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Dynamics of Laterality in the Cuttlefish *Sepia recurvirostra* Through Interactions With Prey Prawns

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Predator-prey interactions based on laterality have recently been observed between fishes and their prey populations. Maintenance of antisymmetric dimorphism by frequency-dependent selection has been reported in fish, but has not been observed in invertebrates. Over 10 years, we investigated long-term changes in the "ratio of laterality" (frequency of righty morphs in a population) in the cuttlefish *Sepia recurvirostra* and its potential prey prawns *Penaeus semisulcatus* and *Metapenaeus endeavouri* in the Visayan Sea, the Philippines. The morphological laterality of cuttlefish and prey prawns was defined by measuring the asymmetry of the cuttlebone and carapace, respectively. Cuttlefish and prey prawns showed morphological antisymmetry, being composed with righty morphs and lefty morphs. The ratio of laterality of cuttlefish and one prey prawn oscillated significantly, but the oscillation was not strongly synchronized. The ratio of laterality of cuttlefish followed that of the prey prawn, indicating that predation biased to each laterality occurred in relation to their laterality. These results suggest that the lateral dimorphism of cuttlefish is maintained through frequency-dependent selection on lateral morphs of the predator cuttlefish and prey prawns. Our findings provide new insight into the ecological significance and antisymmetry maintenance mechanism in relation to interspecific interactions in marine invertebrates.

Key words: lateral dimorphism, antisymmetry, predator-prey interaction, frequency-dependent selection, oscillation, parallel-predation, cuttlebone, Visayan Sea

INTRODUCTION

Behavioral and sensory lateralization is widely observed in various vertebrates (Roger and Andrew, 2002). Studies of lateralization have focused primarily on humans and higher vertebrates, and have mainly examined cerebral lateralization and related behavioral or sensory differentiation between the right and left sides of the body (Bisazza et al., 1998). However, morphological asymmetry (e.g., differences between the right and left sides of behavioral or sensory organs) has not yet been explored in detail (Matsui et al., 2013). Recent studies of laterality in fish have revealed that behavioral laterality is always associated with morphological asymmetry, which is characteristically represented by dimorphism in populations, i.e., each population is composed of both "righty morphs" and "lefty morphs" in differing proportions (e.g., Hori et al., 2017). This morphological asymmetry in animals is categorized as antisymmetry (AS), which shows a bimodal distribution of values of any index, and can be distinguished from fluctuating asymmetry (FA), with a unimodal and symmetrical distribution, or directional asymmetry (DA), with a unimodal distribution shifted from symmetry (Palmer and Strobeck, 1986).

Recent studies of fish laterality have shown that dimorphism is maintained with oscillation in the "ratio of laterality" (proportion of righty morphs), driven by frequency-dependent selection mediated by predator-prey interactions. For example, the lateral antisymmetric dimorphism in the scale-eating cichlids in Lake Tanganyika is maintained despite oscillation of the ratio of laterality (Hori, 1993; Hori et al., 2019). Interactions with prey fishes seemingly caused this oscillation. Recently Hori et al. (2021) demonstrated that oscillation of the ratio of laterality in piscivorous chub, 'hasu' (*Opsariichthys*

uncirostris) and its prey, 'ayu' (Plecoglossus altivelis) in Lake Biwa, Japan, is caused by biased predation between two morphs, i.e., one type of "lateral morph" can prey on the opposite laterality type more frequently than on the same type. When there are more "righty predators" than "lefty predators", "lefty prey" are more exploited. As a result, "righty prey" may increase, leading to an increase of lefty predators. Ultimately, both morphs in predator and prey are maintained with dynamic changes in their proportions in their populations. Theoretical examinations (Takahashi and Hori, 1994, 1998; Nakajima et al., 2004, 2005) support this causation in wild populations.

Given that all fish have antisymmetric laterality (Hori et al., 2017), it would be reasonable to expect that some, and probably many, active aquatic invertebrate taxa, such as Crustacea and Cephalopoda, also exhibit this type of AS and interact with each other in the community. In fact, many crustaceans and mollusks show antisymmetric dimorphism (Palmer and Strobeck, 1986; Frasnelli, 2019) that is sometimes associated with behavioral laterality.

Recently, antisymmetric laterality in aquatic invertebrates has been investigated in depth, especially in crustaceans (Takeuchi et al., 2008; Tobo et al., 2012) and cephalopods (Byrne et al., 2002, 2004; Alves, 2007; Lucky et al., 2012). Some crustacean populations are composed of two types of individuals: righty morphs (right side of the abdomen or carapace is dominant over the left side) and lefty morphs (the opposite situation) (Takeuchi et al., 2008; Tobo et al., 2012). These morphological asymmetries correspond to the behavioral laterality exhibited in "backward escape jumps".

In comparison, studies of asymmetry in mollusks have mainly focused on the coiling pattern of Gastropoda and shell attachment of Bivalvia (Palmer, 2005; Frasnelli, 2017), most of which are categorized as DA. But it is noteworthy that the members of Cephalopoda show lateralized behavior and sensory asymmetry (Palmer, 2005). The extant groups of coleoid Cephalopoda, such as octopuses, squids, and cuttlefishes, have the largest brain and optical systems among invertebrates; they are all powerful marine carnivores, interacting actively with each other and other animals (Hanlon and Messenger, 1996; Wataniki et al., 2000). Several studies have investigated the laterality of octopuses (Byrne et al., 2002, 2004; Frasnelli et al., 2019), cuttlefish (Alves et al., 2007; Jozet-Alves et al., 2012a, 2012b; Schnell et al., 2016, 2019), and squid (Wentworth and Muntz, 1989; Sakurai and Ikeda, 2022) in terms of behavioral lateralization (e.g., turning in a T-maze) or perceptual asymmetry (e.g., differential eye use toward a conspecific vs. a predator), but they have not examined the morphological asymmetry, except for neuroanatomical asymmetry (Wentworth and Muntz, 1989; Jozet-Alves et al., 2012b). Many of these works have found both differential functions between the right and left eyes and biased behavioral tendencies (e.g., turning left more often than right), but few found antisymmetric patterns in such behaviors. Exceptionally, Byrne et al. (2004) showed an antisymmetric pattern of eye use among Octopus vulgaris when watching a dummy of prey (crab decoy) presented outside their tank under experimental conditions. Thus, it can be said that the octopuses have perceptual AS, but the relationship to body morphology has not been investigated, probably due to their soft bodies.

