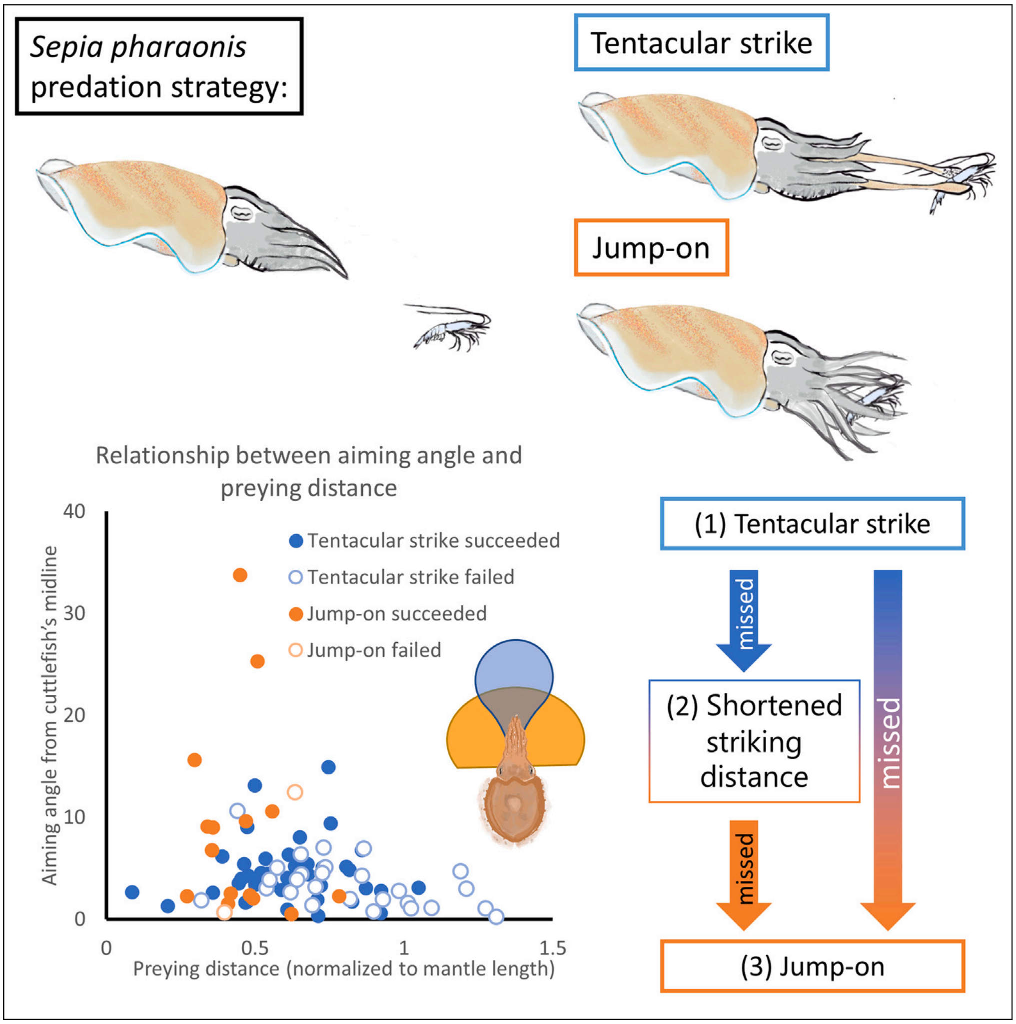
iScience

CellPress
OPEN ACCESS

Article

# Switching by cuttlefish of preying tactics targeted at moving prey



José Jiun-Shian Wu, Chuan-Chin Chiao

ccchiao@life.nthu.edu.tw

### Highlights

Cuttlefish use tentacular strike and jump-on alternately to prey a moving shrimp

These two preying tactics have different operating ranges relative to the prey

Jump-on behavior appears mostly after a miss attack by previous tentacular strike

Cuttlefish adjust preying tactic adaptively depending on prior preying experience

Jiun-Shian Wu & Chiao, iScience *26*, 108122
November 17, 2023
https://doi.org/10.1016/j.isci.2023.108122

iScience

CellPress
OPEN ACCESS

Article

# Switching by cuttlefish of preying tactics targeted at moving prey

José Jiun-Shian Wu[1,2] and Chuan-Chin Chiao[1,2,3,*]

## SUMMARY

**Previous studies have demonstrated that the size of the prey relative to the cuttlefish is important to the choice between tentacular strike and jump-on tactics. In the present study, we investigated the decision-making in the cuttlefish's tactical switch when preying on the same size prey. A servomotor system controlling the movement of a shrimp was used to elicit the cuttlefish's preying behavior. The success rate of prey capture and the kinematics of visual attack were examined systematically. The results showed that the jump-on behavior appeared mostly after a miss attack by previous tentacular strike on a moving shrimp. Compared with a visual attack with tentacles, the jump-on tactic has over a shorter attacking distance and wider attacking angles. Thus, these two different preying tactics have different operating ranges relative to the prey. More importantly, the cuttlefish can adjust their preying tactics adaptively depending on their prior preying experience.**

## INTRODUCTION

Predation is the searching, assessment, pursuit, capturing, and handling of animals to obtain the energy and nutrition required for growth and survival.[1,2] Animal predation can have many strategies, some rely on superior moving speed, endurance, or teamwork to seize their prey, while others may ambush by stealth, by luring, or the use of their surroundings and the notion of unexpected to capture their prey.[3–5] For a successful pursuit, predators need to search for potential prey and then assess the prey's properties prior to engaging in an attack.[6,7] In the case of ambush predators (or sit-and-wait predators), they are usually camouflaged, motionless, and solitary while waiting at a selected site for their prey to come within ambush distance before attacking.[8–12] For successful pursuit predation and especially ballistic interception strategies, the predator must predict the future location of the prey.[13] In such circumstances, the sensory-motor precision is crucial to the predators for the efficient and successful capturing of prey.[14]

In the coleoid cephalopods, the species diversity and the differences in the environments where they live lead to a wide variety of predation behaviors.[15,16] Ambushing, stalking, luring, attacking, and pursuing in disguise, among others are known hunting tactics in cephalopods.[17] In shallow water, the use of vision to detect prey and the ballistic action to capture prey are a predominant predation strategy in cephalopod species.[18,19] In addition, cephalopods have developed one of the most sophisticated central nervous system (CNS) in the marine animals outside the vertebrate lineage.[20] Their expanded and highly centralized CNS allows cephalopods to perform complex behaviors, including various flexible predation behaviors.[15,21,22]

