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Active provisioning of food to host sea anemones by anemonefish

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In mutualistic symbiosis, organisms often provide food to their partners. However, the processes and significance of food provisioning to hosts remain poorly understood. The anemonefish *Amphiprion clarkii*, which prefers larger hosts, has been suggested to provide food to its host the sea anemone *Entacmaea quadricolor*. In the present study, we investigated food provisioning by anemonefish and its effects on the symbiotic relationships. When given foods of various sizes and types in the field, anemonefish selectively consumed small animal food (krill, clams, squid, and fish) and green macroalgae of small size, while providing larger pieces of animal food to their hosts. Additionally, the anemonefish avoided either eating or providing brown macroalgae and sponges to the host anemone, which appeared to be unsuitable as food for both anemonefish and sea anemones. When repeatedly provided small pieces of animal food, the anemonefish initially consumed the food themselves, but upon satiety, increased provisioning to the host. Food provisioning positively influenced the growth of host anemones. These findings suggest that anemonefish actively provide food to host anemones based on the situation, adding to our knowledge of the mutual benefits of symbiosis among partners.

Keywords Symbiosis, Mutualism, Food provisioning, Fish, Sea anemone, *Amphiprion*

Mutualistic symbiosis is an interaction wherein different species living in the same location benefit from each other. In this interspecific relationship, nutritional exchanges between partners may develop to maintain interactions[1]. Most nutritional exchanges occur during endosymbiosis, during which both partners often provide nutrition to others. Corals and zooxanthellae[2,3], rhizobia and plants[4], and animals and their gut bacteria[5,6] are typical examples. Meanwhile, in most cases of ectosymbiosis, one symbiont provides nutrition, whereas the other provides a different form of service[7]. There are only a few known examples in which both symbionts provide nutrition to each other. For instance, the symbiotic system between epiphytes and ants consists primarily of trophic interactions[8–11]; the larvae of the lycaenid butterfly provide nectar to the ants and, in turn, receive food from their partners[12,13]; and the attine ants that cultivate fungi use them as a nutrient resource[14,15]. Thus, although there are few known examples, the provisioning of nutrients plays an important role in maintaining interspecific relationships in ectosymbiosis, and elucidating the function of food and the direction of provisioning is a fundamental approach to understanding the maintenance and evolution of these relationships.

Coral reef areas are highly productive environments but nutrient-poor[16–18]. In such habitats, interspecific interactions often compensate for nutrient shortage. For instance, hermatypic corals benefit from the nutrients discharged by fish and boring bivalves to the coral[19,20]. Similarly, cleaner shrimp, which live in sea anemones and attract client fish near the anemone, may cause anemones to absorb nutrients from clients[21]. Additionally, in goby-shrimp relationships, the excrement of symbiont fish is used as a nutrient source for the shrimp host[22]. The anemone-anemonefish relationship, one of the oldest known examples of symbiosis in the ocean environment, may also involve trophic exchange between partners (reviewed by Karplus[23]). In these relationships, both benefit from each other. Host anemones provide anemonefish with refuge and spawning sites[24–29]. Anemonefish also benefit host anemones by protecting them from predation[27,30–33], by aerating around the host[34], and by removing waste products and unwanted material from the host[27,35]. The size of the host anemone also influences the social structure of the resident anemonefish, with *Amphiprion percula* females colonising larger anemones being able to grow and lay more eggs[36]. Recent studies using stable isotopes have shown that nutrients are exchanged between the anemones (and their endosymbionts, zooxanthellae) and anemonefish[32,37–41]. However, in these examples, the benefit to the hosts was from the uptake of nutrients passively released by the ectosymbionts. It is unclear whether ectosymbionts in coral reef environments actively provide food to their hosts, which may have implications for the evolution and maintenance of this mutualism.

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Recently, an interesting behaviour of Clark's anemonefish, *A. clarkii* was observed in our field research. When a piece of clam was given, the anemonefish sucked it into its mouth and attached it to the host anemone *Entacmaea quadricolor* tentacles (Supplementary Movies S1–S3). Indeed, this behaviour is known since the nineteenth century[42] and can be easily induced in captivity[23,43,44]. The frequency of this behaviour may vary among species of anemonefish[24,25], and is likely related to anemonefish hunger[44], food size[23,44,45], and the presence or absence of competitors[46]. Several hypotheses have been proposed regarding the significance of the food-provisioning behaviour of anemonefish to the host[41,47,48]. One possible explanation is that anemonefish use anemones to tear small pieces of food to eat[42] or to eat food attached to the host later[48]. However, there is no clear evidence supporting these hypotheses[23,25,49]. Furthermore, because this behaviour is rarely observed in the wild[48,49], it may be of little importance in symbiotic relationships[23,30]. Therefore, the significance of the food-provisioning behaviour of anemonefish to the host remains unclear, and the role of food provisioning in forming the relationship between anemones and anemonefish has not been examined.

Here, we hypothesised that anemonefish would benefit their host sea anemone by providing food and investigated the factors affecting this behaviour and its effects on the host sea anemone. Specifically, we investigated whether (i) host anemones consumed the food provided by the anemonefish, (ii) the size and type of food and (iii) the hunger level of the anemonefish affected food-provisioning behaviour, and (iv) food-provisioning behaviour affected the growth of host anemones. Because the reproductive success of anemonefish is correlated with the size of the host anemone[36], increased growth of host anemones through food provisioning may benefit the anemonefish. To our knowledge, this is the first study to demonstrate the effects of food provisioning by anemonefish on host anemones.

