



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

Piero Amodio^{1§}, Paul Andrews^{2§}, Marinella Salemmè^{1§}, Giovanna Ponte^{1,3} and Graziano Fiorito^{1,3}

¹Associazione Cephalopod Research, CephRes-ONLUS, Napoli, Italy; ²St. George University of London, London, UK;

³Stazione Zoologica Anton Dohrn, Napoli, Italy

Summary

The willingness of the cephalopod mollusc *Octopus vulgaris* to attack a live crab is traditionally used as a method to assess the overall health and welfare of octopuses in the laboratory. This method requires placing a crab in the home tank of an animal, measuring the time (latency) taken for the octopus to initiate an attack and withdrawing the crab immediately prior to capture. The same crab is commonly used to assess multiple octopuses as part of daily welfare assessment. Growing concern for the welfare of crustaceans and a review of all laboratory practices for the care and welfare of cephalopods following the inclusion of this taxon in Directive 2010/63/EU prompted a study of the utility of an artificial crab to replace a live crab in the assessment of octopus health. On consecutive days *O. vulgaris* (N=21) were presented with a live, a dead or an artificial crab, and the latency to attack measured. Despite differences in the predatory performance towards the three different crab alternatives, octopuses readily attacked the artificial (and the dead) crab, showing that they can generalize and respond appropriately towards artificial prey. Researchers should consider using an artificial crab to replace the use of a live crab as part of the routine health assessment of *O. vulgaris*.

Keywords: *octopus vulgaris*, Directive 2010/63/EU, animal testing alternative, replacement, crab

1 Introduction

Cephalopods (nautiloids, squid, cuttlefish and octopus) have been utilized for more than one hundred years to explore aspects of their biology, physiology and mostly in neuroscience. These studies provided important information on basic cellular and behavioral processes, including learning and memory, that appears shared among many taxa (Hochner et al., 2006; Borrelli and Fiorito, 2008; Hochner, 2012; Brown and Piscopo, 2013; Huffard, 2013). The inclusion of cephalopods in the legislation of the European Union on the protection of animals for scientific purposes (Directive 2010/63/EU; see: Andrews et al., 2013; Smith et al., 2013) requires the development of guidelines for all aspects of their care and welfare (Fiorito et al., 2014; see also: http://www.cost.eu/domains_actions/fa/Actions/FA1301).

Due to the current limits of breeding cephalopods in captivity (Iglesias et al., 2014), experimental studies should rely on wild born animals. Animals taken from the wild should be allowed to acclimatize to captivity before any experiment starts.

Since the pioneering studies initiated at the end of the 1940s, acclimatization in cephalopods is assessed by measuring the la-

tency to attack a live prey, usually crab or prawn (Boycott, 1954; Messenger, 1968; Duval et al., 1984; review in: Borrelli and Fiorito, 2008; Boal, 2011). In *Octopus vulgaris*, a rapid predatory response triggered by the prey is used as proxy of achieved acclimatization and for assessment of good health status of the individual (Borrelli, 2007). In brief, each animal is presented daily with a live crab (*Carcinus maenas*, Crustacea, Decapoda) attached to a cotton thread (tethered crab). The prey lands on the bottom of the tank in less than five seconds, and it generally walks spontaneously; if the crab stays motionless (freezing behavior) the thread is gently pulled (after about 10 s) to solicit movement. Although motion is not considered a necessary prerequisite for the octopus to respond to a stimulus (Wodinsky, 1971), a moving object (more than one staying still) attracts an octopus' attention, and promotes the attack response (Maldonado, 1963; Packard, 1963). The acquisition of experience in captivity reduces the requirements for a moving object, thus revealing contextual learning and plasticity of behavior (Hochner et al., 2006; Borrelli and Fiorito, 2008; Huffard, 2013).

Once the octopus moves towards the crab, this is promptly removed before capture. The same crab is normally used to

Received January 28, 2014; accepted in revised form June 4, 2014; Epub June 12, 2014; <http://dx.doi.org/10.14573/altex.1401282>

§ These authors mutually contributed to this work

measure willingness to attack in multiple octopuses in a single session and may even be used on consecutive days.