Cuttlefish are active and highly efficient predators, which they typically use the tentacular strike to capture shrimp and fish or jump on the prey with their arm crown to grab a crab.[23–28] Cuttlefish hunting behavior involves three different stages: attention, positioning, and seizure.[25] Both methods of attack highly rely on their binocular vision to estimate the prey location in front of the cuttlefish.[25,29] Previous studies have demonstrated that the size ratio of prey/predator, rather than the locomotory characteristic of the prey, is an important factor in the choice between these two types of visual attack by cuttlefish.[27] For example, small crabs are preferentially captured by tentacular strike and large crabs are jump-on. These two predatory attacks differ in both seizure distance and angle. Tentacular strike affords a longer attacking distance but has a sharper attacking angle, while the jump-on tactic must be performed within a close distance but has a less sharp aiming angle.[25] Previous studies also showed that the frequency of using the jump-on strategy was gradually increased as a result of ablation of the tentacle tips, suggesting an adaptive and flexible behavior.[25,27] In a separate study, it was reported that naive cuttlefish typically jumped on the crab from the front and were often pinched, but in subsequent trials, cuttlefish rapidly improved their prey capture tactics by jumping on the crab from behind.[30] However, it was the crab odor exposure, rather than observing the experienced cuttlefish, which made the improvement in their preying strategy. Hunting behavior was considered as innate and stereotypic behavior in cuttlefish.[31,32] However, it has been suggested that the variability in the stereotyped prey capture sequence of male cuttlefish is related to the personality differences.[33] This implies that between-individual variation may arise from the flexibility of cuttlefish predatory behaviors.

---

[1]Institute of Systems Neuroscience, National Tsing Hua University, Hsinchu, Taiwan
[2]Department of Life Science, National Tsing Hua University, Hsinchu, Taiwan
[3]Lead contact
*Correspondence: ccchiao@life.nthu.edu.tw
https://doi.org/10.1016/j.isci.2023.108122

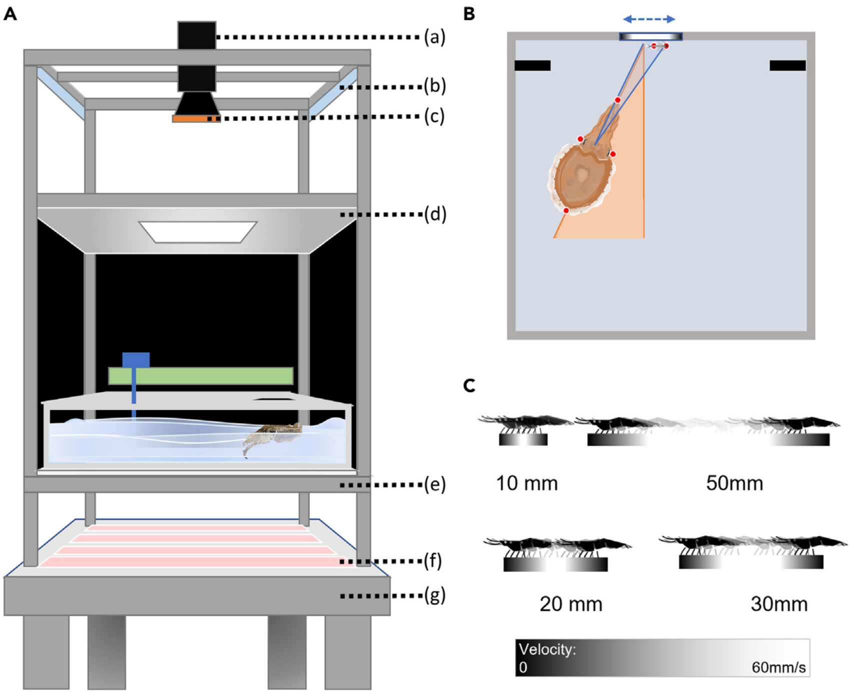


**Figure 1. The experiment setup**

(A) The tank and imaging system for recording cuttlefish predatory behavior. The specific components included (a) a digital camera, (b) white light LEDs, (c) an adjustable neutral density filter, (d) a light diffuser screen, (e) a diffuser plate, (f) infrared LEDs, and (g) a shockproof table.

(B) The schematic diagram showing the motor system for controlling prey movement. The shrimp was attached to a steel rod via a hook, and the back-and-forth movement was programmed via Arduino to control the sliding rail driven by the servomotor. The black bar at both sides represents the prey-starting area which was covered by a black screen to prevent cuttlefish from seeing and accessing the shrimp in this area. The attack angle from the vertical line (the angle formed by orange lines) and the aiming angle from cuttlefish's midline (the angle formed by blue lines) were calculated based on the labeled red points on the cuttlefish and the shrimp.

(C) The cartoon shows the programmed one-dimensional simple harmonic movement of the prey with four different moving distances. The prey movement is controlled by the motor system. The gradient represents the speed profile of the prey, and it was slowed down to zero at both reversal points.

In the present study, we examined the factors other than the size ratio of prey/predator that influence cuttlefish choosing one of these two predatory tactics targeted at moving prey. Specifically, we assessed if cuttlefish could switch between these two tactics depending on the prior outcome of a capture attempt. In other words, how cuttlefish adaptatively adjust their preying strategies based on their previous experience. We also characterized the kinematics of these visual attacks systematically to better understand the trade-off between these two predatory tactics, especially the attacking distance and the attacking angle.

## RESULTS

### Cuttlefish switch from tentacular strike to jump-on after a capture failure

The experimental configuration is based on our previous sutdy and shown in Figure 1.[28] During the experiments, cuttlefish may pay attention to moving prey without a visual attack, use the tentacular strike to capture the shrimp successfully, use the tentacular strike unsuccessfully, use the jump-on tactic to seize the prey, or use the jump-on unsuccessfully. The trial numbers of all five response types for the six individual cuttlefish are shown in Figure 2A. It is apparent that cuttlefish tended to use the tentacular strike more frequently than the jump-on method when faced with a moving shrimp (Table 1; Figure 2B; Chi-square test, $p < 0.0001$, $n = 88$). However, failed tentacular strikes and attention without a visual attack were also frequently observed. This suggests that capturing the moving prey with a tentacular strike is not an easy task and that the jump-on tactic may be reserved for an unsuccessful tentacular strike. To confirm this observation, we analyzed the prior outcome of all the jump-on behavior event, and it was found that 65% of the successful jump-on behavior events occurred after a prior tentacular strike failed, 12% after a prior jump-on failed, and 23% with no prior failure experience (Figure 2C). It is evident that the jump-on tactic was used mostly after failure of tentacular strike (Chi-square test, $p = 0.0194$, $n = 17$).

