Amodio et al.:

The Use of Artificial Crabs for Testing Predatory Behavior and Health in the Octopus

Supplementary Data

Tanks and environment

Following Borrelli (2007), tanks have standardized fittings including a yellowish-brown layer of sand on the bottom and a pair of bricks, set in a corner, that serves as a den (Fiorito et al. 1990; Fiorito and Scotto 1992). The experimental room and environmental factors such as lighting and seawater are as described by Borrelli (2007).

Experimental design and procedures

In the original test, animals were presented either with live crabs alone or in the presence of a novel object (Borrelli, 2007; see also Borrelli and Fiorito, 2008). In the present study and according to this procedure, and during each day of the experiment, an octopus was presented with a live crab, and either the artificial (dummy) or the dead crab (Fig. S1, S2).

Latencies to attack were measured (in seconds) from video recordings of experiments. All experiments were videotaped by remote controlled 3-CCD video cameras (JVC, Model KY-F32, Japan) connected to video-recorders (Panasonic, Model AGDV 2700, Japan) through video timers (For.A, Model VTG-33, Japan) that provided real time (in hundreds of seconds) and allowed to identify each frame (1/25 s). For each experiment the video camera was hidden from the animals' view as it was positioned behind a dark curtain.

In addition, and to control for any potential influence of the tester's behaviour on the performance of the octopuses, the landing time (in s) of the stimuli utilized in the experiment were

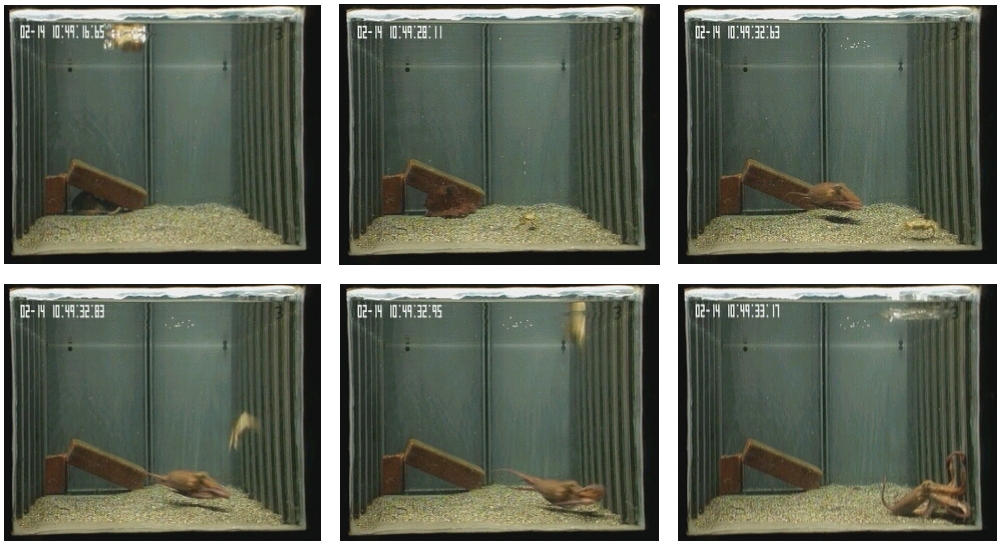
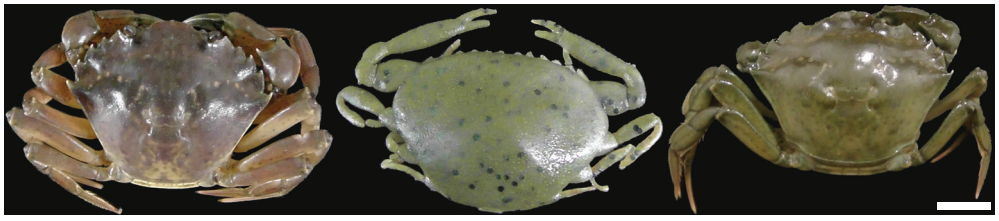


Fig. S1: A sequence of frames (from top left to bottom right) taken from video recordings of the experiment (reference latency, live crab) following a typical acclimatization test (see text for details)



Live crab Dummy crab Dead crab

Fig. S2: Photographs of the stimuli presented to *O. vulgaris* during the experiment to assess predatory performance Live (*C. maenas*: weight: 9 g), dummy (Berkley, Gulp Peeler crab; weight: 6 g) and dead (*C. maenas*: weight: 9 g) crabs. Scale bar = 1 cm.