Over the last five years there has been growing concern regarding the ability of decapod crustacea to experience pain and suffering (Gherardi, 2009; Elwood, 2011; Horvath et al., 2013; Magee and Elwood, 2013). Although the welfare impact on the crab of repeated exposure to a predator has not been assessed, it is likely that this is stressful for the animal. This is supported by the observation that when a species (*Liocarcinus* sp.), other than *C. maenas*, is utilized repeatedly during acclimatization of the octopus, the crab dies (M. Salemm, pers. obs.).

In compliance with the 3Rs principles, here we investigated the use of an artificial crab as a replacement, i.e., a dummy or stimulus model (*sensu* Immelmann and Beer, 1989), for a live crab to assess the willingness of an octopus to attack, and hence the potential for this method to be utilized during acclimatization and daily health monitoring of *O. vulgaris*.

2 Materials and methods

Ethical statement

Experiments complied with national legislation for the care and use of animals for research purposes, and the precautionary principles adopted for cephalopods (Andrews et al., 2013; Smith et al., 2013; Fiorito et al., 2014).

Experimental animals

O. vulgaris Cuvier 1797 (N=21) of both sexes (250–600 g body weight) caught in the Bay of Naples (Italy) were individually identified and housed in an experimental tank (60 x 100 x 50 cm) with running seawater (temperature 19.0°C).

Experimental design and procedures

Octopuses were randomly assigned to two different conditions based on the duration of acclimatization: i.) short (N=9, 5 males and 4 females) lasting five days, and ii.) long (N=12, six for each sex) lasting at least 30 days. During this time, animals were exposed daily to live crab (*C. maenas*) and fed with live crabs every other day, see Tab. S1 at <http://dx.doi.org/10.14573/altex.1401282s>.

To assess the potential for replacing the use of a live crab, we compared the latency of *O. vulgaris* over five consecutive days to attack a live crab or, as alternatives, a freshly dead *C. maenas* or an artificial peeler crab (Berkley Gulp Peeler Crab), see Fig. S1, S2 at <http://dx.doi.org/10.14573/altex.1401282s>. Experiments were designed to compare the time required (latency to attack, s) by an octopus to attack a live crab or its alternative by using a partial modification of the neophobia test (Greenberg, 1983) designed for *O. vulgaris* by Borrelli (2007). Every day two blocks of two trials each were carried out. 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 the artificial one

(dummy). Following Borrelli (2007), 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 four hours (morning and afternoon blocks); inter trial interval was fixed at five minutes. For more detailed information, see supplementary data at <http://dx.doi.org/10.14573/altex.1401282s>.

To further validate the possible use of a stimulus model as a replacement of 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. Octopuses were randomly assigned to two groups and presented every day with either the live or the dummy crab.

During the experiments the investigator was hidden behind curtains located on each tank to minimize interaction with the animals and instructed to present the stimulus at approximate similar distance during trials and days and to all animals (Borrelli, 2007).

Data analysis

Data were not normally distributed and non-parametric statistics were used to analyze the performance of animals (Siegel, 1956; Zar, 1999). Data are presented by median and ranges, and using box and whisker plots. Between-group comparisons were assessed with a Mann-Whitney U test; Wilcoxon matched pairs signed-ranks tests were utilized to test related sample comparisons. All tests were two-tailed and the alpha was set at 0.05; SPSS (rel. 14.0, SPSS Inc. – Chicago, 2005) was utilized for statistical analysis.

3 Results

All octopuses met acclimatization criteria (i.e., well adapted to captivity) at the beginning of the experimental phase. However, short and long acclimatization induced a significant difference in behavioral performance of octopus towards a live crab (Mann-Whitney U test: $Z=4.39$, $N_1=9$, $N_2=12$, $P<0.001$) with the latency being longer and more variable after short acclimatization (Tab. 1; Fig. 1). Despite these differences, the attack performance of *O. vulgaris* towards live *C. maenas* during the five days remained stable and homogeneous (morning vs. afternoon block, pair-wise comparison always not significantly different; data not shown, see Table 1).

