Spawning Aggregation of Beardfish, *Polymixia lowei*, in a Deep-water Sinkhole off the Florida Keys

Rex E. Baumberger¹, Nancy J. Brown-Peterson², John K. Reed³, and R. Grant Gilmore⁴

A large spawning aggregation of *Polymixia lowei*, Beardfish, was documented via video and specimen collection in a deep-water (413 m) sinkhole off Key West, Florida on 5 June 2007. The use of the human-occupied submersible, *Johnson-Sea-Link II*, allowed for *in situ* observations, video documentation, and specimen collection. The maximum density (117 fish m⁻²), average abundance (56 fish m⁻²), and average standard length (152.1 mm) were estimated from video transects. Ovarian histology of the fish collected indicated recent spawning activity. Observations of a deep scattering layer above the sinkhole by echo sounder suggested that *P. lowei* were in the water column during the pre-dawn hours. This represents the first report of aggregating behavior for reproduction in *P. lowei*.

A series of deep-water, marine sinkholes occur along the rim of the Pourtalès Terrace that parallels the Florida Keys in the Straits of Florida. First discovered and described from seismic surveys by Jordan (1954), the sinkholes were further described by Land and Paul (2000) using side-scan sonar aboard the U.S. Navy’s submersible NR-1. They determined that the sinkholes formed in the marine environment from submerged freshwater seeps. Reed et al. (2005) made the first submersible dives inside the sinkholes to describe their benthic biota and habitat, identifying a total of 26 fish taxa. During a recent expedition to map and describe deep-water reefs in the southern Straits of Florida, four additional sinkholes (A, B, C, and #6) were explored for the first time, and *Polymixia lowei* were observed and videotaped in sinkholes A, B, and C.

The Polymixiform *Polymixia lowei* is found on continental shelves at bathydemersal depths of 82–660 m, and is distributed throughout the Western Atlantic from Canada to Uruguay, the Gulf of Mexico, and Antilles (Haimovici et al., 1994; Moore et al., 2003; Nelson, 2006). In Brazil percent frequency of abundance from bottom trawls ranges from 5–10% (Perez and Währich, 2005). The species is usually found over soft bottoms, and its diet consists of benthic invertebrates and small fishes. *Polymixia lowei* reaches a maximum size of 200 mm SL and is most common at around 150 mm SL (Moore, 2002). *Polymixia lowei* is widely collected in low abundances in bottom trawls and gill nets, but pelagic trawls have captured larval and juvenile *P. lowei* in the mesopelagic zone (Kotlyar, 2004), indicating broadcast spawning as the most likely reproductive mode. Due to their characteristic occupation of a deep-water habitat, few *in situ* observations of behavior of *P. lowei* have been published. Gilmore (unpubl.) observed *P. lowei* in the Caribbean and Bahamas between 100 and 700 m, and Reed et al. (2006) recorded *Polymixia* sp. off the southwestern Florida shelf associated with deep-water coral mounds of *Lophelia pertusa*. This report represents the first observation of spawning aggregation in *P. lowei*.

MATERIALS AND METHODS

**Data collection.**—Dives from the R/V Seward Johnson support vessel were made with the human occupied submersible *Johnson-Sea-Link* (JSL) II to conduct video/photographic surveys and collect benthic invertebrates, fishes, and geological samples. The submersible carries four people and dives to 914 m. The front acrylic sphere provides >180° field of view to the observers and is equipped with a manipulator arm for collections with a clam-shell grab, jaw, and suction hose; 12 12.7-L Plexiglas sample buckets; and a CTD data recorder (Seabird SBE 25 Sealogger). The R/V Seward Johnson is equipped with a SIMRAD EQ50 38/50 kHz video echo sounder that allows still image captures of bottom features via computer. The surface ship navigation utilizes differential GPS (Magna-vox MX 200 Global Positioning System, DGPS), and submersible tracking uses Ultrashort Baseline Sonar technology that calculates the submersible’s real time DGPS position relative to the ship throughout each dive. Video transects were recorded on digital mini-DV tapes using an external pan and tilt video camera (Sony DXZ 3000A) with parallel lasers 25 cm apart for scale. Fish were collected using the manipulator arm suction hose and retained in a Plexiglas sample bucket for transport to the surface. The fish were photographed and immediately frozen until laboratory processing.

