, I.L., 2003. Habitat preferences of a seahorse species, *Hippocampus reidi* (Teleostei: Syngnathidae) in Brazil. Aqua 6, 165–176.
- Foster, S.J., Vincent, A.C.J., 2004. Life history and ecology of seahorses: implications for conservation and management. Journal of Fish Biology 65, 1–61.
- Gascoigne, J., Lipcius, R.N., 2004. Allee effects in marine systems. Marine Ecology – Progress Series 269, 49–59.
- Gillanders, B.M., Able, K.W., Brown, J.A., Eggleston, D.B., Sheridan, P.P., 2003. Evidence of connectivity between juvenile and adult

- habitats for mobile marine fauna: an important component of nurseries. Marine Ecology Progress Series 247, 281–295.
- Green, G., Coughanowr, C, 2003. State of the Derwent Estuary 2003: a review of pollution sources, loads and environmental quality data from 1997–2003. Department of Primary Industries, Water and Environment, Hobart, Australia.
- IUCN, 2003. 2003 IUCN Red List of Threatened Species. <a href="http://www.redlist.org">http://www.redlist.org</a> (accessed 21.06.04.).
- Kiddon, J.A., Paul, J.F., Buffum, H.W., Strobel, C.S., Hale, S.S., Cobb, D., Brown, B.S., 2003. Ecological condition of US Mid-Atlantic estuaries, 1997–1998. Marine Pollution Bulletin 46, 1224– 1244
- Krebs, C.J., 1999. Ecological Methodology, second ed. Addison Wesley Longman, Inc., NY, USA.
- Kuiter, R.H., 2000. Seahorse, Pipefishes and Their Relatives. A Comprehensive Guide to the Syngnathiformes. TMC Publishing, Chorleywood, UK.
- Lourie, S.A., Vincent, A.C.J., Hall, H.J., 1999. Seahorses: An Identification Guide to the World's Species and Their Conservation. Project Seahorse, London, UK.
- Moreau, M.-A., Vincent, A.C.J., 2004. Social structure and space use in a wild population of the Australian short-headed seahorse *Hippocampus breviceps* Peters, 1869. Marine and Freshwater Research 55, 231–239.
- Perante, N.C., Pajaro, M.G., Meeuwig, J.J., Vincent, A.C.J., 2002. Biology of a seahorse species, *Hippocampus comes* in the central Philippines. Journal of Fish Biology 60, 821–837.
- Pogonoski, J.J., Pollard, D.A., Paxton, J.R., 2002. Conservation Overview and Action Plan for Threatened and Potentially Threatened Marine and Estuarine Fishes. Environment Australia, Department of Environment and Heritage, Canberra, Australia.
- Price, A.R.G., 2002. Simultaneous 'hotspots' and 'coldspots' of marine biodiversity and implications for global conservation. Marine Ecology – Progress Series 241, 23–27.
- Ross, D.J., Johnson, C.R., Hewitt, C.L., 2003. Variability in the impact of an introduced predator (*Asterias amurensis*: Asteroidea) on soft-sediment assemblages. Journal of Experimental Marine Biology and Ecology 288, 257–278.
- Ross, D.J., Johnson, C.R., Hewitt, C.L., Ruiz, G.M., 2004. Interaction and impacts of two introduced species on a soft-sediment marine assemblage in SE Tasmania. Marine Biology 144, 747–756.
- Vincent, A.C.J., 1994. Operational sex ratios in seahorses. Behaviour 128, 153–167.
- Vincent, A.C.J., 1996. The International Trade in Seahorses. TRAF-FIC International, Cambridge, UK.
- Vincent, A.C.J., Marsden, A.D., Evans, K.L., Sadler, L.M., 2004. Temporal and spatial opportunities for polygamy in a monogamous seahorse, *Hippocampus whitei*. Behaviour 141, 141–156.
- Vincent, A.C.J., Sadler, L.M., 1995. Faithful pair bonds in wild seahorses, *Hippocampus whitei*. Animal Behaviour 50, 1557– 1569.
- Wijnstekers, W., 2003. The Evolution of CITES, seventh ed. CITES Secretariat, Geneva, Switzerland.
- Woods, C.M.C., 2000. Preliminary observations on breeding and rearing the seahorse *Hippocampus abdominalis* (Teleostei: Syngnathidae) in captivity. New Zealand Journal of Marine and Freshwater Research 34, 475–485.