Journal of Sea Research 102 (2015) 33–40

Contents lists available at ScienceDirect

Journal of Sea Research

journal homepage: www.elsevier.com/locate/seares

Spatial distribution patterns and movements of *Holothuria arguinensis* in the Ria Formosa (Portugal)

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ARTICLE INFO

Article history:
Received 2 October 2014
Received in revised form 23 February 2015
Accepted 17 April 2015
Available online 23 April 2015

Keywords:
Sea cucumber ecology
Home range
Behavior
Coastal lagoon
Management

ABSTRACT

Holothurian populations are under pressure worldwide because of increasing demand for beche-de-mer, mainly for Asian consumption. Importations to this area from new temperate fishing grounds provide economic opportunities but also raise concerns regarding future over-exploitation. Studies on the habitat preferences and movements of sea cucumbers are important for the management of sea cucumber stocks and sizing of no-take zones, but information on the ecology and behavior of temperate sea cucumbers is scarce. This study describes the small-scale distribution and movement patterns of *Holothuria arguinensis* in the intertidal zone of the Ria Formosa national park (Portugal). Mark/recapture studies were performed to record their movements over time on different habitats (sand and seagrass). *H. arguinensis* preferred seagrass habitats and did not show a size or life stage-related spatial segregation. Its density was 563 ind. ha^{-1} and mean movement speed was 10 m per day. Movement speed did not differ between habitats and the direction of movement was offshore during the day and shoreward during the night. Median home range size was 35 m^2 and overlap among home ranges was 84%. *H. arguinensis*' high abundance, close association with seagrass and easy catchability in the intertidal zone, indicate the importance of including intertidal lagoons in future studies on temperate sea cucumber ecology since those systems might require different management strategies than fully submerged habitats.

1. Introduction

Increasing demand of beche-de-mer is influencing sea cucumber populations all over the world. The traditional Asian demand of holothurian products for delicacies and medicines has resulted in the depletion of traditional fishing grounds in tropical areas. The fishing pressure is now moving towards temperate areas, including the Mediterranean Sea and Northeastern Atlantic Ocean (González-Wangüemert et al., 2013, 2014, 2015; Toral-Granda et al., 2008). Nowadays, global catch estimates are in the range of 100,000 tonnes of sea cucumbers per year (Purcell et al., 2010).

The overfishing of sea cucumbers in the Indo-Pacific, has resulted in catch of new target species (*Holothuria tubulosa*, *Holothuria polii*, *Holothuria mammata*, *Holothuria forskali* and *Holothuria arguinensis*) from Mediterranean Sea and NE Atlantic Ocean (Aydin, 2008; González-Wangüemert and Borrero-Pérez, 2012; González-Wangüemert et al., 2014; Rodrigues et al., 2015; Sicuro and Levine, 2011). In the last 3 years, the sea cucumber fishery in Northern Turkey has increased rapidly, with 555 Tn in 2011 (80% *H. polii* and 20% *H. tubulosa* plus *H. mammata*) (González-Wangüemert et al., 2014). Sea cucumbers are fished by hookah facilities, a diver catches around 2.000–3.000 individuals per day (Aydin, 2008); the current Turkish fleet (120 vessels) can collect around 720.000 sea cucumbers per day (González-Wangüemert et al., 2014). As consequence, some signals of over-exploitation were already detected, showing loss of the largest and heaviest individuals and genetic diversity (González-Wangüemert et al., 2015). Scarce official information on sea cucumber fisheries from this geographical area is available due to catches are catalogued as "invertebrates" and/or obtained illegally. In Spain, more than 10 companies are exporting sea cucumbers to China (http://www.alibaba.com/countrysearch/ES/sea-cucumber-supplier.html); some of them with US$1–2 millions of total revenue. The main target species are *H. tubulosa, H. forskali* and *H. mammata*. In Portugal, three companies are selling sea cucumbers, mainly *H. arguinensis, H. forskali* and *H. mammata,* offering supply ability among 2.000–50.000 kg/month and prices oscillating among 70–350 euro/kg (http://www.alibaba.com/countrysearch/PT/sea-cucumber.html) depending on quality of product.

