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Temporal and Spatial Site Sharing during Spawning in Snappers *Symphorichthys spilurus* and *Lutjanus bohar* (Pisces: Perciformes: Lutjanidae) in Waters around Peleliu Island, Palau

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Jiro Sakaue, Hiroshi Akino, Manabu Endo, Hitoshi Ida, and Takashi Asahida (2016) Two species of Lutjanidae, *Symphorichthys spilurus* and *Lutjanus bohar*, form spawning aggregation, a large school specifically formed for reproduction. Although they share the same spawning site at the southernmost reef in Peleliu Island, Palau, timing of spawning and their behaviors in the spawning and resting sites differ. Although the spawning behaviors have reported previously, long term and integrated observations documenting the size of the aggregation, exact spawning duration and timing, detailed behavioral profiles, as well as oceanic conditions upon spawning have never been reported. Here, we conducted a comparative study for these species and found behavioral and environmental cues that might be key to differentiate their ecological characteristics. *S. spilurus* begun to aggregate at full moon. Aggregations of *L. bohar* on the other hand, started from four days before full moon. Size of the aggregation was > 50,000 in *S. spilurus*, but about 7,000 in *L. bohar*. Both species migrated from the resting area to the spawning site in a diel rhythm. *S. spilurus* started spawning every half-moon, between the full moon and the new moon, while *L. bohar* spawns on every full moon. The first spawning took place at around dawn but the time shifted. *S. spilurus* spawned only when the current directs toward the southeast (offshore flow), while *L. bohar* spawns only when the current directs toward the southwest (tidal flow). Characteristic swimming behavior was observed for *S. spilurus*, in that, one or few males that could successfully chase the quick-swimming female fish could fertilize the eggs. In contrast, the behavior of *L. bohar*, was in a manner typical of several other lutjanid fish. The comparative and long-term field observation conducted over 10 years identified clear differences in the spawning behaviors of *S. spilurus* and *L. bohar*. Key behavioral and environmental factors found here might be key determinants for the ecology of these species.

Key words: *Symphorichthys spilurus*, *Lutjanus bohar*, Spawning aggregation, Lunar cycle, Current direction, Site sharing.

BACKGROUND

Lutjanid fish belongs to the Perciformes and consists of 17 genera and 109 species (Eschmeyer 2015). Most of these species are distributed in subtropical to tropical regions. Although these fishes are particularly important fisheries resources in tropical regions, little is known about their ecology. It is, however, well known that lutjanid fish form extraordinarily large schools for spawning, thus called spawning aggregations (Carter and Perrine 1994; Claro and Lindeman 2003; Heyman et al. 2005). Because fishing that targets aggregations is efficient, reduction of the resources by fishing the aggregations have been problems especially in the Caribbean Sea (Graham et al. 2008; Claro et al. 2009). It therefore is clear, that systematic and detailed studies on spawning aggregations in relation to their reproduction is of high importance for conservation and sustainable use of these fish species (Sadovy and Colin 2011).

In Palau, many species of reef fishes

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aggregate in great numbers along outer reef slopes, reef projections and along channel sides during certain seasons (*e.g.*, Golbuu and Friedlander 2011; Johannes 1981; Sadovy and Colin 2011). Lutjanid fish in Palau consist of seven genera and thirty species. Some of these including *Symphorichthys spilurus*, *Lutjanus bohar* and *L. fulvus* are known to form massive spawning aggregations (Sadovy et al. 2011). Among them, aggregations of *S. spilurus* and *L. bohar* on the southern end of Peleliu Island, the southernmost extension of the shallow reef around the main Palau group, are remarkable.

The spawning behavior of *L. bohar* is of a typical spawning behavior for lutjanid fishes since the behavioral characteristics of several other lutjanid fishes reported previously are similar to that of *L. bohar*. For example, in *L. kasmira*, Suzuki and Hioki (1979) reported that ten or more fishes in the school join in the courtship activity of the focal pair and ascended spirally all together. For *L. stellatus*, Hamamoto et al. (1992) reported a similar behavioral profile as above, Carter and Perrine (1994) reported spawning behavior of *L. jocu* in which the spawning aggregation became comet shaped cluster, the fish released gametes near surface at apex spawning rush. Heyman et al. (2005) also described for the same species that “several hundred individuals swam up from the main aggregation, leading the entire school into a tight fast-moving spiral rising towards the surface”.

