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Authors: Urata, Makoto, Iwasaki, Sadaharu, and Ohtsuka, Susumu

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Biology of the Swimming Acorn Worm *Glandiceps hacksi* from the Seto Inland Sea of Japan

Makoto Urata^{1*}, Sadaharu Iwasaki², and Susumu Ohtsuka²

¹Marine Biological Laboratory, Graduate School of Science, Hiroshima University, 2445 Mukaishima-cho, Onomichi, Hiroshima 722-0073, Japan

²Takehara Marine Science Station, Setouchi Field Science Center, Graduate School of Biosphere Science, Hiroshima University, 5-8-1 Minato-machi, Takehara, Hiroshima 725-0024, Japan

The enteropneust hemichordate *Glandiceps hacksi* inhabits the muddy bottoms of the intertidal to subtidal zones of Koguno-shima Island, located in the central part of the Seto Inland Sea of Japan. Monthly collections from October 2005 to September 2007 revealed that their spawning occurs once a year, in the latter half of May. Parameters such as density and sex ratio, as well as the type of sediment, were also examined. Worm behavior and type of burrows revealed that *G. hacksi* are infaunal burrowers. Autotomy and regeneration of their posterior regions, and swimming behavior were also observed in an aquarium environment. This is the first comprehensive study on the biology of *G. hacksi*, the swimming acorn worm.

Key words: *Glandiceps*, enteropneust, hemichordate, acorn worm, Seto Inland Sea of Japan, burrow, breeding, swimming

INTRODUCTION

Enteropneusta is a class of the phylum Hemichordata, accommodating ca. 80 species assigned to four families, Harrimaniidae, Spengelidae, Ptychoderidae, and Torquaratoridae (Nishikawa, 1986; Osborn et al., 2012). They are commonly known as acorn worms, mainly soft, benthic animals burrowing in the mud or sand bottom of the sea. Their morphological and developmental characters, such as gill slits, stomochord, and dipleurula-type tornaria larvae, have often been regarded as ancestral characters inherited in deuterostomes. Molecular studies on Harrimaniidae and Ptychoderidae have revealed some homologies and analogies to chordates or echinoderms (Ogasawara et al., 1999; Tagawa et al., 2001; Lowe et al., 2003, 2006). The inter-class or family relationships in Hemichordata were also determined by molecular phylogenetic analysis (Cannon et al., 2009; Osborn et al., 2012).

The family Spengelidae is a small group composed of about 20 species including *Glandiceps qingdaoensis*, recently discovered in China by An and Li (2005). In contrast to the families Harrimaniidae and Ptychoderidae, the ecology and behavior of the Spengelidae are less known. *Glandiceps hacksi* was discovered in Yokohama, in middle Japan (Marion, 1885), followed by further illustrating in details (Marion, 1886), and findings of one large individual in Nagoya and two small one elsewhere in Japan (Spengel, 1893). When six small individuals were discovered from

Surabaya (Java) by the Siboga-expedition (1899–1900), Spengel (1907) described them as *G. malayanus* and distinguished the small individuals previously collected from Japan as *G. eximius* which he had considered to be a younger stage of *G. hacksi*. Recently, many individuals of *Glandiceps* sp. were collected from Shodo-shima Island in the Seto Inland Sea of Japan (Fig. 1) (Yoshimatu and Nishikawa, 1999).

A large-scale swarming of *G. hacksi* in the water column was observed off Sensui-jima Island (originally described “Sesuijima” was probably an erratum), located in the central part of the Seto Inland Sea of Japan (Fig. 1), predawn on September 3 to 5, 1907 (Ikeda, 1908). He reported anecdotal evidence from local fishermen that such unique behavior of *G. hacksi* had taken place under calm sea conditions, during nocturnal periods, from August to the beginning of September. The swarming individuals were characterized by dorsoventrally flattened bodies, dorsoventrally varying body color, and mud-free intestines, suggesting that they may be benthic creepers with their dark dorsal side up, rather than a sediment burrower (Ikeda, 1908). Recently, swarming of *Glandiceps* sp. was similarly recorded from Harima-nada in the Seto Inland Sea of Japan (Fig. 1), from the middle of August to the beginning of October (Yoshimatu and Nishikawa, 1999). On the other hand, Spengel (1909) reported on the swarming behavior of *Glandiceps malayanus* in Surabaya (Java) during the months of April to June 1899. However, the purpose of their swimming behavior and life-style remained uncertain.