Although there are many examples of the laterality in coleoid Cephalopoda, little attention has been paid to the morphological asymmetry of the whole body associated with behavioral laterality.

However, two recent studies on cuttlefish showed antisymmetric morphology associated with behavioral laterality when hunting prey and escaping from predators (Lucky, 2012; Lucky et al., 2012). Unlike other Cephalopoda such as octopuses and squids, cuttlefish have an advantage for studying the morphological laterality; they have a cuttlebone, which is firm enough to examine body asymmetry. Lucky et al. (2012) examined the behavioral laterality of young kisslip cuttlefish, Sepia lycidas, at attacking prey shrimp in the laboratory, and examined the relation of the behavior to their morphological asymmetry. Behavioral tests in the laboratory revealed significant individual differences in turning behavior (either clockwise or counterclockwise toward prey), suggesting behavioral dimorphism in foraging behavior. Morphological asymmetry was examined by measuring the curvature of the cuttlebone. The cuttlebones of some individuals were convex on the right side, while other individuals had cuttlebones convex on the left side. Although the difference in curvature was not large, the frequency distribution of cuttlebone asymmetry was significantly bimodal with no individuals which had a symmetric body, indicating that populations were composed of two types of individuals, i.e., righty morphs and lefty morphs. Moreover, laterality in foraging behavior corresponded with the asymmetry of the cuttlebone; righty morphs tended to turn counterclockwise, and lefty morphs clockwise, when approaching prey. These results showed that cuttlefish display morphological AS in association with behavioral laterality.

In another experiment, Lucky (2012) also examined the escape response of S. lycidas in the laboratory. When young cuttlefish of this species were threatened by sudden presentation of a dummy predator from the front, the same type of lateralized behavior was observed, i.e., some individuals showed a significant bias toward clockwise (rightward) turns, while others showed a significant bias toward counterclockwise (leftward) turns. This lateral bias in escape response strongly corresponded to the direction of asymmetry of the cuttlebone; righty individuals turned clockwise significantly more frequently than counterclockwise, while lefty ones did the opposite. Furthermore, Lucky (2012) conducted a crossing experiment with S. lycidas, and showed that lefty parents produced more lefty offspring, righty parents produced more righty offspring, and pairs of a righty parent and a lefty parent tended to produce intermediate ratios of lefty and righty offspring. This suggests a genetic basis for the laterality of cuttlefish, although the sample size (five couples) was too small to formulate any distinct pattern of inheritance.

In the wild, cuttlefishes are powerful predators that forage mainly on crustaceans, such as crabs, shrimps, and prawns (Hanlon and Messenger, 1996). Crustaceans also exhibit antisymmetric dimorphism in association with behavioral laterality (Takayanagi, 2005; Takeuchi et al., 2008; Teranishi, 2008; Tobo et al., 2012). We can predict that these prey crustaceans will show a similar antisymmetric dimorphism in their escape behavior depending on their laterality. The laterality of cuttlefish may interact with the lateral morphs of prey crustaceans, and the predation success of

cuttlefish and/or prey escape success may depend on the combination of laterality between predator and prey. Success ratios may depend on the current ratio of laterality (frequency of righty morphs) in both predator and prey populations. Consequently, laterality ratios may change over time. We hypothesize that the laterality of cuttlefish has been maintained by frequency-dependent natural selection through predator-prey interactions, in a similar way to the interactions between predator and prey in fish communities (Hori et al., 2019, 2021). However, the laterality of cuttlefish has rarely been surveyed in the wild (but see Schnell et al., 2019), and little is known about the dynamics of their laterality in relation to that of their prey animals.

The purpose of this study is to demonstrate the long-term dynamics of laterality in cuttlefish and its potential prey prawns under natural conditions, and to examine how they interact each other in relation to their laterality. We also discuss the observed dynamics patterns in terms of causation.

