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SUBJECT AREAS:
BEHAVIOURAL ECOLOGY
BIODIVERSITY
ANIMAL BEHAVIOUR
ICHTHYOLOGY

Received
19 April 2013

Accepted
13 June 2013

Published
1 July 2013

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Role of Huge Geometric Circular Structures in the Reproduction of a Marine Pufferfish

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We report that male pufferfishes (*Torquigener* sp., Tetraodontidae) constructed large geometric circular structures on the seabed that played an important role in female mate choice. Males dug valleys at various angles in a radial direction, constructing nests surrounded by radially aligned peaks and valleys. Furthermore, they created irregular patterns in the nest comprising fine sand particles. The circular structure not only influences female mate choice but also functions to gather fine sand particles in nests, which are important in female mate choice. Strangely enough, the males never reuse the nest, always constructing a new circular structure at the huge cost of construction. This is because the valleys may not contain sufficient fine sand particles for multiple reproductive cycles.

Sex differences in size, shape, colouration and behaviour arise from differences in reproductive success caused by competition over mates. Male weapons evolve through contests over females, and male ornaments evolve through sexual selection by female mate choice (sexual selection)^{1,2}. These hypotheses proposed by Darwin have been demonstrated in various organisms, providing an increasingly realistic picture of sexual selection in nature^{3–10}. Although selective mechanisms and characteristics have been studied in fishes, our knowledge is still limited to specific groups: tropical freshwater fishes such as guppy and swordtail (Poeciliidae), which are easy to use for aquarium experiments^{11–13}, and shallow reef fishes such as wrasse (Labridae) and damselfish (Pomacentridae), which are easy to observe underwater^{14–16}.

A geometric circular structure measuring approximately 2 m in diameter was found in 1995 on the seabed near southern Amami-Oshima Island in subtropical Japan. Subsequently, the circular structure was sporadically observed by native recreational divers. The origin of the structure was unclear, and it was unknown whether it had been created by a natural phenomenon or by an organism. The divers named the structure a 'mystery circle'. We identified a small male pufferfish, *Torquigener* sp. (Tetraodontidae) (Fig. 1), measuring approximately 120 mm in total length, constructing the circular structure on the seabed for the first time in 2011. Here we describe how the males construct the structure and discuss reasons for their construction based on underwater observations and the results of a hydrodynamic experiment. Furthermore, we discuss the structure's function and the reasons behind the male's construction of a new structure during each reproductive cycle, instead of repairing and reusing the previous structure.

Results

A total of 10 male reproductive processes (the process of preparing for spawning and subsequently performing egg care) were observed in 2 observation areas set up on the sandy bottom off Seisui and Katetsu (Fig. 2). Two males appeared in each observation area, and no other male was observed in these areas. Although they were not tagged, at least the males at S1 and S3 and K1 and K3 were identified as the same individuals by the presence of lateral body scars in the former and the construction of another new circular structure during egg-care in the latter (Fig. 2). This is to say the actual number of male individuals in the study areas ranged from 4 (in the case that males at S1, S3 and S5; S2, S4 and S6; K1 and K3; and K2 and K4 were the same individuals, respectively) to 8 (in the case that all males except those at S1 and S3, and K1 and K3 were different individuals). Each of the males constructed a circular structure on the seabed (Fig. 3), which required 7–9 days to complete (Fig. 2; K1, K3 and K4). The behavioural patterns of the males changed during construction (Fig. 4).

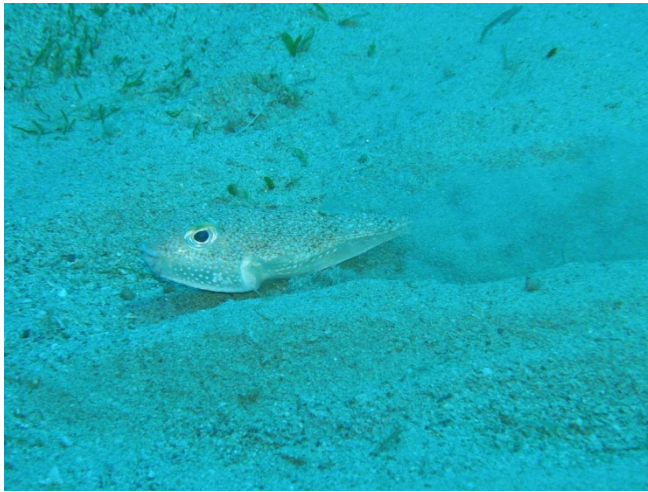


Figure 1 | Male pufferfish, *Torquigener* sp. The male is digging a valley with its fins and body. Photograph by K. Ito at Seisui on 23 April 2012.