Interestingly, adult individuals of *S. spilurus*, except for the spawning season, are rarely observed, and existence of larvae and juveniles also has only been recorded by using plankton-net and light-trap sampling (Leis and Bray 1995). The lack of observation means that the ecology of *S. spilurus* remains largely unknown despite the conspicuous aggregation formed in the spawning seasons. To date, no comprehensive study has been reported on the mode of life of *S. spilurus* to the best of our knowledge.

In contrast to *S. spilurus* adult individuals of *L. bohar* can be consistently observed not only around the Palauan reefs but also other tropical reefs (Öhman et al. 2004; Pistorius and Taylor 2009; Rhodes et al. 2008; Tamelander et al. 2008). Larval and juvenile specimens of *L. bohar* are also recorded from various places (Moyer 1977; Nakamura and Sano 2004).

These clear differences between the two lutjanid fishes strongly suggested that these species ecologically are markedly different even though they share the same site to spawn.

Previous independent studies have shown that the spawning behavior of *L. bohar* is related to the lunar phase and spawning occurs just after dawn (Johannes and Hviding 2000; Sadovy et al. 2011). *S. spilurus* aggregated in huge numbers, and has two reproductive seasons, spring and autumn, based on the timing of aggregation formation observed in Peleliu Island (Sakaue et al. 2011).

Although those observations illustrate discrete behaviors of these fishes, details of the number of individuals of fish within aggregations, timing and rhythm of their spawning in relation to the lunar cycle and other environmental cues have yet to be clarified. Although, long-term and comparative observations of the these fishes are required to identify key behavioral factors which are important in understanding their behavior and ecology, lack of methodology and general measure to record fish behaviors in such extraordinarily large schools of fish has hampered systematic study.

We therefore observed the aggregation and spawning of these two lutjanid species at the southern tip of Peleliu Island, Palau from 2006 to 2015. It is well known that *S. spilurus* aggregates in three different reefs in Palau (*Bkul a Chelas*: Tail top reef, *Butiaur*: Shark city and *Bkul a Omruchel*: Peleliu corner), we chose the site considering geographical conditions such as depth and dimension of the reef so that the long-term and direct observations by divers are possible. We estimated the number of individuals in the schools of the two species and examined relationships between the occurrence of aggregations and the lunar cycle, mode of spawning, as well as the exact timing and rhythm of spawning in relation to physical characteristics of oceanic and tidal currents. Also, we documented precise swimming behaviors, and the nuptial color during the spawning periods.

MATERIALS AND METHODS

Observation site

The southernmost reef point of Peleliu Island, Republic of Palau (Fig. 1) was chosen as the observation site in the present study.

Water temperature, current velocity and direction on spawning site

Current and water temperature were

recorded using a two-dimensional electromagnetic current meter, (model infinity-EM, JFE Advantech, Tokyo). The instrument was positioned at lat / log 6°58'11"/134°13'24" adjacent to the tip of a rocky reef situated at about 23 m deep, an intermediate depth over the entire range at which spawning has been observed (Fig. 2). The data were recorded every minute automatically and a set of 30 continuous data (30 min) was averaged and considered as one data point. The instrument was set during the period when *S. spilurus* and *L. bohar* appeared between the February 2nd 2015 and the April 18th 2015. The data include temperature, current speed, and current direction. A total of 3033 data points (in total 63 days of data) were collected. Days that fish actually spawned were extracted and used for comparison. Normal distribution for each environmental parameter was obtained using a normal probability density function in Microsoft Excel software. Significance