In 2005, a highly dense habitat of *G. hacksi* was found on the mudflat of Koguno-shima (local name Kokuno-jima) Island, located in the central part of the Seto Inland Sea of Japan. Owing to current limited knowledge of the biology of

* Corresponding author. Tel. : +81-848-44-1143;
Fax : +81-848-44-5914;
E-mail : urata@hiroshima-u.ac.jp
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the species, apart from the bizarre swarming behavior in the water column, a biological investigation of this species was undertaken.

MATERIALS AND METHODS

Monthly collections of *G. hacksi* were carried out in the lower intertidal and shallow subtidal muddy sediments over a defined area, the southeastern side of the Koguno-shima, Takehara, Hiroshima, Japan: 34°18'17.0" N, 132°59'04.2" E (Fig. 1), from October 2005 to September 2007. Koguno-shima is a small, uninhabited, island (0.1 km²) located in the central part of the Seto Inland Sea of Japan, where the maximum tidal range is about 3.5 m. Individuals were obtained by digging into intertidal and shallow subtidal muddy sediment by hand at low tide. The subtidal zone outside the collection area was also investigated using a 0.04 m² Ekman-Berge bottom sampler on January 24, 2006, to determine the presence or absence of the acorn worm. Some individuals from the intertidal area were photographed and then either studied in the laboratory, or released back into their natural habitat. The size of living individual was measured at their collar in length and width. The density of burrows and individuals within an area of 1 m² by 20 cm depth was measured on 12 April, 21 June, and 21 September 2006. The maturity of gonads was identified by artificial insemination followed by cleavage and blastula stage, or estimated by trunk color.

To observe worm burrowing, behavior, and regeneration, ten or more individuals were cultured in an aquarium (temperature approximately 21 to 29°C, fed with a unicellular algae, *Chaetoceros gracilis* (I.S.C Co. Ltd.), and using an 1 L plastic bottle filled with mud from their natural habitat for each one or two individuals) at Takehara Marine Science Station, Hiroshima University (Takehara MSS, Fig. 1), or Marine Biological Laboratory, Hiroshima University (Mukaishima MBL, Fig. 1). One individual was cultured in a flat plastic case to observe its burrow. Some individuals were kept in constant light conditions to suppress their sensitivity to light. Swimming action of an intact individual about 13 cm in total body length was observed in a vat of seawater (30 × 20 × 4 cm) without mud and recorded by a video camera (OLYMPUS C-770). Two individuals were fixed in Bouin's solution and sectioned.

Sediment samples were taken from the surface of four sites of tidal flats in Hiroshima prefecture: the habitat on Koguno-shima and three non-habitats, the *Zostera* belt in Koguno-shima, Yoshina, and Jigozen (Fig. 1). Triplicate samples at each site were sieved over the range of 4.75 mm down to 0.075 mm with sedimentation analysis (according to Japanese Industrial Standard A 1204: 2000), fol-

lowed by loss-on-ignition analysis to estimate the amount of organic content. Surface water temperature was simultaneously recorded at the collecting site.

RESULTS

Habitat and density

All collected acorn worms were identified as adult *G. hacksi*, with the exception of one small *Saccoglossus* sp., collected on June 9, 2006. Their collar size, the smallest was 3.4 mm in length, 7.2 mm in width, largest was 8.7 mm in length, 13.8 mm in width and the average was 6.22 mm in length, 9.47 mm in width ($n = 26$), could be distinguished from that of *G. eximius*, 1 to 1.75 mm in length, 2 to 3.5 mm in width. Sections showed the absence of proboscis septum in the front part of the acorn, unlike *G. qingdaoensis*.