Since each octopus was presented with four consecutive tethered crabs (or their alternatives) without being able to capture and feed on any of them, a Wilcoxon matched-pairs signed-ranks test was first performed to measure the potential effect of the repetition of crab presentation on the predatory performance of the animals. Successive crab presentations did not affect octopuses' predatory behavior (live crab morning vs. afternoon: not significant after Wilcoxon signed-ranks test), indicating that the animals continued to readily attack the prey even in the absence of a reward.

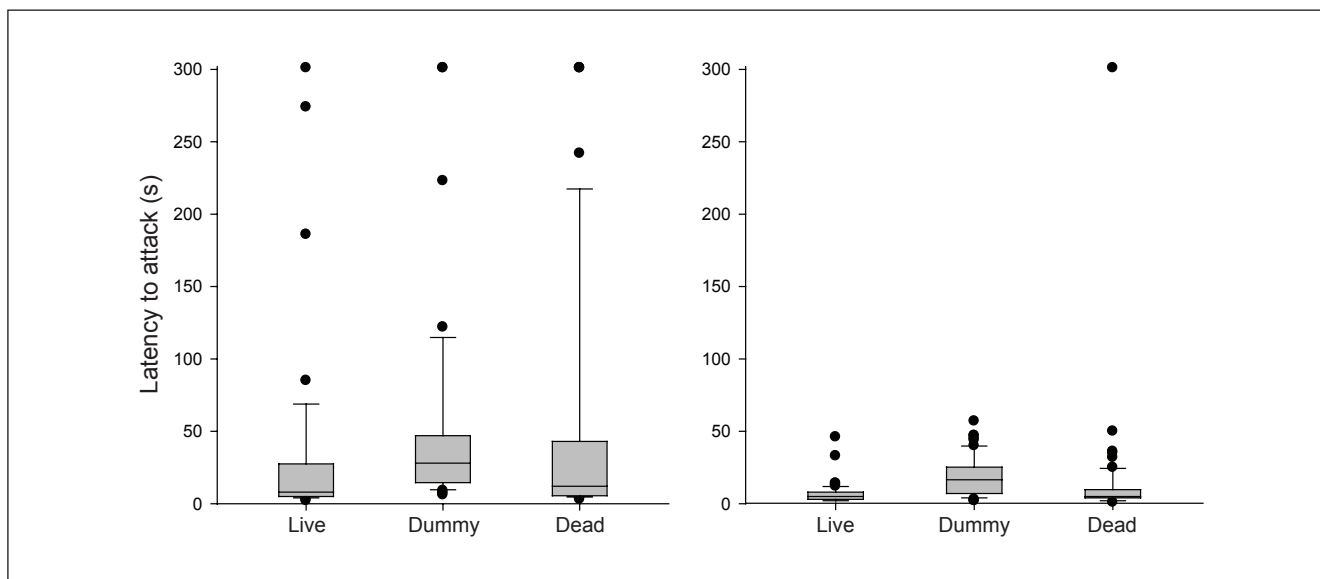


Fig. 1: Box plots showing the latency to attack the crab and their alternatives (dummy and dead) during the experiment by *O. vulgaris* after short (left) or long acclimatization (right)

Boxes represent the interquartile range (25th-75th percentiles), bars within boxes the median values, whiskers the 10th and 90th percentiles, and circles the outliers.

Tab. 1: A summary in tabular form of the performance of *O. vulgaris* (short and long acclimatization) when presented with live, dummy or dead crabs

Behavioral performances are presented as median and ranges (min-max value) of the latencies to attack (in s) over the five days (day 1 - day 5) of the experiment and as overall performance. Values are presented for each pair with values for “live” always in the first row. A value of 301 s correspond to a no-attack. The results of Wilcoxon signed-ranks test (Z and corresponding P) are also provided.