Results

Consumption of provided feed by host anemone

To track the food provided by anemonefish to the host, bait krill tied to a 70 cm string was given to anemonefish at 26 hosts in total. In all cases, the fish attached the krill to the tentacles of the hosts immediately after receiving it. In all eight cases with no video recordings in 2020, the string stretched from the anemone's mouth after 1 h. In 9 of 18 cases videotaped in 2021, host anemones likely brought the krill to the mouth using tentacles 101 ± 17 min (mean ± s.d.) with the shortest time of 4 min after receiving it. In eight and nine cases in 2020 and 2021, respectively, the host anemones likely consumed krill. In the remaining nine cases in 2021, no krill reached the host mouth because of disturbance by other fish or unknown organisms in five cases and pulling out of the string by the anemonefish in four cases (probably because the anemonefish judged the string as a foreign substance for the host anemone). Anemonefish pecked and/or picked up and returned krill attached to the hosts in 10 of the 18 cases in 2021; however, no fish removed or consumed krill (see the dataset for more details). These observations suggest that anemonefish provide food to their hosts.

Size and types of feed and food provisioning by anemonefish

Seven different sizes (3, 5, 7, 10, 20, and 50 mm) of bait krill were randomly and continuously provided to the anemonefish (3–7 mm, five times each; 10–50 mm, twice each in maximum) and observed to determine whether the anemonefish consumed the krill by themselves or provided it to the host anemone. We found that feed size had a significant effect on food provisioning by anemonefish to the host. When the size of feed was smaller than 7 mm, the anemonefish were more likely to consume it themselves, whereas they were more likely to provide the feed if larger than 7 mm to the host anemone (binomial generalised linear mixed model [GLMM], $\chi_1^2 = 112.52$, $p < 0.0001$, $n = 206$ trials from 10 fish; Fig. 1). In particular, anemonefish never consumed feed of 20 or 50 mm in size and provided it to the host. Furthermore, no anemonefish abandoned the krill.

Seven different types of food were provided to each anemonefish to determine the type of feed to be provided to the host sea anemone. When the observer provided the anemonefish with the three types of animal food, a similar tendency was detected in the krill-providing experiment (see Fig. 1). All clams, squid, and almost all fish adjusted to 3 mm in size were consumed by the anemonefish itself, whereas those adjusted to 20 mm were mostly provided to the host sea anemone (Fisher's exact test, df = 2, $p < 0.0001$ for all three food types; Fig. 2). Conversely, in over 60% of the cases, the anemonefish consumed small pieces (3 mm) of the soft-green macroalga *Codium cylindricum* themselves, but rarely consumed or provided the host with large pieces (20 mm) of green macroalgae (df = 2, $p < 0.0001$; Fig. 2). When large pieces (20 mm) of fresh hard tests of the sea urchin *Echinometra mathaei* with the epidermis and tube foot were provided to the anemonefish, in over 60% of cases, the fish gave them to the host. However, the tests were rarely consumed or provided when presented with small (3 mm) pieces (df = 2, $p < 0.0001$; Fig. 2). When the hard brown macroalga *Sargassum hemiphyllum* was administered, all anemonefish abandoned pieces of any size (df = 2, $p > 0.99$; Fig. 2). The results for the sponges were similar to those for the brown macroalgae, except that the anemonefish ate small pieces (3 mm) twice out of the 96 trials (df = 2, $p = 0.50$; Fig. 2).

Hunger level and food provisioning by anemonefish

To examine the relationship between "hunger level" and the occurrence of food provisioning by anemonefish to the host, krill adjusted to 3 mm were continuously given to the anemonefish. The results showed that as the number of feed presentations to the anemonefish increased, the frequency of food provisioning to the host also significantly increased (binomial GLMM, $\chi_1^2 = 187.79$, $p < 0.0001$, $n = 1819$ trials of 21 fish of different groups; Fig. 3). During the first dozen food presentations to the anemonefish, krill was consumed by the anemonefish, and no food provision for the host was observed until the 17th presentation. The first food provisioning was observed between 18 and 76 presentations depending on the individual fish (Fig. 3). The effects of the sex of anemonefish and the time of day of the experiment on the probability of their feeding to the host were not statistically significant (binomial GLMM, male vs female: $\chi_1^2 = 1.88$, $p = 0.17$; AM vs PM: $\chi_1^2 = 0.71$, $p = 0.40$).

