Journal of Experimental Marine Biology and Ecology 447 (2013) 160–164

Contents lists available at SciVerse ScienceDirect

Journal of Experimental Marine Biology and Ecology

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

Special issue: Cephalopod Biology

Measurements of octopus arm elongation: Evidence of differences by body size and gender☆

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ARTICLE INFO

Article history:
Received 12 December 2011
Received in revised form 4 May 2012
Accepted 25 January 2013
Available online 15 March 2013

Keywords:
Arm elongation
Behavior
Biomechanics
Octopus vulgaris

ABSTRACT

This study describes a new method for the measurement of *Octopus vulgaris* arm elongation ability and presents the first preliminary data on sex- and size-related differences. Developing an apparatus targeted to obtain in vivo, non-invasive and direct measurements of one arm at a time, we measured arm elongations of 19 octopuses (*O. vulgaris*) of both sexes to reach an object at the end of a tube. By pulling a prey up the instrument, we encouraged the animal to insert and extend one of its arms to reach it. Results showed behavioural differences among animals different for sex and size, with females performing a higher elongation percentage than males of a similar body size (75%, SD 26%, compared to 61%, SD 33%, *t*-test, $p<0.05$), and smaller-sized octopuses performing a higher average elongation percentage than the bigger ones. Data revealed also that octopuses have a preference of inserting arm L3 into the tube, which is the longest arm, possibly as an energy saving strategy.

The results of this study support the importance of understanding octopus biomechanics using novel techniques and designing dedicated instrumental setups and sensors, thus extending the range of available techniques for comparative and behavioural studies of invertebrates.

1. Introduction

Octopus arms are incredible appendages from a biomechanical standpoint because they perform a rich repertoire of movements, such as changing lengths, twisting, and bending in all directions and at any point along the arm. The body and arms of an octopus totally lack hard elements, which allow this animal to adapt its shape to the environment and squeeze into tiny holes, where the size of its rigid brain capsule is the only limitation (Mather, 2006). Despite the lack of rigid skeletal support, arm stiffness can be controlled and varied to achieve relatively high values of force (Margheri et al., 2012). Octopus arms constitute the majority of its body weight and contain most of the nervous system. The octopus uses its arms to move, defend itself, explore its surroundings and collect food.

Most of the insights on the octopus arms derive from morphological studies (Colasanti, 1876; Cuvier, 1817; Graziadei, 1971; Guérin, 1908; Kier and Stella, 2007; Margheri et al., 2011), or from qualitative descriptions of biomechanics as muscular hydrostat structures (Kier and Smith, 1985). The control of individual octopus arms has been well studied (Gutfreund et al., 1996, 1998; Sumbre et al., 2001, 2005, 2006), as well as the use of arms during different tasks and behaviours in laboratories and directly in the sea (Byrne et al., 2006a, 2006b; Fiorito and Gherardi, 1999; Fiorito et al., 1990; Huffard, 2006; Huffard et al., 2005; Mather, 1998, 2008; Mather et al., 2010). New techniques in the last 20 years, including the use of high-speed cameras (Kier, 1982; Kier and VanLeeuwen, 1997; Stewart et al., 2010) and underwater technologies (DiMarco and Hanlon, 1997), have advanced observational studies pertinent to biomechanics.

Biomechanics have been used in a variety of animal behavioural studies of fish, crows, and spiders (Bels et al., 2003; Blake and Domenici, 2000) to examine the locomotive strategies of walking, swimming, flying, feeding, orientation, communication, and the use of tools (Dickinson et al., 2000; Finn et al., 2009). Additionally, biomechanics aspects of octopus movements have been documented for crawling, jet propulsion or bipedal locomotion (Huffard, 2006; Huffard et al., 2005; Villanueva et al., 1997). The flexibility of these hydrostatic movements has inspired a field of investigations into soft robotics (Calisti et al., 2011), most of which has focused on the reaching movement, which is a fundamental component of more complex octopus arm uses (Sumbre et al., 2005). This rapid development of biomechanical studies on octopus arm motor performance for analyzing animal behaviour calls for new methods to be developed to study these unique animals (Margheri et al., 2012). By contrast, biomechanics measurements on performance capabilities of the octopus arms for animals' different masses and sex have not been published previously.

The mechanical properties of the arm are strictly related to the extreme flexibility and compliance of the arm tissues and play a key role in the animal's unique exploratory and environmental interaction behaviours. Capability of elongation provides octopuses with means

☆ This article is part of a special issue on Cephalopod Biology published under the auspices of CephRes-ONLUS (www.cephalopodresearch.org).

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http://dx.doi.org/10.1016/j.jembe.2013.02.025

for reaching, searching (probing), feeding, capturing prey, and mating. Reaching movement reflects the ability of males to trail the females from a distance, or the capabilities to poke the arm into areas that are likely to contain prey (Mather, 1991). Consequently, elongation may affect the mechanics of movement and the behaviour of the animal. Moreover, because of the high diversity of possible movements and the potential applications to the field of biomimetics or soft robotics (Laschi et al., 2012; Mazzolai et al., 2012), the study of the structure, the use, and the control strategies of the octopus arms poses a challenge both for biological and engineering researchers, and a measuring method to study the efficiency of elongation and reach is long overdue. Indeed, Margheri et al. (2012) presented a novel instrumental setup to measure the active mechanical capabilities of octopus arms (e.g., elongation, shortening, pulling force, and stiffening) specifically for biorobotic design purposes.

When interpreted in a behavioural context, the elongation capability reveals the potential for biomechanical influences on predation, exploration, or animal–animal interaction (mating and aggression). Consequently, the size or the sex of the animal may have an impact on the mechanical efficiency, or different arms may show different performances.

