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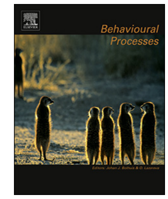

Hunger and satiety determine foraging decissions in land snails: Evidence from the invasive species *Theba pisana*

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ABSTRACT

The foraging behaviour of gastropod molluscs usually involves complex decisions that provide a model for the study of high-order cognitive processes. Land snails tested for food-finding in the laboratory, however, have shown an invariable feeding pattern: novel foods are mostly missed (i.e. just found by chance) whilst familiar foods, due to a type of conditioned attraction, are always located and ingested. This effect, known as *Food-attraction conditioning*, has led to the conclusion that, regardless of their hunger level, land snails are both willing to eat anything at any moment and also blind to the odours of novel foods. An alternative account of these findings emerges from the fact that the snails are usually tested whilst in a moderate state of hunger, so that they benefit from feeding on known foods but not from taking the risk of feeding on those that are unknown. The present experiments suggest that it is the case. Snails of the invasive species *Theba pisana* were tested for food-finding according to their seasonal cycle in a laboratory located in their native Mediterranean region. Subjects collected at the beginning of their aestivation period succeed in locating novel food items after being deprived for a long period (45 days), but ignored a conditioned food when they were sated with this food at the end of their lethargy. The results allow us to conclude that the feeding behaviour of snails is the product of a complex cost-benefit analysis in which their motivational state and the stimuli they perceive (and the memory of such stimuli), are evaluated. Finally, we anticipate that these results will be of use in increasing the efficiency of current baits employed for the protection of crops.

1. Introduction

High-order cognitive processes can be investigated in behaviourally simple organisms. In particular, foraging in gastropod molluscs has been proposed as an adequate model for studying the evolution and cognitive capacities of higher species (Elliott and Susswein, 2002; Jing et al., 2009; Olivera et al., 2015). To guarantee survival, feeding, particularly in those invertebrates labelled as “generalist”, must involve complex decisions about the cost and benefits derived from attempting, or avoiding, the ingestion of a given food. Such decisions depend on the appetitive state (arousal) of the animal, in which sensations provided by the stimuli, the internal state of the subject, and its prior experience, are all integrated (Gillette and Brown, 2015; Hirayama et al., 2012; Sulikowski, 2017). Terrestrial snails represent a significant part of gastropod species and are mostly generalist, but their feeding behaviour could be considered too simple for studying the higher cognitive abilities involved in decision-making processes.

Although land snails may consume different kind of foods (dead plants, decomposing materials or they may even be carnivorous), most species feed on vegetables (Kiss, 2017). Some authors claim that most terrestrial gastropods have evolved species-specific food preferences and that they are able to locate their preferred foods through olfaction (Kiss, 2017). However, according to others, terrestrial snails may represent an exception to that rule as they seem to show no particular preference for any of the available foods (Cowie, 2009). This lack of a feeding predisposition has received strong support from the results of food location tests conducted in the laboratory. Experimental subjects from several species with different feeding habits – e.g. herbivorous as *Helix pomatia* and *Cornu aspersum* (Peschel et al., 1996; Ungless, 2001; Loy et al., 2017), carnivorous as *Achatina fulica* (Chase, 1982; Croll and Chase, 1980) or detritivores as *Theba pisana* (Baker et al., 2012)– hardly ever succeed in finding potential food sources that had been placed just

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several cm away from them and this has led to the conclusion that land snails do not have the innate capacity to recognize food. It is assumed that the posterior tentacles of snails, those used for spatial orientation through smelling (Zaitseva, 2016) are not able to detect novel food odours (Peschel et al., 1996; Teyke, 1995), so that, initially, feeding occurs just by chance and depends entirely on a casual encounter between the snail's mouth and the food source (Baker et al., 2012).

