

Discovery of an Earliest-Stage “Mystery Circle” and Development of the Structure Constructed by Pufferfish, *Torquigener albomaculosus* (Pisces: Tetraodontidae)

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Abstract: Male pufferfish (*Torquigener albomaculosus*) construct “mystery circles”, geometric circular structures with radially aligned peaks and valleys, on sandy seabeds for reproduction. We discovered an earliest-stage mystery circle composed of dozens of irregular depressions. The structure changed to a primitive circular form with radially aligned valleys and a circular depression in the central area on day 2. The number of valleys increased, and the radius of the structure was much smaller than that of the final structure. These features were observed from days 2 to 4, considered the early stage of construction. The mystery circle had radially aligned peaks and valleys with a central flat area, while the radius and the differences in elevation between the peaks and valleys were increasing. These features, which were observed from days 5 to 8, were considered the middle stage of construction. On day 9, the mystery circle was completed. An irregular pattern was formed in the central area, and the radially aligned peaks were decorated with shell and coral fragments. These are the first recorded observations of construction of a mystery circle from the earliest stage.

Keywords: reproductive behavior; spawning ecology; paternal egg care; three-dimensional morphogenesis; Amami-Oshima Island

1. Introduction

Pufferfishes (family Tetraodontidae, Order Tetraodontiformes) are distributed in tropical and temperate regions of the Atlantic, Indian, and Pacific Oceans. They comprise 25 genera with 159 species [1,2]. The reproductive behavior of several species of pufferfish has been reported, with descriptions of the spawning substrate. Groups of *Takifugu niphobles* migrate to intertidal beaches to release their gametes on boulders [3–5]. Females and males of several *Canthigaster* species mate in pairs polygynously and release their gametes on algae [6–9]. In some *Tetraodon* species inhabiting fresh and brackish waters, the male constructs a nest and cares for the eggs until hatching [10,11].

The white-spotted pufferfish *Torquigener albomaculosus*, which has recently been listed as a new species of tetraodontid [12], is distributed in southern Amami-Oshima Island, Japan. We discovered that pufferfish males that have attained 120 mm in total length construct “mystery circles”, which are geometric circular structures with radially aligned peaks and valleys measuring approximately 2 m in diameter [13]. The males construct the structures by digging valleys at various angles in a radial direction with their fins and body. They create an irregular pattern with fine sand particles in the central area and decorate the radially aligned peaks with shell and coral fragments before the day of spawning [13]. The females visit the males’ nests on the sandy bottom, and the females and males mate in pairs and release their gametes on the nest. They mate repeatedly until the female leaves the nest. A male will mate with other females on the same day. After mating, the male stays at the nest site and cares for the eggs deposited on the sandy bottom until hatching. The reproductive ecology of *T. albomaculosus* is unique among tetraodontids in its paternal egg care and mating system of male-territory-visiting polygamy [14].

In our recent underwater research, we discovered earlier construction stages of the circular structures than those reported in the previous study [13], whose shape was quite different from that of the later-stage structures. Here we describe the development of the structure of the mystery circle constructed by the pufferfish *T. albomaculosus* from the beginning of construction until completion.

2. Results and Discussion

The process of construction of the mystery circle was roughly divided into three stages: an early stage with a basic circular shape, a final stage with an irregular pattern in the central area, and a middle stage between the early and final stages [13]. In the present study, we discovered a mystery circle in an earlier stage of construction than has been previously reported. The earliest stage was found on the sandy seabed at a depth of 13 m on the morning of 7 June 2016 (Figure 1A and Table 1). Dozens of irregular depressions were observed in an area of approximately 65 × 50 cm. The male created the depressions by pressing his body against the sandy bottom and flapping his pectoral and caudal fins rapidly (Supplementary Video 1). In the afternoon, the number of depressions was increased, and several tens of depressions were observed in an area of approximately 85 × 75 cm (Figure 1B). The features of this earliest-stage mystery circle were fundamentally different from those of the later stages in that radially aligned valleys and a circular depression in the central area were not yet formed. Why did the male create irregular depressions on the seabed instead of creating valleys from the beginning? The depressions might function as landmarks for the formation of the radial pattern, which is constructed by digging valleys from the outside to the center repeatedly in the later stages of construction.

Table 1. Number of valleys detected from outside (VO) and from inside (VI) the structure and radius of a mystery circle constructed by the pufferfish *Torquigener albomaculosus*.