All individuals were found as infaunal burrowers within 10 cm of the benthic surface. They were found every month during our study period, except February 2007, without any significant changes to their morphology except for the development of gonads. The number of individuals collected is shown in Fig. 3. Estimations of individual density, per habitat, in the intertidal zone, are shown in Table 1. The density ranged from 11 to 24 ind./m² with a mean density of 16.7 ind./m² ($n = 3$). The density in the subtidal zone was 12.5 ind./m², estimated by an Ekman-Berge bottom sampler on January 24, 2006. The eelgrass *Zostera japonica* grew in some parts of the mudflat, but few individuals were collected from this location. Small numbers of other benthic animals were also collected from the habitat of *G. hacksi*. The decapod *Asthenognathus inaequipis*, belonging to the family Pinnotheridae, a possible symbiote with molluscs, polychaetes, echinoderms, and enteropneustes (Ross, 1983), was also collected around the burrow of *G. hacksi* on April 12, June 9, 21, and July 21, 2006, although the possibility of a symbiotic relationship to *G. hacksi* was not determined.

The area consisted of well-sorted mud and fine sand containing a small amount of weakly carbonized vegetable debris. The results of a grain-size distribution curve for the sediment are shown in Fig. 4. The median diameter of sediments, obtained from Fig. 4, was 0.08 mm for the Koguno-shima habitat, 0.06 mm for the *Zostera* belt in Koguno-shima, 0.40 mm for Yoshina, and 0.43 mm for Jigozen. The ignition losses of sediments were 5.32% for the Koguno-shima habitat, 4.51% for the *Zostera* belt in Koguno-shima, 1.73% for Yoshina, and 1.69% for Jigozen.

Breeding and sex ratio

Artificial insemination allowed us to determine the sex of matured individuals by their trunk color, i.e. pink ovaries for a female, white testes for a male (Fig. 2). Gonads filled the lateral body wall and usually ranged from the branchial region to the hepatic region. During the breeding season of 2006, mature individuals identified by artificial insemination were found in January 2006. Nearly all individuals collected on April 12 and May 11 developed gonads estimated by trunk color, and then, the gonads of all individuals emptied until June 21. No mature individuals were collected from the summer to winter. During the breeding season

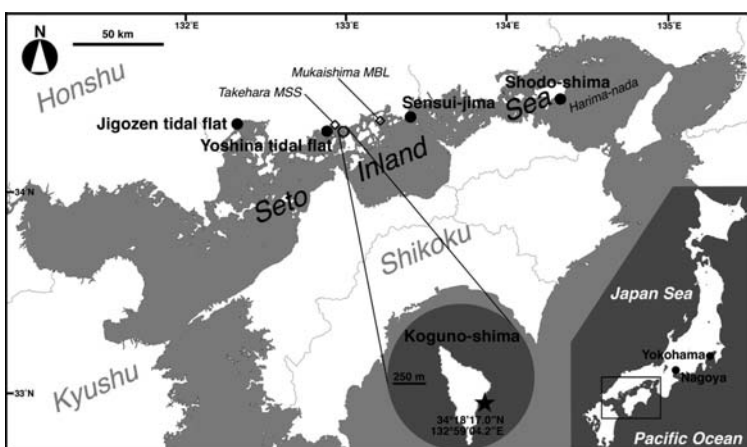


Fig. 1. Map of western Japan, showing the collection site, 34°18'17.0" N, 132°59'04.2" E (asterisk), in Koguno-shima and other tidal flat sites, Yoshina and Jigozen in the Hiroshima prefecture.



Fig. 2. Matured individuals of *G. hacksi*, showing the difference of the trunk color in the male and female. Scale bar = 1 cm.

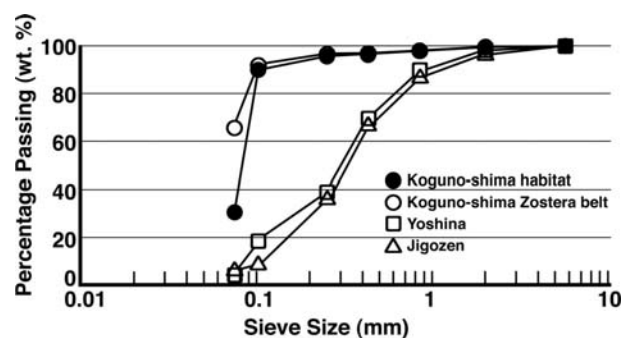


Fig. 4. Grain-size distribution curves of sediments from collection areas in Koguno-shima and neighboring tidal flats in the Hiroshima Prefecture.