However, this random foraging strategy changes dramatically as a result of conditioning, that is, once the novel food is ingested, the snail then associates its smell with its nutritive consequences. This association occurs quite quickly (after just a single feeding trial) and results in a constant and enduring attraction to the food so that the effect, for resembling so much the mirror image of Garcia's food aversion conditioning paradigm, was named as *Food-Attraction Conditioning* (FAC) (Teyke, 1995). In snails, the lowering of the posterior tentacle is a conditioned response that has been used as a measure for assessing basic Pavlovian phenomena and thus their cognitive abilities by (e.g. Loy et al., 2017). Although the relationship between the tentacle lowering response and successful food finding has been questioned (Ungless, 2001), it is assumed that the formation of this appetitive association is the only way land snails locate the food through smell.

Taken together, the absence of an ability to locate novel food, and the attraction shown towards conditioned smells would indicate that memory is the only variable that influences the feeding behaviour of snails. This assessment is supported by the fact that their motivational state does not seem to affect their feeding behaviour since land snails have been reported to feed independently of their sated/hungry state (Teyke, 1995; p. 412). It can be concluded, therefore, that no feeding decisions should be made by snails solely on the basis of foraging: snails will eat anything they found, at random if novel or intentionally if conditioned.

Interestingly, a similar absence of feeding criteria has been challenged by recent findings in the species *Pleurobranchaea californica* (*PBc*). This marine slug was considered an opportunistic and indiscriminate voracious carrion-predator able to feed on almost any meat (e.g. Davis et al., 1974) but, accidentally, it was observed that *PBc* ejected from the mouth what turned out to be the poisonous Spanish slug *Flabellina iodinea* (Noboa and Gillette, 2013). Afterwards, in subsequent tests, *PBc* was found to feed on another safe slug (*Hermissenda crassicornis)* but still avoided the toxic *F. iodinea*. However, this feeding discrimination, rather than being fixed, was completely reversed according to the subjects' motivational state. After being starved for a couple of days *PBc* ingested the entire poisonous slug and in contrast, when fed until satiety, it avoided the safe and nutritive *H. crassicornis*. Thus, the authors concluded that motivational state strongly determines the feeding behaviour of *PBc*, which is activated or inhibited according to both the stimuli it perceives and its prior experience of such stimuli (see also Gillette et al., 2000). Further research has shown that isolated neurones of the *PBc* feeding system had a similar threshold to hungry/sated donors in response to food stimuli, thus allowing the formulation of simple neuronal-network models that simulate such complex decision making (Brown et al., 2018; Hirayama et al., 2012, 2014).

Compared to other invertebrates (such as *P. californica*) that need to feed almost every day and can get hungry after just some days of deprivation (Davis et al., 1977), setting the limits of the motivational state in land snails represents a difficult challenge because, being lethargic species, they can resist long periods of starvation (up to 5 months depending on the species and the season; see, for example, Chevallier, 1992). This could mean that in previous food location studies, land snails were in a moderate state of hunger that favoured the attraction to conditioned odours but not, due to their uncertain consequences, to those that were novel. (As will be discussed later, a review of the literature gives rise to this possibility.) Thus, in order to determine if snails feed on novel and familiar vegetables not only according to their memory but also to the stimuli they perceive and to their motivational state, it will be necessary to induce high and low feeding thresholds in these organisms.

The biological feeding cycle could be used to experimentally induce high motivational levels of both hunger and satiety in land snails. A long period of starvation could be made possible in the laboratory by collecting the subjects at the beginning of their natural lethargy. On the other hand, by collecting them at the end of their inactive season, snails would be expected to ingest a large quantity of food, enough to become fully satiated in the laboratory. This was the basic procedure that we planned to use in the present experiments.

1.1. Theoretical and practical reasons for choosing Theba pisana as experimental subjects

It has been suggested that when studying cognitive processes in the laboratory, the subjects' natural context must be taken into account (Sulikowski, 2017). In our case, this was almost mandatory. Our selection of the white snail *Theba pisana* as experimental subjects was based on the fact that this species is native to the southwest of the Mediterranean (Däumer et al., 2012), where our laboratory is located. In addition, white (or Mediterranean) snails are species whose seasonal movements, breeding, and habitat selection have been extensively studied (e.g. Baker, 1988, 2002, 2008; Johnson, 2011). These investigations have been mainly conducted in order to reduce the economic damage produced by this invasive species in countries around the world, particularly in Australia (e.g. Sanderson and Sirgel, 2002).