Day	Date	VO	VI	Radius (mm)	Figure 1
1	7 June	ne	ne	-	A,B
2	8 June	24	22	603	C,D
3	9 June	u	u	-	E
4	10 June	28	24	607	F
5	11 June	28	28	-	-
6	12 June	28	28	831	G
7	13 June	29	29	-	-
8	14 June	29	29	-	-
9	15 June	29	29	918	H

u: uncountable; ne: non-existent; -: no data.

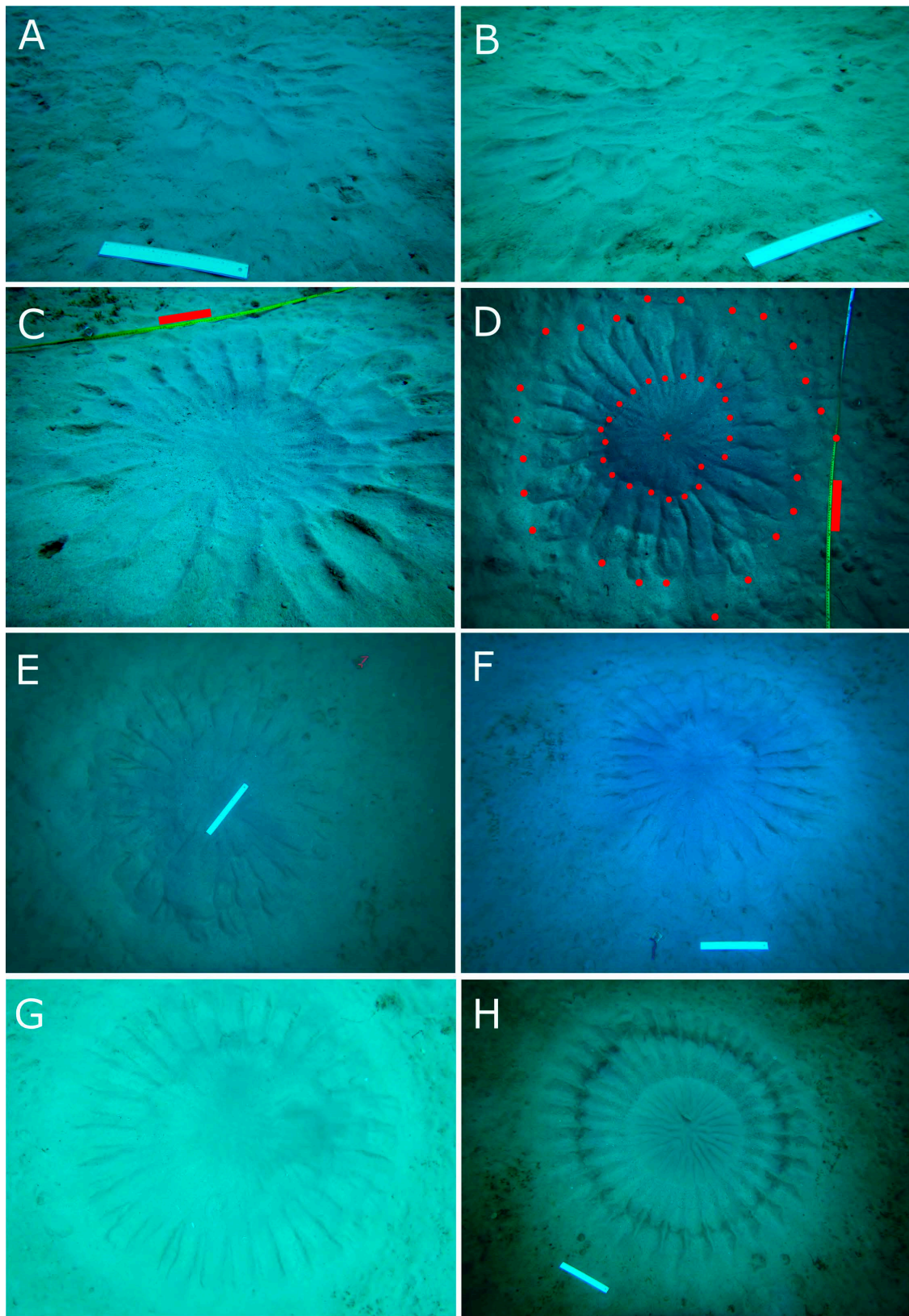


Figure 1. Development of mystery circle constructed by the pufferfish *Torquigener albomaculosus*. The earliest stage (A,B), early stage (C–F), middle stage (G), and final stage (H) of construction are shown. See details in text and Table 1. Scale length in (A,B,E,F,H) is 30 cm. Red bar in (C,D) represents 20 cm. See text for red star and solid circles in (D). Photo by Hiroshi Kawase.

