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ORIGINAL ARTICLE

Aquaculture

Shelters for aquaculture of *Octopus sinensis*: preferences for gap width and horizontal versus vertical plates

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Abstract

It is difficult to keep a large number of cultured octopuses together in the same holding tank due to the high incidence of cannibalism. Appropriate shelters within which the octopuses can more readily avoid their peers are expected to ameliorate this problem. Shelters can be constructed from square plates assembled with appropriate spacing (gap) between them. The purpose of this study was to ascertain the optimum plate orientation and gap width between plates for shelters to maximize the accommodation of octopuses. It was found that the number of shelter occupants increased with the number of gaps available, such that more than half of the individuals in the experimental tank used the same shelter simultaneously. Octopuses tested singly to determine preferences for shelters placed vertically or horizontally preferred the vertical arrangement. Octopuses of more than 300 g tested singly in aquaria given shelters with different gap widths (20–100 mm) were found to not enter shelters with gap widths of 20 or 40 mm. It is concluded that octopus shelters constructed from square vertical plates spaced with gap widths suitable for octopuses are a useful contribution towards maximizing the number of octopuses that can be maintained in culture conditions.

Keywords *Octopus sinensis* · Shelter · Selectivity

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Introduction

Octopus aquaculture has yet to become commercially viable, despite attempts beginning more than 50 years ago (Itami et al. 1963). The East Asian common octopus, *Octopus sinensis* d'Orbigny, 1841, is a medium–large sized octopus growing to a total length of up to 60 cm and a wet weight (w/w) of around 3 kg, which inhabits the continental coastal waters of Japan, Korea, and China, from southern Hokkaido to at least Taiwan (Gleadall 2016). It mainly eats a wide range of crustaceans and molluscs, and shows strong territoriality except during the mating season (Okutani 1994).

The demand for octopus is high in Japan, where they are harvested by octopus pot fishing, bottom trawl, angling, or driftline (Sauer et al. 2019). In 1993–2008, the annual catch of *O. sinensis* in Japan was around 50,000–60,000 t. However, since 2009, it has declined to around 30,000–40,000 t (Ministry of Agriculture, Forestry and Fisheries Statistics Department 2021). In addition, octopuses are vulnerable to low salinity and fluctuations in water temperature, and in the Seto Inland Sea, which is the main production area in Japan for *O. sinensis*, the catch tends to fluctuate significantly due to the effects of precipitation (Yamazaki et al. 2015). Against this background, a large amount of *Octopus vulgaris* (which is closely related to *O. sinensis*) is imported: 100,000–120,000 t per year (Okutani 2013). However, in recent years, the global annual catch of various species of common octopus has fluctuated and overfishing is a problem (Gleadall 2016; Sauer et al. 2019), so it is desirable to establish reliable techniques of aquaculture for these species to augment efforts of maintaining sustainable supplies.

Octopuses are highly territorial and cannibalism occurs when their density in the habitat increases (Ibanez and Keyl 2010). Regardless of the size of the octopus, individuals introduced later are often attacked by those already resident, so adding octopuses to an aquarium that already contains other octopuses should be avoided (Kawamoto 1967). Cannibalism among octopuses of the genus *Octopus* (such as *O. vulgaris*) is well known, with octopuses of smaller size falling prey to larger individuals (e.g., Hernández-Urcera et al. 2014). Therefore, in conventional aquaculture, in order to prevent cannibalism, the number of octopuses in a tank is regulated and the octopuses are temporarily maintained in other tanks before being released into the breeding tank at the same time to minimize territoriality (Inoue 1969).

Normally, for other aquaculture organisms, the incidence of cannibalism can be reduced by methods such as increasing the number of refuge areas and installing shelters, as shown also for octopuses (Chapela et al. 2006). However, in aquaculture experiments, shelters are typically confined to the two-dimensional space at the bottom of the culture tank. Under some circumstances, shelters such as octopus pots (as used by fishermen) may obstruct the circulation of water in the tank, and if an octopus dies inside a pot it may be difficult to detect, risking the deterioration of water quality. In addition, since each octopus pot is occupied by just one octopus, it is necessary to prepare a far larger number of pots than the number of individuals present (e.g., Iglesias et al. 2014). In view of these problems, it is important to design a type of shelter that can be used by large numbers of octopus, preferably within a relatively limited volume.

Therefore, the purpose of the present research is to develop a new type of shelter composed of square adjustable plates, and to examine the arrangement and combination that matches the spacing width with an optimum frequency of utilization of the shelter by octopuses in aquaculture tanks.

Materials and methods

Octopuses

Octopuses (*O. sinensis*) caught in Ise Bay and Mikawa Bay were purchased in October 2019 and March, May, July, and November 2020. In addition, octopuses caught by net between April and September 2020 around the Masaki Lighthouse in Miho, Shizuoka, were used. The captured octopuses were kept in an outdoor aquarium at the Shimizu Campus of Tokai University. They were housed in a large 7.0 kL circular water tank (diameter 3 m, height 1 m) lined with an inert plastic sheet, or a small 0.8 kL square fiber-reinforced plastic (FRP) water tank (150 × 90 × 60 cm). The large tank was supplied with natural seawater from an underground marine aquifer flowing at 8.2 L min^{-1}. A pressure filter (3D-HP610; Eiko Corporation) was installed to remove silt particles. The small tank was supplied from the same seawater source at a flow rate of about 0.6 L min^{-1}. The water temperature during the rearing period was 19.7 ± 0.03 °C. During this period, the octopuses were fed frozen clam, crab, and sand lance to apparent satiety, once a day. For experiments, these octopuses were moved to their allotted experimental tank (where no food was provided).

Observations on the number of gaps utilized simultaneously by octopuses in a shelter with square plates (experiments 1 and 2)

Experiments were carried out using 18 male and 12 female octopuses (w/w 511.2–1901.3 g and 542.6–1288.3 g, respectively), which were held separately in 7.0 kL circular water tanks similar to the large experimental tank. These octopuses were identified as male or female by the presence or absence of a hectocotylus (the modified third right arm of mature males; Gleadall 2016).