Like most land snails, *Theba pisana* remains inactive during cold winters as well as during hot and dry summers (Cowie, 2009). On contaminated land crops the aestivation of white snails results in mass climbing of the plants, which is supposed to avoid the heat of the ground in early summer (Baker and Hawke, 1990; Baker, 2008). Therefore, aggregations of white snails appear on the cereals just at the point of harvesting, which complicates the process (by clogging the machinery), downgrading the grain, and hindering the export of the crops (Baker, 2002).

The current baits used for pest control are inefficient, given that, as already noted, the food preferences of *Theba pisana*, as those of many other land snail species, remain unknown. Moreover, the randomly-based foraging strategy observed in other land snails also seems to protect *Theba pisana* from being attracted by the smells emanating from potentially toxic meals (Baker et al., 2012). In fact, according to the FAC effect already described, if land snails only encounter the food that they have already eaten while a novel food can only be found at random, baits would be expected to be more efficient if they included food that is known to have been previously consumed by snails. In other words, it would be recommendable (though paradoxical) to feed the snails on appetitive foods to subsequently exploit their (conditioned) smell as a way of ensuring that they are able to locate the baits (Baker et al., 2012). However, if the ingestion of a given food depends on decisions made on the basis of the hunger/satiation level, feeding the snails on a given food could result in a loss of its attractive properties whilst a long period of starvation could result in the successful location of a novel food.

2. Experiment 1

The capacity for ejecting prey from the mouth, as well as for vomiting the indigestible parts of those already ingested, would allow *PBc* to take the risk of attacking any prey it smells, even if it is unknown. This, however, is not the case for land snails, which are non-vomiting species. In order to prevent poisoning, in higher vertebrates such as the rat, the absence of the anti-peristaltic reflex makes it necessary to avoid the ingestion of novel flavours, a strategy known as neophobia. Thus, it is possible that neophobic behaviour in snails could have been misinterpreted as an innate incapacity for food detection.

When tested for food location, animals used in previous studies have not been food-deprived for longer than two weeks (Chase, 1982; Croll and Chase, 1977, 1980; Ungless, 2001; Loy et al., 2017). Whilst such a period of food deprivation might be long enough to induce a high level of motivation, as already noted, it must be considered that most land snails can aestivate and/or hibernate for several months. It seems plausible, therefore, that a longer deprivation period, i.e. longer than those previously used, could be needed for snails to show attraction to a novel, and therefore potentially dangerous, meal.

In order to obtain a low motivational feeding threshold, and to ensure the snails' welfare throughout the study, subjects were collected from the field at the beginning of their aestivation period (April), since we considered that, at this time of the year, there should be no major differences between being starved in the wild and in the laboratory. Experimental and control subjects were then tested after being deprived for 45 days or 10 days respectively.

2.1. Method

2.1.1. Subjects and housing

The experiments were conducted in Ceuta, a Spanish town bordered by Morocco and situated on the Southwest coast of the Mediterranean Sea (North Africa). The subjects were 60 adult *Theba pisana* snails (mean shell diameter: 16.1 mm; range: 13.5–19.5 mm) collected from the field. The snails had been observed regularly for weeks before collection and were captured in April, i.e. when the aestivation period had begun. The beginning of the snails' lethargy was assessed by looking for their typical aggregation on the top of the plants they usually climb (which reaches approximately 3 m above the ground in some orange trees), and also by checking for the presence of the epiphragm (a calcareous substance they use to attach themselves to the plant and to prevent dehydration). Snails taken to the laboratory were woken upon arrival by spraying fresh tap water onto them and they were thereafter housed in individual plastic cages (13.5 × 8.5 × 4.8 cm). The cages contained a small amount of water that was renewed every week. Food access in these cages was restricted as will be specified ahead.