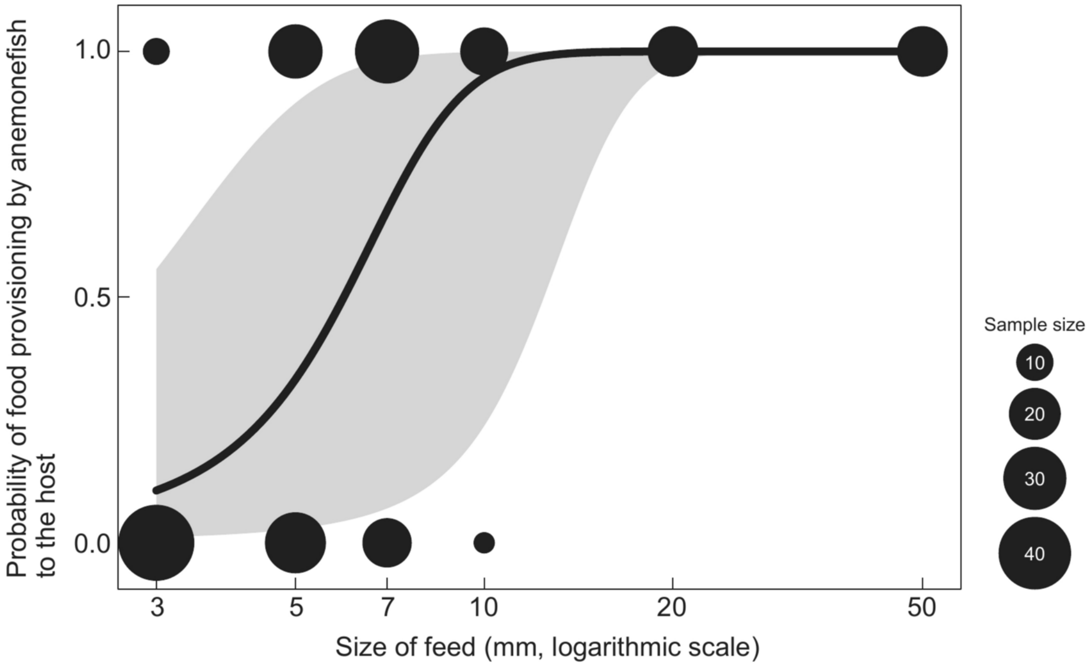

Fig. 1. Size of feed and probability of food provisioning by anemonefish to the host. When the anemonefish consumed the feed, the probability is 0, and when the anemonefish provided the feed to the host anemone, the probability is 1. The probability of food provisioning by anemonefish to the host significantly increased with feed size (3, 5, 7, 10, 20, and 50 mm). The black line represents the regression line with the shaded 95% confidence interval from the binomial GLMM ($n = 206$ trials from 10 fish). The size of the circles indicates the number of occurrences.

Effects of food provisioning by anemonefish on host growth

To examine the effect of food provisioning by anemonefish on host anemone growth, the surface areas of the oral disks of sea anemones were compared among three treatments: (i) provisioning feed to anemonefish ($n = 9$), (ii) direct provisioning feed to hosts ($n = 11$), and (iii) no food provisioning (control, $n = 11$). Two pieces of fish (approximately 50 g in total), which were too large for anemonefish to swallow, were provided to each of the two fed groups at intervals of approximately every 3 days. At the start of the experiment, the surface area of the oral disk did not differ among the three treatments (Fig. 4a; Kruskal–Wallis test, $\chi_2^2 = 1.15$, $p = 0.56$). At the end of the experiment (91–106 days after the start of the experiment), sea anemones in the group fed with anemonefish and the group fed directly to the host were significantly larger than those at the start of the experiment (Fig. 4a; exact Wilcoxon signed-rank test, $V = 0$, $p = 0.004$, $n = 9$ and $V = 0$, $p = 0.005$, $n = 11$, respectively), whereas only slight changes in anemone size were found in the control groups (Fig. 4a; $V = 20$, $p = 0.28$, $n = 11$). Therefore, as predicted, at the end of the experiment, the surface area of the two fed treatments was significantly greater than that of the non-fed treatment (Fig. 4a; Kruskal–Wallis test, $\chi_2^2 = 6.56$, $p = 0.04$). Consequently, the fed anemones showed a higher growth rate than the non-fed anemones (Fig. 4b; Kruskal–Wallis test, $\chi_2^2 = 8.62$, $p = 0.01$). Daily growth was significantly higher in the group fed anemonefish (Fig. 4b; exact Wilcoxon rank-sum test, adjusted $p = 0.009$) and tended to be higher in the group fed directly to the host than in the no-feeding treatment (adjusted $p = 0.07$). Daily growth did not differ between the two fed treatments (adjusted $p = 0.46$).

Food provisioning by anemonefish to the host in natural situations

In the experiments, the observer artificially provided feed to the anemonefish; however, the occurrence of such a situation in nature remains unknown. However, we observed two cases in which anemonefish carried dead animals to host sea anemones in the wild. On 2nd June and 2 July 2022, the anemonefish carried a small portion (3–4 cm square) of a sea cucumber (*Stichopus naso*) body to their host at a depth of 5.5 m. During this period, many dead sea cucumbers were found at the study site, probably because of the high sea temperatures in summer. The frequency of concurrence was 2% (twice in 100 scuba dives).

Discussion

Little is known about the processes and significance of food provision to hosts in mutualistic symbiosis. Using a field experimental approach, we tested predictions based on the hypothesis that anemonefish benefit host sea anemones by providing food. We found that: (i) anemonefish provided food for the hosts, and the host anemones likely consumed the food; (ii) anemonefish provided larger pieces of animal food, but not brown macroalgae, to the host, whereas they fed on animal food and green marcoalgae small enough to their mouth size when foods of various sizes and types were given to the anemonefish; (iii) the probability of food provisioning to the host increased with the satiety of the anemonefish; and (iv) food provisioning resulted in increased growth of the