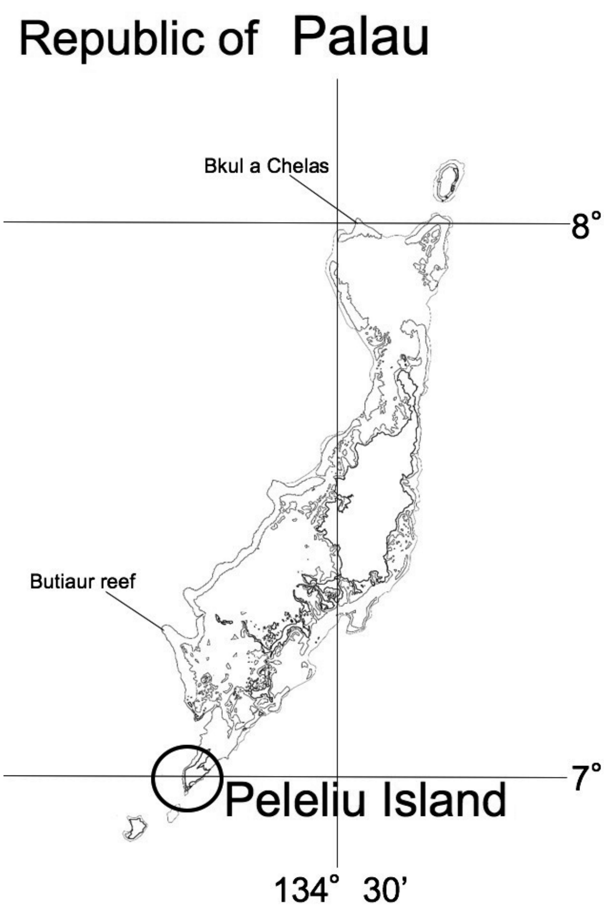

Fig. 1. Location of Peleliu Island and two other sites where *S. spilurus* is known to spawn in Palau.

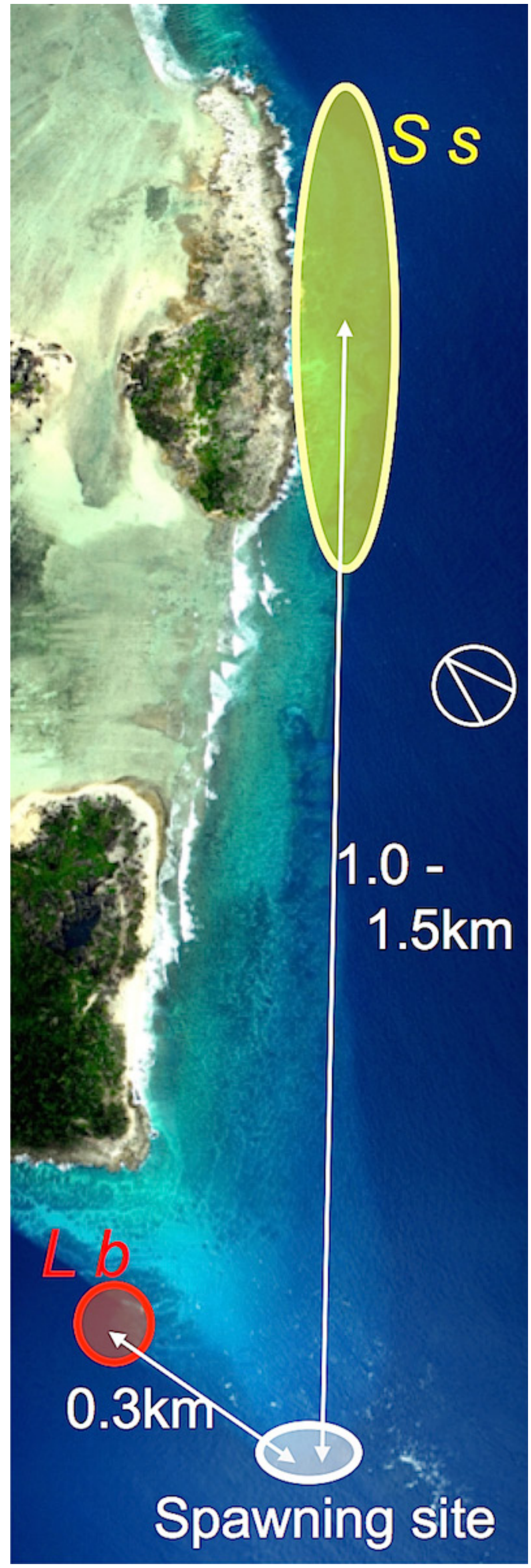

Fig. 2. Observation site: southernmost reef of Peleliu Island. The "resting area" where sub-aggregate schools of *S. spilurus* and *L. bohar* gather are highlighted in yellow and red, respectively. The spawning ground is marked in white circle.

between the mean value of the grand average (average of all data) and that of extracted data points were assessed by unpaired Student's *t*-test using GraphPad Prism software.

General observations and documentation of fish behaviors in relation to spawning

Observation schedule

We carried out systematic observations of the activities of the two lutjanid species of fish from 2006 to 2015 using SCUBA. The behaviors were recorded using compact digital camera equipped with a 24 mm lens in a video mode. Frequency of spawning (occurrence / one minute) and behavioral profiles of fishes were determined by post hoc analysis of the video movies. Intensive spawning defined as "core spawning" time when spawning behaviors occur five or more times per minutes.

One period of observations was made between the emergence and the disappearance of the spawning school, which corresponds to between the full moon and the new moon. More than 50 dives were conducted for each set of observations by the same researcher (JS). One period (between February and May) comprised of four sets of observations. Assistant divers were employed as timekeeper when we count number of fish. The above observations were made when the fishes move from the resting area to the spawning area (Fig. 2). We therefore observed three different sites to assess the dynamic state of diel movement every day in the season. The timing of actual spawning and the behavioral pattern in detail were recorded in the spawning site.