To equate as much as possible the laboratory conditions to those of their natural habitat, the temperature of the colony room in which the snails were housed was kept constant at 23 °C during both the light (22:00 to 10:00) and dark (10:00 to 22:00) phases. At the end of the experiment snails were given free access to food for two days before being returned to a field different to the one from which they were initially collected.

2.1.2. Apparatus and stimuli

The experiments were conducted in a test arena placed in a room dimly lit by a white light (28 W) without air currents. The arena consisted of wood-enclosed transparent glass (75 × 46 cm) that was cleaned before each individual test trial. The stimuli used in the experiments were commercially obtained apple and carrot. The food slices were located at 55° (left and right counterbalanced) and 50 mm from the head of the snails. These parameters were adapted to this species and were based on those used by Ungless (2001) with the garden snail (*Cornu aspersum*).

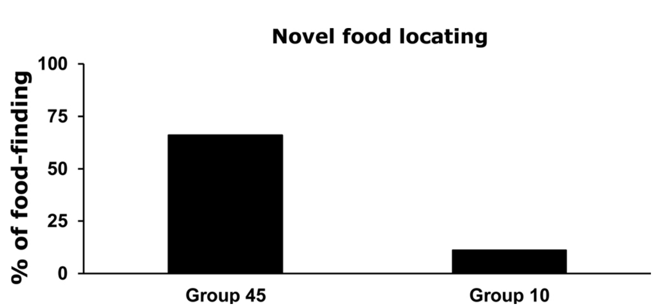

Fig. 1. Percentage of novel food location in two groups of subjects that were maintained in a state of food deprivation for 45 and 10 days.

2.2. Procedure

Manipulation of the subjects was always conducted during the dark cycle, between 10:00 and 15:00, in order to promote activity in this nocturnal species. Before treatment, snails were woken up in a room poorly illuminated with a red light (80 W). Manipulation began by detaching the snails from the walls of the cages and by peeling off any residual epiphragm that remained in their shells. This was conducted to prevent the snails from eating it, a typical behaviour observed in this species. They were then placed on their sides and sprayed with fresh tap water to induce activity. Once the body completely emerged from the shell, snails could be returned to the home cage, where they were allowed to eat or, alternatively, they could be placed in the food locating test arena.

Animals had constant access to commercial rat food during the first three days in their home cages. Group 45 was then food-deprived for 45 days while Group 10 remained deprived for 32 days. Subjects in Group 10 were fed again for three days 13 days before the test. As a result of the feeding schedule, the snails of both groups were tested on their ability to find a novel food source (apple or carrot) on Day 46, i.e. after 45 or 10 days of deprivation.

On the food locating tests successful food location was scored when animals made oral contact, by means of their lips, with the food. Unlike the procedure used in our work, in Ungless (2001), food finding was determined by a spatial criterion, i.e. an approach to the food equal to, or lesser than, 0.5 mm, was regarded as a successful food finding trial. However, based on previous studies in which snails missed a food source when being very close to it (see Peschel et al., 1996, Fig. 1, who considered that fact as an example of how much blind snails are to novel smells) and on observations in our laboratory, where approximately 17% of the subjects that met this spatial criterion did not actually make oral contact with the food (i.e., they missed the food) we decided to choose "kissing" as a more accurate criterion for considering a food-finding trial to be successful. As soon as the snails' lips made contact with the food, they were removed to prevent them from ingesting it. Failure to locate the food was registered when 1) the snails did not kiss the food after two minutes, 2) when they walked past the food for a distance of more than 40 cm away or, 3) when they reached the limits of the arena. If the snails failed to find the food, a second test trial was conducted, so that a negative score represented two missed food locating trials. The slices of food used on the tests were renewed every 30 min. All data were recorded by an experimenter who was blind to the allocation of the groups.