On the morning of the second day of observation (8 June), the configuration of the structure was not very different from that on the previous afternoon. However, by the afternoon it had drastically changed to a structure with radially aligned valleys and a circular depression in the central area (Figure 1C, D and Table 1). The radius, i.e., the distance between the center (Figure 1D, star) and the beginning of the valleys (Figure 1D, outer solid circles), measured 603 ± 48 mm (mean \pm standard deviation, $n = 24$). There were 24 valleys detected from outside the structure (VO) (Figure 1D, outer solid circles) and 22 valleys detected from inside the structure (VI) (Figure 1D, inner solid circles). Because the outer edges of the valleys were irregularly branched (Figure 1E), VO and VI were uncountable on the third day of observation (9 June) (Table 1). On the fourth day of observation (10 June), VO and VI had increased to 28 and 24, respectively (Figure 1F and Table 1), while the radius of the structure (607 ± 49 mm, $n = 28$) was not very different from that on 8 June. From the afternoon of 8 June to 10 June (Figure 1C–F), the mystery circle can be regarded as being in the early stage of construction. During this period, it had a primitive circular form with radially aligned valleys and a circular depression in the central area, the number of valleys was increasing, and the radius of the structure was much smaller than that of the final structure.

On the fifth day of observation (11 June), the number of VI valleys reached 28 and matched the number of VO valleys. The number of VOs was still 28 as on the day before; however, the radius extended to 831 mm on 12 June (Figure 1G and Table 1). The numbers of VO and VI valleys reached 29 on 13 June and remained constant thereafter (Table 1). From 11 to 14 June (Figure 1G), the mystery circle can be regarded as being in the middle stage of construction. During this period, it had the basic form of radially aligned peaks and valleys with a central flat area, while the radius and the differences in elevation between the peaks and valleys were still increasing.

On the ninth day of observation (15 June), the radius reached 918 mm. An irregular pattern was formed in the central area, and the radially aligned peaks were decorated with shell and coral fragments (Figure 1H and Table 1). Thus, the mystery circle was completed. As the two characteristics appear restrictively before mating, it appears reasonable to assume that females visiting male nest sites evaluate the nest characteristics and that these characteristics play an important role in female mate choice [13,14]. These are the first recorded observations of construction of a mystery circle from the earliest stage.

3. Materials and Methods

The behavior of male pufferfishes *Torquigener albomaculosus* constructing mystery circles and the features of the structure were observed by scuba divers off Katetsu ($28^{\circ}08' \text{ N}$, $129^{\circ}20' \text{ E}$), southern Amami-Oshima Island, Japan, from 31 May to 30 June 2016. An observation area of approximately 50×30 m was set up on the sandy bottom at depths of 13 to 17 m, where the water temperature ranged from 24 to 26 °C during the observation period. Two males (A and B) repeatedly constructed mystery circles in each reproductive cycle in the observation area. On 7 June, male A began to construct a new mystery circle (A2) while caring for eggs at a current mystery circle (A1) (Kawase in prep.). The process of construction of mystery circle A2 was observed on one to three dives per day, for approximately 30 to 40 min per dive, every day until completion of the structure. The process of construction of the circle was recorded on a portable digital still camera (Coolpix AW130, Nikon Corporation, Tokyo, Japan) and a video camera (GoPro Hero, Woodman Labs, Inc., San Mateo, CA, USA) for analysis of the structure and of the male's behavior.

Supplementary Materials: Supplementary video 1 is available online: <http://www.mdpi.com/2410-3888/2/3/14/s1>. Digging behavior of male *Torquigener albomaculosus* in the earliest stage. The male presses his body against the sandy bottom and flaps his pectoral and caudal fins rapidly. Video by Hiroshi Kawase.

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perspective of building simulation of mystery circle. Koh Hosoda, Masahiro Shimizu, and Hirofumi Shin participated in the discussion from the perspective of motor patterns of fins of pufferfish. Daisuke Iwai and Yuki Kitajima participated in discussion from the perspective of three-dimensional structure of mystery circle.

Conflicts of Interest: The authors declare no conflict of interest.

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