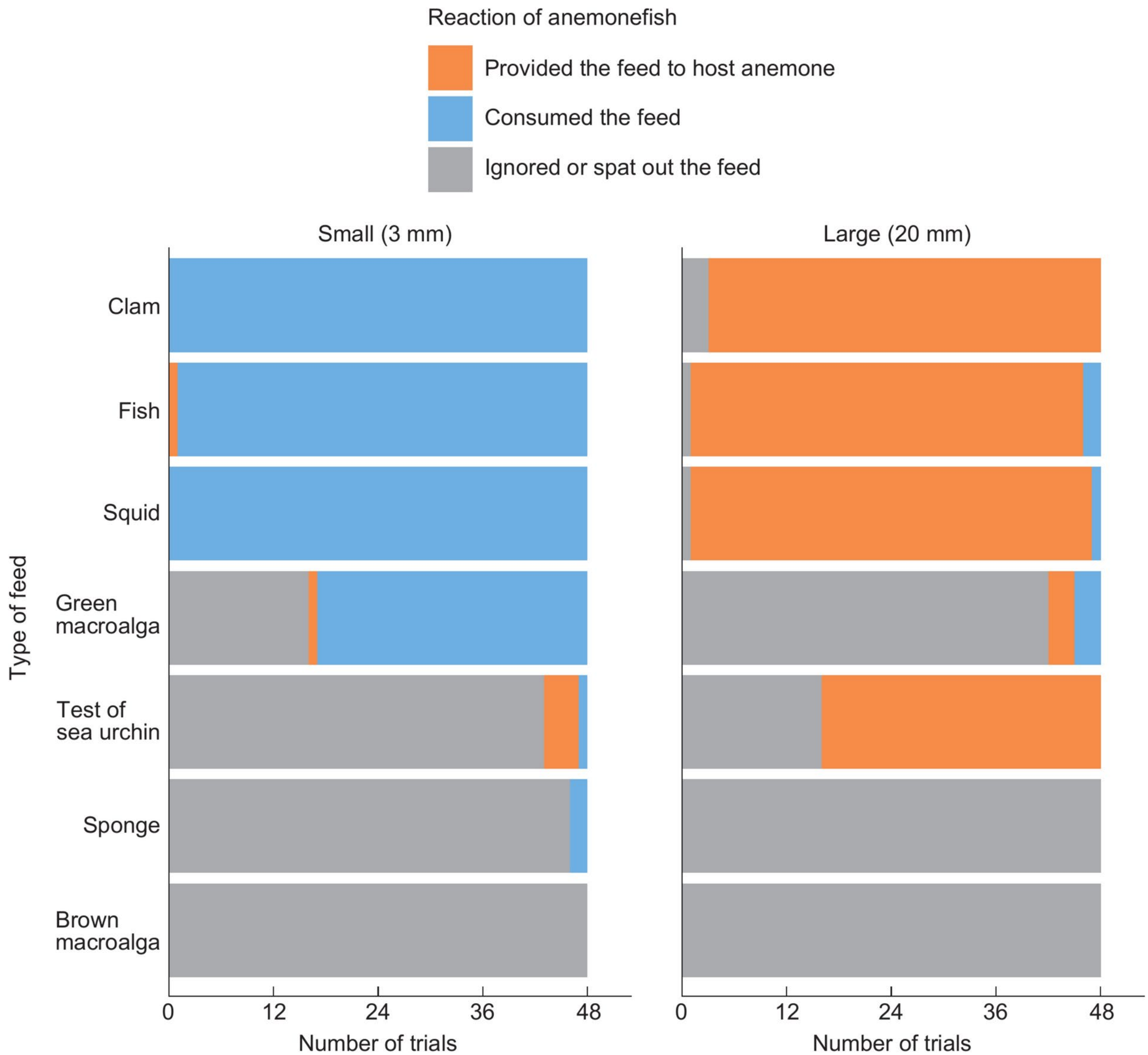

Fig. 2. Food types and food provisioning by anemonefish to the host. Feed was presented in three continuous trials of five types of feed of two different sizes per individual for clam, fish, squid, green macroalgae, and sea urchin test ($n = 16$ fish of different hosts, 480 trials in total) and six trials of two types of feed of two different sizes per individual for brown macroalgae and sponges ($n = 8$ fish of different hosts, 192 trials in total). Reactions of the anemonefish to the feed are shown in different colour bars.

host anemone. Our findings suggest that anemonefish are likely to actively provide food to their hosts depending on the food habits of the hosts and the conditions of the anemonefish. Furthermore, increased growth of host anemones through food provisioning will, in turn, benefit the anemonefish themselves, such as increasing the clutch size of the anemonefish and avoiding predation because of increased concealment among tentacles[30,36,50].

We found that the anemonefish consumed smaller krill and provided larger krill to their hosts. Similarly, anemonefish fed on smaller clams, fish, and squids and provided larger pieces to the host, which agrees with the observations of previous studies[44,45]. These results suggest two alternative interpretations: anemonefish provide food that is too large for it to eat on its own, or select food that is sufficiently large to benefit the host. When anemonefish were given hard fresh sea urchins of two different sizes, they abandoned the smaller tests and provided the larger tests to the hosts. Sea urchin tests are very solid and are probably difficult for anemonefish to swallow and digest; however, host anemones might benefit from digesting the epidermis and tube foot in the test, although this is speculative. Indeed, during the field study, we occasionally found several body parts of sea urchins, such as spines and tests, in the gastrovascular cavities of host sea anemones (Y. Kobayashi, pers. obs.). This suggests that anemonefish choose larger feed items for their hosts, which are considered of greater benefit to the host. In addition, when the green macroalga *C. cylindricum* was provided to anemonefish, they consumed smaller pieces. Although there is no evidence that *C. cylindricum* forms a part of the natural diet of anemonefish, it is edible and digestible for omnivorous anemonefish that forage primarily on copepods and (filament) algae[51–53]. Conversely, the anemonefish mostly ignored larger pieces of green macroalgae and never provided