Holothurians are sensitive to over-exploitation due to a combination of biological (late maturity, low recruitment rate and density-dependent reproduction), and anthropogenic factors (the ease with which shallow water species can be harvested) (Bruckner et al., 2003). Dramatic reductions in sea cucumber abundance might cause concern

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http://dx.doi.org/10.1016/j.seares.2015.04.003
1385-1101/

about their ecological role in bioturbation, nutrient recycling and habitat structuring (Bruckner et al., 2003; Uthicke, 1999, 2001a,b), and also on the food-web, because sea cucumbers are a substantial biomass for some predators as fishes, starfishes and crustaceans (Francour, 1997).

Information on the ecology and behavior of sea cucumbers is relatively scarce (Bruckner et al., 2003; Conand, 1990; Graham and Battaglene, 2004; Navarro et al., 2013, 2014), despite their economical and ecological importance. Studies on the habitat preferences and movements of sea cucumbers have demonstrated their utility on the management of sea cucumber stocks and sizing of no-take zones providing for example, parameters for surveys and restocking (Purcell and Kirby, 2006; Shiell and Knott, 2008).

Movement and distribution of sea cucumber can be influenced by several factors such as substrate type, organic matter availability, light intensity, depth and salinity (Dong et al., 2011; Hamel et al., 2001; Mercier et al., 2000; Navarro et al., 2013, 2014; Shiell and Knott, 2008; Slater et al., 2011). Movement speed is slow with averages of 2–5 m day^{-1} for *Holothuria fuscogilva*, *Holothuria whitmaei*, *Apostichopus japonicus* and *Actinopyga mauritiana* (Graham and Battaglene, 2004; Reichenbach, 1999; Shiell, 2006; YSFRI, 1991) and around 10 m day^{-1} for *Holothuria sanctori* (Navarro et al., 2013).

Behavioral studies on sea cucumbers are focused mainly on tropical species (e.g. Graham and Battaglene, 2004; Mercier et al., 2000; Purcell and Kirby, 2006; Reichenbach, 1999; Shiell, 2006; Shiell and Knott, 2008), but information on temperate species is still scarce (Navarro et al., 2014).

H. arguinensis is a sea cucumber that is distributed along the east North Atlantic from Portugal to Morocco, Mauritania and Canary Islands (González-Wangüemert and Borrero-Pérez, 2012; Rodrigues, 2012), and some individuals have been found in the Mediterranean sea on the south-eastern Spanish coast recently (González-Wangüemert and Borrero-Pérez, 2012). This species is usually associated with sandy/rocky areas and seagrass meadows where it occurs from 0 m to 50 m depth (González-Wangüemert and Borrero-Pérez, 2012; Navarro, 2012; Rodrigues et al., 2015). In Ria Formosa National Park (South Portugal) this species is the most abundant holothurian and can be found in high densities exposed on the intertidal flats during low tide (González-Wangüemert et al., 2013). As was stressed before, *H. arguinensis* is one of the target species caught in Portugal to be exported mainly to Asiatic countries. Nowadays, unless three companies are offering this species to be sold (http://www.alibaba.com/countrysearch/PT/sea-cucumber.html). The combination of its high nutritional value for human consumption (Roggatz, 2012), high densities of its populations and the ease with which this species can be harvested, makes it vulnerable to overexploitation such as another tropical species (Bruckner et al., 2003).

The ecology and behavior of *H. arguinensis* have been studied recently but only on the Canary Islands (Spain), where this species is occupying habitats dominated by volcanic rocks and seagrass at 4–8 m depth (Navarro, 2012; Navarro et al., 2013, 2014; Tuya et al., 2006). These habitats are substantially different from the tidal lagoon habitat of Ria Formosa national park, where only one study on *H. arguinensis* has been conducted to assess the population status of this species through a volunteer program doing visual census (González-Wangüemert et al., 2013). We aim to study the behavior of *H. arguinensis* at small spatial scale in Ria Formosa Natural Park (South Portugal). To achieve this objective, we analyzed the movement patterns of *H. arguinensis,* interpreted its spatial distribution across habitat-diverse areas, studied the home-range of this species and its relationship with the size of individuals, and estimated its density. This information would be very useful to further development of regulations to sea cucumber fishery in Ria Formosa (Natural Park, South Portugal) and along Portuguese coast. Also, these data have a valuable interest to develop the aquaculture biotechnology on *H. arguinensis*, which could supply part of the demand from Asiatic countries, with less impact on wild populations, and allow further restocking programs if they are necessary.