We investigated the curvespine cuttlefish, *Sepia recurvirostra* Steenstrup, a medium-sized cuttlefish (maximum dorsal mantle size, 17 cm), widely distributed on continental shelves in the West Pacific Ocean (Carpenter and Niem, 1998). This species is the main target of cuttlefish fishing in the Visayan Sea, around Panay Island, the Philippines (Fig. 1A–C). In the Visayan Sea, many small local ships catch cuttlefishes and prawns by dragnet at relatively shallow depths offshore around the island. We analyzed temporal changes in the ratio of laterality of this species every 3 months over a 10-year period, by purchasing cuttlefish samples caught with trawling nets by local fishermen. The short survey interval (every 3 months) was based on the lifespan of most tropical and temperate region cuttlefishes, which is 1 year (Okutani, 1995; Hanlon and Messenger, 1996). Some individuals of another large cuttlefish, *Sepia pharaonis* Ehrenberg, were caught in the same net. However, as the number of individuals was small and variable (0–10), it was difficult to collect data over time on this species. Tentative examination of the stomach contents of the curvespine cuttlefish indicated that they fed mainly on medium-sized prawns and crabs, but the species of these prey crustaceans could not be identified as they were crushed into small pieces. In the course of the present study, morphological and behavioral laterality were found in some species of shrimp and crayfish (Takeuchi and Hori, 2008; Takeuchi et al., 2008; Tobo et al., 2013). Based on this information, in the latter half of the study period, we surveyed temporal changes in the laterality of two species of penaeid prawns, the green tiger prawn, *Penaeus semisulcatus* de Haan, and Endeavour prawn, *Metapenaeus endeavouri* (Schmitt), which were the two main species of prawn caught
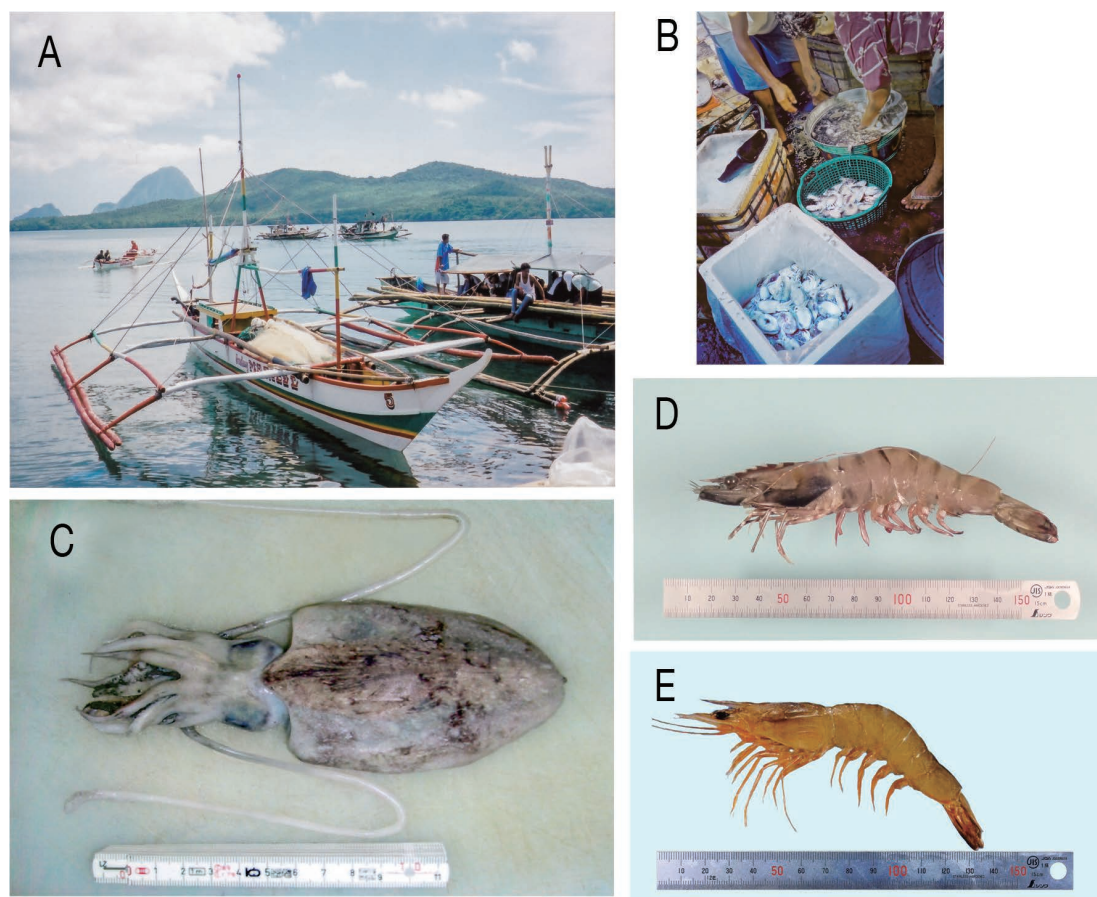

Fig. 1. **(A)** A local fishing boat that trawls for cuttlefish and other marine products using a Danish seine net at the fishing port of Estansia, Panay Is. **(B)** the catch for one morning. **(C)** A curvespine cuttlefish, *Sepia recurvirostra* (scale bar = 13 cm). **(D)** a green tiger prawn, *P. semisulcatus* (scale bar = 17 cm). **(E)** An Endeavour prawn, *M. endeavouri* (scale bar = 17 cm).

in considerable numbers in the same dragnet by the fishermen (Fig. 1B, C). We assumed that these medium-sized prawns were the main prey of the curvespine cuttlefish in this area. The lifespan of these prawns is approximately 1.5–2 years (Motoh, 1999). Although many crabs were also caught in the same net, we did not survey them as prey, because the species and numbers of individuals caught varied too widely from time to time, and it seemed to be difficult to survey their dynamics.

MATERIALS AND METHODS

Sampling of materials

Samples of the curvespine cuttlefish, *S. recurvirostra*, were purchased every 3 months from September 2001 to September 2011 (four times per year, i.e., in March, June, September, and December) from contracted local fishermen based at the Estancia fishing port on the northeast tip of Panay Island. The samples were captured at night using a Danish seine net at 6–24 m depths offshore around Estancia. There was no apparent sex bias in abundance. We aimed to collect > 100 samples per sex, but the samples sizes were sometimes smaller. In the latter half of the study period (5-year period from September 2006 to September 2011), samples of two prawns, the green tiger prawn, *P. semisulcatus*, and Endeavour prawn, *M. endeavouri*, were also collected every 3 months from the same catch as that for the cuttlefish. We aimed to collect > 50 samples of each sex for each species.

All samples were kept on ice on board. They were transported to the Visaya Campus laboratory of the University of the Philippines. After sexing based on the gonads, the cuttlebones were separated, numbered, and dried. A sample lot of cuttlebones from March 2006 was lost due to damage caused by mice during the drying process. Prawns were fixed in 10% formalin for several days and kept in wet conditions. Each time, the samples were sent to the laboratory at Kyoto University, Japan, for further investigation of their morphological laterality and statistical analysis.

