Can preference for crabs in juvenile *Octopus maya* be modified through early experience with alternative prey?

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Abstract

Previous studies on cephalopod feeding have suggested that predetermined preference for certain prey types can be modified by exposing newly hatched individuals to visual or chemical stimuli (imprinting), or by familiarizing them to feed on prey different from those preferred (associative learning). The aim of our study was to determine whether preference to attack crabs in *Octopus maya* could be modified by early experience with or without food reinforcement using palaemonid shrimp, a prey octopuses consume readily, but will reject if crabs are available. We conducted experiments on the attack response of juvenile octopuses that had been either exposed to (visual and chemical stimuli for at least 48 h before and after hatching) or had fed only on palaemonids (16 days after hatching). Octopuses were then presented with crabs and palaemonids simultaneously and attacks on either prey were recorded. Control treatments, where octopuses had to choose between two similar alternatives (no choice) were included in order to discriminate between active and passive selection. Results were analysed by means of asymmetrical χ^2 contingency tables. Both, octopuses that had only been exposed to stimuli from palaemonids and those that fed on shrimp, selectively attacked crabs when first presented with both alternative prey ($\chi^2 = 6.09$ and $\chi^2 = 5.01$, respectively; both $p < 0.05$). These results indicate that preference for crabs in early juvenile *O. maya* could not be modified through experience with other prey. Because octopuses had never been in contact with crabs prior to trials, such preference was not obtained through their short life experience but could be pre-determined.

1. Introduction

Octopuses are versatile predators with morphologic and behavioural adaptations that enable them to search, capture and manipulate different types of prey (Mather, 1991; Rodhouse & Nigmatullin, 1996). In spite of being considered opportunistic generalists, several *in situ* studies have shown octopuses consistently include crabs in high proportions in their diet (e.g., Anderson et al., 2008; Leite et al., 2009), and the overwhelming dominance of crabs in the diet of *Octopus cyanea* in Hawaii has again prompted the discussion on the ecological significance of feeding preferences in octopuses (Mather, 2011).

Experience in early life stages has been shown to play an important role in determining different aspects of adult behaviour, and an important example of the ecological consequences of such experience is concerned with feeding (Immelman, 1975). Previous studies have demonstrated that food preferences in a variety of animal groups can be established or modified through associative learning (e.g., Burghardt & Hess, 1966; Stasiak, 2002) and imprinting (e.g., Darmaillacq et al., 2006; Schausberger et al., 2010). Associative learning requires the association between the stimulus from prey and the predatory action to be reinforced by food, whereas imprinting implies a phase-sensitive learning period in which the stimulus for prey recognition does not involve food as a reinforcing reward (Schausberger et al., 2010).

Behavioural plasticity is central for the survival of individuals encountering environmental heterogeneity (Mery & Burns, 2010), and learning, as a special type of plasticity allows animals to better exploit environmental features unique to certain times and places (Dukas, 2013). Whilst learning is associated with developmental behavioural plasticity and involves changes in the nervous system as a result of experience (Snell-Rood, 2013), often changes in behaviour can be induced rapidly in response to a particular external context and rapidly reversed once the stimulus disappears from the environment. This other form of behavioural plasticity allows individuals to execute behaviour based on an evolved innate mechanism and has been referred to as activational behavioural plasticity (Snell-Rood, 2013).

Amongst cephalopods, previous studies on feeding behaviour have suggested an innate or predetermined nature in prey recognition and preference (Wells, 1958, 1962), which in cuttlefish can be overridden by exposure to alternative food types (Darmaillacq et al., 2006). Laboratory-based studies have demonstrated that *Octopus maya* at 7 days (Portela-Rodríguez, 2011)

and 45 days since hatching (Domingues, 2007) consumed crabs in higher proportions than other crustacean prey offered simultaneously. Whilst the early 7-day *O. maya* hatchlings utilised yolk reserves, hence never experienced external food (Portela-Rodríguez, 2011), 45-day individuals were reared on palaemonid shrimp and had never been in visual, tactile or chemical contact with crab prey (Domingues, 2007). Predetermined food preference has thus been put forward as the behavioural mechanism to explain the disproportionately higher frequency of attacks of *O. maya* on crabs than on palaemonid shrimp when octopuses had a choice of different prey types (Portela-Rodríguez, 2011).

