A novel underwater visual census: Seahorse population survey as a case study

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HIGHLIGHTS
- UVC2 was more effective in finding seahorses, particularly at low density.
- UVC2 might be used to survey other seahorse species populations.
- UVC2 can be used to survey other benthic species.

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ABSTRACT
Two different Underwater Visual Census (UVC1 and UVC2) were performed at three sites in the Ria Formosa, using 30 meter transect belts, and compared for differences in seahorse density and holdfast availability. Each UVC differed in transect placement and total survey area coverage. The observed Hippocampus guttulatus density was significantly higher (P < 0.05) using UVC2 for Site 1 and Site 2, but not in Site 3. No statistically significant differences were found in holdfast availability (P > 0.05) between the two UVC methods. We conclude that the UVC2 method is more effective in quantifying abundance of seahorses compared with UVC1, particularly in low population density scenarios. This method can also be used in assessing other seahorse species’ populations, in similar habitats.

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1. Introduction

The first underwater visual census (UVC) were performed by Brock (1954) and set the basis for most studies on fish ecology using visual techniques. UVCs have since been used to study a wide variety of marine fish species, especially those exploited in fisheries (Russ, 1984; Kulbicki, 1998; Ferreira et al., 2004; Mayo and Jackson, 2006; McClanahan et al., 2009). The non-destructive nature of this technique makes it appealing for fish monitoring as it is fisheries-independent, quick and relatively inexpensive (Watson and Quinn, 1997). UVCs have also been reported to be an appropriate technique for estimating the abundance of shallow-water reef fish and are used worldwide (García-Martín et al., 2004; Floeter et al., 2007; Stobart et al., 2007; Evans et al., 2008; Leopold et al., 2009; Andrefouet and Wantiez, 2010). This method involves systematic data collection in the surveyed area, enumerating the animals and collecting environmental information (Curtis et al., 2004) and it can be used for the estimation of relative abundances, biomass, and distribution of fish populations (Andaloro et al., 2011; Cenci et al., 2011; Pelletier et al., 2011).

However, underwater visual surveys present specific challenges, including restricted surveying time spent underwater, observer mobility and underwater visibility (Charton et al., 2000; Williams et al., 2006). Thus, UVC methodology has been adapted to monitor fish populations depending on the target fishes’ biology, behaviour and surveyed area (Mapstone and Ayling, 1998; Samoilys and Carlos, 2000; Ordines et al., 2005).

UVC has been widely used for surveys of particular fish families and species (De Raedemaeker et al., 2010; Kulbicki et al., 2010), including seahorses (Bell et al., 2003; Curtis et al., 2004; Curtis and Vincent, 2005; Freret-Meurer and Andreata, 2008; Caldwell and Vincent, 2012). Although several UVC techniques have been used to monitor Syngnathid species, from timed swims (Martin-Smith, 2011; Harasti et al., 2014) to permanent transects (Sanchez-Camara and Booth, 2004), none have been tested for the two seahorse species that inhabit the Ria Formosa lagoon. In the early 2000’s, Curtis and Vincent (2005) used UVC to describe the highest seahorse densities ever recorded in the Ria Formosa lagoon or anywhere else in the world. The protocol used in the latter study

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was designed based on this high seahorse density and the wide distribution of the focal species throughout the lagoon. However, a more recent study of the same seahorse populations using Curtis and Vincent (2005) UVC methodology showed a significant decrease in seahorse density: 94% and 57% for Hippocampus guttulatus Cuvier 1829 and Hippocampus hippocampus Linnaeus 1758, respectively (Caldwell and Vincent, 2012). Most seahorse species are found at low densities and are patchily distributed (Perante et al., 2002; Bellet al., 2003; Martin-Smith and Vincent, 2005; FreretMeurer and Andreata, 2008; Haraste et al., 2012) which highlights the importance of a proper UVC method to accurately assess the populations. This study aimed to compare a modified UVC technique designed to survey sedentary fish populations at low densities, with a previously used one, in order to assess seahorse populations in the Ria Formosa lagoon.

2. Material and methods

Two seahorse species occur in the Ria Formosa lagoon (South Portugal), the long snouted seahorse (H. guttulatus) and the short snouted seahorse (H. hippocampus). These species are easily distinguishable by their size and morphological traits, including coronet shape, number of trunk rings (Lourie et al., 2004) and skin colour patterns. The two species share a similar distribution in shallow, coastal waters of the north-eastern Atlantic Ocean and Mediterranean Sea (Lourie et al., 2004). However, they have distinct habitat preferences: while H. guttulatus has been reported to prefer habitat with greater complexity such as seagrass meadows, H. hippocampus is mostly found in low complexity sandy habitats (Curtis and Vincent, 2005).