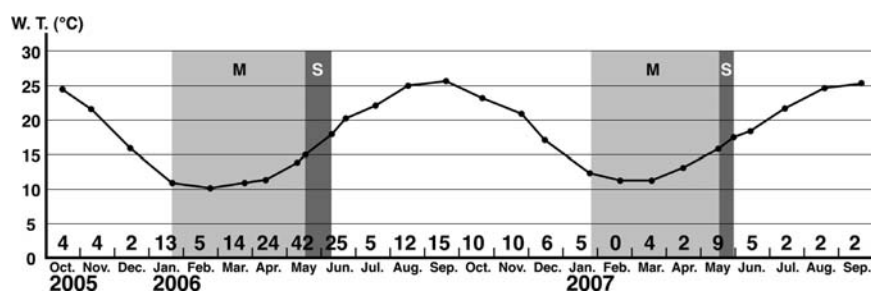


Fig. 3. Breeding season of *G. hacksi* and a change of water temperature (W. T.) around collection sites in Koguno-shima, from October 2005 to September 2007, showing maturing/matured periods (M) and deduced spawning periods (S). The number in the lower part of the graph shows the monthly number of collected individuals.

Table 1. Collection data from 1 m² × 20 cm depth of muddy area in the Koguno-shima intertidal zone.

	Male	Female	Immature	Density (m ⁻²)	Sex ratio (M: F)	Burrow-opening
12-Apr-06	12	11	1	24	1: 0.92	50
21-Jun-06	0	0	11	11	—	21
21-Sep-06	0	0	15	15	—	50

of 2007, mature individuals identified by artificial insemination were found in December 2006, and 19 out of 20 individuals collected on May 11 developed gonads estimated by trunk color. The gonads of all individuals emptied on May 31, and no mature individuals were collected until September 2007. Surface water temperature was approximately 10 to 15°C in maturing periods and increased to about 18°C in spawning periods in both years (Fig. 3, Table 1).

As shown in Table 1, the M : F sex ratio was 1 : 0.92 on April 12, 2006. A significant bias in the sex ratio was not detected (χ^2 test, $P > 0.05$).

Autotomy and regeneration

The soft trunk of worms, especially the hepatic and caudal region, was easily damaged and amputated during handling. In addition, the artificial change of seawater temperature, up to 28°C from 18°C for example, could trigger autotomy, which occurred simultaneously at many cutting points in the trunk. These points seemed to appear at certain constant intervals from the posterior (Fig. 5A). In aquaria, the antero-most piece regenerated its lost posterior part within 10 to 30 days after cutting (Fig. 5B), but the posterior piece did not regenerate. Regeneration proceeded by a morphallaxis process to form the caudal region from the branchial and/or postbranchial region within 10 days, followed by an epimorphosis process to form the posterior trunk, including the hepatic region (Fig. 5B).

Burrows and behavior

On the sediment surface in the Koguno-shima habitat, many holes, approximately 5 to 8 mm in diameter,

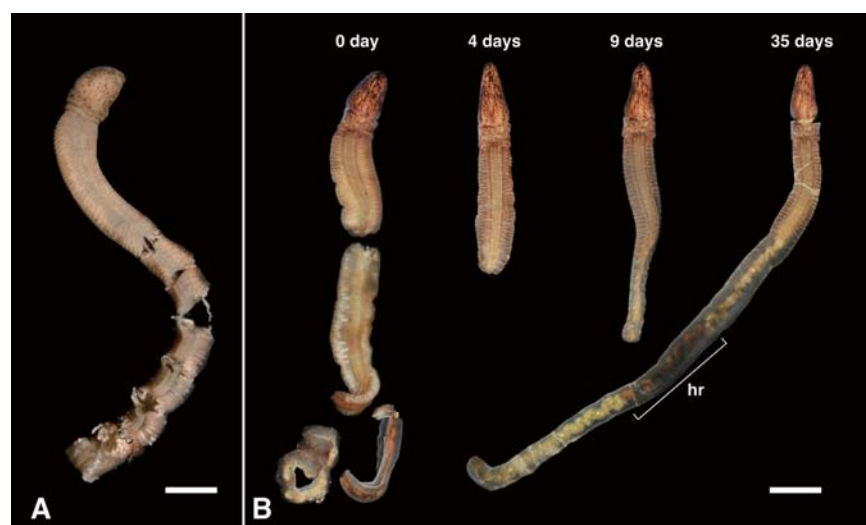


Fig. 5. Autotomy and regeneration of *G. hacksi*. (A) A male individual dismembered by autotomy, ventral view. (B) Tracing of the regeneration of the same individual cultured in aquaria, showing the primary morphallaxis process (0 to 9 days) and secondary epimorphosis process (9 to 35 days) with hepatic region (hr). Scale bars = 1 cm.