In contrast to other octopus species, *O. maya* does not have a paralarval stage previous to benthic settling, but experiences a short post-embryonic phase, during which octopuses undergo considerable morphological, physiological and behavioural changes (Moguel et al., 2010). At hatching, newborns are almost completely developed and hold enough yolk reserves to survive and grow into early juveniles (Boletzky, 2003). Not only feeding, but anti-predatory strategies should develop rapidly during this phase in order for octopuses to satisfy their high energetic demands (Wells & Clark, 1996), and grow out of the size where they are most vulnerable. Consequently, accurate and efficient mechanisms to decode information and facilitate the discrimination of valuable prey from dangerous predators should be critical in the first days of life.

Most studies on prey selection have demonstrated differences in the consumption of alternative prey types (e.g., Iribarne at al., 1991; Darmaillacq et al., 2006), or in the proportions in which prey are consumed with respect to the relative abundance in which they are naturally found (e.g., Ambrose, 1984; Mather, 2011). Whilst these differences constitute evidence of non-random patterns of association between predators and prey, they do not necessarily reflect an active behavioural choice displayed by the predator (see Singer, 2000). Barbeau & Scheibling (1994) suggested that analysis of specifically defined components of predation can be used to determine the relative importance of passive and active prey selection. Passive or mechanical prey selection usually results from physical properties of prey and predator, such as relative abundance, prey vulnerability and locomotion, which determine the probability of encountering, detecting and capturing different types of prey. Active selection, in contrast, may occur at different moments throughout the foraging bout, such as when a predator decides whether to

attack an encountered prey or whether to reject one that is being manipu-
lated. Passive components of predation will influence the consumption of
a certain type of prey whether predators have a choice of other prey types
or not, whilst the active choice of a predator will only become evident under
multiple prey circumstances (Underwood et al., 2004). Thus, active selection
or preference is not simply the consumption of a certain prey type at a rate
other than random (Jackson & Underwood, 2006), but can only be identified
by differences that result from comparing the response of predators in single
and multiple-prey situations (Singer, 2000).

Underwood & Clarke (2005) further described detailed analytical meth-
ods specifically developed to solve problems in choice experiments based on
these comparisons. They explained that testing hypothesis about prey selec-
tion involves the estimation of probabilities of consumption under the null
hypothesis (where no choice is available), and that these estimates have an
associated error that decreases in size with increasing sampling intensity. In
their paper, the authors show that by using maximum likelihood estimators
(rather than those commonly used in χ^2 tests) the imprecision of sampling
to estimate the null expectation is taken into account, turning these novel
procedures more accurate and reliable than common tests.

In the present study we have taken these definitions and methods to exam-
ine the probability of attack by *O. maya* as an active component of predation
that will provide un-confounded evidence of a behavioural preference only
if attacks on alternative prey are observed in frequencies that differ under
choice and no-choice situations. We conducted a series of experiments to
determine whether early experience with an alternative prey either with or
without food reinforcement, could modify the preference to attack crabs ob-
served in early juveniles of *O. maya*.

2. Materials and methods

Juveniles of *O. maya* were obtained from a single female kept in captivity
at the Unidad Multidisciplinaria de Docencia e Investigación-Sisal, Yucatán,
México. Immediately after spawning, eggs were transferred to artificial in-
cubators (without maternal care) following procedures described in Patent
No. WO 2010/030155 A1 (Rosas et al., 2010). Eggs hanging in small clus-
ters were held at $27 \pm 2°C$, 36 ups and constant photoperiod (12:12 h) until
hatching (ca 50 days), and maintained in the absence of any external stimuli
(chemical, tactile or visual), except specified otherwise.

Crabs *Pachygrapsus* sp. (0.6–0.8 cm carapace width, distance between outermost lateral spines) and palaemonid shrimp *Palaemonetes vulgaris* (1.5–1.8 cm total length, distance from tip of the rostrum to tip of telson) were the prey used throughout experiments. Both prey species and size intervals were chosen on the basis of previous studies showing these were the preferred and not preferred prey types, respectively (Portela-Rodríguez, 2011). Palaemonid shrimp have been previously used to rear *O. maya* under laboratory conditions (van Heukelem, 1977), and possess an elongate body shape, escape trajectories and movement that contrast markedly with those of crabs. All prey were captured from coastal lagoons and wetlands in the nearby area either manually or with a drop net.

