**Remarkable spatial memory in a migratory cardinalfish**

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## Summary

The ability to orient and navigate within a certain environment is essential for all animals, and spatial memory enables animals to remember the locations of such markers as predators, home, and food. Here we report that the migratory marine cardinalfish *Apogon notatus* has the potential to retain long-term spatial memory comparable to that of other animals. Female *A. notatus* establish a small territory on a shallow boulder bottom to pair and spawn with males. We carried out field research in two consecutive breeding seasons on territory settlement by individually marked females. Females maintained a territory at the same site throughout one breeding season. After overwintering in deep water, many of them (82.1%) returned to their breeding ground next spring and most occupied the same site as in the previous season, with only a 0.56 m shift on average. Our results suggest that female *A. notatus* have long-distance homing ability to pinpoint the exact location of their previous territory, and retain spatial memory for as long as 6 months.

## Introduction

Spatial learning and memory are essential properties for animals to forage, reproduce, avoid predators, and migrate. Studies suggest that fish are capable of spatial learning and can use information in various different environments (Odling-Smee et al. 2006). In fish, spatial memory can enhance foraging rate (Hughes and Blight 1999), territory defense (Lamanna and Eason 2003), and predator avoidance (Markel 1994). In mammals and birds, the hippocampus plays a crucial role in spatial memory (Healy et al. 2005). Fish also possess a brain structure (telencephalon) that is functionally equivalent to the hippocampus (Salas et al. 1996). Some fish species have the ability to integrate geometric and non-geometric information to orient themselves (redtail splitfin *Xenotoca eiseni*: Sovrano et al. 2002, 2005, 2007; Sovrano and Bisazza 2003; goldfish *Carassius auratus*: Vargas et al. 2004; see reviews by Chiandetti and Vallortigara 2008).

It has been reported that fish use a variety of cues for orientation and navigation. For example, coho salmon (*Oncorhynchus kisutch*), using an olfactory cue, can return to their natal stream 18 months after migration to sea (Cooper and Hasler 1974). Nishi and Kawamura (2005) suggested that the Japanese eel *Anguilla japonica* could use geomagnetic field as their directional guide for long-distance migration. Sticklebacks can associate visual cues with the status of potential food sources and use memorized information to guide foraging behaviour (Hughes and Blight 2000). Furthermore, juvenile Atlantic salmon (parr), *Salmo salar*, can use a coloured visual landmark as a local cue (Braithwaite et al. 1996) and goldfish, *Carassius auratus*, can learn a simple visual discrimination (landmark versus no landmark) to find a hidden food reward efficiently (Warburton 1990).

*Apogon notatus* (Pisces: Apogonidae) is a marine gregarious cardinalfish inhabiting the coastal waters of the northwestern Pacific. Female *A. notatus* start establishing their territories on a boulder bottom more than two months prior to the breeding season, and maintain their territories throughout the breeding season (Okuda 1999) (see Figure 1). Females invite males shoaling above the boulder bottom to their territories to live in pairs for several weeks to months until spawning. After receiving a spawned egg mass in their buccal cavities, males leave the territories to mouthbrood in shoals. Female territorial behaviour is directed nearly exclusively toward potential egg predators (shoaling conspecifics) rather than toward mating competitors (Fukumori et al. 2009), suggesting that the primary function of the female territory is to avoid predation of the egg mass at the moment of spawning. After having several breeding cycles with different males, females abandon their territories in autumn to join large shoals in the water column (Okuda 1999). Thereafter, both males and females migrate to deep water to spend a couple of winter months there (Fukumori et al. 2008).

In the present study, we examined the homing ability of female *A. notatus* from their deep-water habitat to their neritic breeding habitat, by focusing on the positional shifts of territories occupied in two consecutive breeding seasons.

## Methods

We conducted a field survey at Morode Beach, Shikoku Island, Japan, with the aid of SCUBA. We set a quadrat measuring 10 × 20 m on the boulder area at a depth of 3.6-8.5 m and censused *A. notatus* there four or five times per month from April 2000 to

March 2001 (but only once in June 2000). In each census, we counted the number of *A. notatus*, discriminating between territorial females and other fishes based on their positions and behaviour.

To assess how accurately females return to their territories over consecutive breeding seasons, we conducted a follow-up survey of individually marked females from 1999 to 2000. At the beginning of the breeding season in 1999, we caught 139 females in and around the quadrat using seine and hand nets, and marked them with visible implant elastomer (VIE) tags (see Okuda 1999 for details and ethical notes). After marking, we released them at their capture sites.

We plotted the locations of marked territorial females on the quadrat map in 15 weekly censuses conducted from June to October, 1999. To estimate territory size, we measured the area of a minimum convex polygon covering all locations plotted for each female whose locations were plotted at least three times. We also converted these locations into x and y coordinates and averaged the values of each coordinate to determine the centroid of the territory. In the following breeding season, we conducted 21 censuses for marked females found in the quadrat to detemine the centroid of each territory again. We used the distance between the two centroids as an index of their homing accuracy.