During the early stages of construction (on the day of and the day after construction of the new circular structure), each male created a basic circular shape (Fig. 3a). Males dug valleys in the sandy bottom using their pectoral fins, anal fins, and caudal fins while swimming linearly (Fig. 1), and then swam at various angles in a radial direction from the outside of the circle to the inside, forming radially aligned peaks and valleys on the seabed. They passed from the outer zone towards the inside (O-in), the inner zone towards the inside (I-in) or continuously passed through both zones towards the inside (Figs. 4, 5, Supplementary Video 1). Males temporarily suspended fin flapping while moving inwards, thereby forming concavities and convexities in the valleys. They swam in random directions in the central zone (C), stirring the sand to form a flat area (Figs. 4, 5). In this way, a circular structure on the seabed composed of radially aligned peaks and valleys with a flat area at the centre was constructed. The radius of the circular structure and that of the final central zone measured 79–105 cm and 31–50 cm, respectively ($n = 6$, Fig. 2). The number of peak and valley pairs ranged from 24–32 ($n = 10$).

During the middle stage of construction (between the early and final stages of construction), additional male behaviour refined the circular structure (Figs. 3b, 4). Valleys and peaks became more clearly defined as males repeatedly performed O-in and I-in behaviour (Supplementary Video 2). As the males swam in a circular direction and stirred the sand in the central zone (Supplementary Video 3), they flattened the seafloor surface and enlarged the width of the central zone (Fig. 5). Furthermore, when the males passed

through the inner zone, moving outwards (I-out), fine sand particles were stirred up and flowed towards the central zone (Supplementary Videos 4, 5).

During the final stage of construction (on the day of and the day before spawning), the circular structure was completed. The most remarkable change that occurred during this stage was the formation of an irregular pattern in the central zone (Fig. 3c). This pattern was created by flapping the anal fin on the bottom of the central zone (Supplementary Video 6). The pattern was composed of the fine sand particles (Fig. 6a) that had been gathered in the central area. During this stage, I-out behaviour was not observed (Fig. 4); a part of the inner zone where males had been flapping their fins was flattened, and the central zone was further enlarged (Fig. 5). Instead, they displayed flapping behaviour in the outer zone when they swam outward (O-out; Supplementary Video 6). During this behaviour, fine sand particles flowed towards the centre, similar to that during the I-out behaviour. During this stage, males also decorated the radially aligned peaks with shell and coral fragments, and the frequency of the behaviour was significantly higher than that during other stages (Dec; Fig. 4). They chose fragments from the seafloor of the central zone and valleys and placed them on the peaks. Females were observed to visit the male's nest only during this stage of construction. When a female approached the circular structure, the male stirred up the sand particles in the central zone of the nest. When the female entered the nest, the male retreated from the central zone and rushed towards her several times (Supplementary Video 7). The female stayed in the nest for a minute and then left, often visiting the same nest again. Spawning occurred in pairs, and the eggs were released into the central zone of the nest.

After spawning, males remained in the circular structure for 6 days to care for the eggs (Fig. 2; S3, S4, K1 and K2). They did not perform digging or other maintenance behaviour of the radially aligned peaks or valleys during this period. As a result, the structure gradually collapsed and was smoothed to become almost flat by water currents (Fig. 3d). Furthermore, the fine sand particles were dispersed and replaced by coarser sand particles (Fig. 6b), which covered the nest site. After the eggs hatched, males left the nest site and soon reappeared in the observation area for the next reproductive cycle. However, they never returned to an old nest site but instead began to construct a new circular structure at a separate site.

A fluid dynamics experiment using a 1/2 scale model of the circular structure (Fig. 7a) revealed that strings placed upstream were turned towards the inner side (blue arrow), and those that were downstream turned towards the outer side of the structure (green arrow), and those on top of the hills and central zone were turned towards the downstream direction (deep blue arrows). The directions of strings in the valleys situated at the midpoint of upstream