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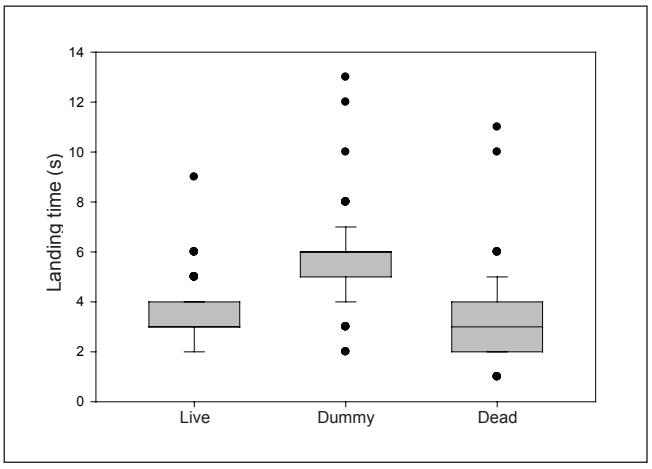

Fig. S3: Box plots showing the landing time of each stimulus (live, dummy and dead crabs) during the experiment

Tab. S1: Tabularized scheme of experimental procedure for the presentation of the stimuli (i.e., live crab or its alternatives: dead or dummy) to octopuses during each of the five days of the experiment
Trials were arranged into two alternative morning-afternoon blocks. A coin flip was utilized to determine the sequence of trials for each day to the animals.

Morning block		**Afternoon block**	
Trial 1	*Trial 2*	*Trail 1*	*Trial2*
Live	Dead	Live	Dummy
Live	Dummy	Live	Dead

measured from video recordings. This value was computed as the difference (in seconds) between the actual time of appearance of the stimulus at the water surface and the time the crab landed on the bottom of the tank (Fig. S3). This data also provided an insight into the hydrodynamics of the three challenges that could contribute to any differences in the response of the animal to the test.

To test for potential fear towards novel objects (i.e., alternatives to the natural prey), each octopus was presented every day with two blocks of two trials each. Within each block, the animal was presented with a tethered crab first: this provides a measure of the octopus' attack performance in "normal" conditions, enhances the animal's attention, and "prepares" it for the actual task, i.e., reference latency (Borrelli, 2007). During the second trial of the block the tester presented to the octopus either the dead crab (dead) or to the artificial one (dummy). Following Borrelli (2007; but see also Borrelli and Fiorito, 2008), the two alternative preys were attached to a cotton thread and always presented in front of the animal, similar to the live crab. Each trial lasted a maximum of five minutes (ceiling latency: 301 s) and a failure to attack within this period was classified as "no attack".

The two blocks were spaced apart by approximately 4 h (morning and afternoon blocks). Before the beginning of the morning block a coin was flipped to assign the type of presentation to each animal at the second trial (dead or dummy). Consequently, two alternative morning-afternoon blocks were possible (Tab. S1).

In all trials octopuses were allowed to start from their home position (i.e., the den in their own tank) before presentation of a stimulus (i.e., live, dummy, dead).

To further validate the possible use of a stimulus model as a replacement for a live crab to monitor octopus well-being, at the end of the five days of the experiment, we exposed nine *O. vulgaris* (Short acclimatization) to an extended acclimatization procedure (i.e., daily presentation of a crab) for 15 successive days (see main text). Octopuses were randomly assigned to two groups and presented every day with either the live or the dummy crab.

Each presentation lasted a maximum of two minutes (ceiling latency: 121 s) and a failure to attack within this period was classified as "no attack". A shorter ceiling latency as a criterion for acclimatization was applied to octopuses that experienced more than 10 days in captivity. This is based on evidence that the contextual learning promotes familiarization to the stimuli (Borrelli, 2007; Maldonado, 1963). Animals were fed with a live crab every other day, in the afternoon.

References

Fiorito, G., von Planta, C. and Scotto, P. (1990). Problem solving ability of Octopus vulgaris Lamarck (Mollusca, Cephalopoda). *Behav Neural Biol 53*, 217-230.

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