A shelter was created by connecting six square black PVC plates with sides of 450 mm, using four screw-threaded PVC rods of 500 mm in length, adjusting the space between the plates with spacers of length 80 mm (Fig. 1). The

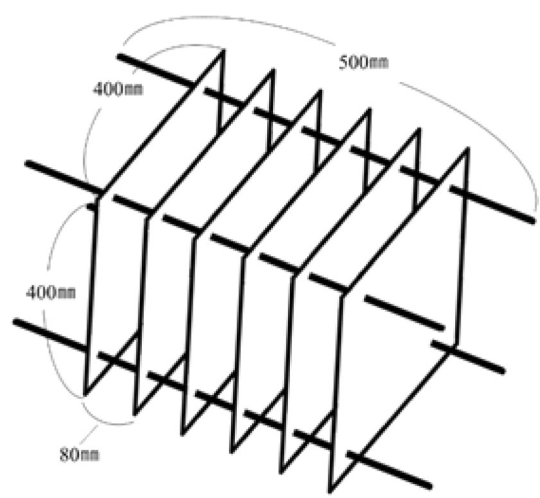


Fig. 1 Shelter with 6 square plates and a gap width of 80 mm. Used in experiments 1 and 2

experiment was conducted under two different conditions, either with just one shelter (experiments 1M and 1F) or with two shelters (experiments 2M and 2F). First, the octopuses were placed in an experimental tank without a shelter and allowed to accommodate to the conditions for about 30 min. Either one or two shelters were then placed in the middle of the experimental tank with the plates oriented vertically. The number of octopuses within the shelter was recorded every 10 min for 60 min.

A total of 40 trials were conducted with male and female octopuses, 10 times each under the two conditions. If an octopus was attached to the outside of the shelter, or if only the arms were placed within the shelter, that octopus was not included as "using" the shelter (Fig. 2). From counting the number of octopuses using the shelter, the gap utilization ratio was calculated using the following formula:

$$\text{Gap utilization ration} = \text{Number of octopuses using shelters}/\text{Number of between - plate gaps in the shelter(s)}$$

The gap utilization ratio is a measure of how many octopuses used in the experiment could use the shelter at the same time.

The wet weight of each octopus was measured at the end of the experiment (wet weight being a more convenient measure than, e.g., dorsal mantle length). For each condition, the experiment was repeated ten times and the results were fitted to a binomial distribution, then the probability of the highest number of individuals using the shelter was examined using a chi-square goodness-of-fit test. The relationship between the passage of time (in 10-min intervals) and the number of individuals using the system was examined by regression analysis using the least-squares method.

This experiment was conducted between 20 and 27 December 2020.

Preferences in shelter orientation (experiments 3–5)

In these experiments, the number of shelter occupants was compared when the shelter plates were placed horizontally or vertically. To take into account the effect of different wet weights of octopus on the selectivity of the shelter, the gap width of the shelter was divided into 40 mm (experiment 3), 60 mm (experiment 4), or 80 mm (experiment 5) for each condition. For the vertical arrangement, the shelter was created by connecting four square black PVC plates with sides of 300 mm with a PVC rod and spacers of total length 400 mm between the two end plates. The number of gaps in this shelter was set to three (Fig. 3a). When placed horizontally, the number of plates was reduced to three, such that there were three gaps including the gap between the bottom of the tank and the first horizontal plate (Fig. 3b). In addition, three other types of shelter were constructed, with gap widths of 40, 60, or 80 mm.

The experimental tank (0.8 kL square FRP aquarium) contained two shelters: one with the plates horizontal and the other with vertical plates. To eliminate any differences in octopus shelter selection criteria depending on the location of the shelters, four different arrangements of shelter pairs

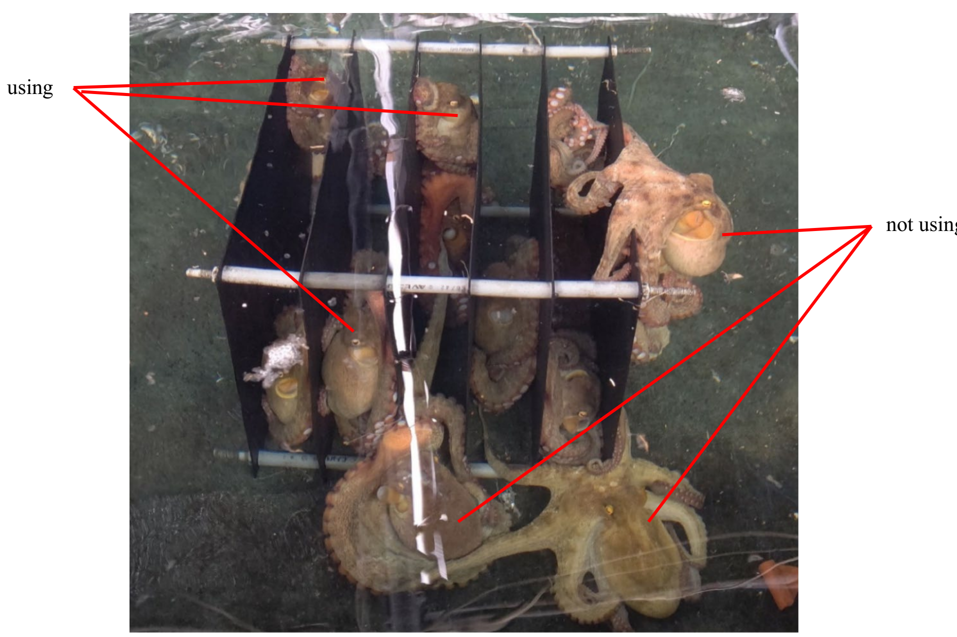


Fig. 2 Shelter use by octopuses. Individuals not entirely between two plates are classified as "not using" the shelter. Used in experiments 1 and 2