	Short acclimatization								Long acclimatization							
	Live vs Dummy				Live vs Dead				Live vs Dummy				Live vs Dead			
	N	median	Z	P	N	median	Z	P	N	median	Z	P	N	median	Z	P
Day 1	9	22.0 (4-106) 27.0 (7-122)	1.60	0.133	9	20.0 (3-301) 7.5 (4-301)	0.12	0.931	12	6.0 (3-11) 18.5 (11-45)	3.06	>0.001	12	6.0 (2-13) 8.5 (2-50)	2.12	0.032
Day 2	9	14.5 (5-301) 20.5 (9-301)	0.18	0.909	9	30.5 (6-227) 19.5 (5-301)	0.70	0.528	12	6.0 (3-18) 20.5 (5-57)	2.47	0.011	12	7.0 (2-33) 7.0 (3-301)	0.77	0.487
Day 3	9	12.0 (4-301) 41.0 (10-301)	1.60	0.128	9	16.0 (5-274) 25.5 (6-301)	2.44	0.014	12	7.0 (3-14) 18.0 (3-47)	2.40	0.012	12	6.5 (4-23) 5.0 (3-19)	0.24	0.835
Day 4	9	10.0 (4-205) 35.0 (10-301)	1.60	0.124	9	9.0 (4-301) 9.0 (3-95)	0.54	0.646	12	3.5 (2-16) 8.0 (2-31)	2.45	0.012	12	4.0 (2-12) 4.5 (2-36)	0.28	0.829
Day 5	9	7.0 (2-26) 29.5 (6-301)	2.31	0.017	9	4.0 (2-18) 14.0 (5-301)	2.03	0.046	12	4.0 (2-46) 15.5 (2-47)	2.58	0.006	12	4.0 (2-18) 4.0 (1-15)	0.49	0.654
Overall	9	8.0 (2-301) 28.8 (6-301)	2.94	0.003	9	8.0 (2-301) 12.0 (3-301)	1.53	0.118	12	5.0 (2-46) 16.5 (2-57)	5.90	>0.001	12	5.0 (2-46) 5.0 (1-301)	1.96	0.046

The presentation of either dummy or dead crabs induced a more variable response in the octopuses, with animals' performance after a short acclimatization markedly variable (Fig. 1). In addition, when alternatives to the live crab were presented, some animals occasionally failed to attack (short acclimatization: in 2 and 3 instances out of 45 trials, dead vs dummy, respectively; long acclimatization: 1 out of 45 trials, dead).

As shown in Table 1, octopuses with a short exposure to captivity did not show a significant difference when attacking the live or the dead crab, but recognized the artificial crab as a potential different prey item on all five days. Comparison between the predatory performance of more experienced (long acclimatization) *O. vulgaris* towards the three different prey alternatives showed highly significant differences (Tab. 1; Fig. 1). The performance over five days (Tab. 1) of the animals under the two conditions revealed that short-acclimatized octopuses performed almost constantly towards one of the two prey alternatives; however, towards the end of the experiment, octopuses started to treat the dummy crab as not a "valid" alternative and attacked it with significantly longer latencies.

In contrast, *O. vulgaris* with longer acclimatization times distinguished between dummy and live crabs over all the five days of the study (Tab. 1; Fig. 1).

Finally, at the end of the experiment, the repeated presentation over the successive 15 days of the artificial crab to octopuses resulted in an improvement in their performance (as indicated by a decrease in latency to attack), but animals never reached a level similar to that when presented with a real crab (median, range (s)=8.8, 5.9 and 12.0; 21.5, 11.0 and 40.9; live vs dummy, respectively).

4 Discussion

The primary aim of this study was to assess whether it was possible to replace the use of a live crab with an artificial one in the test of predatory behavior widely used as part of daily health and welfare assessment of octopuses in captivity. Our results show that whilst *O. vulgaris* would attack the artificial crab, the animal appears to be able to differentiate between the live, artificial and dead crabs. Additionally, the latency and incidence of attack is affected by the period of acclimatization (5 or 30 days, i.e., short or long), an observation that has implications for guidelines for the care and welfare of cephalopods (Fiorito et al., 2014). In particular, while the predatory performance towards the live prey resembled the one established as a criterion for acclimatization, the presentation of a dead and, more evidently the artificial, crab resulted in a more variable response, with latencies that were relatively higher.