**Fish assemblage.**—Density estimates of the fish aggregation on the bottom of the sinkhole were calculated using CPe 3.5 point count software (Kohler and Gill, 2006). Still images of the aggregation were derived from randomly selected video frame grabs. Total filming of the aggregation consisted of seven minutes of video, 168 seconds of which was deemed suitable for scoring. Appropriate photos for scoring were well illuminated wide angle images, which were grabbed every five seconds, providing non-overlapping frames. The software was used to calculate the area of the entire image based on the scale of the laser dots, or other reference of known length in each image. A total of 30 individual frames were digitized and used for analysis. The mean and maximum fish densities were calculated based on observer counts of number of fishes in each frame divided by the software-calculated area. The standard lengths of fish were estimated by using the video camera’s parallel lasers. Fish located between the two laser points were measured by calculating the ratio of distance between laser points versus fish standard length. Standard...
length was also estimated utilizing the length/area calculating tool in CPCe 3.5. Fish from the center of the image frame were selected to reduce the effects of image distortion, and fish oriented perpendicular to the line of sight were used to accurately measure length. Five fish per frame were measured, for a total of 150 measures of standard length. This software has been used previously for estimating densities of benthic, deep-water communities, as well as measuring area and length in quadrat sampling (Kohler and Gill, 2006; Reed et al., 2006). Standard lengths of the sampled specimens were also recorded.

**Histological methods.**—The ovaries were removed from the sampled frozen specimens of *P. lowei* and allowed to thaw in 10% neutral buffered formalin. The ovaries remained in fixative for two weeks prior to histological processing. The whole ovaries were placed into individually labeled cassettes, rinsed overnight in running tap water, dehydrated in a series of graded ethanols, and embedded in paraffin following standard histological procedures. Ovarian tissue was sectioned at 4 μm and stained with Hematoxylin 2 and Eosin Y (Richard Allen Scientific) for histological inspection. Histological ovarian phases were assigned following Brown-Peterson and Heins (2009). Identification of post-ovulatory follicles (POFs) followed Hunter and Macewicz (1985) and Hunter et al. (1986).

**RESULTS**

**Description of the sinkholes.**—Five sinkholes on the outer edge of the Pourtalès Terrace were surveyed with an echosounder during the expedition; four of these were ground-truthed for the first time during five submersible dives (Sinkholes A, B, C, and #6). They did not appear to be currently active since the bottoms were filled in with sediment, and there was no evidence of temperature or salinity anomalies at the bottom, indicating no fresh water seeps from the Floridian Aquifer. Sinkhole B (24°15′21″N, 81°47′35″W, Fig. 1) is located 20.6 nautical miles (38.1 km) south of Key West. The maximum depth found by submersible was 413 m, and the top rim ranged from 269 to 296.5 m; maximum vertical relief was 38.1 km south of Key West. The maximum depth found by submersible was 413 m, and the top rim ranged from 269 to 296.5 m; maximum vertical relief was approx. 15–30 m thick. This was not visibly apparent during the descent of the dive at 0815 hr, two hours after the echosounding, but upon entering the sinkhole to a depth of 396 m, a reflecting layer approx. five meters thick was identified with the submersible sonar. As the apparent bottom (405 m) came into view, the submersible occupants recognized thousands of fish making up the sonar reading, and discovered the actual bottom seven meters deeper at 412 m. The entire bottom (as far as visible with submersible lights, >20 m) was covered with fish, which were later identified as Beardfish, *Polymixia lowei* (Fig. 1). The submersible traveled 158 m along the bottom of the sinkhole before reaching the vertical slope where the fish sightings ended.

Based on the CPCe point count software analysis of 30 individual video images, estimates of density ranged from 31 to 117 fish m⁻², with a mean of 56 fish m⁻². The total number of *P. lowei* recorded in still images was 3795 fish and the average number of fish per image was 127. Due to variation in the amount of camera zoom, individual frame area ranged from 0.6 to 5.8 m². The fish ranged in size from 92 to 197 mm SL, mean 152.1 mm SL (± 18.9 SD, n = 152). Most of the fish were swimming near the bottom, and some were seen touching the bottom with their barbels; however, feeding was not observed. The aggregation decreased in density as the submersible moved through the area. During a second dive in Sinkhole B the next day, 6 June 2007, few fish were observed. *Polymixia lowei* was observed on dives in Sinkholes A and C over the next two days. Dive JSL II #3593 conducted in Sinkhole A (7 June 2007, 461 m) revealed a less dense school of *P. lowei* of approximately 150 fish. Only three *P. lowei* were observed during the Sinkhole C dive on 6 June 2007 (JSL II #3591).