2.3. Results

After being sprayed with water, the subjects took a few minutes to wake up. Their appearance and motility seemed completely normal regardless of the level of deprivation. One subject from Group 45 and three from Group 10 were discarded because they were not active enough (2 subjects) or because, despite waking up normally, they did not move at all during the test (2 subjects).

The results obtained on the location test are displayed in Fig. 1. As can be seen, 11% of the subjects found the novel food after 10 days of deprivation, a result that was expected on the basis of previous studies (e.g. Teyke, 1995; Loy et al., 2017). However, 66% of the snails successfully located the novel food when they had remained food-deprived for 45 days. These differences (the rejection level adopted here and in subsequent analyses was $p < .05$) were statistically significant (Squared chi = 16.643; $p < .05$). No significant differences were found between the groups according to whether the food tested was carrot or apple, or whether it was presented to the left or to the right of the animals (both Squared chis < 1)[1] . These results show that snails that have been food deprived for a short period of time (i.e., 10 days) do

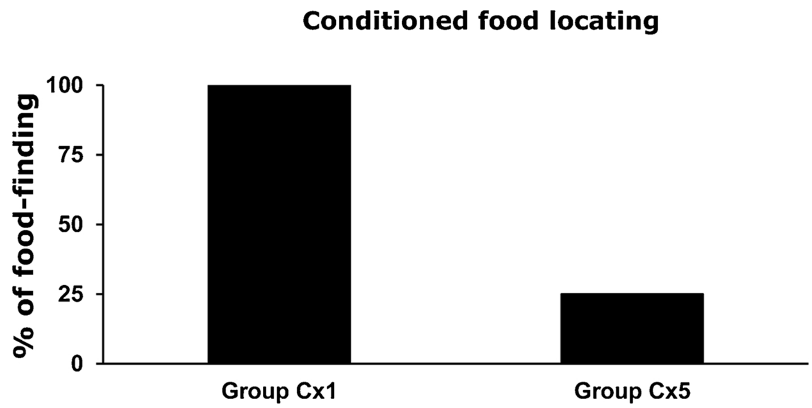

Fig. 2A. Percentage of conditioned food location. Group Cx1 received one day of access to carrot and was then deprived for 5 days. Group Cx5 was given access to carrot for 5 days and was then deprived for 24 h.

not locate a novel food, which is in agreement with previous findings (e.g. Teyke, 1995). In contrast, when deprived for a longer period, snails become more successful at finding a novel food source. This shows that the motivational levels have an effect on novel food finding, contrary to the general claim that privation has no effect on successful food finding (Teyke, 1995).

3. Experiment 2

The results obtained in previous Experiment 1 reveal that hunger makes snails locate and eat unknown food items, but it remains to be clarified whether the opposite state (satiety) could just produce the contrary result: the avoidance of a known food. A review of the literature could indicate that such a possibility has been disproved, i.e. that in spite of being satiated snails are attracted to conditioned foods. In some studies using the food location test, FAC has been observed in snails tested the very next day after (i) being collected from the field (Teyke, 1995) or (ii) being feed for weeks in the laboratory (Chase and Croll, 1981; Peschel et al., 1996; Friedrich and Teyke, 1998). Thus, if animals have had free access to food, it is reasonable to suppose that they were fully sated at the time of the food location test. However, this suggestion is only speculative. Snails used in these studies, whether in the field or in the laboratory, could have either been simply resting or in a state of lethargy. Additionally, regardless of whether they were resting or active, the amount of time since they last ate remains unknown, so it is difficult to determine to what extent these snails were sated. In fact, some results obtained using procedures that, unintentionally, involved feeding the subjects during long-lasting food-location test trials, could be taken to indicate that snails are not attracted to conditioned food when in a state of satiety.