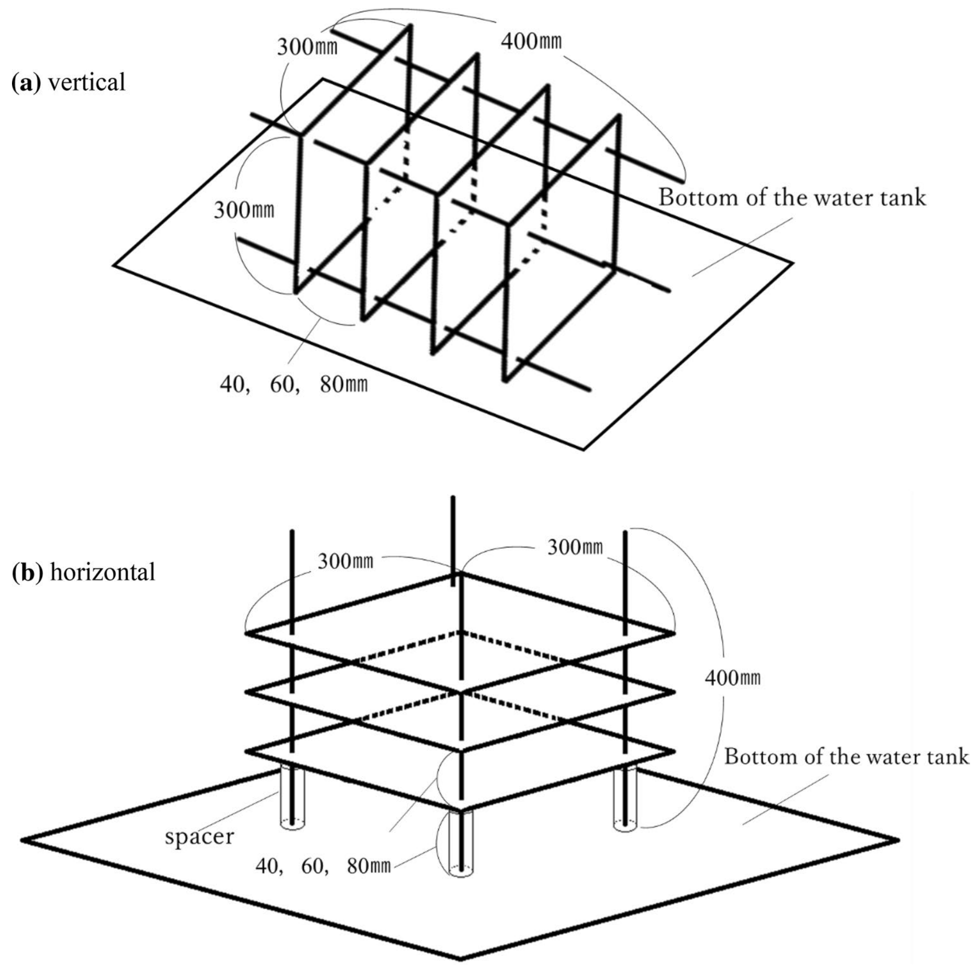


Fig. 3 Shelter with plates placed perpendicular to the bottom of the aquarium. **a** When the plate is placed vertical at the bottom of the tank. **b** When the plate is placed horizontal at the bottom of the tank

were used (Fig. 4). Each octopus used in the experiment was observed once in each of the four different shelter pair arrangements.

In experiments using a shelter with a gap width of 40 mm, 21 octopuses of wet weight (w/w) 48.8–897.6 g were used; for a gap width of 60 mm, 47 octopuses (w/w 13.6–1109.7 g) were used; and for a gap width of 80 mm, 23 octopuses (w/w 63.0–897.6 g) were used. For each experiment, the octopus was monitored continuously for 1 hour with a video camera (Panasonic HC-V550M) mounted above the tank. The octopus was considered to have selected the shelter placed in that orientation when the same gap was used continuously for 30 min or more (different from the previous experiment, where gap occupancy was counted every 10 min). To determine whether octopuses prefer the shelter plates to be placed horizontally or vertically, the number of times each octopus used a shelter was examined for significant differences using the Wilcoxon signed rank test.

This experiment was conducted during two periods: from 9 October to 30 November 2019 and from 8 June to 7 August 2020.

Gap width preference (experiment 6)

To investigate the shelter gap width preferred by the octopuses, 51 individuals (w/w 11.1–1109.7 g) were tested. The shelter for this experiment was constructed by connecting six square black PVC plates with sides of 300 mm with four screw-threaded PVC rods of length 400 mm. The five gaps between the plates were spaced at 20, 40, 60, 80, and 100 mm (Fig. 5). To eliminate any selection effects due to location of each gap size within the shelter, the octopuses were tested under five different gap arrangements (Fig. 5). Shelter and octopus were housed in a 0.8 kL square FRP tank. Five observations were made for each octopus used in the test: once for each of the five different gap arrangements. The experimental tank was monitored continuously for 1 hour with a video camera. The octopus was considered to have selected the shelter if it used the same gap continuously for more than 30 min during the hour. The wet weight of the individual was measured at the start of the experiment. The results were analyzed to determine if there was a correlation between the use of each gap width and the wet