All the procedures of this study were performed in accordance with the Regulations on Animal Experimentation of Kyoto University. As the regulations do not stipulate how to treat dead invertebrates purchased from fishermen, no ethical approval was required for this study.

Measurement of morphological laterality in cuttlefish

The length of each cuttlebone was measured by Vernier calipers to the nearest 0.05 mm. The laterality of each cuttlebone was quantified by measuring its curvature using a digital microscope (VHX-100; Keyence) following Lucky et al. (2012). Each cuttlebone was placed on the microscope stage and maintained in a strict horizontal position using a two-dimensional gonio stage and a level scope for measurements (Hata et al., 2013). The anterior and posterior ends of the cuttlebone were designated as the tip of the median sulcus (anterior) and base of the spine (posterior), respectively, on the monitor of the digital microscope (Fig. 2A). A perpendicular line was drawn at the midpoint of the longitudinal line between the two ends, and the distances from the right and left sides of the cuttlebone to the intersection (CB_R and CB_L, respectively) were measured to the nearest 0.01 mm. To prevent measurement errors, the measurements were repeated three times for each cuttlebone, mounting them independently on the stage in each time, and the median value was used for the analysis.

To quantify the asymmetry of each cuttlefish, we calculated an index of asymmetry (*IAS*) as $2 \times ([CB_R - CB_L]/[CB_R + CB_L]) \times 100$. Individuals with a positive *IAS* value for the cuttlebone, which were convex on the right side, were defined as righty morphs, and those with a negative *IAS* value for the cuttlebone, which were convex on the left side, were defined as lefty morphs (Lucky et al., 2012).
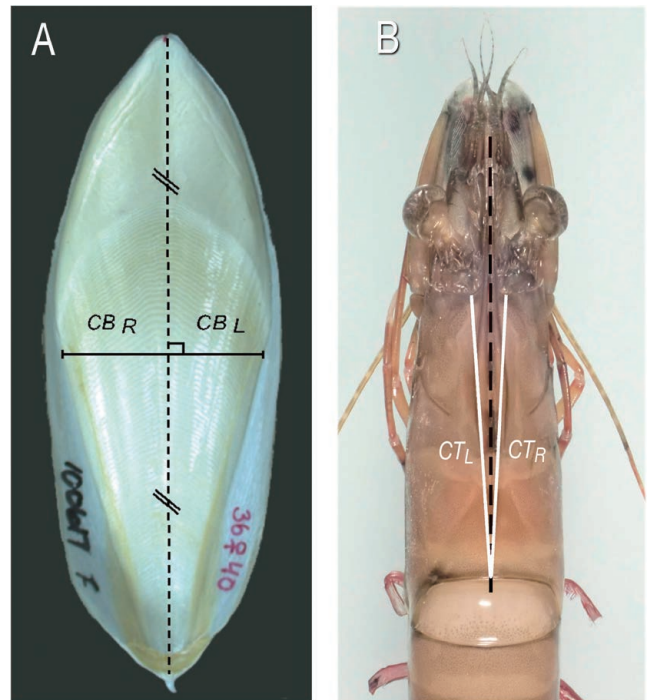

Fig. 2. Data points for measuring laterality. **(A)** Cuttlebone (ventral view of *S. recurvirostra*) and **(B)** prawn cephalothorax (dorsal view of *P. semisulcatus*). CB_R and CB_L are the lengths of the right and left sides at the midpoint of the cuttlebone, respectively, and CT_L and CT_R are the lengths of the left side and right sides of prawn cephalothorax. See text for details.

Measurement of morphological laterality in prey prawns

The prawns of each species were sexed based on the first pair of pleopods. The length of each individual was measured from the tip of the rostrum to the end of the thoracic segment by Vernier calipers to the nearest 0.05 mm. Prawn laterality was quantified according to Tobo et al. (2012), based on the difference in length between the right and left sides of the cephalothorax for each individual (Fig. 2B). While maintaining a strict horizontal position of each sample, the distances from the right orbital cavity (CT_R) and left orbital cavity (CT_L) to the posterior end of the dorsal sagittal line in the carapace of each individual were measured using a digital microscope. Each individual was measured in triplicate, and the median value was used for the analysis. We calculated the *IAS* as $2 \times ([CT_R - CT_L]/[CT_R + CT_L]) \times 100$, to quantify the asymmetry of each prawn (which was essentially the same as the asymmetry calculation method used for the cuttlefish).

Test for the type of asymmetry

Morphological differences between the right and left sides of the body in animals can be categorized as FA, DA, and AS (Palmer and Strobeck, 1986). To define the type of asymmetry of the traits measured here (the *S. recurvirostra* cuttlebone and prey prawn cephalothorax), we applied the likelihood method. The *IAS* was approximated to a distribution by combining the normal *IAS* distributions of lefty and righty individuals; the average and standard deviations of these normal distributions are μ_1, μ_2, σ_1, and σ_2, respectively. Appropriate values for parameters were obtained by the most likelihood method in three models; the FA model (one parameter: $\mu_1 = \mu_2 = 0$, $\sigma_1 = \sigma_2$), the DA model (two parameters: $\mu_1 = \mu_2$, $\sigma_1 = \sigma_2$), and the AS model (three parameters: $\mu_1 = -\mu_2$, $\sigma_1 = \sigma_2$, ratio of righty morph). To judge the best fit model, we calculated Akaike information Criteria (AIC) for the three models using

their maximized likelihood values and degrees of freedom. The model with the lowest AIC was considered to show the distribution of each *IAS*. Model fitting of the *IAS* distribution was applied to 737 cuttlebones (338 males and 399 females) of *S. recurvirostra* collected in 2009, 277 individuals (152 males and 125 females) of the green tiger prawn in 2009, and 399 individuals (200 males and 199 females) of Endeavour prawn collected in 2009. The analysis was performed using the ISAD package (ver. 1.1; Hata et al., 2013) in R. Finally, we used the Wilcoxon rank sum test to compare the distribution pattern of the *IAS* between male and female cuttlefish.