Although a live crab has been used to assess willingness to attack in *O. vulgaris* (even for aversive training paradigms) for over 60 years, the method itself has been subjected to little formal study (Boycott, 1954; Maldonado, 1965; Borrelli and Fiorito, 2008; Cartron et al., 2013). Consistent with Borrelli (2007; Borrelli and Fiorito, 2008), here we demonstrate that under the same acclimatization conditions (see below), the latency to attack a live crab is relatively consistent over days

and from morning to afternoon. Although the octopus is not "rewarded," the predatory response is not extinguished. These results support the validity of the use of predatory behavior as part of the daily assessment of health in octopus. These data with a live crab provided a suitable stable baseline against which the response to an artificial or a dead crab could be assessed objectively. Although octopuses would attack both artificial and dead crabs, the response was more variable than for the live crab and occasionally animals failed to attack (see above). Overall, the latency to attack a live crab is shorter than for an artificial or a dead crab (only for short acclimatized animals) and this may reflect the fact that movement is known to promote predatory behavior in *O. vulgaris* (Packard, 1963; but see Wodinsky, 1971). Monitoring the attack response to either live or artificial crabs over 15 days revealed that whilst both were attacked, the latency to attack the live crabs remained relatively stable, while that for the artificial crab decreased although it never matched the performance seen with the live crab.

The differences in latency between attacking a live and an artificial crab do not invalidate the use of an artificial crab provided that appropriate values are selected for assessment of "normal attack" as is also the case when a live crab is used.

Our study used two different acclimatization times and this revealed that it has an effect on attack latency that has not previously been reported (but see Borrelli and Fiorito, 2008). With short periods (5 days) of acclimatization the latency to attack all three "crabs" was longer and more variable than after 30 days of acclimatization. It has been reported that recently caught octopuses are reluctant to attack and explore novel objects placed in the tank (Hochner et al., 2006). However, in the short acclimatization group, whilst the latency to attack the live and dead crabs was similar, both were shorter than for the artificial crab. Consideration should be given for the time taken for the artificial crab to reach the tank floor (see Fig. S3 at <http://dx.doi.org/10.14573/altex.1401282s>) which is longer than for either the live or dead crab and could contribute to the longer latency to attack, although *O. vulgaris* attack crabs in mid-water as well as on the tank floor (Hochner et al., 2006; Borrelli and Fiorito, 2008). However, the artificial crab is lighter than either the live or the dead crabs and may not have the same hydrodynamic properties as a real crab in its descent to the tank floor. This observation may suggest that the type of movement displayed by a potential prey item in the water column may also be important rather than movement *per se* and requires further study. In the short acclimatization group there was also some indication that the octopuses could also distinguish between live and dead crabs (Tab. 1: days 3 and 5). In the long acclimatization group the latency to attack the artificial crab was significantly longer on all five testing days than for the live crab, but on one day (day 1) animals distinguished live from dead crabs. These preliminary indications that octopuses have some capacity to distinguish live from dead crabs are intriguing and require further study.

The finding that duration of acclimatization affects predatory behavior shows the importance of stating acclimatization protocols in the methods sections of publications and particu-



larly those investigating any aspect of cephalopod behavior, as in the ARRIVE guidelines (Kilkenny et al., 2010). As most cephalopods in research are obtained from the wild (Smith et al., 2013; Fiorito et al., 2014) adequate acclimation to the laboratory environment is pivotal to study design and interpretation.

This study shows that an artificial crab could replace the use of a live crab to assess the willingness to attack as an index of overall health and well-being in the octopus. The use of an artificial crab avoids exposing a live crab repeatedly to a potentially stressful situation. In addition, the predatory behavior test can be more readily standardized with an artificial crab as they can be used all year round, which may not be possible with live *C. maenas* necessitating a change of species and/or of the size of the individual crab utilized for different octopuses due to seasonal differences. Additionally, using the artificial crab removes the possibility that an octopus being tested prior to transport, drug administration by gavage or about to undergo a scientific procedure where food deprivation is required, will capture the crab and ingest it, thus confounding the study.

Each laboratory would need to set criteria for “normal values,” taking into account the period of acclimatization utilized. Unless there is a compelling scientific justification to use a live crab (especially in repeated testing), we recommend that those responsible for daily care and welfare of cephalopods assess the use of an artificial crab in their own experimental setting. As most laboratories feed their *O. vulgaris* using a live crab, measurement of attack latency can readily be compared with an artificial (or dead) crab. Although we have focused on the use of an artificial crab to measure predatory behavior in *O. vulgaris*, we believe this method has applicability to cuttlefish, possibly using an artificial crab or prawn. Finally, as for any other investigation based on the study of stimulus models, a better designed artificial crab may improve the “quality” of the presentation to the octopuses (and other cephalopod species).