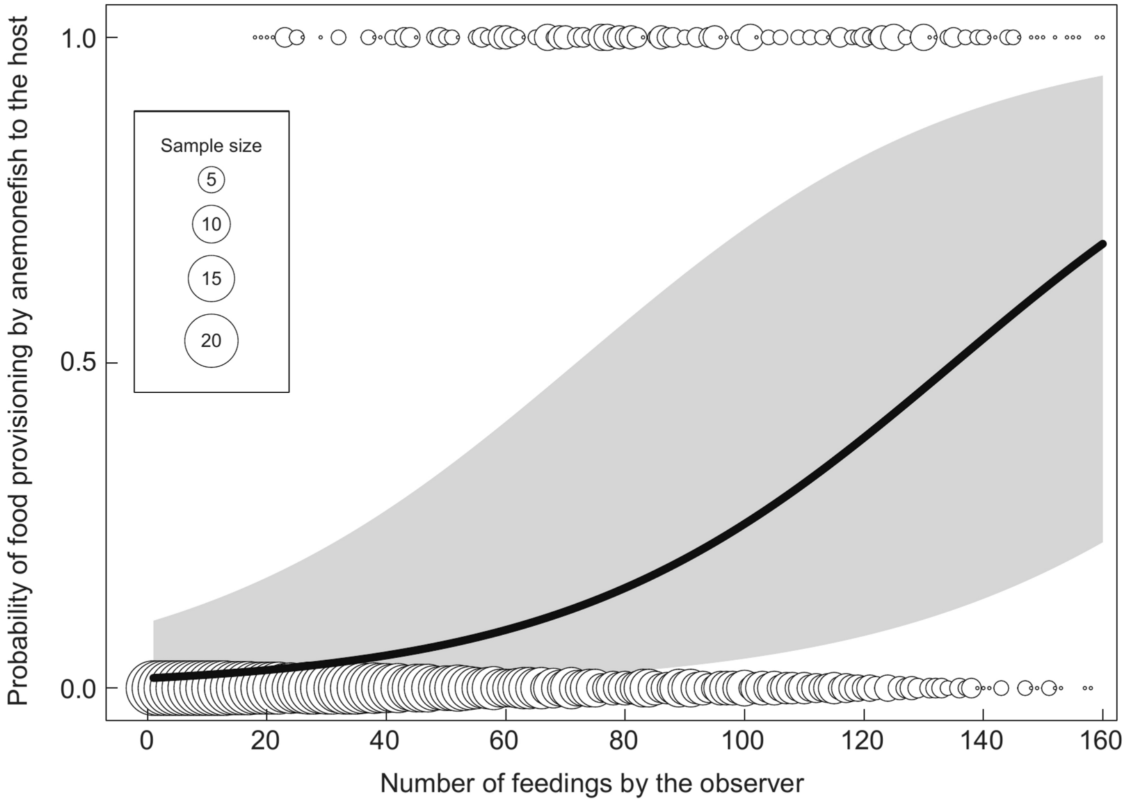

Fig. 3. Hunger level and food provisioning by anemonefish to the host. When the anemonefish consumed the feed krill of 3 mm in size, the probability is 0, and when the anemonefish provided the feed to the host anemone, the probability is 1. The probability of food provisioning by anemonefish to the host significantly increased with the number of feedings. The black line represents the regression line with the shaded 95% confidence interval from the binomial GLMM ($n = 1819$ trials from 21 fish). The size of the circles indicates the number of occurrences.

them to the host, indicating that the anemonefish selected food for the carnivorous host, the sea anemone[54]. Anemonefish neither consumed nor provided sponges and brown macroalgae *S. hemiphyllum* to hosts of either size, which is presumably unsuitable as food for both anemonefish and their hosts. Collectively, anemonefish may select food items and provide the host with suitable feed that is beneficial to the host.

When small pieces of krill were repeatedly presented to the anemonefish, the anemonefish first ate the feed, but they began to provide krill to the host anemone later, as observed in a previous study[44]. This indicates that anemonefish behave selfishly and cooperate with the host depending on their body condition. Furthermore, this may be one of the reasons why food-provisioning behaviour is rarely observed in the wild because food provisioning to the host does not occur when fish are hungry. It is also important to note that the anemonefish never abandoned their food, even when their stomachs were full, reflecting the high strength of the connection between sea anemones and anemonefish. These results also suggest that anemonefish recognise the ultimate benefits of food provision to their hosts. The next step would be to present objects that could be harmful to the anemone and objects that could be beneficial to the anemone, but that the anemonefish had never seen before, and to observe the behaviour of the anemonefish.

The reason why anemonefish provide feed to their hosts is not well understood despite studies being conducted over a long time[23]. Previous studies have proposed that the host is used to make the food finer for consumption by anemonefish[42] or that anemonefish forage later[48]. However, these assumptions were rejected by our food-tracking experiment: the host sea anemone likely consumed feed provided by the anemonefish, and the anemonefish did not remove or consume feed and frequently returned krill to the host tentacles when they picked them up. These observations strongly suggest that anemonefish actively provide food for their hosts. Furthermore, our host growth experiment revealed that food-provisioning behaviour by anemonefish increased the growth rate of the host anemone. The host anemone provides refuge and nest sites to lay eggs for the anemonefishes[24,25,29,50,55]. Females of the clown anemonefish, *A. percula* which settle on larger hosts, grow larger and lay more eggs[36]. These results suggest that host size is important for the resident anemonefish, *A. clarkii* and influences their fitness. Thus, it is plausible that anemonefish increase their fitness by growing their hosts through the provision of food. Furthermore, anemonefish are long-lived dwellers[56,57], and their symbiotic relationship with sea anemones, which appears to continue for a long period, makes food provisioning meaningful. To date, there are only a few examples of symbionts that provide feed to their hosts to improve habitat quality, such as