Time series analysis of the ratio of laterality of cuttlefish and prey prawns

In total, 2892 curvespine cuttlefish cuttlebones were examined in males (cuttlebone length; $\bar{X} \pm$ SD = 76.8 $\pm$ 10.9 mm) and 2897 in females ($\bar{X} \pm$ SD = 77.2 $\pm$ 8.4 mm) over the entire study. The study examined 617 male (carapace length; $\bar{X} \pm$ SD = 15.5 $\pm$ 3.3 mm) and 579 female ($\bar{X} \pm$ SD = 15.3 $\pm$ 3.2 mm) green tiger prawns and 751 male (carapace length; $\bar{X} \pm$ SD = 17.3 $\pm$ 3.8 mm) and 731 female ($\bar{X} \pm$ SD = 16.8 $\pm$ 3.9 mm) Endeavour prawns.

The ratio of laterality (frequency of righty morphs in each population) was calculated for each sampling time as follows: $N_R/(N_R + N_L)$, where N_R and N_L are the numbers of righty and lefty morphs of both sexes, respectively. We analyzed the temporal variation (periodicity analysis) of the ratio of laterality in cuttlefish and prey prawns using the Fourier transform method of Yasugi and Hori (2011).

Relationship between the laterality of cuttlefish and prey prawns

There is no simple, formalized statistical method for examining the relationship between two time series of ratios that change periodically. To examine the temporal relationship between the laterality of predator cuttlefish and prey prawns, we plotted the ratio of laterality of the cuttlefish against that of each prawn species directly according to Hori et al. (2019, 2021). In this diagram, when the plots scatter on a straight line of slope +1, it means that the ratios of laterality of cuttlefish and prawn change synchronously. In contrast, when the plots scatter on a line of slope –1, the ratios of the two animals change in the opposite manner, i.e., when the prawn ratio increases, the cuttlefish ratio decreases. This means that the ratio of cuttlefish follows the change in that of prawn with a time lag of 1/2 cycle. When the plots align counterclockwise on the circumference of a circle, it means that the ratio of laterality of cuttlefish follows the change in that of prawn with a time lag of 1/4 cycle. When they align clockwise on the circumference of a circle, it means that the ratio of the prawn follows the change in that of cuttlefish with a time lag of 1/4 cycle. Of course, when the plots are scattered without coordination, it means that there is no relationship between the changes in the two ratios. Although this graphical method may not be statistically rigid, it can estimate any possible relationship between the two changing ratios. Refinement of this method is desirable from a statistical perspective.

RESULTS

Morphological laterality of cuttlefish and prey prawns

The numbers of lefty and righty morphs were not significantly different between the sexes in

the curvespine cuttlefish, *S. recurvirostra* (151 lefty and 187 righty in male, 178 lefty and 221 righty in female; Fisher's exact test, $P = 1$), in green tiger prawn, *P. semisulcatus* (87 lefty and 65 righty in male, 74 lefty and 51 righty in female, Fisher's exact test, $P = 0.80$), or in Endeavour prawn, *M. endeavouri* (110 lefty and 90 righty in male, 108 lefty and 91 righty in female; Fisher's exact test, $P = 0.92$; Fig. 3). Additionally, the distribution pattern of the *IAS*s did not differ significantly between the sexes in the cuttlefish (Wilcoxon rank sum test, $W = 69989$, $P = 0.37$), green tiger prawn ($W = 9186.5$, $P = 0.63$), or Endeavour prawn ($W = 19765$, $P = 0.90$). The AS model had the best fit in terms of the frequency distribution of *IAS*s for the cuttlefish and its two potential prey prawns (Fig. 3).
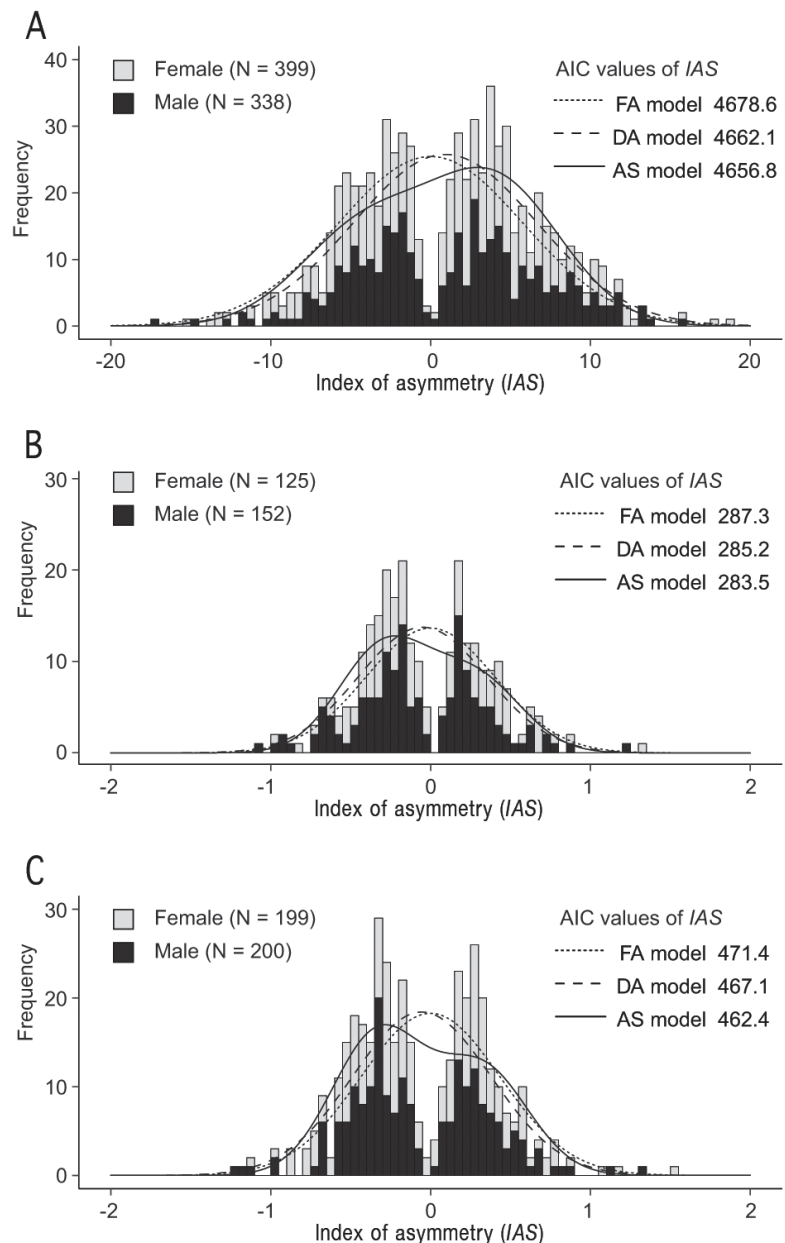