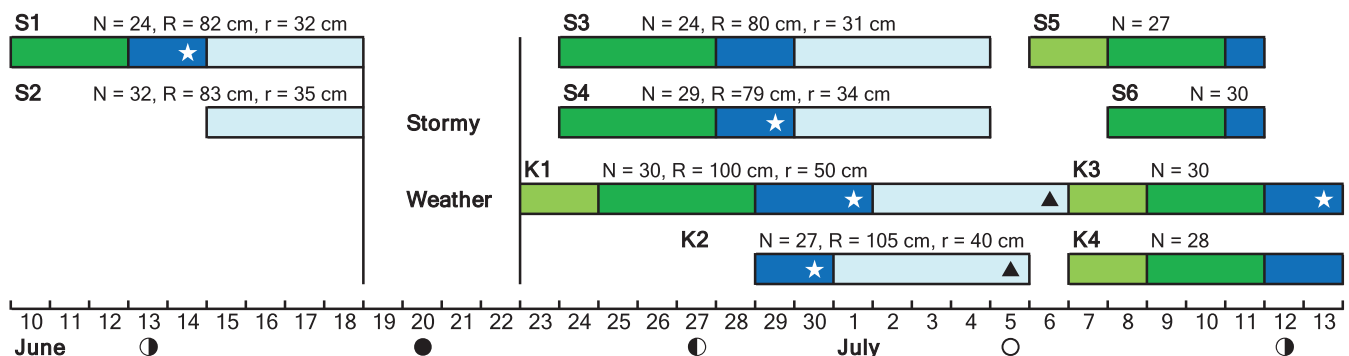


Figure 2 | Reproductive process of male pufferfish. S1–6 and K1–4 indicate circular structures found at Seisui and Katetsu, respectively. Columns of light green, green and blue indicate the duration of the early, middle and final stage of circular structure construction, respectively. Refer the text for the definitions of the 3 construction stages. Light blue columns indicate the duration of egg care. White stars indicate spawning, solid triangles indicate hatching. N, R, and r indicate the number of peaks and valley pairs, radius, and radius of the central zone of the circular structure, respectively.