References

- Andrews, P. L. R., Darmaillacq, A.-S., Dennison, N. et al. (2013). The identification and management of pain, suffering and distress in cephalopods, including anesthesia, analgesia and humane killing. *J Exp Mar Biol Ecol* 447, 46-64. <http://dx.doi.org/10.1016/j.jembe.2013.02.010>
- Boal, J. G. (2011). Behavioral research methods for octopuses and cuttlefishes. *Vie et Milieu-Life and Environment* 61, 203-210.
- Borrelli, L. (2007). Testing the contribution of relative brain size and learning capabilities on the evolution of *Octopus vulgaris* and other cephalopods. PhD, Stazione Zoologica Anton Dohrn, Napoli, Italy; Open University, London, UK.
- Borrelli, L. and Fiorito, G. (2008). Behavioral analysis of learning and memory in cephalopods. In J. J. Byrne (ed.) *Learning and Memory: A Comprehensive Reference* (605-627). Oxford, UK: Academic Press.
- Boycott, B. B. (1954). Learning in *octopus vulgaris* and other cephalopods. *Pubblicazioni della Stazione Zoologica di Napoli* 25, 67-93.
- Brown, E. R. and Piscopo, S. (2013). Synaptic plasticity in cephalopods; more than just learning and memory? *Invert Neurosci* 13, 35-44. <http://dx.doi.org/10.1007/s10158-013-0150-4>
- Cartron, L., Darmaillacq, A.-S. and Dickel, L. (2013). The “prawn-in-the-tube” procedure: What do cuttlefish learn and memorize? *Behav Brain Res* 240, 29-32. <http://dx.doi.org/10.1016/j.bbr.2012.11.010>
- Duval, P., Chichery, M. P. and Chichery, R. (1984). Prey capture by the cuttlefish (*Sepia officinalis* L.). An experimental study of two strategies. *Behav Process* 9, 13-21. [http://dx.doi.org/10.1016/0376-6357\(84\)90004-4](http://dx.doi.org/10.1016/0376-6357(84)90004-4)
- Elwood, R. W. (2011). Pain and suffering in invertebrates? *ILAR J* 52, 175-184. <http://dx.doi.org/10.1093/ilar.52.2.175>
- Fiorito, G., Affuso, A., Anderson, D. B. et al. (2014). Cephalopods in neuroscience: Regulations, research and the 3Rs. *Invert Neurosci* 14, 13-36. <http://dx.doi.org/10.1007/s10158-013-0165-x>
- Gherardi, F. (2009). Behavioural indicators of pain in crustacean decapods. *Annali dell'Istituto Superiore di Sanità* 45, 432-438. <http://dx.doi.org/10.1590/S0021-25712009000400013>
- Greenberg, R. (1983). The role of neophobia in determining the degree of foraging specialization in some migrant warblers. *American Naturalist* 122, 444-453. <http://dx.doi.org/10.1086/284148>
- Hochner, B., Shomrat, T. and Fiorito, G. (2006). The octopus: A model for a comparative analysis of the evolution of learning and memory mechanisms. *Biol Bull* 210, 308-317. <http://dx.doi.org/10.2307/4134567>
- Hochner, B. (2012). An embodied view of octopus neurobiology. *Curr Biol* 22, R887-R892. <http://dx.doi.org/10.1016/j.cub.2012.09.001>
- Horvath, K., Angeletti, D., Nascetti, G. and Carere, C. et al. (2013). Invertebrate welfare: an overlooked issue. *Annali dell'Istituto Superiore di Sanità* 49, 9-17.
- Huffard, C. L. (2013). Cephalopod neurobiology: An introduction for biologists working in other model systems. *Invert Neurosci* 13, 11-18. <http://dx.doi.org/10.1007/s10158-013-0147-z>
- Iglesias, J., Fuentes, L. and Villanueva, R. (2014). *Cephalopod Culture*. Berlin, Germany: Springer. <http://dx.doi.org/10.1007/978-94-017-8648-5>
- Immelmann, K. and Beer, C. (1989). *A Dictionary of Ethology*. Cambridge, Mass, USA: Harvard University Press.
- Kilkenny, C., Browne, W. J., Cuthill, I. C. et al. (2010). Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. *PLoS Biol* 8, e1000412. <http://dx.doi.org/10.1371/journal.pbio.1000412>
- Magee, B. and Elwood, R. W. (2013). Shock avoidance by discrimination learning in the shore crab (*Carcinus maenas*) is consistent with a key criterion for pain. *J Exp Biol* 216, 353-358. <http://dx.doi.org/10.1242/jeb.072041>
- Maldonado, H. (1963). The positive learning process in *Octo-*