were observed (Fig. 6A). These holes opened slopingly and were liable to collapse. Because individuals in aquaria made holes similar to these (Fig. 6B), it was inferred that these were their burrow openings. Results of the estimation of the number of openings of burrows within 1 m² quadrat are shown in Table 1. No fecal mounds were identified in the

field, although individuals in aquaria sometimes made a mound consisting of fecal pellets that was fragile, cylindrical, and 2 or 3 mm in width (Fig. 6B). Usually, the gut of an individual contained some grayish mud and/or fragile blackish feces, clearly distinguishable from their burrowing mud.

When individuals were put in an aquarium, some indi-

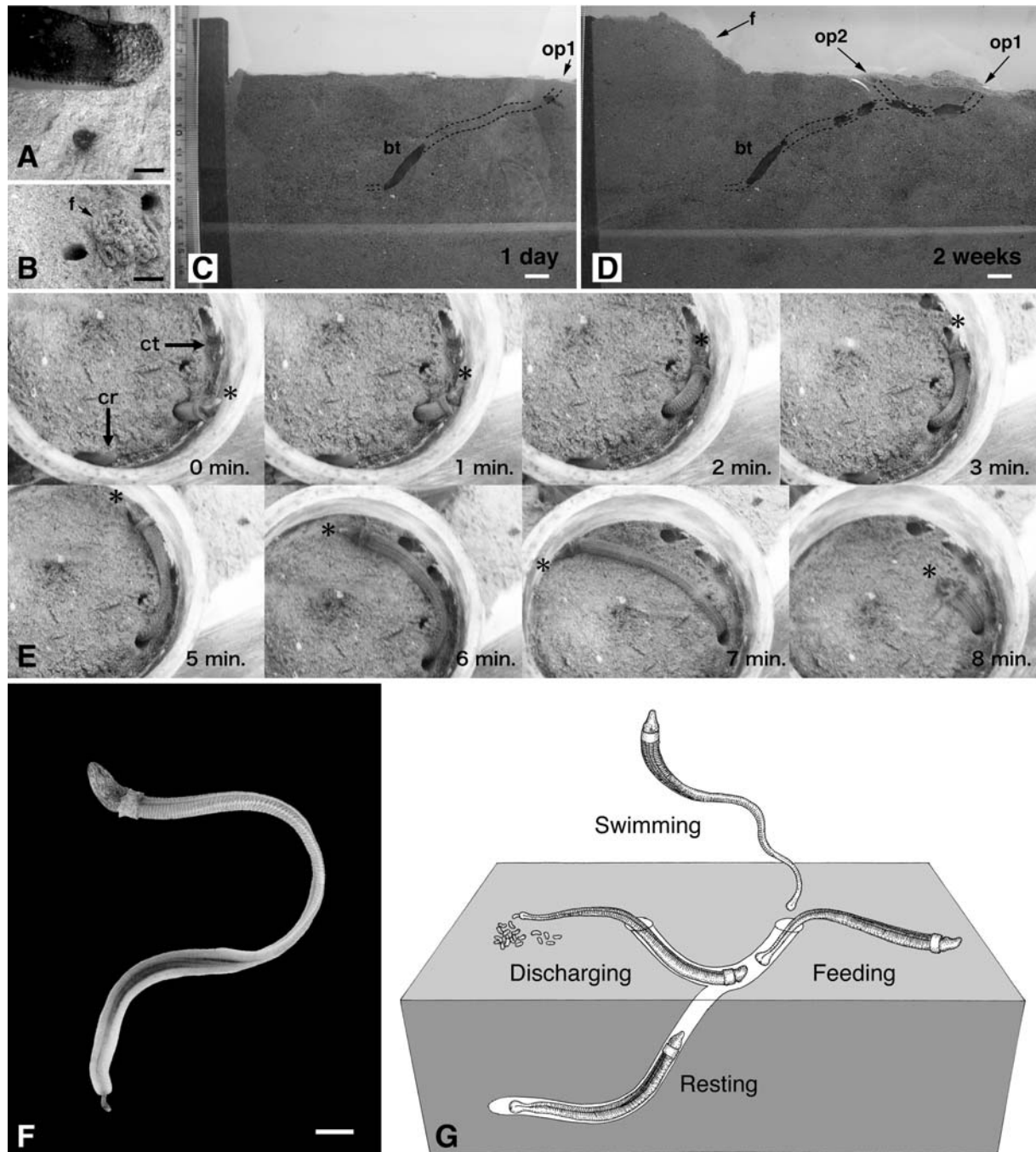


Fig. 6. Burrows and behavior of *G. hacksii*. (A) A presumed burrow opening of *G. hacksii* in the Koguno-shima habitat, with the photographer's finger added for size comparison. (B) Burrow openings and fecal pellets (f) in aquaria. (C, D) The construction process of a burrow by one individual in aquaria. Firstly there is a base tunnel (bt) with a single opening (op1) at Day One (C), with the number of openings increasing afterwards (op1, 2), and the base tunnel is maintained after two weeks (D). Note the mound of feces (f) formed far from the opening. (E) The anterior body extension process in aquaria. Asterisks show the top of the extending anterior body. Extended caudal region (cr) and crawling trace (ct) of other individuals also visible. (F) An individual adopting a swimming form and discharging fecal pellet. (G) The putative lifestyle of *G. hacksii*. Scale bars = 1 cm.

viduals crawled on the surface of the sediment for a few minutes with their dorsal side upward, but all individuals finally burrowed into sediment approximately 5 cm in depth (Fig. 6C). Burrows were relatively stable and possessed an additional opening (Fig. 6D). Fecal pellets were discarded at a place apart from the opening (Fig. 6B, D). In addition, an extended posterior region and a crawling trace from an opening were sometimes observed (Fig. 6E). Under dark conditions, individuals sometimes extended an anterior region of the body from the opening and retracted it by light stimulation. Some individuals cultured in artificial constant light condition for half a year also extended and moved the body horizontally around the opening with their posterior end hooked into the burrow (Fig. 6E).