The first experiment examined the effect of sensory experience, both visual and olfactory, without food reinforcement on the preference to attack crabs in newborn *O. maya*. Eggs kept in one artificial incubator were exposed to chemical and visual stimuli from palaemonids from the first day of hatching onwards. Amongst octopuses that hatched on the second day, 42 individuals were randomly selected and further exposed for another 48 h. Previous studies have shown that newborn *O. maya* almost never attack prey during the first 2–3 days after hatching (DAH), and can survive by exclusively using yolk reserves up to 5–7 DAH (Moguel et al., 2010). However, in order to rule out any possibility of food reinforcement by octopuses consuming shrimp, these were kept in various transparent acrylic chambers (20 palaemonids per chamber) with perforations throughout the exposure period. After exposure, all prey were removed and octopuses were kept without food or any external stimuli until day 7 after hatching (DAH), thus assuring that yolk reserves had been exhausted when selection trials of experiment 1 begun.

The second experiment studied the effect of experience with food reinforcement on further attack response in *O. maya*. Forty two *O. maya* of the same age (1 DAH) were randomly selected and individually placed in square plastic containers (Figure 1). Here, octopuses were offered 3 palaemonid shrimp daily from day 1–14 DAH, and were allowed to consume prey throughout this period. Shrimp consumption was registered and consumed items replaced once a day. From day 1–3 DAH only 1 octopus was observed to consume 1 shrimp, and only octopuses with 4 DAH started to consume shrimp in a consistent manner. From day 4–14 DAH shrimp consumption never exceeded 1 shrimp per day, and only occasionally were the 3 shrimp
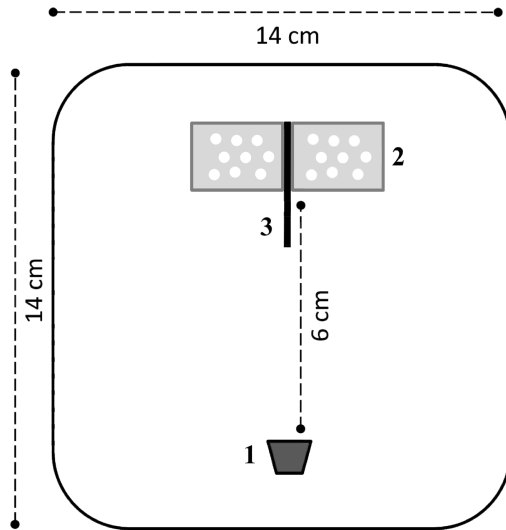
Figure 1. Diagram of the plastic square containers used in experiments on the attack response of early *O. maya* when presented with crabs and palaemonid shrimp in different treatments. (1) Opaque plastic cone (1.5 cm diameter and 2 cm long) fixed on the floor of the container facing the chambers, which served as a refuge for octopuses from which they received a complete and similar view of prey kept in either chamber. (2) Transparent acrylic chambers (4 (width) × 2 (length) × 10 (height) cm) with small perforations allowing visual and the diffusion of chemical stimuli from prey. Chambers were separated by an opaque plastic sheet (3) that helped discriminate in the case of attacks coming from the front or behind the chambers.

found intact. Measures of the amount of food ingested in any one catch were not registered, but octopuses feeding on palaemonid shrimp survived well after 14 days, indicating they received enough food throughout the learning phase. Feeding stopped 48 h prior to selection trials in order to standardize hunger levels and guarantee a response to stimuli. These trials were conducted when octopuses were 16 DAH.

Selection trials were carried out in square plastic containers (approx. 1 l) with two lateral openings covered with mesh (1.2 mm mesh size) to assure constant water flow (Figure 1). Containers were in turn placed in 65-l aquaria connected to a closed water recirculation system that filtered and maintained water in conditions similar to those described for incubation and hatching. This setup allowed octopuses to be kept in constant water conditions, and prevented the presence of any external stimuli (chemical, tactile or visual)

that could be associated with food before and during the attack response experiments.