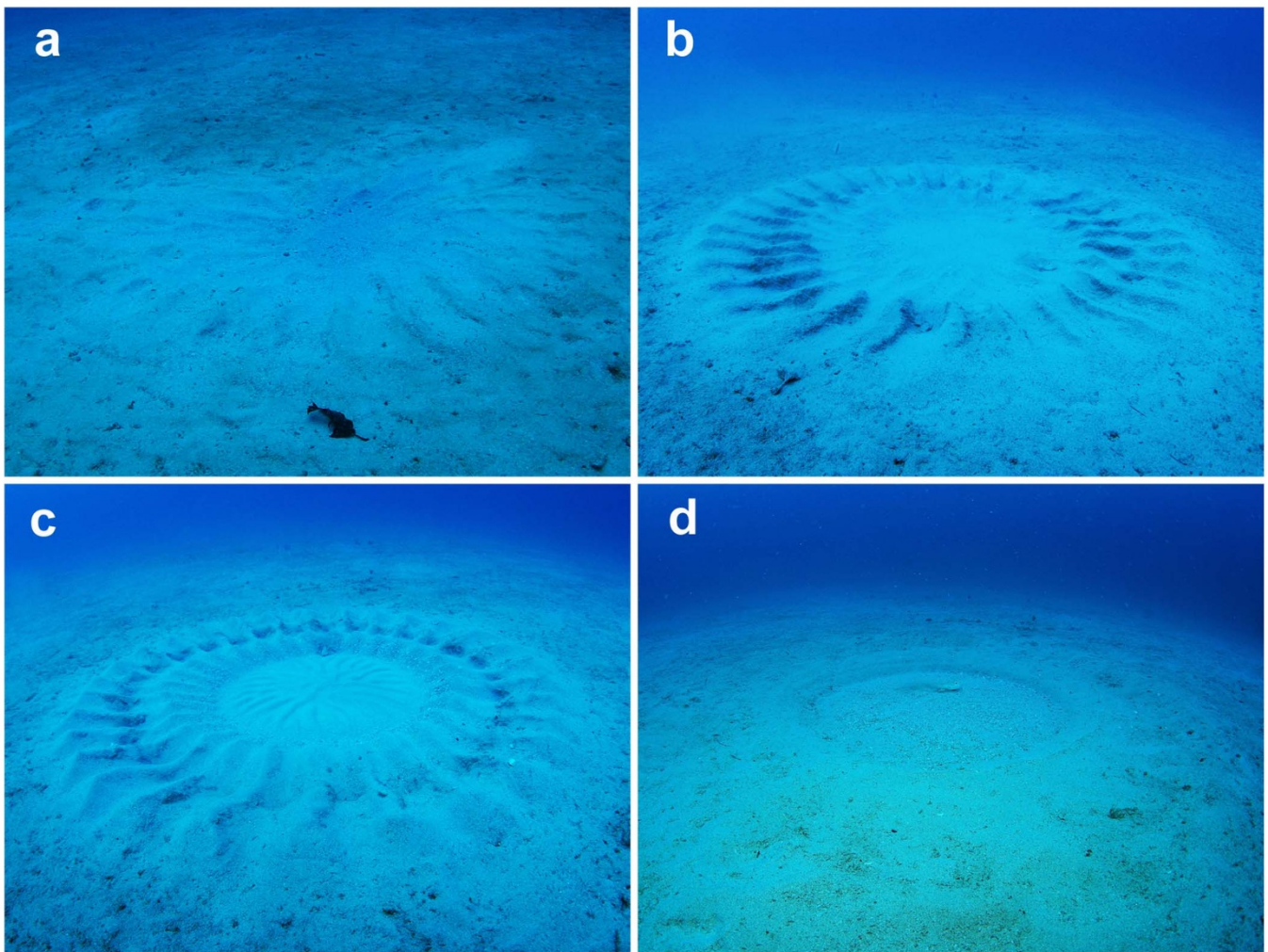


Figure 3 | Changes in the circular structure constructed by male pufferfish. (a) Early stage; (b) middle stage; (c) final stage; and (d) after spawning of the same circular structure of K1 in Figure 2. Photograph by Y. Okata on 23, 27, 29 June, and 6 July 2012, respectively.

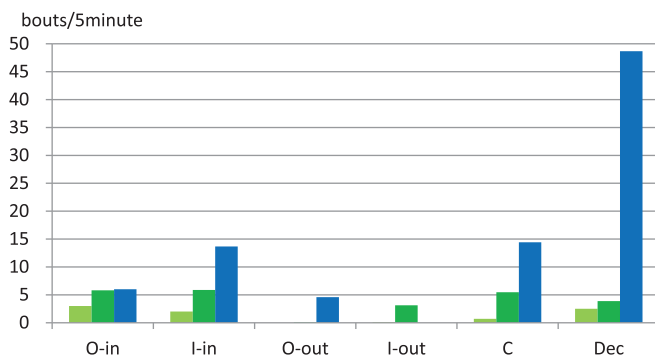


Figure 4 | Changes in behavioural pattern frequency during construction of a circular structure. Columns in light green, green and blue indicate the duration of the early, middle and final stage of circular structure construction, respectively. See text for the definition of the 3 construction stages and the abbreviations of O-in, I-in, O-out, I-out, C and Dec. Data is based on the observations of K3 in Figure 2. O-in: $P = 0.198$ (N. S.), $H = 3.236$; I-in: $P < 0.001$, $H = 15.676$; O-out: $P < 0.001$, $H = 15.973$; I-out: $P < 0.001$, $H = 25.805$; C: $P < 0.001$, $H = 28.956$; Dec: $P < 0.001$, $H = 21.253$, Kruskal–Wallis test.

and downstream were unclear (light blue arrow). These results revealed that the water flows in a central direction in the valleys at the upper reaches of the water current (Fig. 7b, blue arrows), while water flows out of the circular structure in the valleys at the lower reaches of the water current (Fig. 7b, green arrows). There is little water flow in the valleys situated at the midpoint of the upper and lower reaches of the current. The direction of water flow on top of the peaks and the centre of the circular structure is the same as the current direction (Fig. 7b, deep blue arrows). The current speed in the central zone was reduced by 24.2% from that of outside the circular structure.

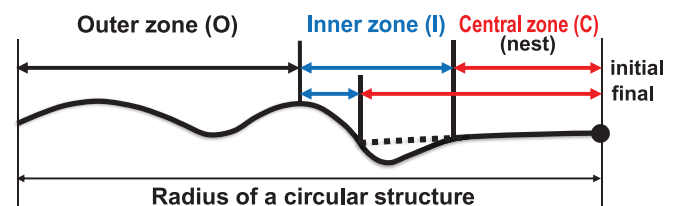


Figure 5 | Sectional view of the radially aligned valley in the circular structure. As construction proceeded, the width of the central zone enlarged (dotted line).