### Cuttlefish use different prey-aiming strategies for the tentacular strike and jump-on behaviors

In our previous study, it was observed that cuttlefish are able to attack moving prey from different directions with a tentacular strike.[28] In the present study, we compared the prey-aiming behavior between the two different tactics. It was apparent that tentacular strike has a wider

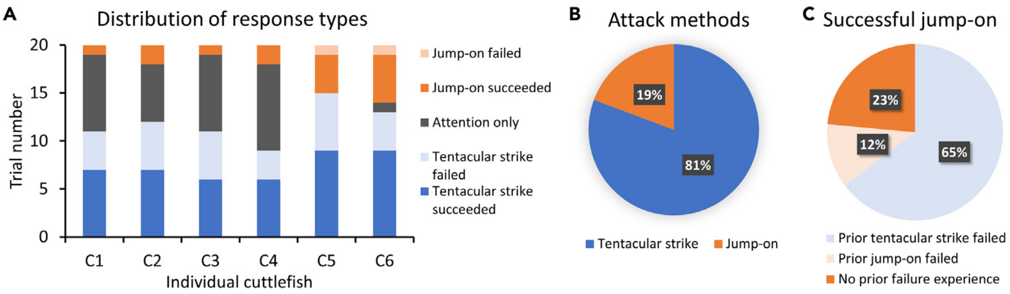


**Figure 2. The jump-on behavior occurred mostly after failure of tentacular strike**

(A) The trial number of response types (including successful tentacular strike, failed tentacular strike, attention only without any attack, successful jump-on, and failed jump-on) for six individual cuttlefish in all valid experiments.

(B) The percentage of two attack methods. The proportion of the tentacular strike was significantly larger than the jump-on. (Chi-square test, $p < 0.0001$, $n = 88$).

(C) The percentage of the successful jump-on behavior occurred with a prior tentacular strike failed, a prior jump-on failed, and no prior failure experience. It is evident that the jump-on tactic was used mostly after failure of tentacular strike (Chi-square test, $p = 0.0194$, $n = 17$).

attack angle from the vertical line (the line perpendicular to the axis of the prey movement) but a narrower aiming angle from cuttlefish's midline (Figure 3A). This was similar for both successful and failed tentacular strikes. However, for the jump-on attack (succeeded and failed), the attack angle from the vertical line was much smaller, but the aiming angle from cuttlefish's midline had a much greater tolerance in terms of angle (Figure 3A). This suggests that the tentacular strike requires more precise prey-aiming along the cuttlefish's body axis during the attention phase,[25] and the jump-on attack is more concerned with the attack direction relative to the prey.

To examine the convergent eye movement during these two different visual attack tactics, we also analyzed the relationship between left and right eye angles during the seizure phase for a successful tentacular strike, a failed tentacular strike, and a jump-on attack (succeeded and failed).[25] It is evident in most cases that the left and right eye angles from cuttlefish's midline are similar during the seizure phase for both tactics (Figure 3B). This suggests that depth perception and stereopsis are both equally important when the cuttlefish is adopting a tentacular strike and when the cuttlefish is adopting the jump-on tactic.

## Cuttlefish change the preying distance after an unsuccessful attack

To catch the prey successfully, cuttlefish adjust their distance from the prey by roughly one mantle length during the positioning phase of visual attack.[25] Interestingly, we found that the average preying distance in a successful tentacular strike was about 0.6 mantle length (Figure 4A). This may indicate that capturing moving prey requires a closer distance than capturing stationary prey. More importantly, when cuttlefish switched to the jump-on tactic, the preying distances were further decreased significantly ($p = 0.0019$; Figure 4A). If we compare successful and unsuccessful tentacular strikes, it is apparent that the preying distances in the trials where there was a failed tentacular strike are significantly larger than those when there was success ($p = 0.0012$; Figure 4B). This suggests that a successful tentacular strike on moving prey requires a shorter prey distance. To examine if cuttlefish would adaptatively shorten the preying distance immediately after a failed visual attack, we compared the preying distances of an unsuccessful visual attack (including both tentacular strikes and jump-on attacks) and its next attack (including both successful and unsuccessful tentacular strikes, as well as successful jump-on attacks). It was found that the preying distance right after an unsuccessful visual attack was significantly reduced ($p = 0.0011$; Figure 4C). Interestingly, we also noticed that after an unsuccessful tentacular strike, if cuttlefish switched to the jump-on tactic and caught the prey successfully, then the preying distances were also significantly reduced ($p = 0.0020$; Figure S1A and Video S1). However, after an unsuccessful tentacular strike, if cuttlefish continued to use the tentacular strike and caught the prey successfully, then, although the preying distances were also reduced, this was not statistically different ($p = 0.0938$; Figure S1B and Video S2); furthermore, if the cuttlefish failed again at its next attack, the preying distance was not significantly changed ($p > 0.9999$; Figure S1C and Video S3). Finally, after an unsuccessful jump-on, if the cuttlefish remained using the jump-on tactic and caught the prey successfully, the preying distances were also not significantly changed (Figure S1D and Video S4). These results suggest that the preying distance of cuttlefish right after an unsuccessful visual attack depends on tactical mode and the attack outcome.

## Cuttlefish's jump-on tactic is used as an alternative mode to increase successful seizure

While cuttlefish were able to track and catch moving prey in our previous study,[28] the successful rate of the visual attacks for different types of moving prey is not known. In the present study, we found that cuttlefish made more unsuccessful tentacular strikes when the prey moved back-and-forth at a shorter range (10 mm and 20 mm) than when the prey moved at a longer range (30 mm and 50 mm), with the successful rate being 54%, 50%, 65%, and 83% for the 10 mm, 20 mm, 30 mm, and 50 mm, respectively (Table 1). However, when cuttlefish used the jump-on tactic, the successful rates were similar across different prey's moving ranges, with only two unsuccessful jumps across all 17 trials (Table 1). This suggests that cuttlefish find it more difficult to effectively target moving prey for a tentacular strike when the prey changes its moving direction too fast. In addition, when cuttlefish switched to the jump-on behavior, the success rate was significantly increased regardless of

**Table 1. Trial number for each attack type and outcome in all prey-moving conditions**

| | Prey-moving distance in a simple harmonic movement | | | | |
|---|---|---|---|---|---|
| | 10 mm | 20 mm | 30 mm | 50 mm | Total events |
| Tentacular strike | | | | | 71 |
| Success | 13 | 6 | 15 | 10 | |
| Failure | 11 | 6 | 8 | 2 | |
| Jump-on | | | | | 17 |
| Success | 4 | 3 | 4 | 4 | |
| Failure | 0 | 1 | 1 | 0 | |

the frequency of prey's moving direction change. This implies that the jump-on tactic was used as an alternative for increasing the successful seizure.