In the study by Teyke (1995), *Helix pomatia* snails were fed on either potato or carrot before they were tested in an open field arena where a piece of each food source was available. The location of both substances was tested for 11 consecutive days during which snails were kept unfed. Throughout this test phase, it was observed that approximately 80% of the subjects were successful at locating the item of food that they had been fed on, and that, in contrast, naive snails did not locate any of the food items (see Teyke, 1995, Fig. 2A). Different results were obtained in a subsequent experiment in which, after having been conditioned to one particular substance (by feeding the snails on this substance), the food locating abilities of the snails were again tested across 11 consecutive days during which they had free access to a different type of food in their home cages. In this second experiment, most of the snails successfully located the food during the first three days of the test phase, but, after the 4th or 5th day, a significant number of snails failed to find the food (see Teyke, 1995, Fig. 2B). Although it could be argued that having access to a second food source resulted in the loss or abolishment of the association between the smell and the ingestive consequences of the conditioned food, a more parsimonious explanation would be that having free access to a second food rendered the conditioned food unnecessary. That is to say, it is possible that the snails avoided or missed the food source simply because they were satiated.

The objective of this experiment was to determine if in a very low state of hunger (24 h of deprivation), a conditioned food item would still result in being attractive for snails. To test this question, *Theba pisana* snails captured at the end of their aestivation were tested to locate food under different conditions of experience with food (5 or 1 day). According to predictions based on the phenomenon of FAC (see also Croll and Chase, 1980), successive experiences of the smell and the positive internal effects of the carrot should result in a higher score for locating food in snails with 5 days access to food. Contrary to this prediction, however, satiation with carrot could result in these subjects ignoring the food on the finding test.

3.1. Method

3.1.1. Subjects

Subjects were 25 adult *Theba pisana* snails (mean shell diameter: 16.3 mm; range: 18.0–14.9 mm). The snails were randomly divided into two groups: Group Cx1 (n = 13) and Group Cx5 (n = 12).

3.2. Procedure

The snails were housed in individual boxes containing water. On Day 1, a slice of carrot was placed in each of the cages. The carrot was removed 24 h later for animals in Group Cx1 whereas snails in Group Cx5 were allowed to feed on it for 4 further days. For Group Cx5 the carrot was renewed every day. Carrot was also used on the location test, which was run on Day 6 for groups Cx1 and Cx5. Any other procedural details were identical to those already described in Experiment 1.

3.3. Results

Most of the snails in both Cx1 and Cx5 groups ate the carrot when it was available. In Group Cx5, a visual inspection of the surfaces of the carrot slices revealed that snails gnawed on it more on Days 1 and 2 than on Day 5. Indeed, two subjects in Group Cx5 did not eat on Day 3, and three of them refused the food on Day 5.

The results collected during the test are displayed in Fig. 2A. It is clear that all the snails in Group Cx1 "kissed" the food, but only 4 out of 12 snails (i.e., 33%) did so in Group Cx5 (Squared Chi = 10.860;

[1] Here, and in the rest of the tests, no effect of the food location (left or right) was found (Squared chis < 1).

$p < .05$). After feeding on carrot for five consecutive days, the fact that almost 67% of the subjects in Group Cx5 did not approach the food can hardly be interpreted as a failure to associate the smell of the carrot with its ingestive consequences. The idea that just associating the odour of a given food source with its positive internal effects leads to FAC (as stated by Teyke, 1995) is not entirely accurate. Rather, as our results show, intentional feeding does not only depend on the snail's memory but also on their hunger levels.

To further explore this idea, we decided to invert the treatment given to the subjects in a second phase of the experiment; Group Cx1 was fed on carrot whereas Group Cx5 was maintained in a state of food-deprivation. Our prediction was that the opposite pattern of results is found after reversing the treatment. Thus, snails in Group Cx1— which successfully located the carrot after having consumed it for one day— would now be expected to not find it after been sated, whereas snails in Group Cx5 that missed the carrot in the previous test would be expected to locate it if conditioning trials were suspended, i.e. after been food-deprived.

3.4. Experiment 2b

After Test 1, subjects in Group Cx1 were given access to the carrot for 7 consecutive days (hereafter Group Cx1 + Cx7) while those that had received the carrot for 5 days were kept deprived for 7 days (hereafter Group Cx5 + Cx0). This treatment was followed by a deprivation period of 24 h for all the snails, after which the carrot location test was repeated.