Fig. 3. Histogram of the index of asymmetry (*IAS*) for **(A)** *S. recurvirostra*, **(B)** *P. semisulcatus*, and **(C)** *M. endeavouri*. Lines over each histogram are the probability distributions of the three models fitted to the *IAS* values.
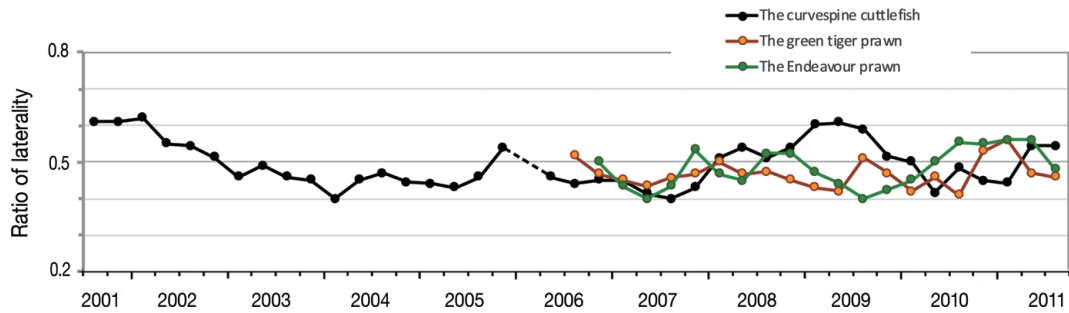
Fig. 4. Temporal changes in the ratios of laterality of the cuttlefish and two prey prawns from 2001 to 2011 in the Estancia fishing area in the Visayan Sea, the Philippines.
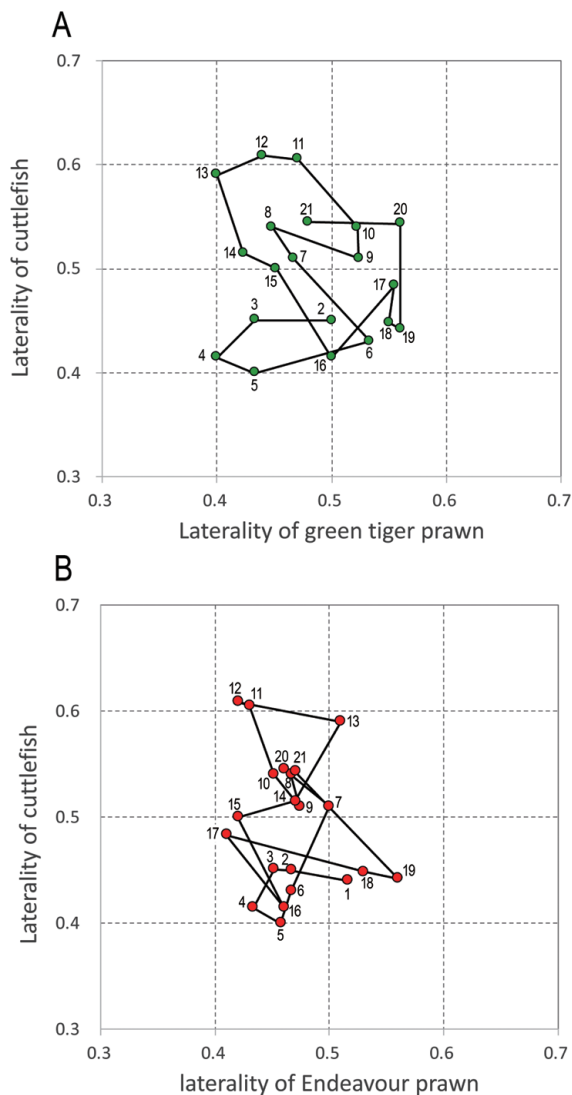

Fig. 5. Relationships of the laterality ratios of the cuttlefish *S. recurvirostra* and two prey prawns, **(A)** *P. semisulcatus* and **(B)** *M. endeavouri*. Suffix number indicates the sampling time from 1 September 2006 to 21 September 2011.

Temporal changes in the ratio of laterality of cuttlefish and prey prawns in the field

The total numbers of lefty and righty morphs of cuttlefish and the two prey prawn species at each sampling time are listed in Supplementary Table S1. The ratios of laterality (frequency of righty morphs) in cuttlefish and the two prey prawn species changed over the 10-year period, ranging between 0.40 and 0.62 (Fig. 4). Fourier transform analysis showed that the ratio of laterality oscillated significantly with two periods, at 3.4 and 2.5 years ($P < 0.001$), in the curvespine cuttlefish, and with one period, at 2.5 years in the green tiger prawn ($P < 0.01$). However, no significant oscillation was identified ($P > 0.05$) in the Endeavour prawn (see Supplementary Fig. S1A–C).