### Cuttlefish shorten the preying distance to gain a wider coverage of the moving prey when using the jump-on behavior

Based on the analyses mentioned previously, it was apparent that the tentacular strike has a wider range of prey distance but a narrower range of prey aiming angle (Figure 5A). In contrast, the jump-on tactic has a narrower range of preying distance but a wider range of prey aiming angle (Figure 5A). In essence, when cuttlefish switched from the tentacular strike to the jump-on tactic, they shortened the preying distance to gain a wider coverage of the moving prey, which increases the prey capture success rate. However, this strategy is dynamic, and it depends on prior attack outcome. When cuttlefish have made an unsuccessful tentacular strike, they would either shorten their striking distance for another tentacular strike or switch to the jump-on tactic, both to increase the successful capture rate (Figure 5B).

## DISCUSSION

It is well known that cuttlefish typically use either tentacular strike or jump-on to capture their prey, depending on the prey type.[23–25] It has also been reported that the size of the prey relative to the mantle length of the cuttlefish is of importance when choosing between these two types of visual attack.[27,34] In the present study, we have shown that cuttlefish are flexible when capturing the same moving prey and use the two different tactics alternately (Figure 2C; Figure S1). Specifically, the cuttlefish's preying strategy is dependent on their prior preying outcomes, and the jump-on tactic is used mostly after failure of the tentacular strike (Figure 2C). While tentacular strike is the most adopted preying tactic when catching a stationary shrimp,[25] it is relatively difficult for cuttlefish to seize a moving prey with this visual attack because the target is quite moving fast, and this is particularly true when the prey changes its moving direction too fast (Table 1). In the present study, we frequently observed that cuttlefish failed with a tentacular strike, or they showed attention to the moving shrimp but did not initiate a tentacular attack (Figure 2A). After an unsuccessful tentacular strike, cuttlefish tended to switch their preying tactic to the jump-on behavior (Figure 2C), perhaps because this visual attack is easier in terms of aiming at a moving target. Our results show when cuttlefish switch to the jump-on behavior, the successful rate is significantly increased regardless of the frequency of prey's direction change when moving (Table 1) and this supports the aforementioned interpretation. Although the jump-on tactic seems to be a better and more efficient preying tactic for cuttlefish to catch a moving shrimp, the total trial number of jump-on events (17) was significantly less than that of the tentacular strike (71). This may imply that the jump-on tactic is more energy consuming or not typically used at this prey/predator size ratio.[27] In general, this observation suggests that cuttlefish can adapt the preying tactic according to their prior preying outcome. This also implies that the jump-on tactic is used as an alternative to increase the success rate in terms of prey capture. This flexibility is important for ambushing predators (or sit-and-wait predators) to capture their prey successfully.

Although both visual attacks were able to be used interchangeably by cuttlefish to capture fast-moving shrimp in the present study, the prey aiming and attacking behaviors between these two tactics are different. It was observed that the tentacular strike has a wider attack angle but a narrower aiming angle, while the jump-on tactic has a smaller attack angle but a larger aiming angle (Figure 3A). For the tentacular strike, it is essential to align the prey along the cuttlefish's body axis during the attention phase.[25] This is similar to the tongue projection of salamander when catching walking prey.[13] However, cuttlefish can use tentacular strike to attack a moving shrimp from almost any angle to the prey,[28] and this flexibility gives them an advantage when catching prey from the side or behind. In contrast, with the jump-on tactic, cuttlefish must attack the prey in front of them, not from the side, thus the attack angle is limited. Besides, this tactic does not require precise aiming at the prey, because arm grabbing has a wider capture area than tentacular strike. This gives the cuttlefish a different advantage when catching the prey because it requires less precise aiming. This advantage might be important to cuttlefish when they are seizing fast-moving prey during which prey aiming could be a challenge task.

Aside from this visual attack aspect, the capabilities of handling/holding prey could be another important feature while the cuttlefish determine the striking strategy.[32] It is apparent that the physical coverage of the prey in the seizure phase was different between two types of visual attacks. Specifically, the width of the paired tentacular clubs at the tentacular strike was significantly smaller than the width of the open arm crown at the jump-on (Figure S2). However, the tentacular club of cuttlefish has big suckers, while the suckers on the eight arms are much

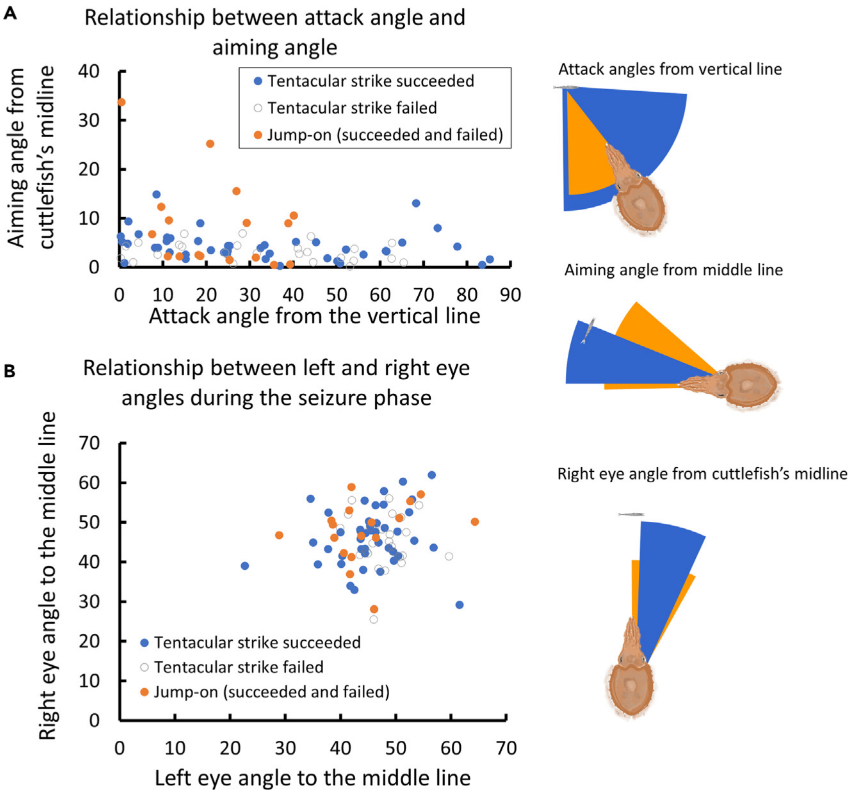


**Figure 3. Tentacular strike and jump-on behaviors use different prey-aiming strategies but similar convergent eye movements**

(A) The relationship between attack angle and aiming angle for successful tentacular strike, failed tentacular strike, and jump-on (succeeded and failed). The attack angle from the vertical line and the aiming angle from cuttlefish's midline are illustrated on the right.