Each experiment consisted of 42 trials with $N = 14$ different individuals of *O. maya* (independent replicates) assigned to each of 3 treatments. Trials were carried out by presenting an individual octopus with prey in one of treatments: (i) only crabs (no-choice treatment 1; NCH1), (ii) only palaemonid shrimp (no-choice treatment 2; NCH2) and (iii) both crabs and palaemonids simultaneously (choice treatment; CHO). Three prey items were placed in each of 2 transparent acrylic chambers with perforations, allowing prey to move easily and facilitate visual and diffusion of chemical stimuli. Chambers were located 6 cm in front of and equidistant to the subject (Figure 1), and prey were randomly placed on the right and left chamber. The number of attacks on prey in either chamber was registered during a 30-min period following prey presentation. An octopus was considered to attack a prey when it crawled slowly on the bottom of the aquarium or dashed with its arms trailed behind towards a chamber and embraced it, often attempting to introduce the tip of the arms through the perforations. If octopuses did not respond to prey stimuli (stayed hidden in the refuge or did not touch and embrace the chamber), the result of the trial was registered as a 'nonresponse' and included in the statistical analysis. Octopuses in treatments NCH1, NCH2 and CH of experiment 1 (7 DAH) contained 0.11 ± 0.016, 0.12 ± 0.009 and 0.11 ± 0.018 g of wet weight (mean $\pm$ standard deviation), respectively; whereas in treatments NCH1, NCH2 and CH of experiment 2 (16 DAH) octopuses the wet weight content was 0.18 ± 0.065, 0.19 ± 0.047 and 0.17 ± 0.073 g, respectively.

To test that *O. maya* actively selected palaemonid shrimp (i.e., its preference for crabs had been modified through early exposure to other crustacean prey), the difference in the frequency of prey attacked under the CHO treatment should be greater than that in NCH treatments. If attack frequency on crabs and shrimp were similar in the CHO compared to NCH treatments, then *O. maya* would attack both prey types in a similar number regardless of whether different alternatives were available. The latter result would thus denote that attacks on both prey types were merely the result of passive components of selection related to prey detection. Data from each experiment were analysed using maximum likelihood estimators and χ^2 in asymmetric contingency tables following procedures described in Underwood & Clarke (2005; Table 1). Because the options in the NCH treatments were similar, m_1

Table 1.

Asymmetric contingency table for the statistical analysis of the number of attacks by early *O. maya* when presented with crabs (preferred) and palaemonid shrimp (not preferred prey) in different treatments.

Treatment	Attack response		No response	Total
	Crabs	Palaemonids		
NCH1	m_1	–	$1 - m_1$	M_1
NCH2	–	m_2	$1 - m_2$	M_2
CHO	n_1	n_2	$1 - m_1 - m_2$	N

For treatments NCH1 (only crabs presented) and NCH2 (only palaemonids presented), m_1 and m_2 are the number of attacks on either prey; for treatment CHO (both crabs and palaemonids presented simultaneously), n_1 and n_2 are the number of attacks on each prey species, respectively; M_1, M_2 and N are the total number of octopuses assigned to each treatment (14).

and m_2 were obtained by adding attacks on crabs and palaemonids in both chambers, respectively (Table 1).

3. Results

From a total of 42 octopuses in the first experiment, 4 died before selection trials had begun; 28 of those used in trials (73.7%) presented an attack response; and only 10 (26.3%) showed no response to either prey species (Table 2). Similar proportions of octopuses attacked crabs (9 of 13) and palaemonids (9 of 12), when stimuli from either prey species was the only alternative present (in treatments NCH1 and NCH2, respectively; Table 2, Figure 2). However, when presented with stimuli from both prey species simultaneously (CHO), a higher proportion of octopuses attacked crabs (9 of 13) than palaemonids (1 of 13; Table 2; Figure 2).

The χ^2 test showed that the proportion of attacks on crabs with respect to those on palaemonids was significantly higher when octopuses were presented with a choice than when each prey species was presented separately ($\chi^2 = 6.09$; $p < 0.05$; Table 2). The terms that most contributed to the χ^2 value were those corresponding to attacks under CHO treatments, indicating amongst them lay the greatest differences between expected and observed frequencies (Table 2). Therefore, attack response of *O. maya* was preferentially directed to crabs and not palaemonids, in spite of them having been in close visual and chemical contact with the latter during 2 DAH.

Table 2.

Observed and expected (in parentheses) attack frequencies by *O. maya* on crabs (preferred) and palaemonid shrimp (not preferred prey) presented in different treatments.