Fig. 4 Four combinations of shelter positioning. Used in experiments 3, 4, and 5

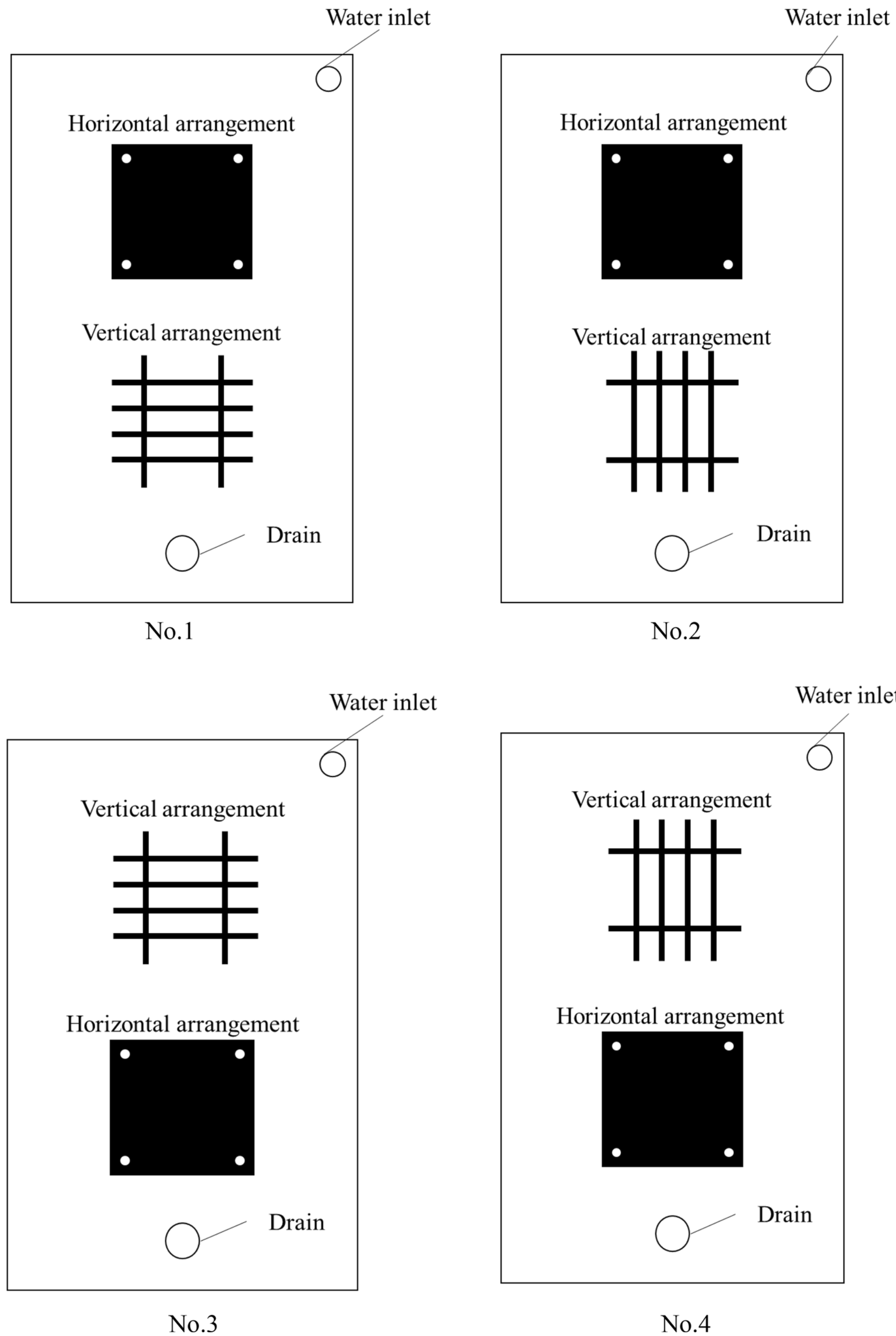
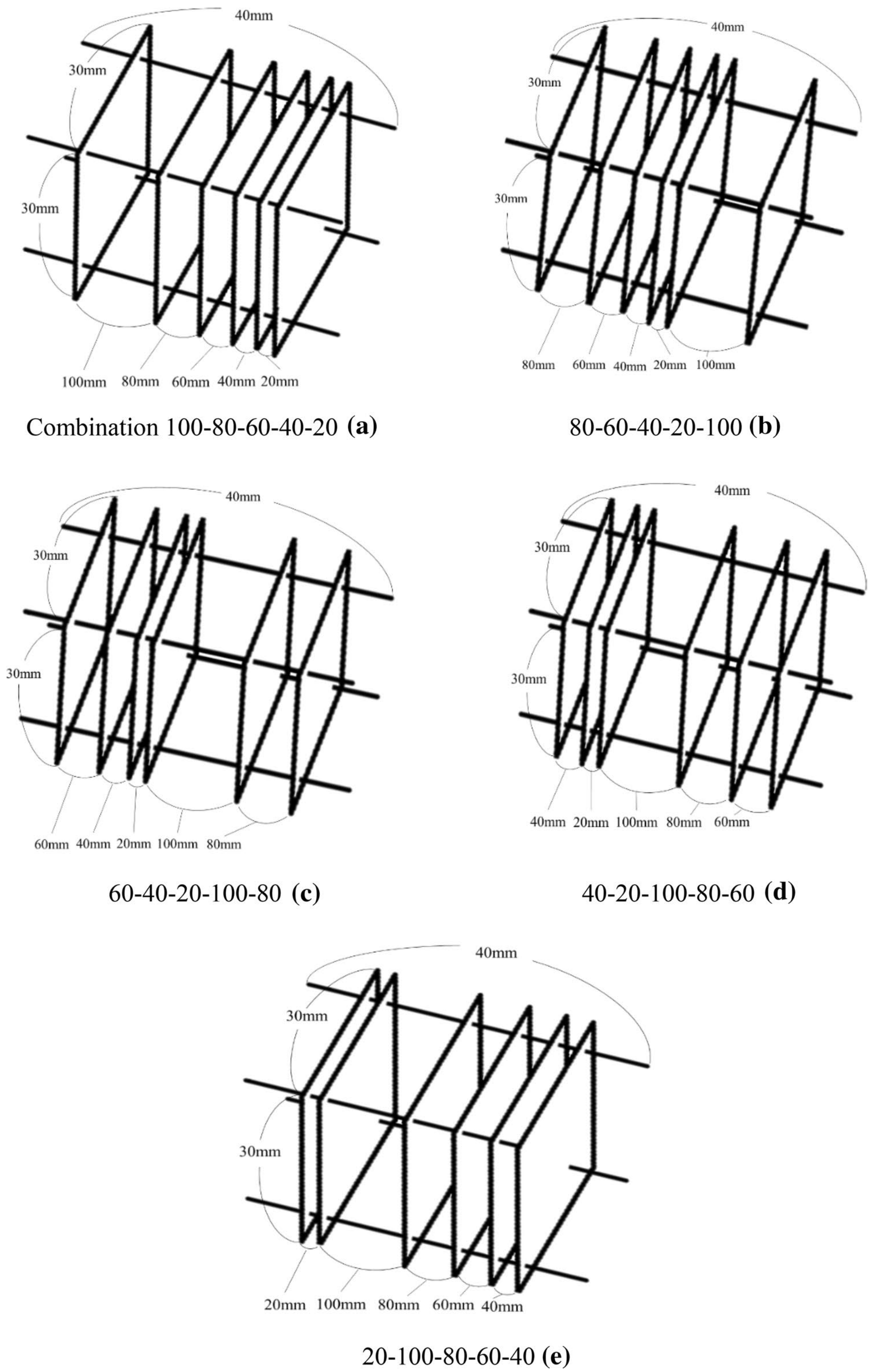


Fig. 5 Shelter with plates separated by different gap widths of 20–100 mm. Used in experiment 6



weight of the octopus by determining the correlation coefficient and using a *t* test.

This experiment was conducted from 10 October to 29 November 2019 and 14 April to 2 July 2020.

Results

Observations on the number of gaps utilized by multiple octopuses in a shelter with square plates (experiments 1–2M, 1–2F)

Experiment 1M: one shelter was placed in an aquarium containing 18 male octopuses. In a shelter with five gaps: after 10 min with the shelter, 2–7 octopuses were using the shelter, and after 60 min there were 3–9 octopuses using it. In ten experiments repeated with the same octopuses, each time the experiment was run, the proportion of individuals using the shelter was calculated and fitted to a binomial distribution. The resulting binomial probability revealed that the highest number of individuals using the shelter for this experiment was 4, 10 min after the start of the experiment, reaching a maximum of 7 at 40 min, and tending to remain constant thereafter (until the run was completed at 60 min after introducing the shelter). This demonstrated that the number of octopuses using the shelter tended to increase with time, reaching a maximum at about 40 min ($R^2 = 0.84$, $n = 6$, $p < 0.01$, Table 1).

Examining the number of octopuses occupying the shelter at each 10-min interval during the 60-min experiment revealed that, in 10 of a total of 60 observations, half of the octopuses used the shelter at the same time, up to a maximum of nine octopuses. In this experiment, the gap utilization ratio was 1 or more on 45 of 60 occasions, with a maximum of 1.8. Therefore, it was confirmed that several octopuses can utilize one gap at the same time (Table 1).

Experiment 2M: when two shelters were placed in the aquarium with the same 18 male octopuses (so the number of gaps available is 10), 4–11 octopuses used one of the shelters within the first 10 min, and after 1 hour, there were 9–15 octopuses using them. As found when testing with a single shelter, the proportion of individuals using a shelter during the 1-hour duration of the experiment was calculated in ten repeated experiments and fitted to a binomial distribution. The resulting binomial probability revealed that the highest number of octopuses using a shelter was 6 at 10 min after the start of the experiment, and reaching a maximum of 11 after 50 min, at which it remained until the end of the experiment. This confirmed that the number of octopuses using a shelter increased with time ($R^2 = 0.94$, $n = 6$, $p < 0.01$, Table 1).