(B) The relationship between left and right eye angles during the seizure phase for successful tentacular strike, failed tentacular strike, and jump-on (succeeded and failed). The right eye angle from cuttlefish's midline is depicted on the right.

smaller.[35] This morphological difference suggests that the tentacular strike with its enlarged suckers on the clubs may be more suitable for the rapid retraction of moving prey.[36]

It has been reported that cuttlefish use stereopsis to strike at prey.[29] The ability to extract depth information from the disparity between left and right visual fields is essential for a successful visual attack by cuttlefish.[25] Our findings show that the left and right eye angles from cuttlefish's midline are similar during the seizure phase for both tentacular strike and jump-on (Figure 3B), and this suggests that depth perception is equally important for both tactics. Although the prey aiming behavior during the jump-on tactic does not require the same precision as the tentacular strike (Figure 3A), estimating the prey distance for a successful arm grabbing still requires accurate depth computation. Thus, convergent eye movement is an equally important visuomotor behavior for both visual attacks.

Attacking distance, or depth information, is crucial to predation.[37] Predators need to estimate the risk and the distance before deciding the best timing for an attack.[38,39] Interestingly, the information on estimated prey distance is used differently for cuttlefish when attacking a stationary prey and when attacking a moving prey with tentacular strike tactic. While cuttlefish normally adjust their distance from a stationary prey to roughly one mantle length during a successful tentacular strike,[25] we found that the average attacking distance from a moving prey was only about 0.6 mantle length (Figure 4A). Furthermore, when we compared the preying distances between failed and successful tentacular strikes, it was found that the distances in the unsuccessful trials were significantly larger than those in the successful trials (Figure 4B). This suggests that cuttlefish tend to overestimate the striking distance for a moving target, which can result in a missed shot. However, cuttlefish can quickly shorten the preying distance after an unsuccessful attack to increase the capture rate (Figure 4C). This can be achieved by decreasing the tentacular strike distance or by switching to the jump-on tactic in their next visual attack attempt (Figure S1; Figure 5B).

In summary, our results demonstrate that the tentacular strike has a wider range of preying distance but a narrower range of prey aiming angle, while the jump-on tactic has a narrower range of preying distance but a wider range of prey aiming angle (Figure 5A). In other words, cuttlefish shorten the preying distance for wider coverage of moving prey to increase the success rate of prey capture, and this preying tactic switch is dynamic and depends on the prior attack outcome. This is similar to the tennis racket's sweet spot, in which cuttlefish are able to choose different prey tactics in order to achieve an efficient prey capture (Figure 5A inset). Our findings thus add to the knowledge on the degree of flexibility of cuttlefish hunting behaviors long considered as hard wired.

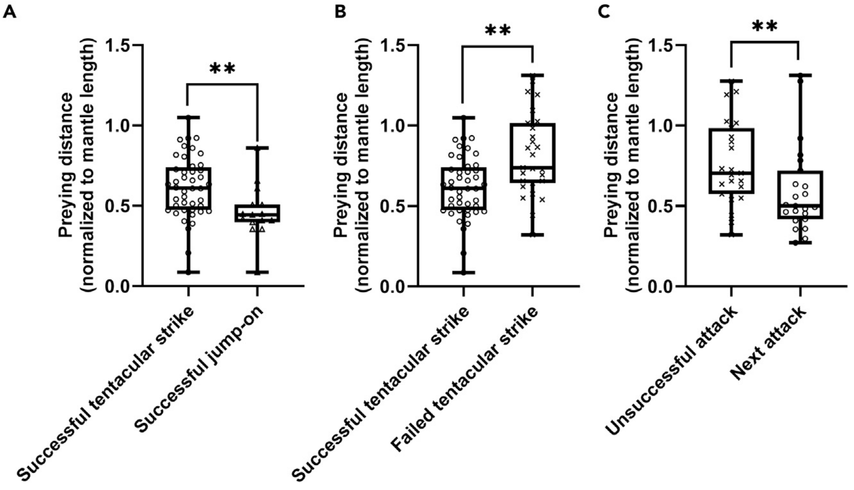


**Figure 4. Preying distances of cuttlefish are different depending on attack tactics, attack outcome, and prior experience**

(A) Preying distances in successful tentacular strike and successful jump-on were statistically different (Mann-Whitney U test, p = 0.0019, n = 44 and 15).

(B) Preying distances in successful tentacular strike and failed tentacular strike were significantly different (Mann-Whitney U test, p = 0.0012, n = 44 and 27).

(C) Preying distances right after unsuccessful visual attack (including both tentacular strike and jump-on) were significantly reduced (Wilcoxon matched-pairs signed rank test, p = 0.0011, n = 23). Next attack could be successful or failed attack. Box-plot shows medians and lower/upper quartiles.

## Limitations of the study

The present study was conducted when the cuttlefish were juvenile, and their behaviors may have changed when they became adults. Despite this, our results showed that cuttlefish's predatory behavior was plastic and depended on their prior preying experiences. In the current study, the movement of the prey was reduced to one dimension, and we only analyzed two-dimensional movement of cuttlefish. Indeed, both the

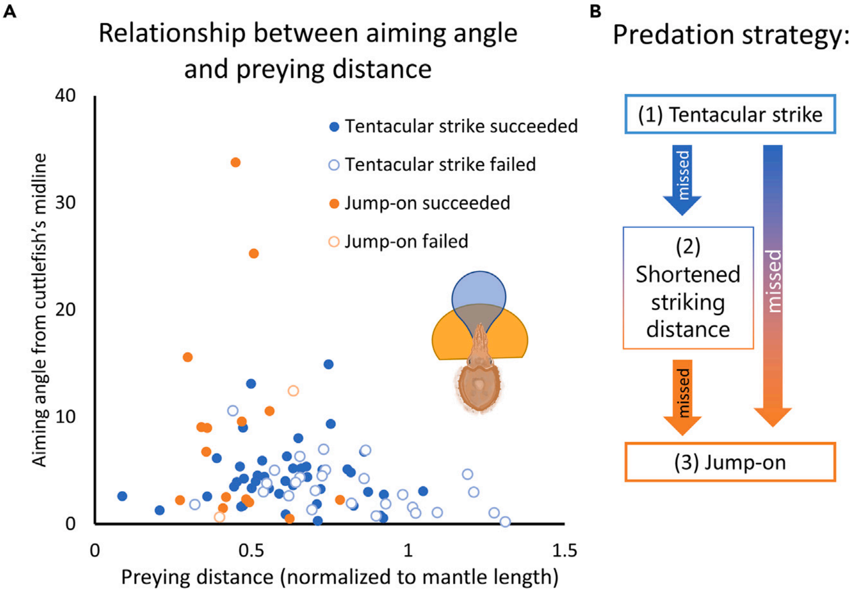


**Figure 5. Tentacular strike and jump-on have different aiming angles and preying distances, and cuttlefish's predation strategy is dependent on the prior attack outcome**

(A) The relationship between cuttlefish's aiming angle and preying distance for successful and failed tentacular strike, as well as successful and failed jump-on. Compared with visual attack with tentacles, the jump-on tactic has shorter attacking distances and wider attacking angles. The cartoon depicting this observation is shown on the right.