Treatment	Attack response		No response	Total
	Crabs	Palaemonids		
Experiment 1				
Only crabs (NCH1)	9 (9.87)	–	4 (3.13)	13
Only palaemonids (NCH2)	–	9 (7.72)	3 (4.28)	12
Both prey (CHO)	*9 (5.41)*	*1 (4.59)*	3 (3)	13
Total				38
Experiment 2				
Only crabs (NCH1)	11 (11.56)	–	3 (2.44)	14
Only palaemonids (NCH2)	–	11 (10.11)	3 (3.89)	14
Both prey (CHO)	*8 (4.80)*	*1 (4.20)*	5 (5)	14
Total				42

Experiment 1: octopuses with 7 days since hatching had been imprinted with visual and chemical stimuli from palaemonids. Experiment 2: octopuses with 16 days since hatching had been fed on palaemonids. Italicised values are those terms that most contributed to the χ^2 value in each contingency table.
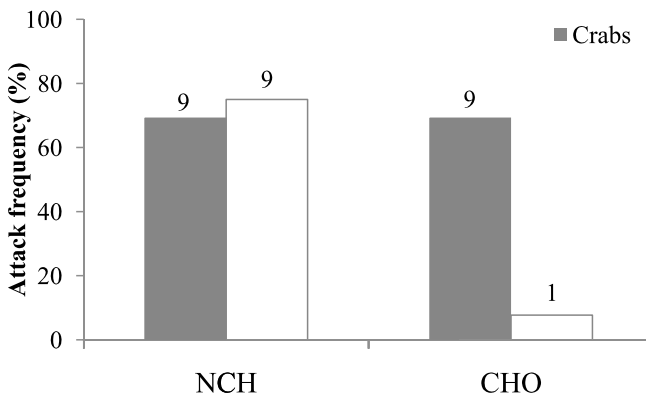
Figure 2. Frequency (%) of *O. maya* that attacked crabs (preferred) and palaemonid shrimp (not preferred prey) presented in treatments with a choice (CHO) or no choice (NCH) of alternative prey species. The numbers of octopuses showing an attack response are given above each bar. Octopuses were 7 days since hatching and had been exposed to visual and chemical stimuli from palaemonid shrimp.
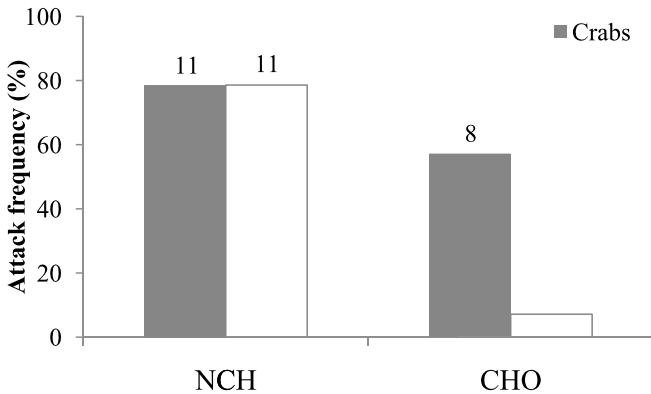
Figure 3. Frequency (%) of *O. maya* that attacked crabs (preferred) and palaemonid shrimp (not preferred prey) presented in treatments with a choice (CHO) or no choice (NCH) of alternative prey species. The numbers of octopuses showing an attack response are given above each bar. Octopuses were 16 days since hatching and had been fed palaemonid shrimp.

In the second experiment, from a total of 42 octopuses, 31 (73.8%) made an attack, whereas 11 (26.2%) showed no response to either prey species (Table 2). Eleven of 14 octopuses attacked crabs and the same proportion attacked palaemonids, when stimuli from each prey species were the only alternative present (in treatments NCH1 and NCH2, respectively; Table 2; Figure 3). But, when octopuses were presented with stimuli from both prey simultaneously (CHO), 8 of 14 *O. maya* attacked crabs and only 1 of 14 attacked palaemonid shrimp (Table 2; Figure 3).

Here again, results of the χ^2 test showed that the proportion of attacks on crabs was significantly higher when octopuses were presented with a choice than when each prey species was presented separately ($\chi^2 = 5.01$; $p < 0.05$; Table 2). The terms that most contributed to the χ^2 value were also those corresponding to attacks under CHO treatments, indicating here lay the greatest differences between expected and observed frequencies (Table 2). Thus, juvenile *O. maya* preferred to attack crabs rather than palaemonids in spite of having learned to feed exclusively on the latter during the first 14 DAH.