**Gonad analysis.**—Two specimens of *P. lowei* were collected from Sinkhole B using the manipulator’s suction hose (U.S. National Museum [USNM] catalog number 393552). Specimen one was a female, 172 mm SL; specimen two was also female, 157 mm SL; both had meristic counts concurrent with Moore (2002). The ovaries of both fish were histologically classified in the regressing reproductive phase. No vitellogenic oocytes were present in the ovaries of either specimen, although there were several cortical alveolar oocytes. Gonads from both fish showed POF that were estimated to be ≤ 24 h (Fig. 2). Large lamellar spaces suggest that the gonads were much larger at one time. A few atretic oocytes were present but were not common. Both ovaries showed a rather homogeneous population of primary growth oocytes, suggesting that spawning was over for the year for these individuals. There was no evidence of multiple or batch spawning.

**DISCUSSION**

Submersible ground truthing recorded depths similar to Jordan (1954), who reported a maximum depth of 439 m based on echosounder records. Fossilized dugong bones similar to those collected at Sinkhole B have been identified previously from the Jordan Sinkhole (Reed et al., 2005) and were identified as *Metaxytherium floridanum* (Dugonidae, Sirenia), a species common in the middle to late Miocene, 6.7–14.2 Ma b.p. (Domning, 1999). The fishes observed, which were similar to those reported for other deep-water sinkholes and deep-water coral reefs of the southeastern U.S.
Reed et al., 2005, 2006; Ross and Quattrini, 2007, 2009), indicate the sinkhole habitat is important for mid-slope fishes. The large aggregation of *P. lowei* documented in Sinkhole B was exceptional. This was the first videotaped documentation of a large aggregation of this fish. Numerous observations of this species during 356 JSL submersible dives from 1980 to present in the Florida Straits, Bahamas, Greater and Lesser Antilles, and Central America reveal that this species is usually a solitary benthic forager and has not been observed to school (Gilmore, unpubl.). Although numerous individuals have been videotaped in situ, their relative disposition has typically been within 5–10 m of one another and not in the same attitude or swimming direction. The present observations were unprecedented as thousands of individuals were observed in the water column in an apparent spawning aggregation. *Polymixia lowei* was not recorded during previous submersible dives in South Florida sinkholes (Reed et al., 2005). Individual *P. lowei* have been documented off western Florida on reefs consisting of 15-m tall carbonate boulder piles encrusted with corals (*Lophelia pertusa*, Stylasteridae, and Antipatharia) and sponges at depths of 414 m (Reed et al., 2006). These observations suggest sinkholes may be the preferred habitat for spawning behavior, while foraging is conducted in soft bottom habitats where barbels used for locating benthic infauna are more important.

Many fishes that inhabit the upper slope aggregate to spawn near bottom features such as seamounts and banks (Koslow et al., 2000), and many aggregations show site fidelity from year to year. In Florida, Gag, *Mycteroperca microlepis*, Scamp, *Mycteroperca phenax*, and Snowy, *Epinephelus niveatus*, groupers utilize deep pinnacles and shelf edge reefs on the western coast and the Oculina coral reefs on the eastern coast as spawning sites on the outer shelves (Gilmore and Jones, 1992; Koenig et al., 2000). Orange Roughy, *Hoplostethus atlanticus*, aggregate over seamounts in the South Pacific (Bull et al., 2001) as do Alfonsino, *Beryx splendens* (Lehodey et al., 1997). Alfonsino are broadcast spawners with pelagic larvae and juveniles. However, in the Atlantic, they spawn multiple times over a longer interval during the summer to fall and stay in their normal home range for reproduction (González et al., 2003). Gonadal analysis of Alfonsino showed highest GSI in summer in the Atlantic (González et al., 2003) and during the southern ocean summer off New Caledonia (Lehodey et al., 1997), but in both cases, the fish were not sexually dimorphic.

![Fig. 1](https://example.com/fig1.jpg)
Similarly, *P. lowei* is broadcast a spawner with pelagic larvae and juveniles (Kotlyar, 2004). During video review, no evidence of sexual dimorphism was observed in *P. lowei*, and the fish were within a narrow size range, as in *B. splendens* (Lehodey et al., 1997). The fact that all observed *P. lowei* exhibited sizes near the published maximum (Moore, 2002), and that the specimens collected were reproductive females, indicate that the fish observed were mature adults where reproduction is most likely to occur (Merrett, 1994).