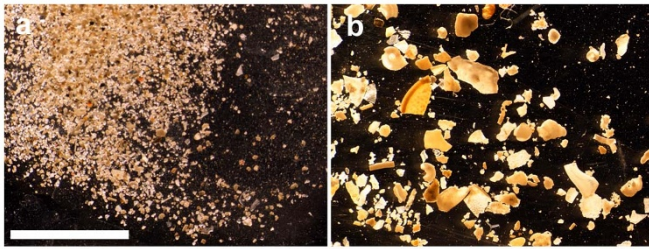


Figure 6 | Photomicrographs of the sand particles at the nest site. Collected on the day before spawning (a) and hatching (b). White bar = 5 mm.

Discussion

There are few studies on the reproductive behaviour of pufferfishes (Tetraodontidae) based on underwater observation, with the exception of *Canthigaster* species. *C. valentini* and *C. rostrata*, tropical species inhabiting shallow coral reefs, mate in pairs and deposit demersal eggs on the algae, which are not tended by both parents^{17,18}. Egg release on sandy bottoms has also been observed in some species of leatherjackets (Monacanthidae) and triggerfishes (Balistidae), which are closely related to Tetraodontidae^{19–22}. Nest sites are constructed on sandy bottoms for mating in some fish species. Nest sites of the yellow-margin triggerfish *Pseudobalistes flavimarginatus* are crater-like depressions in the substratum, 1.5–2.5 m diameter, with small piles of coral rubble. Males deepen and widen their nest sites and construct egg chambers in these piles. Both parents care for the eggs released in the chambers²³. Males of some species of Tanganyikan cichlid are known to construct crater-shaped mounds, which females only visit to have their eggs fertilized^{24–26}. However, in the pufferfish species investigated in this study, the nest exhibited 3 unusual characteristics that have never been reported in fish. First, radially aligned peaks and valleys were created outside the nest site; second, the peaks were decorated with shell fragments; and third, fine sand particles were gathered in the nest site to create an irregular pattern. All 3 characteristics were completed and maintained before mating, when females visited the nest site, and they collapsed thereafter. Therefore, it appears reasonable to assume that females visiting male nest sites evaluate nest characteristics and that these characteristics play an important role in female mate choice. However, the definite factors affecting this choice remain unknown, i.e. size of the circular structure; symmetric properties, peak height, number of radially aligned peaks and valleys; size, colour, number and arrangement of the ornaments; and the design, amount and quality of the fine sand particles in the irregular central pattern.

Furthermore, the radially aligned structure surrounding the nest plays an important role in gathering the fine sand particles and depositing them in the nest site. When males dig valleys on the sandy seabed by flapping their fins, sand particles are stirred up and the smaller particles are deposited on the valley surfaces. The width of the valleys matches the male's body width; the peaks on both sides of the valleys are effective in preventing the stirred particles from being scattered. During this time, the fluidity of the sand particles is increased by the presence of water between the particles, reducing friction (liquefaction). In addition, a fluid dynamics experiment showed that water flows in a central direction in the valleys at the upper reaches of the water current. Thus, water flows towards the centre in half of the valleys, regardless of current direction. In this way, the fluid fine sand particles gathered in the valleys were propelled towards the central zone by the male's I-out and O-out behaviour. However, because the fine sand particles that accumulate in the nest site are fluid, they disappear without continuous re-supply.

Nest sites are important for breeding and are usually retained and reused during the next spawning cycle^{16,18,23}. Male *P. flavimarginatus* establish nest sites, and males repeatedly return to the same nest site for every reproductive cycle. The nest site is an important resource for the males, and severe male–male aggression results over these resources²³. It is quite obvious that the cost of constructing the nest site is extremely high in the species in our study because it took 7–9 days to complete the structure. In that case, why do males not reuse nests for the next reproductive cycle but always construct a new circular structure at a huge cost? Males could construct new structures because the valleys may not contain sufficient fine sand particles for multiple reproductive cycles; a single reproductive cycle consumes a large proportion of the available fine sand particles. Thus, males must construct a new circular structure at a separate site where sufficient fine sand particles are available.

Methods

The study pufferfish is thought to be a new species of the genus *Torquigener* (Tetraodontidae, Tetraodontiformes) because distribution of small spinules and the body colour pattern do not match that of any other fish of the genus *Torquigener* (K. Matsuura, personal communication). Thus far, the fish has only been found in the coastal waters off southern Amami-Oshima Island, the subtropical region of Japan. Despite our extensive underwater SCUBA search at less than 30 m water depth off the Island, the fish has been found, in small numbers, in very restricted areas off Seisui (28°07'N, 129°19'E) and Katetsu (28°08'N, 129°20'E).