Underwater surveys were carried out in the Ria Formosa lagoon (36° 59’ N, 7° 51’ W) and three locations were selected and surveyed on 3 occasions (Fig. 1). Surveys were performed in May and June 2012.

The surveyed sites were chosen due to their differences in habitat characteristics (Table 1). A GPS unit (Garmin® GPSMap 78s) was used to accurately determine the location of each study area and during the site delineation. The same bearing was taken while laying each transect so that the same area could be consistently covered on each sampling occasion. On each sampling occasion, the two survey techniques were performed by SCUBA simultaneously in order to minimize bias derived from individual seahorse’s daily movement. First, the UVC method (Caldwell and Vincent, 2012) was used and hereafter described as UVC1. In this methodology, three 30 m belt transects were randomly placed >5 m apart, and a 1 m wide strip on each side for each transect was covered by two divers (Fig. 2(a)), for a total area of 180 m$^{-2}$ per site. The second UVC method (specifically designed for this study) was then performed after the completion of UVC1, hereafter described as UVC2. UVC2 consisted of placing two 30 m belt transects in parallel and 2 m wide strips on each side were surveyed, covering a total area of 240 m$^{-2}$ (Fig. 2(b)). Surveys were carried out by 2 divers in each UVC method. Both divers performed the two UVC methods in order to minimize observational bias. All surveys were carried out at slack high tide when turbidity is lowest, visibility greatest and to minimize effects of the water current. At each sampling site, the two methodologies were performed over a 2 h period. Whenever a seahorse was found, information on species, sex, substrate type, holdfast type and overall bottom coverage (in a 1 m$^2$ diameter area at each seahorse location) was recorded. Habitat complexity was considered low if bottom coverage (i.e. number of holdfasts available), was less than two holdfasts m$^{-2}$; medium for 2–10 holdfasts m$^{-2}$; and high for more than 10 holdfasts m$^{-2}$. Holdfast distribution was considered patchy when the distance between holdfasts was greater than a 3-m radius. In each sampling, human related activities were recorded to determine their impact in each site. Sites were considered as highly impacted by human activities when two or more activities were observed during all the sampling occasions (i.e., fishing, boat traffic and anchoring); medium impact when at least two of the activities were observed in at least half of the sampling occasions; and low impact when less than two activities were observed in less than half of the sampling occasions. Seahorse abundance was calculated for each UVC at each site, and the Student $t$-test was used to test for differences in abundance (Zar, 1999). For each site, the 3 surveys were pooled together for the statistical analysis.

3. Results

The observed overall mean ± S.D. seahorse abundances (both species pooled together) using UVC1 was 12.0 ± 4.0, 3.0 ± 1.0 and
Table 1
Description of each surveyed site regarding depth (in meters); hydrodynamics (water flow); substrate type; habitat complexity (holdfast availability); holdfast distribution; and human impact.

<table>
<thead>
<tr>
<th>Location</th>
<th>Depth (m)</th>
<th>Hydrodynamics (m s⁻¹)</th>
<th>Substrate</th>
<th>Habitat complexity</th>
<th>Holdfast distribution</th>
<th>Human impact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>5–6</td>
<td>High (0.5–1)</td>
<td>Sand</td>
<td>High</td>
<td>Patchy</td>
<td>High (fisheries and boat anchoring)</td>
</tr>
<tr>
<td>Site 2</td>
<td>3–4</td>
<td>Medium (0.3–0.7)</td>
<td>Mud</td>
<td>Medium</td>
<td>Patchy</td>
<td>Medium (occasional fisheries)</td>
</tr>
<tr>
<td>Site 3</td>
<td>2–3</td>
<td>Low (0.1–0.5)</td>
<td>Sand</td>
<td>Low</td>
<td>Homogeneous</td>
<td>Low (no human activity)</td>
</tr>
</tbody>
</table>

Fig. 2. Design of the two UVC methodologies tested. (a) Previously used methodology (UVC1) and (b) new proposed methodology (UVC2). Arrows indicate diver’s direction during survey.

11.0 ± 1.0 seahorses per transect, whereas abundances using UVC2 were 23.0 ± 1.0, 21.0 ± 8.0 and 14.0 ± 5.0 seahorses, at Site 1, 2 and 3, respectively. *H. guttulatus* and *H. hippocampus* densities at each site, using the two different UVCs are presented in Fig. 3. The observed *H. guttulatus* densities with the two UVC protocols were significantly different in Site 1 (t(4) = 4.492, P < 0.05) and Site 2 (t(4) = 4.053, P < 0.05) but not significantly different at Site 3 (P > 0.05). No significant differences (P > 0.05) were found between protocols in Site 1 and Site 3 for *H. hippocampus*. No *H. hippocampus* was found at Site 2. No statistical differences (P > 0.05) were found in gender or size for both species at all surveyed Sites. In all UVC performed only seahorse adults were found.