Relationship between the laterality of cuttlefish and prey prawns

Plotting the temporal relationship between the ratios of laterality of cuttlefish and each prey prawn species showed that the cuttlefish ratio appeared to follow the green tiger prawn ratio in a counterclockwise direction in the time series (Fig. 5A). A similar pattern was observed between the ratio of cuttlefish and Endeavour prawns, but the tendency was less clear (Fig. 5B). This suggests that the laterality of cuttlefish may interact mainly with that of the green tiger prawn.

DISCUSSION

Morphological asymmetry of the cuttlefish and two prawn species

Our results demonstrate that populations of the curvespine cuttlefish, *S. recurvirostra,* show morphological AS, i.e., they are composed of righty and lefty morphs, in both sexes. This verified the results of Lucky et al. (2012) for the kisslip cuttlefish, *Sepia lycidas*. Lucky et al. (2012) also indicated that the AS of the kisslip cuttlefish corresponds to behavioral laterality; righty morphs attack a prey shrimp from the lateral side by moving rightwards, while lefty morphs do this by moving leftwards. We suspect that the same type of behavioral laterality would be seen in the curvespine cuttlefish.

Byrne et al. (2004) indicated that the European common octopus, *O. vulgaris,* showed an antisymmetric pattern of eye use at targeting prey under laboratory conditions. Frasnelli et al. (2019) suggested that the visual lateralization of *O. vulgaris* was context-dependent and was not consistently seen at a population level but indicated that each individual had a righty or lefty preference, i.e., behavioral AS, although the authors did not use this term. It is possible that the octopus shares the same type of laterality as the cuttlefish found here. However, these octopus studies did not investigate morphological asymmetry.

Alves et al. (2007) studied the side-turning behavior of

adult and subadult European common cuttlefish, *S. officinalis*, in a T-maze, and found that 53% of 15 individuals tested preferentially turned right, 20% turned left, and the remaining 27% showed no preference. Jozet-Alves et al. (2012a, 2012b) studied the relationship between the side-turning preferences in a T-maze and the anatomical and neurochemical difference in right- and left-brain systems of juvenile *Sepia officinalis* (30–45 days post hatching). They found a significant correlation between the size of some brain parts and bias in the turning direction, and claimed that the cuttlefish showed asymmetry of the neural structures associated with behavioral bias at a population level. The difference, however, was continuous and unimodal rather than bimodal and 60% of individuals (12/20) showed a preference for left turns, 15% preferred right turns, and 25% showed no preference. Note that the individuals tested were from eggs laid by several females. Therefore, it is difficult to conclude that the cuttlefish shows brain and behavioral lateralization at a population level.

Under laboratory conditions, Schnell et al. (2016) found that *S. officinalis* sub-adults were significantly more likely to use the left eye to scan for predators and the right eye for prey attack. Although they claimed that the cuttlefish showed laterality at a population level, 20–40% of individuals always showed the opposite type of eye use. The individuals tested were also from eggs laid by several females. Schnell et al. (2019) also studied eye use of giant Australian cuttlefish, *Sepia apama*, derived from one population during behavior in both the field and laboratory, and claimed that it showed population-level lateralization toward left-eye preference, although 20–40% of individuals showed the opposite or no preference. Moreover, it is possible that the proportions of majority- and minority-type individuals in a population in the field might change periodically, as discussed below. Thus, the conclusion that the cuttlefish shows population-level eye-use lateralization seems premature. Furthermore, as these studies on cuttlefish, like those on octopus, did not investigate the morphological asymmetry except for sensory and nervous system asymmetry, it is difficult to examine the relationship between eye-use lateralization and the antisymmetric pattern in cuttlebones further. This remains a future challenge.

Our results also indicated that populations of two species of prawns, the green tiger prawn and Endeavour prawn, show morphological AS. Takeuchi et al. (2008) indicated that two species of atyid shrimp, *Limnocaridina latipes* and *Neocaridina denticulate*, are composed of righty and lefty morphs, and showed that when threatened righty morphs jump back rightwards, while lefty ones jump back leftwards. Tobo et al. (2012) demonstrated the same type of morphological and behavioral AS in the crayfish, *Procambarus clarkia*. It may be reasonable to expect that the two species of prawns show the same type of behavioral laterality when attacked by cuttlefish. If so, the lateralities of the cuttlefish and prey prawns may affect each other and lead to dynamism in the ratios of laterality in each population.

Temporal changes in the laterality of cuttlefish and potential prey prawns

Our results showed that the ratios of cuttlefish and prey prawns changed periodically around an equilibrium point of 0.5 throughout the study period. The Fourier transform analysis indicated that the ratios of the cuttlefish oscillated with 3.4- and 2.5- year periods ($P < 0.01$), and the green tiger prawn did so with a 2.5-year period ($P < 0.01$). The ratio of the Endeavour prawn also showed a non-significant periodical change over a 2.5-year period. Fourier transform analysis is used to detect a periodicity which is assumed to be a constant, although if the period of oscillation changes, periodicity may not be detected. However, there is no reason for the ratios of laterality to oscillate with a fixed periodicity according to the dynamics of laterality, and many factors may modulate periodicity, including the change in the ambient temperature and abundance of generalist predators or alternative prey. In this study, the ratios of laterality of the cuttlefish and prey prawns changed more or less periodically. Although various factors may affect the periods of oscillation, the ratio of laterality itself of one species was only affected by the ratio of laterality of the others (either conspecific or interspecific). Thus, there seemed be a correlation between the periodical changes in the ratios of the cuttlefish and its potential prey prawns.