Out of a total of 60 observations, on 38 occasions more than half of the octopuses were using a shelter at the same time, up to a maximum number of 15. In this experiment, the gap utilization ratio was observed to be 1 or more on 24 of 60 occasions, with a maximum value of 1.5. With two shelters, the number of gaps being used increased compared with when there was only one shelter, so the number of users per shelter tended to be lower (Table 1).

Experiment 1F: when using female octopuses, one shelter was housed in an aquarium containing 12 female octopuses. After the first 10 min, 2–6 octopuses were using the shelter and after 60 min, 3–9 octopuses. As with male octopuses,

Table 1 Number of octopuses using the shelter

Number of shelter sets	Experiment Elapsed time	1st run	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	Number of octopuses with the highest binomial probability
One set (Experiment 1M)	10 min	7	4	6	4	4	2	3	2	3	5	4
	20 min	8	6	7	3	6	5	3	3	5	9	5
	30 min	8	5	7	4	6	4	5	6	8	9	6
	40 min	8	7	9	3	6	7	6	7	8	9	7
	50 min	9	8	7	3	6	8	8	9	8	9	7
	60 min	9	9	8	3	5	8	8	8	8	9	7
Two set (Experiment 2M)	10 min	7	6	5	5	7	11	6	4	8	7	6
	20 min	8	6	7	6	10	13	9	6	8	8	8
	30 min	9	10	9	8	9	14	12	8	9	8	10
	40 min	9	9	9	8	10	15	11	8	9	9	10
	50 min	10	12	10	10	9	14	14	8	9	10	11
	60 min	10	12	11	11	10	15	12	9	9	11	11

Total number of octopuses: 18 males. Binomial probability: The number of octopuses, obtained from the binomial distribution when the number of shelter users is highest at each 10-min interval throughout the hour-long experiment. In experiment 1, the gap utilization ratio was > 1 for more than five individuals; and in experiment 2, it was > 1 for more than ten individuals

Table 2 Number of octopuses using the shelter

Number of shelter sets	Experiment Elapsed time	1st run	2nd	3rd	4th	5th	6th	7th	8th	9th	10th	Number of octopuses with the highest binomial probability
One set (Experiment 1F)	10 min	4	4	5	4	5	3	4	6	4	3	4
	20 min	4	5	5	3	5	5	6	6	4	3	4
	30 min	4	5	6	4	5	6	6	5	6	4	5
	40 min	4	5	7	4	5	7	6	6	6	6	5
	50 min	4	6	7	5	5	8	6	6	6	6	6
	60 min	5	7	5	5	5	9	5	6	7	6	6
Two set (Experiment 2F)	10 min	8	3	4	6	7	4	6	6	6	7	6
	20 min	7	4	6	8	8	5	8	8	8	8	7
	30 min	6	6	8	8	10	8	9	10	8	9	8
	40 min	8	5	8	8	11	9	9	10	8	9	9
	50 min	7	6	8	9	12	8	11	9	9	9	9
	60 min	9	9	9	9	12	8	11	10	8	9	10

Total number of octopuses: 12 females. Binomial probability: The number of octopuses, obtained from the binomial distribution when the number of shelter users is highest at each 10-min interval throughout the hour-long experiment. In experiment 1, the gap utilization ratio was > 1 for more than five individuals; and in experiment 2, it was > 1 for more than ten individuals

the proportion of individuals using the shelter at each 10-min time interval was calculated for ten repeated experiments and fitted to a binomial distribution. The resulting binomial probability revealed that the highest number of individuals using the shelter for this experiment was four at the first 10-min interval after the start of the experiment, reaching a maximum of six at 50 min where it remained until the end of the experiment, again demonstrating that the number of octopuses using the shelter increased with time after its introduction to the tank ($R^2 = 0.91$, $n = 6$, $p < 0.01$, Table 2).

Counting the number of shelter users every 10 min over the 60 min duration of the experiment showed that more than half of the octopuses used the shelter at the same time in 25 of 60 experiments. The maximum number of octopuses using the shelter at the same time was nine. The gap utilization ratio was observed to be 1 or more on 43 of 60 occasions, with a maximum of 1.8. Therefore, as with male octopuses, it was confirmed that several octopuses can utilize one gap at the same time (Table 2).

Experiment 2F: when two shelters were placed in the aquarium with the same 12 female octopuses (so the number of gaps available is 10), 3–8 octopuses used one of the shelters within the first 10 min, and after 1 h there were 8–12 octopuses using them. As with experiments using a single shelter, the number of octopuses using a shelter was calculated and fitted to a binomial distribution. The resulting binomial probability showed that the highest number of octopuses using the shelter was 6 at 10 min after the start of the experiment, increasing to 9 at 40 min, and reaching the maximum of 10 at 60 min The number of octopuses using the shelters therefore increased throughout the experiment ($R^2 = 0.94$, $n = 6$, $p < 0.01$, Table 2).

Out of a total of 60 observations, on 54 occasions more than half of the octopuses were using a shelter at the same time. The maximum number of octopuses using a shelter simultaneously was 12, confirming that all octopuses participating in experiment 2F occupied a shelter at the same time. The gap utilization ratio was observed to be 1 or more on 9 of 60 occasions, with a maximum value of 1.2. Due to the increase in the number of gaps compared with when only one shelter was present, the proportion of octopuses using a shelter tended to be relatively low (Table 2).

In these four experiments (experiments 1–2M, 1–2F), there was no significant difference in the behavior of males and females. The number of octopuses using the shelter tended to increase over time and, as the number of gaps available was increased, the number of octopuses using a given gap at the same time tended to decrease.

Preferences in shelter orientation (experiments 3–5)

Experiment 3: considering occupancy of vertical versus horizontal orientation of the shelter plates when the gap width was 40 mm, 15 of 21 octopuses used either the vertical shelter, the horizontal shelter, or both. The breakdown of the behavior of these 15 octopuses is as follows. After four trials of the experiment, one octopus used the vertical plate shelter on all four occasions, but no octopuses used the horizontal shelter. Of the octopuses using the shelter 3 or 4 times out of four trials, nine octopuses used the vertical shelter more often and five octopuses used the horizontal shelter more often. There was no significant difference between the number of users of the vertical or horizontal shelters (Wilcoxon signed rank test: $z = 1.39$, $p > 0.05$, Fig. 6a).