## Results

### Breeding behaviour

Female *A. notatus* started to establish their territories in March, and the number of territorial females increased until May when the earliest spawning was observed (Figure 2). Thereafter, the number of territorial females was relatively constant until August but declined drastically in September, the final month of the breeding season. After the last spawning, females abandoned their territories to join shoals consisting of both sexes in the water column. Shoals were near the breeding ground from September to November. However, in December when the water temperature drastically decreased (Fukumori et al. 2008), most of *A. notatus* disappeared from the breeding ground (Figure 2).

Homing Behaviour

Of 139 marked females, 118 established territories and their locations were repeatedly plotted on the quadrat map during the 1999 breeding season. Their breeding territories were 0.27 ± 0.38SE $m^2$ (N = 118). Of 117 marked females found at the last census of 1999, 75 (64.1%) were found again in and around the same quadrat in the following breeding season. Most of them (82.1%) occupied the same site as in the previous season, with only a 0.57 ± 0.06SE m shift (Figure 3). This means that most fish returned to 20-30 cm of their previous breeding territory. The longest shift observed was 3.0 m.

## Discussion

Spatial memory ability has been reported in a variety of animal species. Some studies have suggested that memory capacity is determined by particular ecological conditions and life history demands (Mackney and Hughes 1995; Clayton 1998; Healy et al. 2005; Odling-Smee et al. 2006). Grey squirrels can remember the precise location of their food storage using a visual cue, and their spatial memory lasts for 62 days at most (Macdonald 1997). The nutcrackers are able to accurately relocate the caches they had made using visual cues and memory persists for 9-11 months (Balda and Kamil 1998; Gibson and Kamil 2009). In fish, spatial memory duration usually ranges from 8 to 330 days (Aronson 1971; Milinski 1994; Brown 2001). Lindauer (1963) reported that bees remember the color of a feeding place over several months. Furthermore, several species of wood ants (genus *Formica*) have been shown to exhibit high degrees of site or route fidelity based mainly on visual memories of environmental landmarks (Rosengren 1971; Rosengren and Fortelius 1986).

Homing behaviour has been observed in some cardinalfishes. The Banggai cardinalfish, *Pterapogon kauderni*, have the ability to home 40 m away from the original location of their group within 24 h of experimental translocation (Kolm et al. 2005). In three Australian cardinalfishes, *Apogon doederleini*, *Cheilodipterus artus*, and *Cheilodipterus quinquelineatus*, adult fish were able to return to their reefs within 3 days after being experimentally moved 2 km away (Marnane 2000). An isotopic study revealed that *A. notatus* overwinter in a deep-water habitat more than 600 m away from their breeding ground (Fukumori et al. 2008). This means that *A. notatus* also have long-distance homing ability.

Fish use several cues for orientation during migration: e.g., olfactory cue (coho salmon *O. kisutch*: Nevitt et al. 1994; five-lined cardinalfish *C. quinquelineatus*: Døving et al. 2006), the earth’s magnetic field (blue shark *Prionace glauca*; stingray *Urolophus halleri*: Kalmijn 2000), and polarized light stimulus (juvenile rainbow trout

*Oncorhynchus mykiss*: Parkyn et al. 2003). A magnetic cue is useful for long-distance cruising during ocean migration, while olfactory and visual cues provide migrators spatial information on local environments. It is well known that salmonids use the earth's magnetic field as an orientation cue during ocean migration, while they also use olfactory and visual cues when approaching their natal stream and breeding ground (Atlantic salmon *S. salar*: Hansen et al. 1993). In the Australian cardinalfish, *C. quinquelineatus*, individuals can discriminate between conspecifics from their own reef and those from other reefs by scent, suggesting that their homing behaviour is based on an olfactory cue (Døving et al. 2006). Fukumori et al. (2009) indicated that female *A. notatus* establish their territories on the basis of the physical characteristics of the breeding ground, such as boulder size and structural complexity. Female *A. notatus* seem to use visual cues for pinpoint homing, based on the memory of detailed spatial structure around their territories, although they may use magnetic and/or olfactory cues to navigate in open water.

Recently, it has been reported that the medial and lateral pallia of teleost fish have functions analogous to the hippocampal pallium and pallial amygdala of mammals (Broglio et al. 2005). Some fish may have the potential to retain long-term spatial memory, as suggested in the present study.

In conclusion, in *A. notatus*, the period during which females are away from their territories is approximately 6 months: 3 months of shoaling after territory abandonment and 3 months in deep-water habitat in winter. Such long-term spatial memory is high among hitherto reported fish. In addition, females possess the ability to pinpoint the exact location of their previous territory. Future work will address the mechanistic basis for this kind of spatial memory.