Pelagic eggs provide transport off the shelf, and are common in species less vulnerable to predation and those with infrequent or transient spawning, as in Lutjanidae and Serranidae (Domeier and Colin, 1997). Transient spawning aggregations, as defined by Domeier and Colin (1997), occur during specific times of year and persist from days to a few weeks rather than year-round, moon phase is a more likely contributor to formation of the aggregation. A full moon occurred on 1 June 2007, and no scattering layers were observed during the day. The bottom transect for Sinkhole B was conducted pre-dawn (0600 hr), similar to times reported for spawning in Alfonsino, due to their diel migrations into the middle pelagic, where summer temperatures can be higher (González et al., 2003). Water temperature is not a likely trigger for spawning in *P. lowei* due to the depth they inhabit and their benthic feeding nature, which does not require vertical migration. Therefore, moon phase is a more likely contributor to formation of the aggregation. A full moon occurred on 1 June 2007, four days prior to our observation of the spawning aggregation of *P. lowei*. This, coupled with the dramatic decrease in fish over the next three days of submersible dives, fits the Domeier and Colin (1997) model for *E. striatus*. The migration of solitary individuals to sites defined by specific bottom features for spawning aggregation suggests both lunar phase and bottom habitat are important for determining the spawning site in *P. lowei*.

While the idea of a preferred habitat for spawning could explain the large number of *P. lowei* observed in the sinkhole during this dive, the scattering layer during the echosounder transect suggests that spawning behavior occurred at night in the water column. No scattering layers were observed during the day. The bottom transect for Sinkhole B was conducted during the 2007 cruise; none recorded a scattering layer as observed on 5 June 2007, when the aggregation was discovered. *Polymixia lowei* was not recorded during sixteen JSL-II submersible dives on the Pourtales Terrace from 28 May 2007 through 11 June 2007, and no scattering layers were visible on ascent or descent (Reed, unpubl.). It is likely the layer observed on the echosounder prior to the submersible dive was the vertically migrating spawning group and that spawning behavior was taking place off the bottom. When dawn arrived, it is possible the spent individuals returned to the bottom and were captured by the sinkhole, preventing them from dispersing. This is supported by the declining density of fish observed during the other four submersible dives in the immediate vicinity over the next three days. During trawl and acoustic surveys in 1996, researchers off the coast of Brazil identified deep acoustic targets, (DATs, Madureira et al., 2005), which occurred as scattering layers from 70–170 m above the bottom (470 m) during the night. *Polymixia lowei* were collected in bottom trawls beneath the DATs at relative abundances ranging from 5.8 to 43.5%. The relative abundance of *P. lowei* in Brazil. The relative abundance of *P. lowei* was highest at dusk and lowest during the day. The bottom transect for Sinkhole B was conducted pre-dawn (0600 hr), similar to times reported for sampling that contained 13.2% *P. lowei* in Brazil. The observed diurnal behavior in Brazil supports the conclusion that the scattering layer recorded by the R/V Seward Johnson sonar contained the spawning aggregation of *P. lowei* that was later observed in Sinkhole B.

Similar behavior has been reported for related species. Pankhurst (1988) conducted echosounder traces of Orange Roughy on Ritchie Bank, New Zealand, which showed fewer fish in the water column during midday than midnight on...
two days of traces (27–28 June 1986). The figures suggested some crepuscular activity as well, similar to the echo sounder trace during this aggregation. The lack of echosounder returns of any aggregation on subsequent days in June 2007 suggests the end of spawning activity may have occurred on the day of this discovery, supported by the gonadal analysis with recent POFs and the absence of late developmental stage eggs, both of which concur with the fish being spent.

The histological data strongly support a recent spawning event, although the initial freezing of the specimens resulted in some disruption to the ovarian tissues. Freezing causes damage to the POFs, creating difficulties in determining their age and condition. Additional visible in lamellar spaces could be deteriorated POFs resulting from freezing. Despite this, the POFs appeared to be recent, probably due to cold bottom temperatures (10 °C) delaying POF degradation. Thus, POF of *P. lowei* classified as <24 h based on the Hunter and Macewicz (1985) scale, which was developed for anchovy POF from warmer waters, may actually be >24 h. Regardless, the presence of recent POF in combination with observations of large aggregations of fish suggests a recent spawning event.

The density estimates, observations, and histological data indicate aggregating behavior in *P. lowei* for reproductive success and suggest that sinkholes may be essential habitats for spawning aggregations to occur off of Florida. The full moon on 1 June 2007 may have represented an environmental cue for formation of the aggregation and spawning that occurred four days later. The scattering layer observed in the pre-dawn hours corroborated by recent POF indicates the likelihood of nighttime spawning, which could explain why this behavior has not been documented. We conclude that *P. lowei* aggregate for reproduction in large numbers at night in the water column similar to *Hoplostethus atlanticus* (Bull et al., 2001) and *Beryx splendens* (González et al., 2003). The geomorphology of the sinkhole allowed the spawning aggregation to be preserved for subsurface observations and could also be a distinctive bottom feature *P. lowei* use for aggregating.

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