4. Discussion

Our results show that in spite of being exposed to visual and chemical stimuli from palaemonids (experiment 1) or being fed with this shrimp species (experiment 2), early *O. maya* still preferred to attack crabs when these were first

presented as an alternative prey (Table 2). Furthermore, octopuses readily attacked palaemonids, a prey that is not favoured normally, when these were the only prey available, and the proportion of attacks on crabs and palaemonids under no choice treatments were very similar (Figures 2 and 3). That octopuses attacked crabs as soon as they became available in spite of having never been in contact with this prey, suggests that preference to attack crabs was not obtained through life experience, but could be the result of innate biological processes.

Recent accounts of the original concept of imprinting consider that generalisation and irreversibility (Immelman, 1975; Bolhuis, 1991) cannot be regarded as specific characteristics of imprinting. Restriction to a sensitive period (usually early in life) and the stability of the response gained through that period, however, have been mentioned as the two main criteria that hold true for this phenomenon (Immelman, 1975; Schausberger et al., 2010). Whilst the reversibility of preferences has been clearly established in certain types of imprinting (e.g., filial; Bolhuis, 1991), there is agreement in that the first stimulus to which the young animal is exposed may exert a greater influence on preferences than subsequent stimuli. It could be argued that *O. maya* was not imprinted due to the lack of stimuli from palaemonids, either in opportunity or quantity, or because of poor visual detection by octopuses as a result of a modified polarization reflection caused by the acrylic glass of chambers holding prey (Shashar et al., 2000). If a modified polarization reflection had impeded prey detection differentially or in a similar manner, then the overall frequency of trials with no response from octopuses would have been much higher than the one registered throughout this study (approx. 25%). Further evidence that the exposure phase enabled *O. maya* to detect and readily recognize palaemonids as an accessible prey is the high proportion in which octopuses attacked shrimp when these were presented alone (in NCH treatments) and its similarity to that on crabs under the same circumstances (Figure 1).

During early post-hatching development, *O. maya* goes through complex morpho-physiological adjustments where young hatchlings gradually change into full juveniles (Moguel et al., 2010). Some of these adjustments suggest important changes in predatory capacity and feeding behaviour (Avila-Poveda et al., 2009; Moguel et al., 2010), whilst others imply critical transformations in the source of metabolic energy, the maturity of the digestive gland, enzyme activity and growth (Briceño et al., 2010; Moguel

et al., 2010). These changes are in close correspondence with the transition from necto-benthic to fully benthic habits, and could correspond to a sensitive period for imprinting to occur (i.e., a developmental stage in which certain kinds of experience result in specific behaviours in a way these would not result if such experience came at a different stage; Immelman, 1975). In the present study, not only were *O. maya* in close contact with visual and chemical stimuli from palaemonids during this period (first experiment), but they captured, manipulated and received an energetic reward for attacking this prey type during the first two weeks since hatching (second experiment). This considerable exposure, however, did not induce a behavioural change in octopus preference thereafter, since *O. maya* consistently preferred to attack crabs than shrimp as soon as it had a choice (in CH treatments).

Several reviews on the study of preferences (e.g., Underwood et al., 2004) explain that when offered a choice amongst two options, to prefer one cannot be unequivocally distinguished from rejecting the alternative. Prey items may be consumed more frequently because they are desirable, palatable, easy to detect or capture, or because the alternatives are undesirable, unpalatable, difficult to detect or capture. A tempting explanation for results in experiment 2 could be that *O. maya* attacked crabs more frequently in selection trials because previous exposure to palaemonids resulted in a mechanism of negative reinforcement, which discouraged octopuses from subsequently attacking palaemonid shrimp. However, the high frequency of attacks on palaemonids registered under no-choice trials contradicts the possibility of octopuses being negatively reinforced in relation to this prey. If failure to capture shrimp during the learning phase would have resulted in negative reinforcement, then attack frequency on shrimp under no choice control treatment in selection trials would have been much lower than 79%. Moreover, differences in attack frequency observed throughout this study could have not been caused by prey properties alone (i.e., prey behaviour or unknown chemical cues), since such prey features were present both in no-choice (control) and choice situations, but only elicited a disproportionally higher attack frequency on crabs in the latter. Given the diverse nature of properties operating in a predator–prey system that can determine the predatory response, the use of appropriate no-choice control treatments is essential in studies on behavioural preference of prey.