Interaction between the cuttlefish and prey prawns

Figure 5A shows that the ratio in cuttlefish followed that in the green tiger prawn, in which counterclockwise rotation was prominent. That is to say, when the frequency of righty morphs of the green tiger prawn increased, the frequency of righty morphs of cuttlefish tended to increase slightly later, and when the former decreased, so too did the latter. This suggests that some consistent tendency in predator-prey interaction is involved with regard to their laterality.

Behavioral laterality inevitably leads to biased directions of attack (Nakajima et al., 2007; Yasugi and Hori, 2012) and evasion (Izvekov et al., 2009; Yasugi and Hori, 2011, 2012). As the behavioral laterality determines the side to which the individual can get more effective results of attacking (Nakajima et al., 2007; Takeuchi and Hori, 2008; Yasugi and Hori, 2012) and evasion (Yasugi and Hori, 2012), such biases should affect the likelihood of each attack's success or failure, depending on the combination of laterality between the predator and attacked prey. Given that both predator and prey have laterality, two situations can occur in each predation event, i.e., "parallel-predation" and "cross-predation" (Nakajima et al., 2007; Yasugi and Hori, 2012, 2016). In parallel-predation, a predator catches a prey of the same laterality (righty predator catches righty prey, and lefty predator catches lefty prey), while in cross-predation, a predator catches a prey of the opposite type of laterality. If either type of predation is predominant in the predator-prey interaction, the ratio of laterality in both the predator and prey populations should change in a particular manner. However, if either type of predation occurs randomly (at a similar frequency), the ratios of laterality in predator and prey populations will not be affected.

In predator-prey interactions in fish, Yasugi and Hori (2011, 2012, 2016) reported that cross-predation was predominant in pursuit-type piscivores, whereas parallel-predation was predominant in ambushing-type piscivores. This seems to be applicable to the interactions between fish predators and shrimp or crab prey (Takayanagi, 2005; Takeuchi, 2008), and between crab predators and crab prey

(Teranishi, 2008).

Cuttlefishes are generally ambush predators (Hanlon and Messenger, 1996), although when they target prey, they sneak around it from the right or left side (Lucky et al., 2012). When prawns notice an approaching enemy, they must turn to face it and escape by jumping back, like shrimp (Takeuchi et al., 2008) and crayfish (Tobo et al., 2012). Thus, we suspect that, when cuttlefishes prey on prawns, parallel-predation may predominate over cross-predation. If this is the case, when righty morph frequency for one prawn species increases, cuttlefish righty morphs will catch more prey and also increase in frequency, which may in turn increase the frequency of prawn lefty morphs; thus, the ratio of laterality in the cuttlefish changes according to that of the prawn prey. This was the situation that we observed in the field data. Thus, we suspect that parallel-predation is predominant between cuttlefish and prey prawns.

Although we found a significant relationship between oscillations in the laterality ratio of cuttlefish and one prey prawn, the oscillation periods did not coincide well with each other. This may be because the cuttlefish dynamics were also greatly affected by the interaction with crabs, which are another main prey of cuttlefish (Hanlon and Messenger, 1998). Although many crab species are found at the sampling site in the Visayan Sea, they are not fishery targets and are abandoned at hauling the net. Therefore, it was difficult to specify the prey species and obtain sufficient samples. The 3.4-year oscillation period identified in the cuttlefish might be related to interactions with their crab prey. Further detailed studies are needed on the interactions between cuttlefish and crab populations. Another possible reason for the weakly synchronized oscillation could be the interactions between cuttlefish and their enemies. The kisslip cuttlefish showed antisymmetric laterality in its escape behavior from a dummy predator (Lucky, 2012). Some teleost fishes and mammals are potential predators of cuttlefish (Hanlon and Messenger, 1998). Therefore, there is a possibility that the ratio of cuttlefish laterality might be driven by the laterality of predators, though it is difficult to identify predators specialized to cuttlefish at the study site at present.

As laterality in cuttlefish has a genetic basis (Lucky, 2012), as in shrimps (Takeuchi et al., 2008), temporal changes in the ratio of laterality of cuttlefish and prey prawns suggest that the genotype frequency both in predator and in prey may also change. Even if each population seems to have a stable population size, the present work suggests that the genetic characteristics underpinning laterality in each predator and prey species population are changing drastically. Each predator and prey population is continuously evolving to maximize its efficiency of hunting or escape (Pianka, 1978; Matsuda et al., 1993, 1994, 1996). However, as Lessard (1984) and Lively (1993) indicated theoretically, this evolution does not progress unidirectionally, but rather progresses cyclically, and polymorphic populations are maintained in a stable state in a community (Nakajima et al., 2004, 2005). Therefore, we suggest that the laterality of these invertebrates contributes to the stability of the community structure and biodiversity in aquatic communities. We hope that our viewpoint of laterality of animals with relation to the stability of aquatic communities will be explored in more depth in the future.

COMPETING INTERESTS

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

NSL collected the samples, measured specimens, analyzed the data, and wrote the manuscript. KJLT, MBT, RI, and KY collected samples and reviewed the manuscript. MY analyzed the data and edited the manuscript. MH conceptualized and organized the study, collected the samples, and reviewed and edited the manuscript. All authors read and approved the final manuscript.

SUPPLEMENTARY MATERIALS

Supplementary materials for this article are available online. (URL: https://doi.org/10.2108/zs220022)

Supplementary Figure S1. Estimation of the significant oscillation period by the Fourier transformation method. **(A)** *S. recurvirostra*, **(B)** *P. semisulcatus*, and **(C)** *M. endeavouri*. The method of periodicity analysis by the Fourier transform follows Yasugi and Hori (2011).

Supplementary Table S1. Laterality of the cuttlefish *S. recurvirostra* and the prey prawns *P. semisulcatus* and *M. endeavouri* measured at each sampling time. Main values are the numbers of righty vs. lefty morphs of both sexes, and values in parentheses are the ratios of laterality (frequency of righty morphs).

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