3.4.1. Results

Most of the 12 subjects in Group Cx1 + Cx7 ate the carrot throughout the entire seven-day period (just one subject did not eat it on the third day), but, as in Experiment 2, a lower intake of carrot was observed on the last day of treatment compared with the first day.

The results obtained in Test 2 are depicted in Fig. 2B. As can be noted, the opposite pattern of results to that obtained in the previous test was found: most of the subjects (91%) in Group Cx5 + Cx0 (i.e. those that ignored the food on Test 1), succeeded in finding the food without receiving more conditioning trials. On the contrary, the snails that initially succeeded in finding the carrot after just one conditioning trial (Group Cx1 + Cx7) did not approach this food after receiving 7 additional conditioning days. These differences were statistically significant (Squared chi = 7.354; $p < .05$).

The reversal of the effect obtained on the first test shows, firstly, that the poor performance on food location in Group Cx5 + Cx0 on Test 1 was not due to a loss of the snails' capacity to identify the smell as a signal for food. Secondly, the fact that Group Cx1 + Cx7 (i.e., the group that showed a higher locating rate on the first test) did not approach the food after receiving seven additional conditioning days, confirms that the attraction to a conditioned food is not observed if the snail is not sufficiently hungry.

4. General discussion

4.1. Conclusions

It has been claimed that food location abilities of snails are limited to already known food and are not dependent on their state of hunger (Teyke, 1995; p. 412). Contrary to this assumption, the results obtained show that, under certain food deprivation conditions (i.e., 45 days), the white snail is able to locate a novel food source intentionally rather than at random (Experiment 1) and that, when sated with a given food, 24 h of deprivation are insufficient for the organism to show attraction to its smell, even if the food is appetitive and has been conditioned (Experiment 2). By manipulating the level of hunger, it has been shown that *Theba pisana* are not "blind" to the odours of novel foods and that they are not always attracted to those that have been conditioned. The fact that a novel food is only located if snails are hungry enough, and that some level of hunger is also needed for a conditioned food to become attractive, implies that foraging is a decision in which hunger levels play a critical role. In other words, for snails not too hungry, ingestive thresholds are higher so that novel foods, which can be perceived as aversive, are avoided. However, in starved snails the thresholds are much lower and avoidance converts to approach. The fact that snails decide whether to eat a given food or not implies that they may be mislead. Further, on the basis of the evidence presented here, currently used baits might be expected to be more effective if they are dispersed throughout the crops when snails have shaken off their lethargic state.

4.2. Discussion

The FAC effect has been confirmed by numerous reports and even by our own results in Experiment 1, where subjects with just 10 days of deprivation showed a very low score on the novel food-finding test. Such results could be explained in terms of neophobia: being deprived of food for a relatively small amount of time leads to higher thresholds which in turn result in the avoidance of the food. Thus, snails in a moderate state of hunger (7–15 days of deprivation) completely ignore a novel food -which could be in fact detected but subsequently rejected- but they are attracted to a familiar or conditioned food. This strategy would allow snails to feed only on already known nutrients when they are not too hungry, as a way of protecting themselves from potentially toxic foods. On the other hand, if hungry enough, they can decide to take the risk of locating and ingesting unknown nutrients rather than die by starvation. Further investigation would be needed to confirm that neophobia is the reason why snails missed novel foods (e.g., by measuring the quantity of the novel food they ingest), but these results rule out the hypothesis that snails do not have the capacity to recognize at distance a novel odour as a potential food source, thus being similar of most of gastropods (Kiss, 2017). The results obtained in Experiment 2, i.e. that a conditioned food can be intentionally missed, further