Previous studies have suggested that cephalopods living in a changing environment would benefit from acquiring food preferences that were in

accordance with relative prey abundance at the moment of hatching (Darmaillacq et al., 2006; Guibe et al., 2012), and thus rely on peri-natal learning mechanisms to identify and hunt for appropriate prey. Whether through imprinting or associative learning, learning constitutes a valuable mechanism for the acquisition or improvement of feeding skills in a variety of marine animals (Dukas, 2013). Food imprinting, in particular, would result advantageous in situations where only cues of the prey are locally present or where the prey is difficult to catch or ingest for younger and smaller but not older and larger life stages (Schausberger et al., 2010). Such a mechanism would allow individuals to optimise their foraging behaviour later in life without having experienced the prey earlier (Schausberger et al., 2010). As with food imprinting, however, to possess a pre-determined food preference would also provide such an advantage. Indeed, a behaviour like that of *O. maya*, in which predators attack prey in accordance with a pre-determined preference whenever the favoured prey is present, but readily consume unfavoured prey when these are abundant would confer flexibility in foraging and allow predators to adjust to unpredictable changes in food quantity and quality.

The persistence of *O. maya* to attack crabs, regardless of never having been in contact with this prey type, allows to hypothesise that preference for crabs is probably not influenced by visual or chemical cues perceived during early life stages but has an innate nature. Previous authors have suggested that innate preferences can result advantageous for individuals that will increment survival chances, grow faster and reproduce more efficiently if they can discriminate spatial patterns associated with predators, food, and sexual partners (Kelber, 2002; Dormont et al., 2010). Amongst nectar-feeding insects, preference for those components in flower patterns on which food return depends greatly (e.g., radial flower patterns that serve as nectar guides) appears to be innate, whereas preference for those that vary spatially or temporally (e.g., flower colour) can be modified through training (Kelber, 2002). Whilst some of the advantages in recognising spatial patterns associated with food could be maximised if they occurred early in life, to rely on early experience and associative learning to access food also involves important risks for the individual. These risks are related not only to the inaccuracy in finding adequate food sources or discriminate especially valuable prey (Hughes, 1979), but more importantly, to mistakes in the identification and timely escape from dangerous predators. Whilst successful outcomes of the attack on the right prey may result in a high energy return, the consequences of errors

could be fatal and will bring about a null adequacy for individuals that have failed to identify a deadly threat (Barbosa & Castellanos, 2005).

Learning from experience constitutes a mechanism based on trial and error, which requires relatively long periods of time. Time minimization, however, has been proven an efficient predatory strategy amongst marine invertebrates (Hughes & Seed, 1981; Leite et al., 2009), through which individuals optimize foraging and reduce exposure to predators and other risks (Mascaró & Seed, 2001). In addition, fast growing individuals, especially under exponential growth modes as those reported for juvenile *O. maya* (Briceño et al., 2010), will gain quick advantage over others as they attain a size conveying protection. To preferentially attack prey that can be unmistakeably identified as food may result in fast and successful foraging bouts that reduce time spent searching and testing alternative prey.

Octopus maya has a restricted geographic distribution, occupying relatively stable, low energy habitats: sand/lime soft bottoms with occasional rocky reefs, frequently covered with *Thalassia* spp. and macroalgae (Solis-Ramírez, 1997). As other cephalopods, octopuses do not have parental care (Hanlon & Messenger, 1996) and juveniles are compelled to search attack and handle their own prey since hatching. Juveniles of *O. maya* possess morphological and physiological features enabling them to feed on similar prey types as adult octopuses (van Heukelem, 1977; Moguel et al., 2010). Throughout this and other studies, *O. maya* with less than 20 DAF have been observed readily consuming live palaemonids and amphipods during experiments, as well as fresh bivalves during preliminary trials (Portela-Rodríguez, 2011). However, it may be that some small prey types readily consumed by juveniles are not suitable for adults (i.e., amphipods, tanaidaceans, nematodes), whereas others consumed by adults are not available is small sizes where juvenile octopuses are found (i.e., penaeid shrimp, lobsters).