Experiment 4: when the gap width of the shelter was 60 mm, 43 of 47 octopuses tested used either the vertical shelter, the horizontal shelter, or both. The breakdown of the 43 octopuses is as follows. After four trials, six octopuses used the vertical shelter on all four occasions. Of the octopuses using the shelter 3 or 4 times out of four trials, 24 octopuses used the vertical shelter more often; seven octopuses more often used the horizontal shelter; and six octopuses used vertical and horizontal shelters equally. Thus, the number of octopuses that preferred vertical plates was significantly higher than that of octopuses preferring a horizontal arrangement (Wilcoxon signed rank test: $z = -4.01$, $p < 0.05$, Fig. 6b).

Experiment 5: with a gap width of 80 mm, the same tendency as in experiment 4 was observed. Of the 23 octopuses used in this experiment, 22 used either vertical or horizontal shelters, or both: after four trials, two octopuses used the vertical plate shelter in all four trials; of those using the shelter over 3 or 4 trials, 14 used the vertical shelter more often; three preferred the horizontal shelter; and three used the vertical and horizontal shelters equally. Thus, the number of octopuses that preferred vertical placement was significantly higher than the number of octopuses preferring horizontal placement (Wilcoxon signed rank test: $z = -2.97$, $p < 0.05$, Fig. 6c).

Use of horizontal gaps

In this series of experiments, 60.7% of the octopuses used the horizontal shelters with the gap width set at 40 mm (experiment 3), 34.7% at 60 mm (experiment 4), and 40.4% at 80 mm (experiment 5). In all three experiments, the gap formed between the lowest plate in the shelter and the tank floor was used most often: 94.1% for 40 mm gaps (experiment 3), 97.0% for 60 mm (experiment 4), and 85.7% for 80 mm (experiment 5). The second and third storeys were used by octopuses only three times where gaps were 80 mm (experiment 5), and once each for gaps of 40 mm and/or 60 mm (experiments 3 and 4).

Gap width preference (experiment 6)

Considering occupation of the 20 mm gaps, 12 octopuses (w/w 11.1–253.7 g) used the shelter at least once and up to five times out of five trials; larger octopuses, though, did not use the shelter with such relatively narrow gaps, indicating a negative correlation between octopus wet weight and shelter use for the 20 mm gap ($r = -0.527$, $n = 51$, $t = 4.342$, $p < 0.05$, Fig. 7a).

For the 40 mm gaps, 26 octopuses (w/w 11.1–695.2 g) used the shelter at least once and for up to four of the five trials. As with 20 mm gaps, there was a negative correlation between the wet weight of the octopus and the number of times the shelter was used, and the octopuses of larger size (w/w) did not use the 40 mm gap at all ($r = -0.506$, $n = 51$, $t = 4.110$, $p < 0.05$, Fig. 7b).

Considering the gap widths of 60, 80, and 100 mm, the numbers of octopuses using the shelter 1–3 times out of 5 trials were 26 (w/w 36.6–1054.6 g), 31 (w/w 13.6–1109.7 g), and 31 (w/w 36.6–1054.6 g), respectively. In these experiments, there was no correlation between octopus wet weight and the number of times the shelter was used, particularly for gaps of 60 or 100 mm (60 mm: $r = 0.179$, $n = 51$, $t = 1.272$, $p > 0.05$, Fig. 7c, 100 mm: $r = 0.287$, $n = 51$, $t = 2.099$, $p > 0.05$, Fig. 7e), although with gaps of 80 mm there was a tenuous correlation between wet weight and number of uses ($r = 0.296$, $n = 51$, $t = 2.172$, $p < 0.05$, Fig. 7d). In short, gaps larger than 60 mm tended to be used regardless of the size of the octopus.

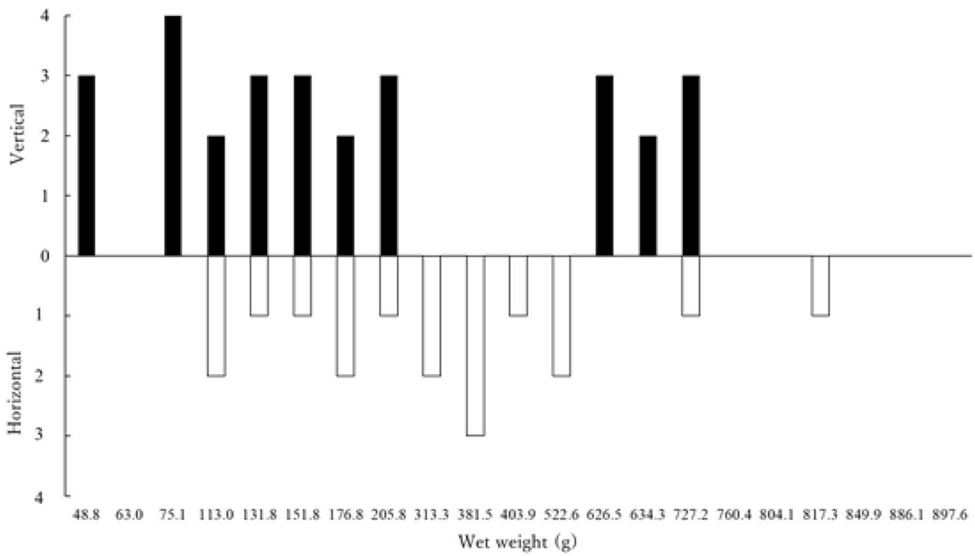
Discussion

Octopuses use various objects in the water as hiding places. In the Mediterranean, the percentage of hiding places chosen by *O. vulgaris* in nature was examined and it was found that about two-fifths of the octopuses examined used discarded human objects such as bottles, a similar number used holes in the substrate, a fifth of them hid under stones, and a few used shells (Katsanevakis and Verriopoulos 2004).

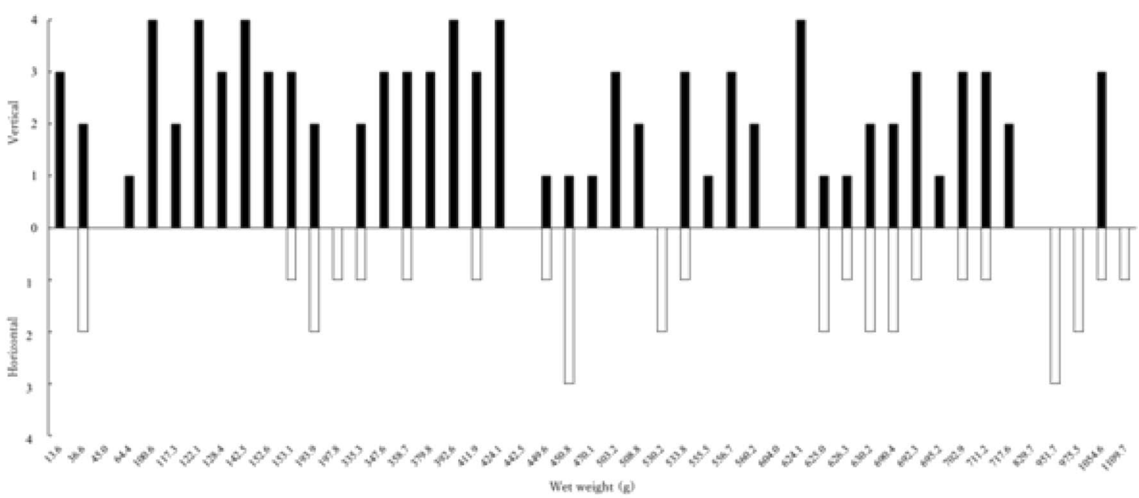
Artificial shelters for octopuses have been installed in the Seto Inland Sea in Japan for the purpose of creating spawning grounds, and it is known that octopuses are using them (Shinohara 2000). In the Aegean Sea, *O. vulgaris* has been found to make ample use of artificial concrete shelters submerged in the sea (Ulaş et al. 2019). Shelters made from PVC piping have also been used for the same purpose, and are known to allow females to lay eggs (Mereu et al. 2018). This emphasizes that octopuses are mollusks without a shell, and so require a hiding place as a means of protection and prefer enclosed spaces. Thus, octopus pots are used not only for fishing for octopus, but also for rearing them.