(B) The predation strategy and its dependence on prior attack outcome are illustrated as a flow chart. (1) Cuttlefish usually use tentacular strikes to attack the moving shrimp. (2) If the attack failed, cuttlefish would either shorten their striking distance or (3) jump on and grab the prey to increase the successful capture rate.

shrimp and the cuttlefish could move in three dimensions during predation. Since we considered cuttlefish as benthic animals, and shrimps were predominately moving in one dimension, thus our simplified experimental design and data analysis were close to their natural predation conditions.

## STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
    - Lead contact
    - Materials availability
    - Data and code availability
- EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS
- METHOD DETAILS
    - Experimental setup
    - Experimental procedure
- QUANTIFICATION AND STATISTICAL ANALYSIS
    - Image analysis
    - Statistical analysis

## SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.isci.2023.108122.

## ACKNOWLEDGMENTS

This study was supported by a research grant from the Ministry of Science and Technology, Taiwan MOST-110-2311-B007-007-MY3 (to C.-C.C.). We thank Mr. Yen-Chen Lin for helping in the experimental setup and instrumentation.

## AUTHOR CONTRIBUTIONS

Conceptualization: J.J-S.W. and C.-C.C.; Methodology, J.J-S.W.; Writing-Original Draft: J.J-S.W.; Writing-Review & Editing: J.J-S.W. and C.-C.C.; Sample collection: J.J-S.W. All authors reviewed the whole work and approved the final version of the manuscript.

## DECLARATION OF INTERESTS

The authors declare no competing interests.

Received: April 15, 2023
Revised: July 31, 2023
Accepted: September 29, 2023
Published: October 6, 2023

## REFERENCES

1. Ridley, M. (1995). Eating and not being eaten. In Animal behavior: an introduction to behavioral mechanisms, development and ecology (John Wiley & Sons), pp. 140–163. https://doi.org/10.1002/9781444313802.ch6.
2. Kramer, D.L. (2001). Foraging behavior. In Evolutionary ecology: concepts and case studies, C.W. Fox, D.A. Roff, and D.J. Fairbairn, eds. (Oxford Academic), pp. 232–246. https://doi.org/10.1093/oso/9780195131543.001.0001.
3. Perry, G. (1999). The evolution of search modes: ecological versus phylogenetic perspectives. Am. Nat. *153*, 98–109. https://doi.org/10.1086/303145.
4. McDaniel, R.R., Jordan, M.E., and Fleeman, B.F. (2003). Surprise, surprise, surprise! A complexity science view of the unexpected. Health Care Manag. Rev. *28*, 266–278.
5. Lang, S.D.J., and Farine, D.R. (2017). A multidimensional framework for studying social predation strategies. Nat. Ecol. Evol. *1*, 1230–1239. https://doi.org/10.1038/s41559-017-0245-0.
6. Stephens, D.W., Brown, J.S., and Ydenberg, R.C. (2008). Foraging: Behavior and Ecology, 2nd Edition (University of Chicago Press).
7. Commons, M.L., Kacelnik, A., and Shettleworth, S.J. (2013). Foraging: Quantitative Analyses of Behavior (Taylor & Francis). https://doi.org/10.4324/9780203781685.
8. Morse, D.H. (1984). How crab spiders (Araneae, thomisidae) hunt at flowers. J. Arachnol. *12*, 307–316.
9. Caraco, T., and Gillespie, R.G. (1986). Risk-sensitivity: foraging mode in an ambush predator. Ecology *67*, 1180–1185. https://doi.org/10.2307/1938673.
10. Fouts, W.R. (1995). The Feeding Behavior and Associated Ambush-Site Characteristics of the Pacific Angel Shark, Squatina californica, at Santa Catalina Island, California (M.S. California State University).
11. Fouts, W.R., and Nelson, D.R. (1999). Prey capture by the pacific angel shark, Squatina californica: visually mediated strikes and ambush-site characteristics. Copeia *1999*, 304–312. https://doi.org/10.2307/1447476.
12. Scharf, I., Nulman, E., Ovadia, O., and Bouskila, A. (2006). Efficiency evaluation of two competing foraging modes under different conditions. Am. Nat. *168*, 350–357. https://doi.org/10.1086/506921.
13. Borghuis, B.G., and Leonardo, A. (2015). The role of motion extrapolation in amphibian prey capture. J. Neurosci. *35*, 15430–15441. https://doi.org/10.1523/JNEUROSCI.3189-15.2015.