Swimming

Although we did not observe any swimming individuals of *G. hacksi* around Koguno-shima, collected individuals quite often exhibited a body wiggle and readily engaged in swimming when removed from the sediment. Swimming behavior was often induced by artificial light or physical stimulation. Individuals could crawl out from their burrow and swim away within several seconds. This swimming ability was observed irrespective of seasons or size of individuals.

During swimming, the proboscis forewent peristalsis movement and remained fixed in a pear shape. In addition, the trunk became more flattened dorsoventrally, and individuals swam forward by wiggling the trunk (Fig. 6F), with an intermittent clockwise rotation. Swimming usually finished in a short time, followed by the body returning to its benthic form. Four episodes of swimming recorded under aquaria conditions revealed that swimming speed and wiggling cycle was 45 to 55 mm/sec and 1.80 times/sec, respectively. The average swimming time was 71 seconds and the longest was 165 seconds. Under the bright conditions in the field, released individuals immediately began to burrow into mud after reaching the sediment, even if they had started swimming.

DISCUSSION

Glandiceps species have been usually collected from depths of 10 m or more, e.g. *G. talaboti* from about 350 m (Marion, 1885) and *G. abyssicola* from 4572 m (Spengel, 1893). In this study, the estimated habitation density of *G. hacksi* in intertidal zone is comparable to previous reports that show the density of ptychoderid and harrimaniid acorn worms could amount to a few tens of individuals/m² (Nishikawa, 1977; Larsen, 1979). The results also show the restriction of the habitat to a small area containing a high ratio of silt and fine sand in comparison with the neighboring typical tidalflats. This suggests a muddy sediment environment is the typical habitat for this species or genus. Because rootstalks of eelgrass seemed to impede the burrowing of *G. hacksi*, other unknown large habitat may extend to subtidal zone.