There are several previous reports on the use of artificial shelters in octopus farming. In a study using gardening pots as shelters instead of octopus pots in octopus aquaculture, it was shown that octopuses used the shelters to the same extent as typical octopus pots and that there was no effect on growth (Kadowaki et al. 2018). In addition, an experiment to verify the effectiveness of a rearing tank using discarded tires as shelters reported that they were used by octopuses to the same extent as PVC shelters and survival rates were good (Kwon and Kim 2015). Thus, even in captivity, octopuses

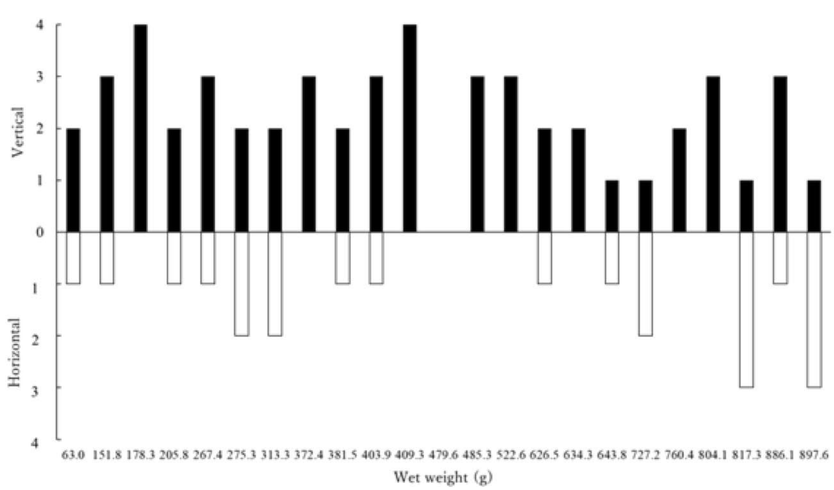
(a) Gap width is 40 mm.



(b) Gap width is 60 mm.



(c) Gap width is 80 mm.



◂**Fig. 6** Shelter use by individuals of different size for perpendicular and horizontal gaps with a uniform gap width of 40–80 mm. Upper scale (black bars): Number of octopuses using vertical shelters. Lower scale (white bars): Number of octopuses using horizontal shelters. **a** Gap width is 40 mm. Results of experiment 3. **b** Gap width is 60 mm. Results of experiment 4. **c** Gap width is 80 mm. Results of experiment 5. Shelters used: Fig. 3a, b

can use a variety of materials as shelters, and these are useful to control cannibalism and by providing octopuses with a relatively secure refuge to protect their soft body.

The type and configuration of shelter used in the present study contributes to resolving problems with individual pots and, in addition, the octopuses are easy to observe. According to the results of experiments 1 and 2, when a large number of octopuses were kept in the tank with the shelter design used in this study, it was confirmed that one gap could be used by several octopuses at the same time. It is known that octopuses are asocial in the wild, tending to occupy their own individual territory and avoiding contact with other octopuses (Hanlon and Messenger 2018). *Octopus briareus* was found to use PVC shelters even when they were adjacent to each other, but there was never more than one octopus occupying the same shelter (Duncan et al. 2021). However, reports exist of high local octopus densities in wild populations of some octopus species (Scheel et al. 2017; Aronson 1986). These high densities have been attributed to habitat and prey availability (Scheel et al. 2017), as well as relaxation of predation pressure (Aronson 1986). In the experiments of the present study, simultaneous use of a single gap by several octopuses occurred often, and it was confirmed that octopuses can use one shelter at high density. In this situation, there is a risk of cannibalism due to their close contact with each other. However, no cannibalism was observed during this series of experiments. In other experiments, when octopuses were kept in suspended cages, it was mentioned that cannibalism may not have occurred because the males and females were kept separately and there were more shelters (Chapela et al. 2006). In their experiments, the difference in the size of the octopuses used was relatively small, and the fact that the males and females were kept separately may be a factor in suppressing cannibalism. However, in the present study, the effect of the shelter itself as a factor to suppress cannibalism requires further investigation.

The effectiveness of such shelters with partitions that form gaps through which animals can reliably shelter has also been confirmed in other marine organisms. For example, in the rearing of the fishes *Sebastes oblongus* and *Epinephelus akaara*, the use of shelters with perforated plates in the sea has been devised for releasing juvenile fishes, enabling them to avoid predation because larger fishes cannot enter the shelter (Miyagawa et al. 2012). The shelter created in the present study may deter cannibalism by other octopuses and protect them because the width of the gap can be adjusted to an optimal size depending on the size of the octopuses present.

Placement and appropriate spacing of shelter panels

The shelter arrangement preferred by the octopuses was verified in experiments 3–5M. When the plates were placed vertically and horizontally with gap widths of 60 and 80 mm (experiments 4M, 5M), there was a significant preference for vertical placement. When the shelter was placed horizontally, there was a tendency to use mainly the gap formed between the lowest plate and the tank bottom. The reason why this trend was observed is that octopuses can swim by spraying water from a funnel, but most of them crawl along the seafloor (Hanlon et al. 2018). Therefore, when the shelter dividers were arranged vertically, each plate and each gap was in contact with the bottom of the tank, so any gap could be used and the octopus could easily recognize it as a shelter.

There are various parameters that affect an organism's choice of hideout. The catfish *Silurus (parasilurus) asotus* dislikes vibrations but the flow of water is also important; therefore, in culture conditions suitable hideouts are selected at sites where there is a significant current flow but distant from the water inlet (Narita et al. 2000). Also, for the salmonid fish *Salvelinus leucomaenis pluvius*, it is important that swimming effort is minimized and that the shelter conveys safety while enabling food to be obtained close by (Yamashita et al. 2016).