2. Materials and methods

2.1. Study area

Ria Formosa Natural Park is a tidal lagoon, consisting of tidal flats and salt marshes protected by a belt of dunes extending for 55 km along the south coast of Portugal (Sprung, 1994). Total surface of the lagoon is about 10,000 ha and the average depth is 3–4 m with a tidal amplitude of about 1.30 m at neap tide and 2.80 m at spring tide. Channels are up to 20 m deep (Malaquias and Sprung, 2005; Sprung, 2001). Habitats are covered mainly by either sand, mud or seagrass (intertidal: *Zostera noltii*, subtidal: *Zostera marina, Cymodocea nodosa* (Malaquias and Sprung, 2005)). *Z. noltii* biomass is high in the intertidal, showing low oscillations during the year (Asmus et al., 2000). Sandy habitats are generally associated with intertidal seaweed communities, consisting mainly on *Ulva* spp. and *Enteromorpha* spp. (Asmus et al., 2000).

Experiments were carried out in the intertidal zone of the Ria Formosa close to Praia de Faro (Fig. 1), covering an area along the coast from the high shore level to the end of the intertidal zone. The area was selected because of its high holothurian abundance (González-Wangüemert et al., 2013). Transects (60 m of length each one and parallel to the waterline) were walked during periods of aerial exposure and percentage of coverage by either seagrass, seaweed or sand was estimated for every cell in a 1 m^2 grid of the study area. Transects were separated 2 m from each other and the observer recorded at both sides of the transects for 1 m distance. Although quadrants were not used for coverage estimates, the observer error was minimized by the use of only one sampler. A principal component analysis (PCA) was then used to summarize habitat variability across the study area by using spatial distribution of coverage information already described above. Each cell of the study area, can be then classified, interpreting the first component of the ordination. Analysis was performed using GRASS GIS v.6.4.2 (Neteler et al., 2012), and variables were represented by 1×1 m raster maps of each coverage type.

2.2. Mark/recapture experimental designing

A preliminary study was done and the methods retained were used in order to optimize the sampling design. The study was performed during two periods at the beginning and end of April 2013. Captures were made during periods of aerial exposure (between 2 h before low tide and 1 h after low tide) for 5 consecutive days (10 low tides per period). Tidal height at low tide varied from 0.57 to 0.70 m (first period) and 0.36 to 0.60 m (second period) (Instituto Hidrográfico: http://www.hidrografico.pt/previsao-mares.php). During each sampling, the whole study area was searched and all holothurians encountered were marked *in situ* and immediately released at the same spot where captured. Marking was done by scratching a code on their dorsal surface with a surgical scalpel (Mercier et al., 2000; Navarro et al., 2013, 2014; Reichenbach, 1999). The wound usually heals within 10 days, leaving a scar with the shape of the mark (Shiell, 2006; Supplementary Fig. S1). Scratched marks are visible up to a month with no indication of any considerable behavioral change (Mercier et al., 2000; Reichenbach, 1999). Other tagging methods such as glued tags, coloring agents, PIT tags and T-bar tags were considered less effective and often more invasive (Conand, 1990; Kirshenbaum et al., 2006; Navarro et al., 2014; Purcell et al., 2008; Shiell, 2006). Stress caused by handling and marking could result in a higher activity during the initial hours (Shiell, 2006). Reducing this effect by postponing the sampling for several hours after marking (Navarro et al., 2013, 2014), was not deemed effective since the specimens were also handled during recaptures in those cases where the marks were difficult to read directly. Observations started directly after marking and the effect of handling was reduced by working *in situ* (Navarro et al., 2014). Recaptured individuals were directly released without remarking since repeated marking could increase the chance of