To investigate these unknown octopus features, in this study we examined how and how much octopuses extend their arms to reach an object at the end of a tube, describing a new method for the measurement of octopus arm elongation during reaching movements, and presenting for first preliminary data on sex- and size-related differences.

We designed and developed instruments to be used to directly measure arm elongation in a non-invasive manner in order to compare and determine differential animal performance. The apparatus is a graduated transparent Plexiglas® tube attached to a support plate and is used to measure the performance of individual arms. The animals elongate an arm up the graduated tube to reach a piece of bait used as a reward, which allows the measurement of arm extension. 12 males and 7 females of *Octopus vulgaris* were exposed to this setup and measured using an experimental protocol that was supported by high-resolution video recordings.

2. Materials and methods

2.1. Subjects

19 octopuses (*O. vulgaris*, 12 males and 7 females, Dorsal Mantle Length range 72.5–122.4 mm, longest arm lengths range 320–565 mm) were collected from the Bay of Naples, Italy, and maintained in $50 \times 50 \times 40$ cm glass tanks with artificial sea-water. The water is circulated continuously in a closed system and filtered by active carbon, mechanical and biological filters. The animals were held at a temperature of 17 °C in a 12 h light/dark cycle. One day before an experiment, the selected octopus was moved to a bigger aquarium ($80 \times 80 \times 60$ cm) at a temperature of 18–20 °C.

2.2. Ethics statement

No specific permits were required for the described studies.

2.3. Apparatus and video recording setup

The measuring apparatus is based on a graduated tube in transparent Plexiglas, joined to a supporting plate (see Margheri et al. (2012)). It can be introduced inside the tank, with the plate located on the tank base to maintain the position. The tube can be fixed to the tank wall with the opening outside of the water. The set-up has been designed to allow octopus to insert one of its arms for measuring into the tube, maintaining the remaining arms and the body outside. The tube has the role both of physical guide to drive the protrusion of one arm and of direct measuring instrument, thanks to a graduated scale on it and the option to add sensors (e.g. load cell, Margheri et al., 2012). The tube is sufficiently long so that no octopus can reach the end (Margheri et al., 2012), thus to ensure that measurements are documenting the maximum range possible. The movements were filmed by two Super VHS PAL synchronized video cameras positioned ~90° apart (temporal resolution 20 ms, shutter speed 1/250). Recorded videos were used to measure the arm length and to identify which arm is used to execute the task.

2.4. Experimental procedure

Octopuses were trained to extend their arms toward a target (an ~1 cm^3 portion of sardines, anchovies, or crab) inside the measuring apparatus. Consecutive training sessions were repeated for ~3 days. The bait was positioned at 3 different points in the tube during each session: proximal (at the base of the tube), medial (100–150 mm distance) and distal (200–250 mm distance depending on the octopus size). The bait position was moved when the animal achieved 5 correct trials. After the training, the elongation capability of each animal was measured for 2–3 days during a single measurement session with 15–20 trials per session. The tube was presented to the animal with the bait in a medial-distal position, and the prey was pulled up as the octopus inserted one arm inside the tube, which induced the animal to elongate its arm and reach the maximum length to catch the prey (Fig. 1 and Supplementary Movie S1). To compare different arms and animals, the measured elongation of each arm was normalised to a reference length measured during animal swimming by jet propulsion. During this movement, the animal swims headfirst with the arms trailing behind during the central part (Villanueva et al., 1997), allowing the measurement of arm in a straight position in vivo. A dedicated aquarium with geometric references on the facets was used for the jet propulsion movement recordings. Each octopus was moved to the aquarium 30 min prior to an experiment. Two cameras were positioned with viewing angles that were normal to the tank facets (i.e., one camera facing the frontal side and one camera facing the lateral side). Bait or white tissues motivated the octopus to swim from one side of the tank to the opposite side in a virtual plane that was perpendicular to the frontal camera optical axis. All of the octopuses were recorded 2 or 3 times under the same environmental conditions during jet propulsion movements in both the directions. A total of 55 trajectories together with 392 arm reference length measurements were recorded for all 19 animals. The reference length of each arm, measured from the arm tip and head base, was calculated with a geometric pinhole camera model and considered the position of the plane of motion, from digitized frames. Animal training and experiments

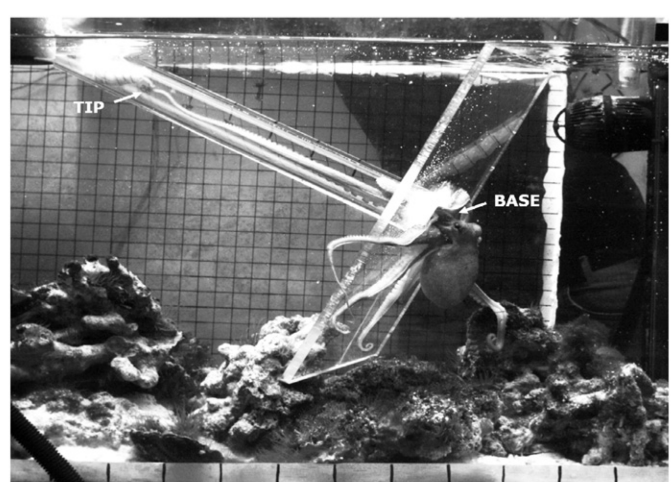

Fig. 1. Example of a frame utilized to measure octopus' arm elongation. Here the animal is using its second left arm (L2). See also Supplementary Movie S1.