The seahorse holdfasts at sighting are presented in Fig. 4. The most abundant holdfast available at Site 1 was shells while Site 2 and Site 3 had higher holdfast availability of *Ascidia* sp. Regardless of the UVC methodology used, no statistical differences were found (P > 0.05) in holdfast use.

4. Discussion

Considering the low mobility, reduced home range behaviour and patchy distribution reported for seahorse species, the UVC methodology needs to be appropriately selected to match the species and site specificities, as reported for other species (De Grolamo and Mazzoldi, 2001; Harvey et al., 2004; Colton and Swearer, 2010). Our results indicate higher seahorse sightings when using the UVC2 methodology developed in this study, but only for one of the two seahorse species found in the study site, *H. guttulatus*. This may be explained by the patchy distribution reported for *H. guttulatus* (Curtis and Vincent, 2006) related to habitat constraints such as low holdfast availability and the different habitat preferences of the two species. As seahorses rely on their prehensile tail to grasp on holdfasts to enhance their cryptic behaviour and cope with high hydrodynamic environments (Curtis and Vincent, 2006), this behaviour is also responsible for their low mobility and small home ranges (Curtis and Vincent, 2006; Caldwell and Vincent, 2013). Therefore, ensuring that the areas with available holdfasts are also represented in the survey is of utmost importance, as they usually sustain higher probabilities of seahorse occurrence. Failing to survey these areas, might skew and bias any calculated seahorse densities, thus leading to misleading evaluations with regard the actual status of the targeted populations.

Considering that both seahorse species that occur in the Ria Formosa have different habitat preferences, this fact should be considered as part of the UVC methodology design. Although both species preferred using covered microhabitats as holdfasts, *H. hippocampus* was observed more often on bare surfaces than *H. guttulatus* (Curtis and Vincent, 2005). This could explain why there were no statistical differences in abundance for this species when using the two different UVC methods. In contrast, *H. guttulatus* has been reported to be associated with higher habitat complexities and to be a holdfast generalist. Therefore, UVC2 seems to be more suitable for surveying *H. guttulatus* populations as it focuses on target areas that correspond to this species’ occurrence.

Within the Ria Formosa lagoon, high holdfast densities are patchily distributed, confined to small areas and usually located close to the main channels. These areas create an environment that

Fig. 3. Seahorse density (seahorse m⁻²) of the two seahorse species, *H. hippocampus* (HH) and *H. guttulatus* (HG), in Site 1 (a), Site 2 (b) and Site 3 (c), using two different UVCs (—UVC1 and —UVC2). Statistical differences (P < 0.05) are presented by (*). Bars represent standard deviation.
seems to be suitable for seahorse settlement and therefore should be included in the sampling areas, when performing a population census using UVC techniques. The summed effect of seahorse patchiness and the randomness of the transect positioning and direction of UVC1 used by Curtis and Vincent (2005) and Caldwell and Vincent (2012), may underestimate the number of seahorses occurring in these important areas, thus overlooking seahorses within the population and providing potentially biased abundance information. Nevertheless, this method is suitable for surveying areas with high holdfast availability, evenly distributed holdfasts, and in sites with high seahorse density. This seems to be the case of Site 3, where habitats with homogeneous holdfast distribution might not have been influenced by the UVC technique used. At this site, seahorses were found scattered throughout a wide area and therefore the effect of using different UVC on overall seahorse sightings was not statistically different. In contrast, UVC2 seems to be more appropriate in low density scenarios as it was designed to survey any sampled area evenly, thus removing any possibility to miss holdfast patches present in the designated survey location. Moreover, this method may be more appropriate than UVC1 for long term monitoring populations as the same survey area is monitored in every survey event, allowing a more accurate perspective on population trends and evaluation of habitat changes. Considering the observed fluctuations in seahorse populations within the

Fig. 4. Seahorse holdfast use (%) at sighting time, using UVC1 (□) and UVC2 (●). Bars represent standard deviation.

Ria Formosa lagoon in the last 10 years (Curtis and Vincent, 2005; Caldwell and Vincent, 2012), we conclude that the UVC2 method is more appropriate for a long-term monitoring programme at this location as it was shown to cope better with low density population scenarios. This method is also likely to have wider applicability for surveying seahorses that globally tend to be found at low densities (Perante et al., 2002; Bell et al., 2003; Martin-Smith and Vincent, 2005; Freter-Meurer and Andreata, 2008; Harasti et al., 2012).

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