14. Rossel, S., Corlija, J., and Schuster, S. (2002). Predicting three-dimensional target motion: how archer fish determine where to catch their dislodged prey. J. Exp. Biol. *205*, 3321–3326. https://doi.org/10.1242/jeb.205.21.3321.
15. Hanlon, R.T., and Messenger, J.B. (2018). Cephalopod Behaviour, Second edition (Cambridge University Press).
16. Chung, W.S., Kurniawan, N.D., and Marshall, N.J. (2022). Comparative brain structure and visual processing in octopus from different habitats. Curr. Biol. *32*, 97–110.e4. https://doi.org/10.1016/j.cub.2021.10.070.
17. Rodhouse, P.G., and Nigmatullin, C.M. (1996). Role as consumers. Philos. Trans. R. Soc. Lond. B Biol. Sci. *351*, 1003–1022. https://doi.org/10.1098/rstb.1996.0090.
18. Kier, W., and Leeuwen, J. (1997). A kinematic analysis of tentacle extension in the squid Loligo pealei. J. Exp. Biol. *200*, 41–53.
19. Villanueva, R., Perricone, V., and Fiorito, G. (2017). Cephalopods as predators: A short journey among behavioral flexibilities, adaptions, and feeding habits. Front. Physiol. *8*, 598. https://doi.org/10.3389/fphys.2017.00598.
20. Budelmann, B.U. (1995). The cephalopod nervous system: What evolution has made of the molluscan design. In The nervous systems of invertebrates: an evolutionary and comparative approach, O. Breidbach and W. Kutsch, eds. (Springer Science & Business Media), pp. 115–138. https://doi.org/10.1007/978-3-0348-9219-3_7.
21. Chung, W.S., López-Galán, A., Kurniawan, N.D., and Marshall, N.J. (2023). The brain structure and the neural network features of the diurnal cuttlefish Sepia plangon. iScience *26*, 105846. https://doi.org/10.1016/j.isci.2022.105846.
22. Montague, T.G., Rieth, I.J., Gjerswold-Selleck, S., Garcia-Rosales, D., Aneja, S., Elkis, D., Zhu, N., Kentis, S., Rubino, F.A., Nemes, A., et al. (2023). A brain atlas for the camouflaging dwarf cuttlefish, Sepia bandensis. Curr. Biol. *33*, 2794–2801.e3. https://doi.org/10.1016/j.cub.2023.06.007.
23. Wilson, D.P. (1946). A note on the capture of prey by Sepia officinalis L. J. Mar. Biol. Assoc. U. K. *26*, 421–425. https://doi.org/10.1017/S0025315400012248.
24. Wickstead, J. (1956). An unusual method of capturing prey by a cuttlefish. Nature *178*, 929. https://doi.org/10.1038/178929b0.
25. Messenger, J.B. (1968). The visual attack of the cuttlefish, Sepia officinalis. Anim. Behav. *16*, 342–357. https://doi.org/10.1016/0003-3472(68)90020-1.
26. Messenger, J.B. (1977). Prey-capture and learning in the cuttlefish. Sepia. Symp. Zool. Soc. Lond. 347–376.
27. 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. https://doi.org/10.1016/0376-6357(84)90004-4.
28. Wu, J.J.S., Hung, A., Lin, Y.C., and Chiao, C.C. (2020). Visual attack on the moving prey by cuttlefish. Front. Physiol. *11*, 648. https://doi.org/10.3389/fphys.2020.00648.
29. Feord, R.C., Sumner, M.E., Pusdekar, S., Kalra, L., Gonzalez-Bellido, P.T., and Wardill, T.J. (2020). Cuttlefish use stereopsis to strike at prey. Sci. Adv. *6*, eaay6036. https://doi.org/10.1126/sciadv.aay6036.
30. Boal, J.G., Wittenberg, K.M., and Hanlon, R.T. (2000). Observational learning does not explain improvement in predation tactics by cuttlefish (Mollusca: Cephalopoda). Behav. Process. *52*, 141–153. https://doi.org/10.1016/S0376-6357(00)00137-6.
31. Shinzato, S., Yasumuro, H., and Ikeda, Y. (2018). Visual stimuli for the induction of hunting behavior in cuttlefish Sepia pharaonis. Biol. Bull. *234*, 106–115. https://doi.org/10.1086/697522.
32. Sugimoto, C., and Ikeda, Y. (2013). Comparison of the Ontogeny of Hunting Behavior in Pharaoh Cuttlefish (Sepia pharaonis) and Oval Squid (Sepioteuthis lessoniana). Biol. Bull. *225*, 50–59. https://doi.org/10.1086/BBLv225n1p50.
33. Zoratto, F., Cordeschi, G., Grignani, G., Bonanni, R., Alleva, E., Nascetti, G., Mather, J.A., and Carere, C. (2018). Variability in the "stereotyped" prey capture sequence of male cuttlefish (Sepia officinalis) could relate to personality differences. Anim. Cognit. *21*, 773–785. https://doi.org/10.1007/s10071-018-1209-8.
34. Blanc, A., du Sel, G.P., and Daguzan, J. (1999). Relationships between length of prey/predator for the most important prey of the cuttlefish *Sepia officinalis L.* (Mollusca : Cephalopoda). Malacologia *41*, 139–145.
35. Tehranifard, A., and Dastan, K. (2011). General morphological characteristics of the Sepia Pharaonis (cephalopoda) from Persian gulf, Bushehr region. In International Conference on Biomedical Engineering and Technology IPCBEE (IACSIT Press).
36. Omura, A., and Ikeda, Y. (2022). Morphology of the suckers for hunting behavior in broadclub cuttlefish (Sepia latimanus). Ecol. Res. *37*, 156–162. https://doi.org/10.1111/1440-1703.12278.
37. Cooper, W.E., and Martín, J. (2016). Predator-prey distance and latency to flee from an immobile predator: functional relationship and importance. Curr. Zool. *62*, 117–122. https://doi.org/10.1093/cz/zow035.
38. Martín, J., Luque-Larena, J.J., and López, P. (2009). When to run from an ambush predator: balancing crypsis benefits with costs of fleeing in lizards. Anim. Behav. *78*, 1011–1018. https://doi.org/10.1016/j.anbehav.2009.07.026.
39. Domenici, P., and Ruxton, G.D. (2015). Prey behaviors during fleeing: escape trajectories, signaling, and sensory defenses. In Escaping from predators: an integrative view of escape decisions, D.T. Blumstein and J.W.E. Cooper, eds. (Cambridge University Press), pp. 199–224. https://doi.org/10.1017/CBO9781107447189.009.

## STAR★METHODS

### KEY RESOURCES TABLE

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---|---|---|
| Biological samples | | |
| *Sepia pharaonis* | Wild (I-Lan,Taiwan) | N/A |
| Software and algorithms | | |
| ImageJ | NIH, version1.54f, open-source of software | RRID: SCR_003070 |
| StreamPix | Version 7.0; NorPix Inc., Canada | RRID: SCR_015773 |
| MATLAB | Version 2022a; MathWorks, United States | RRID: SCR_001622 |
| GraphPad Prism | Version 7.0; GraphPad, United States | RRID: SCR_002798 |
| Cuttlefish Experimental System V6 Firmware | This study | DOI: 10.5281/zenodo.8363146 |

### RESOURCE AVAILABILITY

#### Lead contact

Further information and requests for resources should be directed to and will be fulfilled by the lead contact, Chuan-Chin Chiao (ccchiao@life.nthu.edu.tw).

#### Materials availability

This study did not generate new unique reagents.