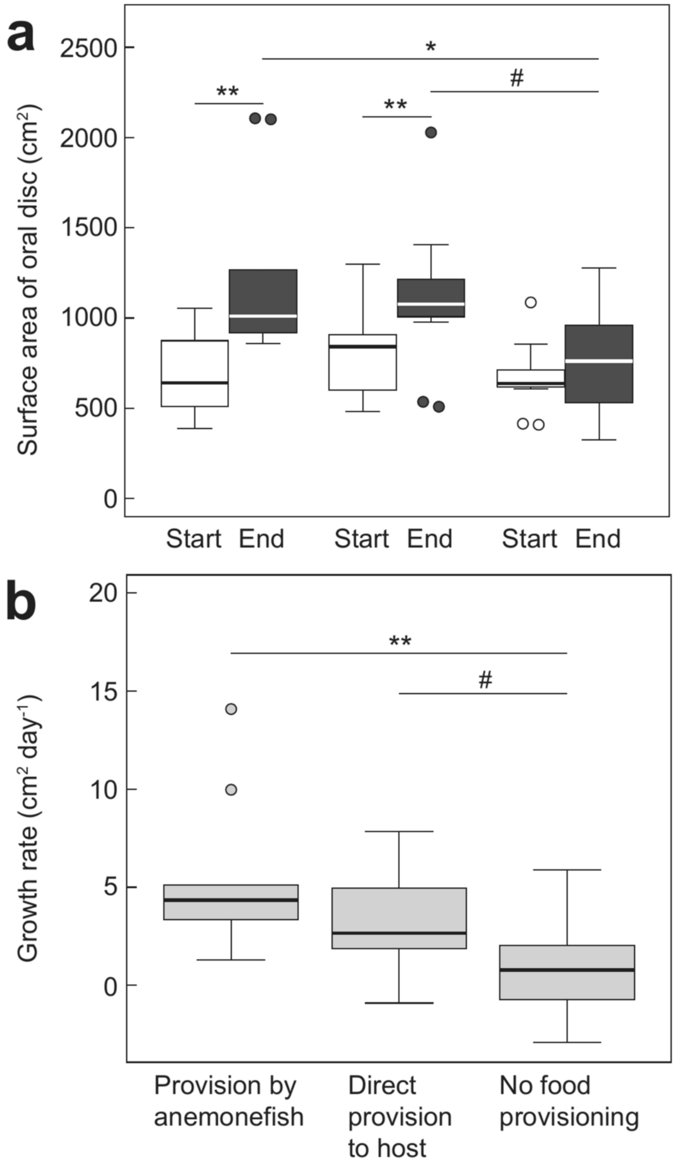

Fig. 4. Growth of sea anemones in the food-provisioning experiment. (**a**) Surface area (cm^2) of oral disk at the start and at the end of the experiment (91–109 days after the start of the experiment). (**b**) Growth rate per day (cm^2 day^{-1}). $^{**}p<0.01$, $^{*}p<0.05$, $^{\#}p=0.07$ by exact Wilcoxon rank-sum tests and Benjamin–Hochberg correction methods, or by exact Wilcoxon signed-rank tests.

Fijian ants and *Squamellaria* spp.[10]. Therefore, this study provides a rare example of the relationship between organisms through the provision of food.

Clark's anemonefish, *A. clarkii*, occasionally forages on the tentacles and eggs of the host sea anemone[41,58] (Y. Kobayashi and S. Awata, unpublished data). Furthermore, zooxanthella-derived nutrients (i.e., from tentacles) accumulate in the liver of anemonefishes[41]. If anemonefish utilise the host anemone as a food resource, this behaviour is costly to the host. Food provisioning may compensate for these costs by allowing the growth of anemones. In this case, the relationship between the two is similar to that in cultivation. Cultivation is well known for its connection between ants and fungi[14] and is also found in algal-farming damselfish and algae[59,60] and mysid-shrimp-domesticating damselfish and algae[61]. In these examples, the symbiont partner acts only as a food resource, but the host anemone also serves as a "home" for the anemonefish. No such complex relationships exist in other mutualistic symbioses. This study suggests that anemonefish improve host quality by providing food to their host anemones in a situation-dependent and proactive manner depending on the host's food habits and their own conditions. The predation of host anemones by resident anemonefish is an interesting topic for understanding the evolution of cultivation.

Until now, food provisioning by anemonefish for the host anemone has rarely been observed in the wild and is regarded as having little importance in their symbiotic relationships[23,30,48,55]. However, dead animals may frequently appear after bad weather, such as storms and typhoons, or during cold winters and hot summers.

Furthermore, these animals are often consumed by other animals. Normally, field studies are conducted during good weather and temperatures; therefore, food-provisioning behaviour is rarely observed in nature. In the present study, we observed such behaviour in the wild at an occurrence rate of 2% in summer, during which time many dead sea cucumbers were found at the study site, probably because of the high sea temperature. If field studies are carried out after storms and drastic changes in water temperature occur, food-provisioning behaviour by anemonefish to the host may be more frequently observed.

Future studies on the relationship between feeding behaviour and external factors, such as the presence or absence of competitors for food and the condition of the symbiotic partner (size and condition of the host), will contribute to the understanding of food provisioning among anemonefish and their host sea anemones.