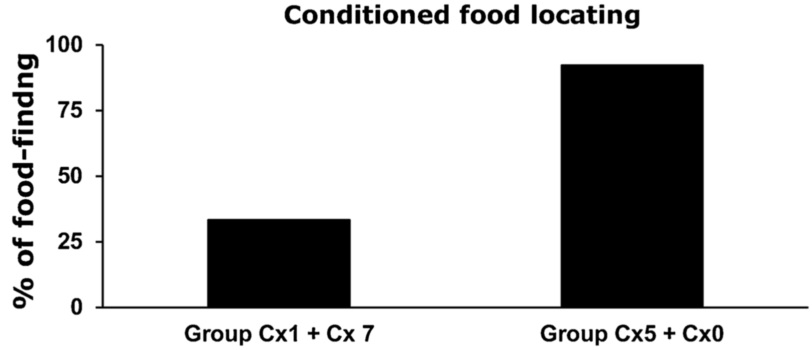

Fig. 2B. Percentage of conditioned food location. Subjects previously in Group Cx1 were later fed on carrot for 7 days (Group Cx1 + Cx7) and then deprived for 24 h. Subjects previously in Group Cx5 had no access to carrot for 7 days and thus remained food-deprived (Cx5 + Cx0).

support the exclusion of this hypothesis since they showed that missing food on location tests might not always be taken as evidence for a failure to detect odours.

Taken together, our results have important implications for using baits to protect crops against snails. The fact that no prior experience (conditioning) is necessary for *Theba pisana* to be attracted by a given smell could be used for the development of novel baits as well as for a better deployment of those currently in use. Firstly, if, as we have shown, these snails are able to locate novel food, they could also show preferences for certain substances that could make current baits more attractive. In addition, our results also suggest that baits might be most efficient if they are used at the end of aestivation, i.e. when snails are hungrier and more willing to eat novel substances. Further, the strategy of feeding the snails on a particular food so that the baits become more likely to be found by the snails (Baker et al., 2012) is questioned by the results obtained in Experiment 2; using conditioned stimuli does not guarantee that the baits will become attractive, particularly if the snails have just been fed on them for several days.

Another question that it is related to food finding in snails is the role played by the posterior tentacles (PTs) in both food detection and the acquisition of FAC. PTs are assumed to be the ones that enable snails to perceive odours at distance (Zaitseva, 2016; Kiss, 2017), but according to previous research, these tentacles are not necessary for FAC learning to occur. Friedrich and Teyke (1998) observed that PTs under anaesthesia after lidocaine application in animals making contact with a novel food does not prevent FAC but, in following tests, such a treatment does prevent further location of that food. The authors therefore concluded that PTs are just necessary for the expression, but not for the acquisition, of conditioning. However, if as we have shown, snails detect novel food at distance, this must be by mean of the sensory capacities of PTs so that these tentacles could play a critical role for the formation of FAC; after a long period of food deprivation, PTs would allow snails to locate a novel food so that subsequent ingestion of the food and FAC can take place. Therefore, the idea that these organs are not necessary for the acquisition of FAC would need to be revised. Alternatively, it might be supposed that snails are able to locate food at distance using organs other than their PTs.

Finally, we would like to highlight the importance of considering ecological factors when research is conducted on the behavioural capabilities of animals. By running the experiments in April and September, when *Theba pisana* is, respectively, at the beginning and at the end of its aestivation phase in the North coast of Africa, we tried to ensure that the experimental subjects would be able to withstand a long deprivation phase in Experiment 1 (e.g., at the time of snails release in July 2017, after the experiment was conducted, *Theba pisana* living in the wild were observed to remain in aestivation, which lasted until October, the same year), and that they would be hungry enough to eat until becoming sated in Experiment 2. The place and time of the year in which these experiments have been conducted could have had an important influence on the results reported. It is possible that different results might have been obtained if the data had been collected in a different season, in a different place, or with a different snail species, even if the same procedure was used.

The results obtained extend the evidence showing that behavioural processes should be investigated according to the particular ecological characteristics of the species under study (see Loy et al., 2017; Sulikowski, 2017; Timberlake, 1984). When such ecological variables are not carefully taken into consideration, i.e. when behavioural processes are investigated outside of the particular ecological conditions of the species, we run the risk of underestimating their cognitive abilities, as was the case for this invertebrate species.

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