#### Data and code availability

- Video data reported in this paper will be shared by the lead contact upon request.
- All original code has been deposited at Zenodo and is publicly available as of the date of publication. DOIs are listed in the key resources table.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

### EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Sub-adult pharaoh cuttlefish, *Sepia pharaonis* (mantle length, 6–10 cm), were reared from eggs collected at I-Lan, Taiwan. These cuttlefish eggs were transported to the National Tsing Hua University and maintained in close-circulation aquarium systems in the laboratory (700 L each; water temperature, 23°C–25°C). After the eggs had hatched, the animals were housed individually in plastic containers (45 cm × 23 cm × 24 cm) inside an aquarium with sea water exchange. They were fed live post-larval white shrimp, *Litopenaeus vannamei*, and post-larval freshwater shrimp, *Neocaridina denticulate*, twice a day at least. The photoperiod of the aquaculture system was a 12/12 h light/dark cycle and used six ceiling full spectrum LED lights (7.5 W each). In total, ten cuttlefish were used during the present study, but only six animals gave attention to the moving prey. The excluded cuttlefish were either only preyed once and showed no interest in the next trial or expressed startle responses when the moving prey started. All the procedures in this research were approved by the Institutional Animal Care and Use Committee of the National Tsing Hua University (Protocol # 108047).

### METHOD DETAILS

#### Experimental setup

The configuration of the imaging system is based on previous study and shown in Figure 1A.[28] To enhance image contrast and to reduce ambient light intensity, infrared illumination invisible to the cuttlefish was provided from below to create a cuttlefish silhouette against a light background. The experimental tank was made of thick acrylic (35 cm × 38 cm × 12 cm). The bottom of the tank had a sheet of brown paper and a semi-transparent film attached on the outside as a diffuser, and the inside walls of the tank were covered with a matted surface film to reduce light reflection. A set of white LED lights (15 W) with a plastic diffuser was used to provide even illumination from the left and right sides to avoid reflection. A highspeed monochromatic 10GigE camera (HT-4000-N, Emergent Vision Technologies, Canada) with a 35 mm lens (HF-3514V-2, Myutron Inc., Japan) was fixed to the top of the tank using a rack that included a two-axis manual translation stage (ThorLabs, Newton, NJ, United States). This allowed flexible maneuvering of the camera. In addition, an adjustable neutral density filter, which was made up of two circular polarizers, was placed in front of the lens to reduce the light intensity within the visible range. This also removed ripples and reflections from the water surface, which had the effect of improving the image quality significantly. The entire system was placed on a shockproof table to stabilize the image and enclosed within a black tent to eliminate environmental disturbance during the experiments.

The motor control system, which provided programmable one-dimensional horizontal movement of a prey target, is illustrated in Figure 1B. The system consisted of a stepper motor (WLC stepping motor, Taiwan), a ball screws with a sliding rail that was connected to a steel rod with a hook at one end for attaching the prey. The stepper motor was connected to a programmable Arduino board (UNO, Somerville, MA, United States) and a custom firmware (Cuttelfish Experiment System v6 firmware, key resources table) is used to control the movement of the prey back and forth in one dimension. The prey would come out from the corner (the prey-starting area) then was moved to a random position before starting simple one-dimensional harmonic movements. Note that the freshwater shrimps *Neocaridina denticulate* (size range 25–30 mm) were used as the prey in the present study, and they were fast frozen before the experiment to maintain the freshness. The moving patterns are represented as a cartoon and are shown in Figure 1C. To prevent any vibration produced by the servomotor from affecting the stability of image acquisition, the motor control system was placed on a separate table next to the shockproof table used for the imaging system. In addition, to prevent the cuttlefish from seeing the steel rod and the sliding rail, the motor control system was covered with a black cloth and only the prey was visible to the cuttlefish in the experimental tank.

The monochromatic images were acquired using a digital camera (HT-4000-N, Emergent Vision Technologies) with an image size of 2048 x 2048 pixels at a speed of 179 frames per second. The images were recorded in Seq format on the high-speed SSD card (NVMe M.2; Samsung, Korea). Image preview was conducted using StreamPix (7.0; NorPix Inc., Canada) and ImageJ (1.52a; National Institute of Health, United States) and further processing was done using MATLAB (MathWorks, Natick, MA, United States).

### Experimental procedure

To motivate the cuttlefish to prey on the moving prey, the animals starved overnight for 8–10 h before experimentation. Each cuttlefish was placed in the experimental tank and allowed to acclimatize for at least 30 min. After the cuttlefish had settled down, which was judged by a reduction in ventilation rate (less than 60 times per min) and slow fin movement, the moving prey was made to appear and to start the back-and-forth movement pattern. The prey moving pattern (Figure 1C) was randomly selected in each trial. Similar to our previous study, cuttlefish typically expressed interest in feeding on the moving prey within 2 min[28] Thus, the response of cuttlefish to the presence of the moving prey was recorded for 120 s or until the cuttlefish captured the prey. If the cuttlefish did not respond to the moving prey at all in three consecutive 120 s recordings, the trial was aborted for the day. Only when the cuttlefish showed attention to the moving prey, the trial was considered a valid one. If the cuttlefish made a successful visual attack on the moving prey, it was allowed to finish eating the shrimp and rest for at least 10 min before starting a new trial. Individual cuttlefish were tested no more than four trials per day, and they were allowed to rest for at least one day before the next experiment. At this developmental stage, cuttlefish could eat more than five shrimps per meal, thus the hunger level was consistent in each trial. Seawater was constantly agitated by providing air bubbles at the corner of the tank throughout the experiment. After each trial of the experiment, fresh seawater was flowing into the tank and replaced the seawater present at the rate of 800–1000 mL/min for at least 10 min to ensure the oxygen and temperature levels remain constant.

## QUANTIFICATION AND STATISTICAL ANALYSIS

### Image analysis

The recorded videos were used to track the various body parts of the cuttlefish in sequence. The key frames of videos were selected manually at the three stages of visual attack, namely attention, positioning, and seizure.[25] The kinematics of the visual attacks were analyzed using these key image frames. The images were sometimes enhanced in terms of sharpness and contrast in order to label the specific body parts of the cuttlefish and the shrimp (red dots in Figure 1B) for extracting key parameters, such as the attack angle and the preying distance. The x-y coordinates of these labeled points were derived using ImageJ (National Institute of Health). The key parameters including the aiming angle, the attack angle, the left/right eye angle, and the preying distance were all calculated using the custom written program of MATLAB (MathWorks).

### Statistical analysis

All statistics were performed in Prism (GraphPad, Boston, MA, USA). The statistical detail of each test (n and p values) was described in each figure legend. The Chi-square test was used to assess the proportion of predation methods and prior experiences in Figures 2B and 2C. To give an appropriate analysis, the non-parametric analyses were adopted in this study for small sample number of cuttlefish. The Mann-Whitney U test was used to compare the preying distance under three conditions, namely successful attack, jump-on attack, and missed attack in Figures 4A and 4B and Figure S2C. The Wilcoxon matched-pairs signed rank test was used to compare the preying distance between an unsuccessful attack and the next one in Figure 4C, Figure S1A, Figure 1B, and Figure S1C.