Methods

Study site and animals

We conducted fieldwork with scuba from August to October 2020 and from June to October 2021 at Morode Beach, Ainan, Japan (33°00′16″N, 132°30′19″E). The study site consists mainly of a boulder zone containing coral communities and a sandy bottom, with a high density of anemone-anemonefish clusters in the boulder zone at depths of 3–10 m. Breeding pairs of the Clark's anemonefish *Amphiprion clarkii* were associated with bubble-tip anemone, *Entacmaea quadricolor*, and no other *Amphiprion* species were found at the study site. Only young anemonefish inhabited another species of sea anemone, *Antheopsis koseirensis*. At the study site, the social group of anemonefish mainly consisted of a breeding pair, and one juvenile (rank 3, see Buston 2003[62]) that does not participate in reproduction was found in approximately 10% of the breeding groups. A total of 112 social groups were used in the experiment over the two seasons, including groups with and without juveniles. The same groups were never used again during the same season, although several groups overlapped between the seasons.

Tracking of food provided by anemonefish to the host

There are two possible reasons why anemonefish carry food to their sea anemone hosts: host feed and food storage[48]. First, if anemonefish carry food to feed the host, the host will eventually consume it. Second, if anemonefish take food to the host for storage, they will consume the food after attaching it to the host. To assess these two alternative explanations, we monitored whether the host anemone consumed food carried by the anemonefish. One end of the string (thickness: 4 mm; length: 0.7 m) was tied to thawed frozen krill (purchased from a supermarket), whereas the other end was not tied to anything. An observer presented the bait to the anemonefish. In all cases, the anemonefish immediately carried the bait to the host and attached it to the tentacles. In 2020, we checked the baits after one hour by eye ($n = 8$). In 2021, we video-shot anemones with bait ($n = 18$) using a tripod-mounted handycam (JVCKENWOOD, GZ-RX680, Yokohama, Japan), GoPro Hero 7, or 8. The shooting time was 1–2 h. The videos were analysed in the laboratory using the video annotation software ELAN. From the videos, we recorded the time when the string was found to stretch from the anemone's mouth after it was provided by the anemonefish, and the number of times the anemonefish pecked, picked up, or returned the krill to the host.

Feed size and food provisioning by anemonefish to the host

To investigate the relationship between feed size and food provisioning by anemonefish to the host, we presented feed of different sizes to 10 individual anemonefish living symbiotically on different hosts. Freshly thawed krill (approximately 50 mm long) purchased from a supermarket was cut into 3, 5, 7, 10, and 20 mm long pieces. Cut krill (3–20 mm) and whole krill (50 mm) were presented to each anemonefish in random order, five times each for 3, 5, and 7 mm, and twice each for 10, 20, and 50 mm (21 trials in total per fish). We recorded whether the anemonefish consumed feed or carried the krill bait to their hosts. One fish left the host during the experiment; therefore, in this case, data from 17 trials were used for analysis.

Food type and food provisioning of anemonefish to the host

Anemonefish feed primarily on copepods and (filament) algae[51–53], while their host sea anemones are known to be capable of preying on fish and other animals[54]. If anemonefish benefit from the host anemone through food provisioning, they would select food for the host anemone according to the host's feeding habits. Seven different types of food were provided to each anemonefish to determine the type of feed to be provided to the host sea anemones. Clam (*Ruditapes philippinarum*), fish (*Saurida macrolepis*), and squid (*Todarodes pacificus*) were purchased from supermarkets. Sea urchins (*Diadema setosum*), two types of algae (green macroalgae: *Codium cylindricum* and brown macroalgae: *Sargassum hemiphyllum*), and purple sponges (*Haliclona permollis*) were collected at the study site. The feed was frozen until the start of the experiments. Sea urchin spines were removed from the body and crushed, and fresh tests with the epidermis and tube foot were used for the experiments. All prepared feeds were cut into two different sizes, with the longest sides of 3 mm and 20 mm. Feed was presented by the observer to the anemonefish continuously for six trials of two types of feed with two different sizes per individual anemonefish ($n = 8$ fish from different hosts, 192 trials in total) for brown macroalgae and sponges, and three trials of five types of feeds with two different sizes per individual anemonefish ($n = 16$ fish from different hosts, 480 trials in total) for other food items. The behaviour of the anemonefish was recorded.

Sea anemones are carnivores[54], whereas anemonefish are omnivorous, feeding on both zooplankton and (filament) algae[25,51–53]. If an anemonefish carries feed to its host, the following results can be predicted: (i) As clams, fish, and squids are animal foods that can be used as food resources by both anemonefish and anemones, the anemonefish would provide these large-sized feeds to the host and would consume them if the feeds were small. (ii) *C. cylindricum* is a soft-green macroalgae. Although there is no evidence that *C. cylindricum* forms a part of the natural diet of anemonefish, it is edible and digestible to omnivorous anemonefish[51,52]. Therefore, the anemonefish is likely to eat *C. cylindricum*, but would not provide it to the host because green macroalgae are probably not suitable as a food source for anemones. (iii) As the fleshy test of the sea urchin is considered too difficult for anemonefish to eat, whereas the host anemone can digest the epidermis and tubefoot, the anemonefish may provide the sea urchin test to the host. Occasionally, sea urchin spines and tests have been observed in the gastrovascular cavity of host sea anemones (Y. Kobayashi, pers. obs.). (iv) *S. hemiphyllum* is a brown macroalga. Because *S. hemiphyllum* is hard and contains carbohydrates[63], it is indigestible by anemonefish. Therefore, *S. hemiphyllum* is not a food resource for anemonefish or carnivorous sea anemones. (v) Sponges generally have chemical defences against predatory animals such as fish, crustaceans, and sea urchins (reviewed by Paul and Puglisi[64]), and both anemonefish and their hosts are predicted to be inedible.