Brachyuran crabs, by contrast, are ubiquitous, diverse and abundant in a wide range of sizes (Bliss, 1990); they do not use neurotoxins as a defence mechanism (Bliss, 1990); and often share benthic habitats with juvenile and adult octopus (Hernandez et al., 2012). Moreover, crabs constitute a food item with an unrivalled nutritional value (Rosas et al., 2012) that appears to be based on an increased lipid absorption efficiency obtained from crab tissue, rather than on overall measures of energy uptake per unit time (Onthank & Cowles, 2011). Preference for crabs may be explained by the need to satisfy specific nutritional requirements of certain fatty acids, as it could

be suggested by the close relation between dietary levels of arachidonic acid (ARA 20:4n − 6) and mantle growth in *Octopus vulgaris* (García-Garrido et al., 2011). These features together with a distinctive movement in direction, mode and velocity of crabs (Vidal-Gadea et al., 2008) may be consistent with the recognition of spatial patterns associated with valuable food. To be able to recognize a valuable prey at the moment of hatching and preserve such ability through adulthood might provide newly hatched *O. maya* with an advantage. Such an advantage will be maximised if octopuses prefer to attack crabs whenever they are present, but pursuit other prey when crabs are scarce, rather than only specializing on even highly valuable prey types.

Many studies have accounted for the extraordinary learning capacity of cephalopods (e.g., Hanlon & Messenger, 1996; Hochner et al., 2006), and results herein do not contradict these findings. Our results only evidence that early learning did not modify attack preference, as the first decisive action taken by octopuses was to attack crabs as soon as these were detected. Whether the predatory skills used by juvenile *O. maya* in the present study improved as a result of experience cannot be determined because these behavioural components were not measured. Consequently, learning could have taken place affecting other components of predation such as feeding efficiency through improvement of prey detection, capture or handling.

Food preference amongst adults reflects experience acquired throughout life (e.g., Boal et al., 2000; Cole & Adamo, 2005), whereas accumulated experience in recently hatched individuals is rather limited. Consequently, most of the feeding behaviour of early juveniles should be determined by genes (Boletzky, 2003). Experiments with cuttlefish have suggested that long-term memory in juveniles (8 days) could be limited, whereas 30-, 60- and 90-day-old individuals increase memory retention by learning (Dickel et al., 2000). Results consistent to those presented here but carried out with *O. maya* of 45 DAH (Domingues, 2007) suggest it is unlikely that even older octopuses could be trained to switch their prey preferences. However, it would be interesting to see whether older *O. maya* could improve the performance of feeding skills on unfavoured prey species, especially if this is caused by increased memory retention. That late juvenile octopuses possess greater memory retention than early ones would be in favour of the idea that learning processes become relatively more important as individuals become older and more experienced.

In her recent review on behavioural plasticity, Snell-Rood (2013) states that environmental variation may (i) produce changes in behaviour that result from the differential activation of an underlying neural network (activational or 'innate' behavioural plasticity Mery & Burns, 2010), or (ii) trigger the development of different developmental trajectories resulting in an array of behavioural expressions (developmental behavioural plasticity). Because developmental plasticity involves changes in the nervous system as a result of experience, it includes the various forms of learning (Dukas, 2013). Whilst activational plasticity is an immediate response to environmental variations, developmental plasticity requires developmental changes to take place (Snell-Rood, 2013). Consequently, the underlying trial and error associated with developmental plasticity is costly in time energy and exposure, but is otherwise more likely to contribute to the integration of behavioural traits in adults (Kasumovic, 2013).

In this context, our findings support the idea that the decision to attack prey in *O. maya* is a behavioural expression unmodified by previous experience with prey. However, prey capture and handling, may constitute components that result from developmental plasticity involving changes in neural networks. Future research, directed towards the understanding of behavioural plasticity in *O. maya* when feeding on both favoured and unfavoured prey would produce information useful for aquaculture and bait-fishing of this commercially important species. In addition, it may help to unravel the reasons why octopuses so inflexibly prefer crabs given that behaviours compliant to a changing environment have so often proven advantageous.

Octopus maya in the present study were obtained from a single wild female, that most probably carried sperm from multiple males (Sánchez-Ponce, 2011; see also Quinteiro et al., 2011). As many as 5 potential males (Sánchez-Ponce, 2011) mating with a single female decreases the possibility of all experimental subjects being full siblings. However, it is likely that even half siblings of *O. maya* share a genetic component to their behaviour (as reported for *O. bimaculoides*; Sinn et al., 2001). The use of a single batch of eggs was a restriction imposed by the purpose and experimental procedures of the present study (i.e., difficulty to control the age and time of exposure to palaemonids with precision) that limits the extent to which our results represent the whole of the population. Bearing in mind such limits, the present research constitutes a modest contribution to our understanding of the behavioural basis underlying predatory response in early octopuses,

and additional research is needed in order to both confirm the consistency in the behavioural preference for crabs and broaden our conclusions to include other aspects of the feeding behaviour in *O. maya*.

Acknowledgements

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