- pus vulgaris. *Zeitschrift für Vergleichende Physiologie* 47, 191-214. <http://dx.doi.org/10.1007/BF00303120>
- Maldonado, H. (1965). The positive and negative learning process in *Octopus vulgaris* Lamarck. Influence of the vertical and median superior frontal lobes. *Zeitschrift für Vergleichende Physiologie* 51, 185-203. <http://dx.doi.org/10.1007/BF00299293>
- Messenger, J. B. (1968). The visual attack of the cuttlefish, *Sepia officinalis*. *Anim Behav* 16, 342-357. [http://dx.doi.org/10.1016/0003-3472\(68\)90020-1](http://dx.doi.org/10.1016/0003-3472(68)90020-1)
- Packard, A. (1963). The behaviour of *Octopus vulgaris*. *Bulletin de l'Institut océanographique (Monaco) Numéro spécial 1D*, 35-49.
- Siegel, S. (1956). *Nonparametric Statistics for the Behavioral Sciences*. Tokyo: McGraw-Hill.
- Smith, J. A., Andrews, P. L. R., Hawkins, P. et al. (2013). Cephalopod research and EU Directive 2010/63/EU: Requirements, impacts and ethical review. *J Exp Mar Biol Ecol* 447, 31-45. <http://dx.doi.org/10.1016/j.jembe.2013.02.009>
- Wodinsky, J. (1971). Movement as a necessary stimulus of octopus predation. *Nature (London)* 229, 493-494. <http://dx.doi.org/10.1038/229493a0>
- Zar, J. H. (1999). *Biostatistical analysis*. Upper Saddle River, N.J.: Prentice Hall

Correspondence to

Marinella Salemm, PhD
Associazione Cephalopod Research, CephRes
Via dei Fiorentini 21
80133 Napoli, Italy
Phone: +39 339 1316246
e-mail: cephres@cephalopodresearch.org

Addendum

Addendum to Evidence for the Detection of Non-Endotoxin Pyrogens by the Whole Blood Monocyte Activation Test

Nina Hasiwa^{1,2}, Mardas Daneshian¹, Peter Bruegger⁴, Stefan Fennrich⁵, Astrid Hochadel², Sebastian Hoffmann⁶, Felix E. Rivera-Mariani³, Christoph Rockel⁷, Stefanie Schindler⁸, Ingo Spreitzer⁹, Sandra Stoppelkamp⁵, Kranthi Vysyaraju³, and Thomas Hartung^{1,3}

¹CAAT-Europe, University of Konstanz, Konstanz, Germany; ²AtaX-Advice, Konstanz, Germany; ³Johns Hopkins University, Bloomberg School of Public Health, CAAT, Baltimore, USA; ⁴Novartis, Basel, Switzerland; ⁵Department of Thoracic, Cardiac and Vascular Surgery, Tübingen University Hospital, Tübingen, Germany; ⁶seh consulting + services, Paderborn, Germany; ⁷Enzler Hygiene AG, Center for Hygiene, Pratteln, Switzerland; ⁸Animalfree Research, Zürich, Switzerland; ⁹Paul-Ehrlich Institut (PEI), Langen, Germany

Regarding this t⁴ report, which appeared in ALTEX (2013), 30(2), as co-author Thomas Hartung would like to acknowledge that the respective patents referred to, which he holds as the co-inventor of the whole blood pyrogen test, were at the time of the article licensed to Biotest Pharmaceuticals, Germany, and he received royalties from sales of this test. The license and associated royalty payment obligations were in the meantime transferred to Merck-Millipore, France.

<http://dx.doi.org/10.14573/altex.1410081>