Hunger level and food provisioning of anemonefish

To investigate the relationship between "hunger level" and the occurrence of food-provisioning behaviour, an experiment was conducted in which krill with the longest side adjusted to 3 mm were continuously presented to the anemonefish. The observer repeatedly presented the fish with krill until it stopped responding and recorded whether it consumed the food itself or provided food to the host anemone. The experiment was conducted using 21 anemonefish inhabiting different hosts.

Effects of food provisioning by anemonefish on host growth

If food provisioning by the anemonefish benefits the host anemone, the growth rate of the latter is expected to increase. To test this hypothesis, we examined the growth rate of anemones by manipulating the food provisioning by anemonefish to host sea anemones. Before the experiments, the host anemones were numbered individually. Numbered anemones were assigned to one of the three treatments. The three treatments were as follows: (i) observer-provisioning feed to anemonefish ($n = 9$), (ii) direct provisioning of feed to the hosts ($n = 11$), and (iii) no food provisioning (control, $n = 11$). The two fed groups were provided with two pieces of fish (approximately 50 g in total) of jack mackerel (*Trachurus japonicus*) or lizard fish (*Saurida macrolepis*), which were too large for anemonefish to swallow, at intervals of approximately every 3 days. The experimental period was 103 days (± 4 s.d., range = 91–106 days). During the experiment, one juvenile anemonefish was observed in four of the 31 numbered anemones. The nutrients excreted by resident anemonefish nourish host anemones[37,41]; however, the presence or absence of juveniles likely did not affect host growth (see the dataset), and the data of these four anemones were included in the analyses. The body sizes of the anemones were measured at the start and end of the experiment. The body size of the host anemone was determined by measuring the long and short diameters (cm) of the anemone's oral disk with a fibreglass folding rule (Shinwa Rules, Niigata, Japan) and calculating the surface area (cm^2) according to Hattori and Yanagisawa[65] (long diameter × short diameter × 4^{-1} × π). The growth rate of the anemones per day was calculated using the surface area of the sea anemones at the start and end of the experiment and on the experimental days (cm^2 day^{-1}).

Food provisioning by anemonefish to the host in nature

In 2022, during the fieldwork of another study, we observed the food-provisioning behaviour of anemonefish under natural conditions at the same study site. During this period, the anemonefish carried dead animals to the host anemones without manipulation. In this study, we describe the occurrence of such events.

Statistics and reproducibility

Statistical analyses were performed using R version 4.0.3 (https://www.r-project.org). The significance level was set at $p < 0.05$ for two-tailed tests. The relationship between food provisioning by anemonefish to the host and feed size or hunger level was analysed using a binomial GLMM because multiple trials were conducted on the same individual fish. The feed size model was constructed with feed size as an explanatory variable, food provisioning by the anemonefish to the hosts (provided to the hosts: 1; eaten by the anemonefish: 0) as a response variable, and individual ID as a random effect (206 trials from 10 individual fish). The hunger level model was constructed with the number of feedings by the observer as the explanatory variable, food provisioning by anemonefish to the hosts (provided to the hosts: 1; eaten by the anemonefish: 0) as the response variable, and individual ID as a random effect (1819 trials from 21 individual fish). Furthermore, because a female dominance hierarchy in anemonefish[66] and the daily rhythm[67,68] may affect individual anemonefish activity, we also examined whether the sex of the anemonefish and the time of day were related to food provisioning. We constructed models that included sex and time of day (morning or afternoon) as explanatory variables.

To examine the type of feed the anemonefish provided to the host anemone, we examined whether the proportion of the three behaviours (fish providing the feed to the host anemone, consuming the feed themselves, and ignoring or spitting out the feed) differed between the small (3 mm) and large (20 mm) feeds. Fisher's exact test (3 × 2 cross-tabulation of the reaction of anemonefish and feed size) was used to analyse each of the seven types of food.

To determine the effect of food provisioning by anemonefish on the growth rate of the host, we compared the surface area (cm^2) of sea anemones at the start and end of the experiment and the growth rate (cm^2 day^{-1}) among the three treatment groups using the Kruskal–Wallis test: (i) provisioning feed to anemonefish ($n = 9$), (ii) direct provisioning feed to the hosts ($n = 11$), and (iii) no food provisioning (control, $n = 11$). The exact Wilcoxon rank-sum test and Benjamin–Hochberg correction method were used for multiple comparisons. Furthermore, we compared the surface areas of the sea anemones before and after the experiment within each treatment group using the exact Wilcoxon signed-rank test.

Data availability

The datasets analysed during the current study are available in Zenodo (https://zenodo.org/records/11055773).

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Author contributions
Conceptualisation, Y. Kobayashi, S.A.; Field experiments, Y. Kobayashi; Data analysis, Y. Kobayashi, Y. Kondo, S.A.; Writing—original draft, Y. Kobayashi, Y. Kondo, S.A.; Writing—review and editing, all authors; Funding acquisition, Y. Kobayashi, S.A.; Resources, S.A.; Supervision, S.A.

Declarations

Competing interests
The authors declare no competing interests.

Ethical approval
No fish or sea anemones were captured, tagged, or sacrificed in this study, and this study relied on behavioural data collected non-invasively from animals. The fieldwork adhered to the guidelines for the Use of Animals in Research and was approved by the Osaka City University Animal Care Committee and local fisheries cooperative associations. The protocol for this study was not subject to the university's ethical regulations; therefore, approval from the ethics committee was not required, and the study was carried out using a method that did not cause stress to the target organisms.

Additional information
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