In *Octopus tehuelchus*, the type of shelter and the size of the octopus were found to be related to the size and volume of the shelter, with larger and safer shelters being important mainly for females (Narvarte et al. 2013). When predation pressure from enemies (such as other octopuses and moray eels) increases, *O. tetricus* will either block the entrance to the shelter with rocks or coral to improve its defenses, or move to a shelter that matches its size (Anderson 1997). Thus, it is thought that the size of the shelter is a factor in the octopus's ability to protect itself. Therefore, the shelter needs to be of a size appropriate for the octopus to be comfortable using it.

Experiment 6M in the present study tested the gap size preference of octopuses of different sizes (w/w). The only hard parts of an octopus are its beaks and cartilaginous cranium, so it can pass through very narrow gaps. The relationship between body size and the gap through which it can pass has been verified by incorporating an escape ring into an octopus cage (for *Enteroctopus dofleini*) to allow smaller individuals to escape as a method of resource management. When the w/w of *E. dofleini* was 3 kg, the escape success rate was 50% when the diameter of the escape ring was 55 mm, but much less for rings smaller than that (Nagano et al. 2019). The inner diameter of the ring through which an

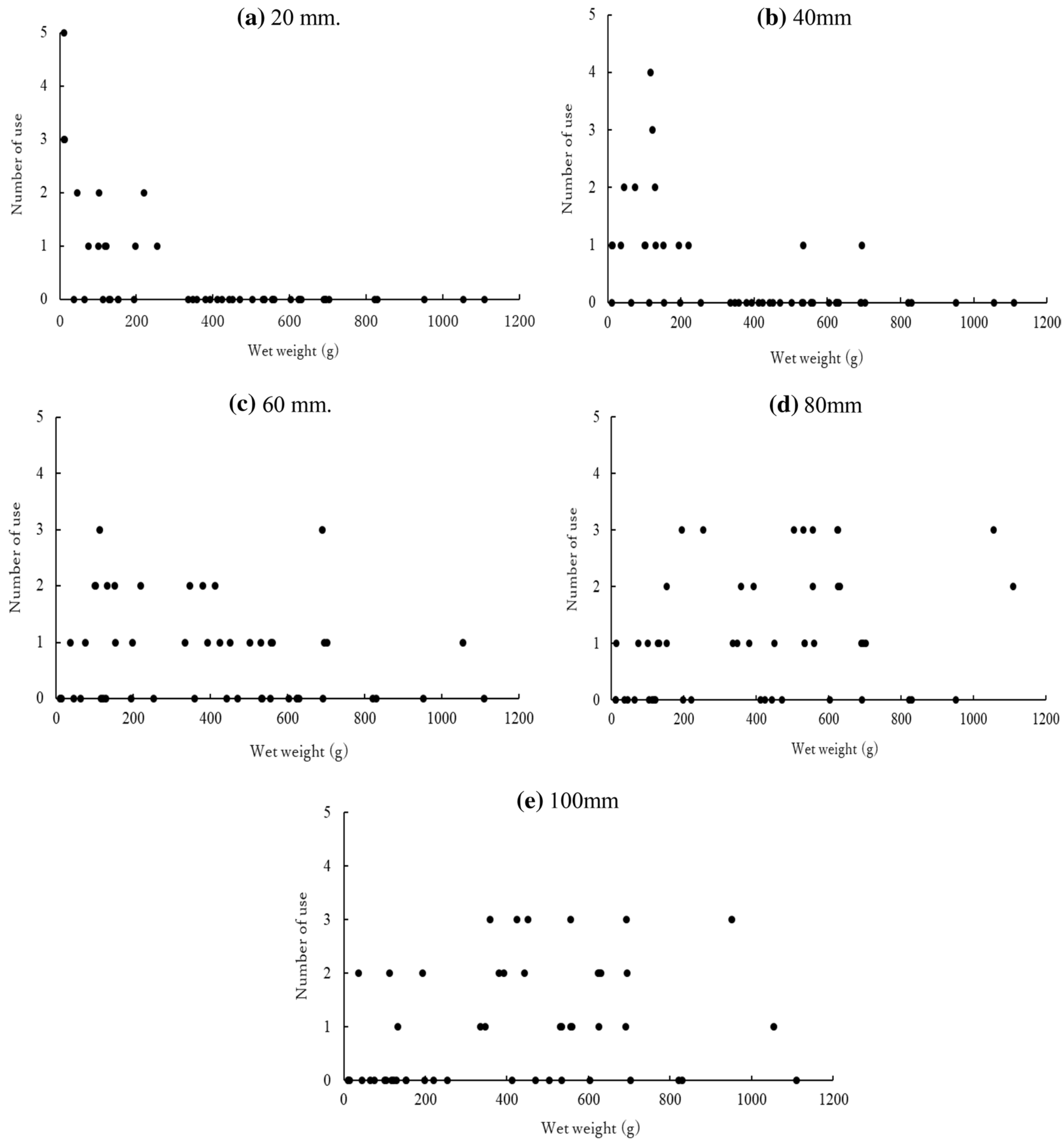


Fig. 7 Number of octopuses using each gap in shelters with five different gap widths. Shelters used: Fig. 5 (one for each type). **a** Gap width is 20 mm. **b** Gap width is 40 mm. **c** Gap width is 60 mm. **d** Gap width is 80 mm. **e** Gap width is 100 mm. Results of experiment 6

octopus can escape at a w/w of 300 g or less is about 27 mm; and when the diameter of the ring is 35 mm, an octopus of about 700 g cannot escape (Tateishi et al. 2012). It is also known that when the mesh size of the catch net is 55 mm, octopuses weighing 1000 g or less can pass through; and when the mesh size is 75 mm, octopuses of 2000 g can pass through (Shin et al. 2008). Thus, it is clear that there are differences in the gaps through which an octopus can pass depending on its size, and these differences affect the use of octopus pots.

With *O. vulgaris*, the size of the octopus caught depends largely on the size of the octopus pot, and it has been clarified that tubular clay octopus pots with an entrance diameter of 140 mm and a depth of 325 mm do not capture octopuses weighing more than 2800 g (Sobrino et al. 2011).

In conclusion, using gaps of different width between the shelter plates in the present experiments revealed that octopuses with a wet weight of about 300–1000 g did not enter shelters with gap widths of 20 or 40 mm, which apparently are considered to be too narrow by octopuses of this size range. This study has clarified that a reasonably large number of octopuses can simultaneously use shelters constructed of square plates of certain gap widths, with the plates oriented vertically. By adjusting the number and size of gaps between adjacent plates according to the size of octopuses present, this type of shelter is potentially of use for high-density culture of octopuses.

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