As reviewed by Hadfield (1975), and Nishikawa (1986), previous studies on the complete development and regeneration of acorn worms have been limited to Ptychoderidae and Harrimaniidae. The clarification of the breeding season and regeneration of *G. hacksi* will serve as useful information in future developmental studies. Our data clearly show

that the spawning season of *G. hacksi* is once a year, in the latter half of May. The spawning may be correlated to changes in seawater temperature, such as the sudden rise in seawater temperature during May, from 15 to 18°C, as shown in Fig. 3. Inducing a change to seawater temperature has been a useful method for inducing artificial spawning in other acorn worms (Colwin and Colwin, 1962; Tagawa et al., 1998; Urata and Yamaguchi, 2004; Miyamoto and Saito, 2007). However, it is still unclear what triggers the induction of spawning in nature. Asexual reproduction was previously reported in some ptychoderid species and the whole process of asexual reproduction, fragmentation, and subsequent morphogenesis, was recently described in *Balanoglossus simodensis* (Miyamoto and Saito, 2010). We observed body fragmentation by autotomy in *G. hacksi*, but did not identify subsequent morphogenesis. The regeneration is well described in ptychoderid acorn worms, mainly on the regeneration from a posterior piece to the anterior (Rychel and Swalla, 2008). Miyamoto and Saito (2010) showed that the trunk piece of *B. simodensis* could regenerate posterior parts, while Humphreys et al. (2010) and Tweedell (1961) also documented the occurrence of posterior regeneration in *Ptychodera flava* and the harrimaniid acorn worm *Saccoglossus kowalevskii*, respectively. Our observations reveal a regeneration process in Spengelidae for the first time, with its remarkable ability to regenerate the posterior similar to these reports, suggesting that it may be a common feature of acorn worms. As shown in Fig. 6E, the caudal region, which regenerated immediately, functioned as an anchor during the extension of their anterior body. Therefore, the foremost purpose of the regeneration for *G. hacksi* may be a recovery mechanism in response to the stresses of daily body action and feeding.

As shown in Fig. 3, we performed monthly collection without a drastic seasonal change in the number of individuals following breeding or a previously reported swimming season. If *G. hacksi* is a stable benthic animal, the swimming swarms may not be an annual migration event, but rather caused by a temporary change to environmental conditions, for example, a decrease in dissolved oxygen content that is not uncommon in muddy sediment environments. The constant swimming ability and the dual mode (burrowing and swimming) potential of *G. hacksi* also suggest a quick response to changes in environmental conditions. On the other hand, the identification of their spawning season in the latter half of May is completely disparate from the previously reported swimming season of *G. hacksi*, from August to the beginning of September. Therefore, as Ikeda (1908) suggested by their sexual immaturity, it is clear that swimming behavior was unrelated to the breeding season. Our observations of their swimming pattern support and expand upon previous studies (Ikeda, 1908; Yoshimatu and Nishikawa, 1999). However, the purpose of their swimming behavior in nature requires further investigation.

As described in the Introduction, Ikeda (1908) suggested that *G. hacksi* is a benthic creeper. Contrary to his expectation, our investigation supported that they are infanal burrowers, as proposed by Spengel (1909). However, the observations of their body extension, crawling traces, and their sloping burrow openings suggest that they occasionally crawl out from their burrows (Fig. 6). The observa-

tion of anterior body extension behavior under simulated nocturnal conditions, suggests this behavior in nature occurs primarily at night. Feeding is likely to be the primary purpose of this epifaunal mode owing to their blackish feces consisting of microorganisms such as diatoms and dinoflagellates (Ikeda, 1908), which suggests filter and/or deposit feeding. Cameron (2002) investigated the feeding behavior of the acorn worm *Harrimania planktophilus* and suggested that the filter-feeding pharynx is evolved prior to the evolutionary divergence of the hemichordate-echinoderm clade from the chordates. He also mentioned *G. hacksii* as a potential filter-feeder. Interestingly, many acorn worms are found crawling on deep-sea floors (Osborn et al., 2012). It is now recognized that crawling, or gliding, is one life mode of the acorn worm. *G. hacksii* has, as it were, triple mode that was partially shared with the deep-sea acorn worms.

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