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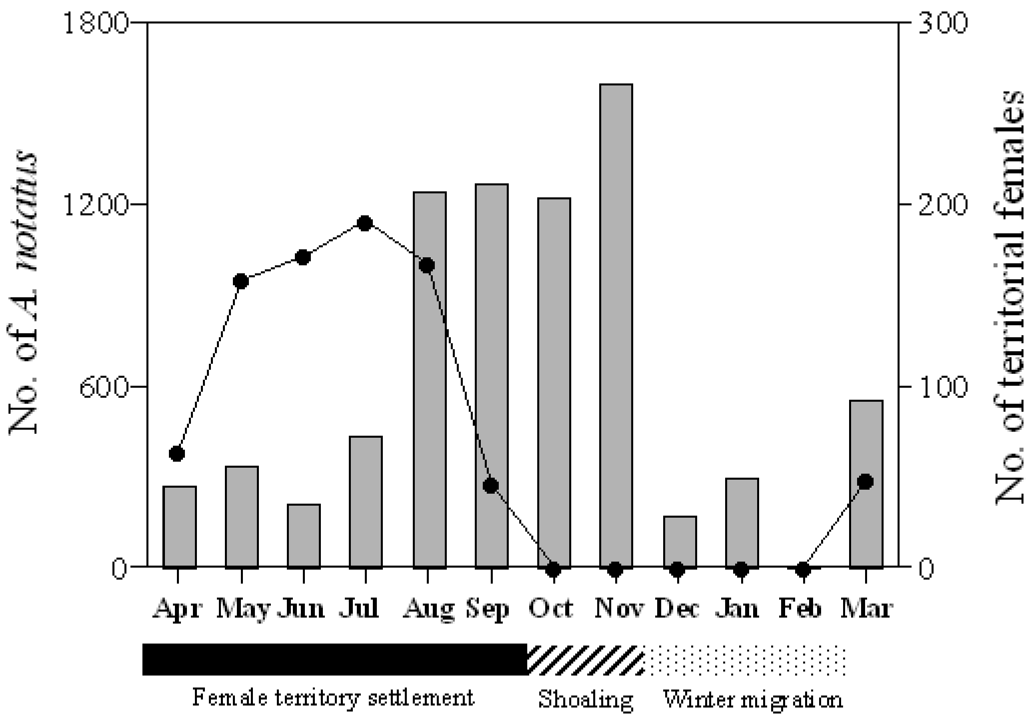
## Figure legends

**Fig. 1** Shallow boulder habitat of *A. notatus* at Morode Beach, Japan. There are three pairs (black arrows) in the photograph (Photo by S. Oguri). See text for details.

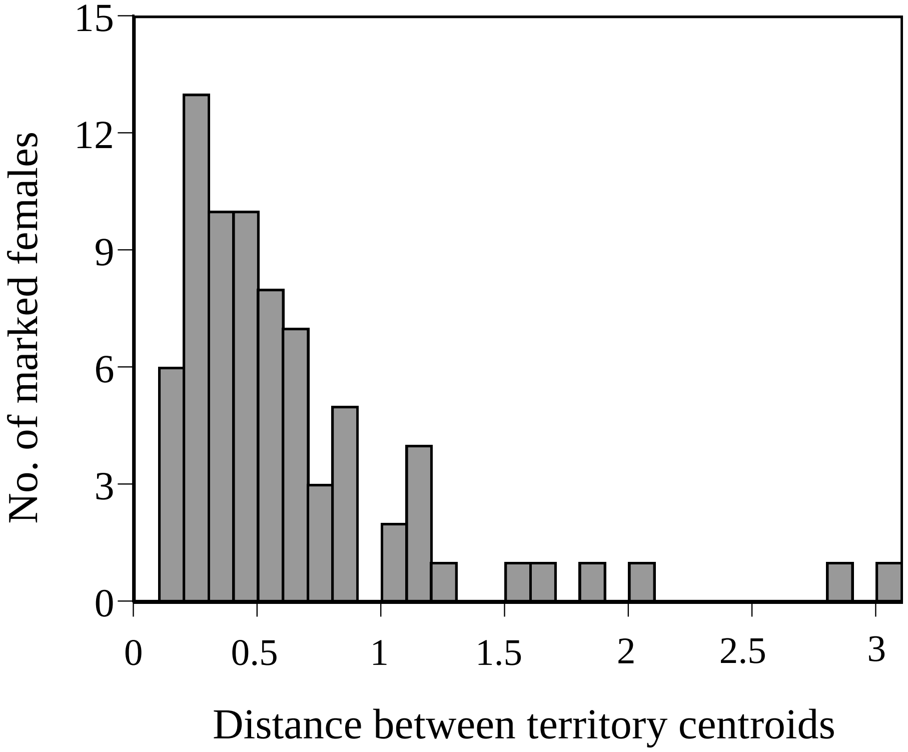
**Fig. 2** Monthly changes in the total number of *A. notatus* (grey bars) and the number of territorial females (closed circles) in the study quadrat. Black, horizontal hatched, and dotted bars indicate periods of female territory settlement, shoaling, and winter migration, respectively.

**Fig. 3** Frequency distribution of distance (m) between centroids of territories settled by each marked female in two consecutive breeding seasons.

Fukumori K. et al. Fig. 1




Fukumori K. et al. Fig. 2




Fukumori K. et al. Fig. 3