Regular observations (1–3 times/week) were made from April to September in 2011 and 2012 to check for the presence/absence of the circular structures and the pufferfishes at Seisui and Katetsu. An observation area of approximately 50 m × 30 m was set up on the sandy bottom of Seisui (23–28 m water depth) and Katetsu (18–23 m water depth) for intensive surveys conducted from 11 June to 13 July 2012.

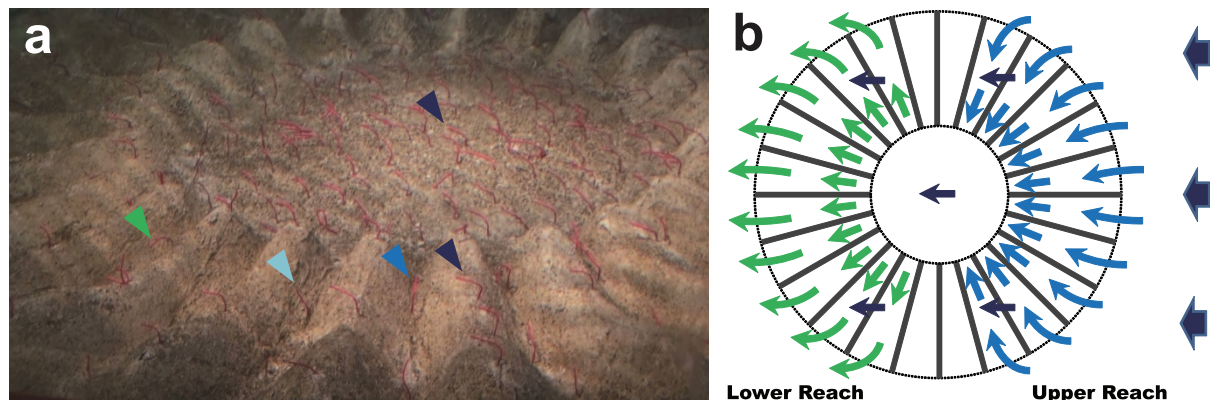


Figure 7 | Water flow experiment with 1/2-scale model of the circular structure. (a) Model of the circular structure with red strings for determining the direction of water flow. (b) Schematic diagram of the circular structure and directions of water flow. See text for the meaning of arrows.



Underwater observations (1–3 times/day, approximately 30–40 min/dive, at various times between 5:00–21:00) were performed every day, except during stormy weather, at one or both sites. Most observations were made in front of the circular structures, and male and female behaviour was recorded on portable digital still cameras and video cameras for analysis of behavioural patterns. Monitoring video cameras were also set up in front of the structures to record behaviour over a longer period of time at the same angle. The video footage recorded at Katetsu (18 m water depth) from 7 to 13 July 2012 was used to clarify the construction process, and 10–50 min of video were analysed each day. The water temperature was 24°C–27°C, and the current reached approximately 0.8 m/s in the observation area during the period of intensive survey.

The hydrodynamic experiment was performed at Chiba Experiment Station, Institute of Industrial Science, the University of Tokyo, on 20 July 2012. A 1/2-scale model of the circular structure (diameter, 1 m) with strings for determining the direction of water flow was set in a circulating water channel with a wind blower and wave maker (length, 25 m; width, 1.8 m; depth, 2 m; maximum speed of water current, 1 m/s), and the direction of water flow in the circular structure was observed. Current speed inside and outside the circular structure was measured with an electromagnetic current meter.

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Acknowledgements

We would like to thank A. Ida, H. Ida, H. Enomoto, T. Matsushita, and T. Kawasaki of NHK for field observations at Amami-Oshima Island. We thank K. Kinoshita and H. Itakura of the University of Tokyo for the circular structure flow experiment. K. Matsuura of National Museum of Nature and Science provided us with taxonomic information of *Torquigener* sp. W. Gladstone, University of Technology, Sydney gave us helpful comments on the manuscript. This study was supported by JSPS KAKENHI Grant No. 22570032.

Author contributions

Y.O. discovered the circular structure for the first time. K.I. monitored the structures and pufferfishes during breeding periods. H.K., Y.O. and K.I. conducted intensive surveys in 2012. H.K. analysed field observation data and videos and considered the functions of the circular structure.

Additional information

Supplementary information accompanies this paper at <http://www.nature.com/scientificreports>

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Kawase, H., Okata, Y. & Ito, K. Role of Huge Geometric Circular Structures in the Reproduction of a Marine Pufferfish. *Sci. Rep.* **3**, 2106; DOI:10.1038/srep02106 (2013).



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