We also interviewed guide divers who dived around the observation site to record additional information. The contents of interview are as follows: (1) if they saw any objective fish; (2) if yes, behavioral characteristics of the fish. These data were used to support our direct observations to complete day-long behavior profiles of the object fishes.

Estimation of school size

We have previously, briefly reported the method to estimate the number and behaviors of individual fish within the school of spawning aggregate (Sakaue et al. 2011), however, no detail was given. Here, we describe the detailed procedure as follows: A counting zone that is 3 m in diameter and of arbitrary height was set in the area so that all the fishes in the school pass. We used a signal float made of yellow plastic as a size marker (Fig. 3a). The float was anchored with diving weights, and installed temporarily when we counted fish. The number of fishes that passed through the zone at the given time n_1 (generally 10 sec.) was counted. The time (t_1 sec.) for all the fish in the school to pass through the zone was recorded simultaneously. The total number of fish in the moving school (n_{tm}) is given by the equation:

$$n_{tm} = n_1 \times t_1/10 \text{ sec.}$$

More than 10 counts were averaged to estimate the size (number of individuals) of the school (Fig. 3). In the case when the size of the school was too large to finish the counting within our dive time (about 35 min), we swam in a counter direction to the school. In this case, the speed of the fish relative to the diver was measured, and nt_m was multiplied by factor F given below:

$$F = V_r/V_f$$

Where V_f is the actual swimming speed of the fish, V_r is the swimming speed of the fish relative to the diver (observer).

The above method was not applicable when a small school remained largely stationary in the resting area (Fig. 4). If this occurred, we roughly estimated the population of fish as follows. First, the number of individuals within about 5 square meters, n_2, was counted from the photograph (Fig. 3b). Then we estimate the area of the whole aggregation, a_a using natural markers in the location (*e.g.*, corals, rocks), and the top and bottom depth of the school. Finally the number of fish in the stationary school (n_{ts}) was given by the equation below:

$$n_{ts} = n_2 \times a_a/5$$

We usually applied this method when the aggregation size was small (about ≦ 300) (Fig. 3b). To minimize the intrinsic error from image overlaps in both methods, we counted as many fish as possible by enlarging the photographs. The total number of fish was given as the sum of n_{tm} and n_{ts}.

Collection of eggs

We collected the eggs in the spawning cloud

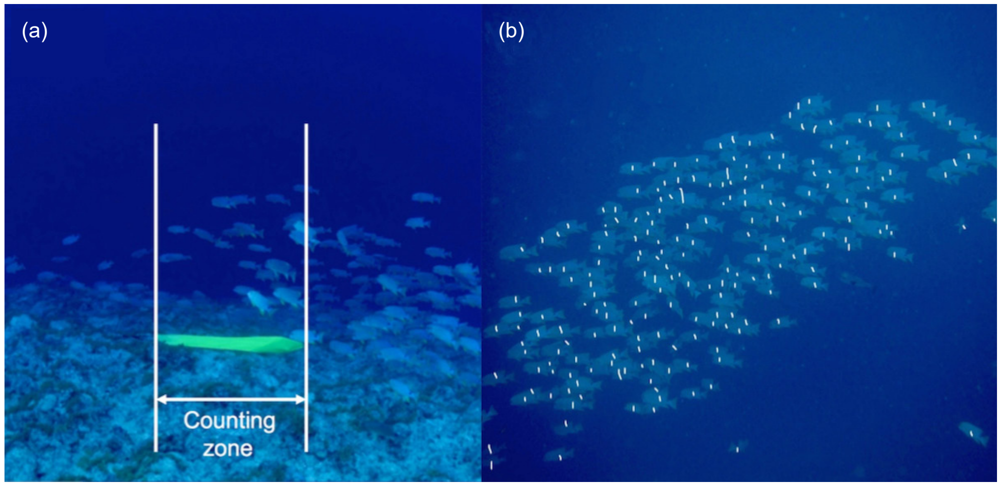

Fig. 3. The counting zone: (a) A commercial signal float (arrow, 3 m in length) was set to define the counting zone. The size of school was estimated by counting the number of fishes that passed the zone in a given time multiplied by the total time that the entire fishes took to pass the zone. (b) Some school that was not participated in the migration group. In this case the school was photographed to estimate the gross population (a dot on fish indicates the fish was counted).

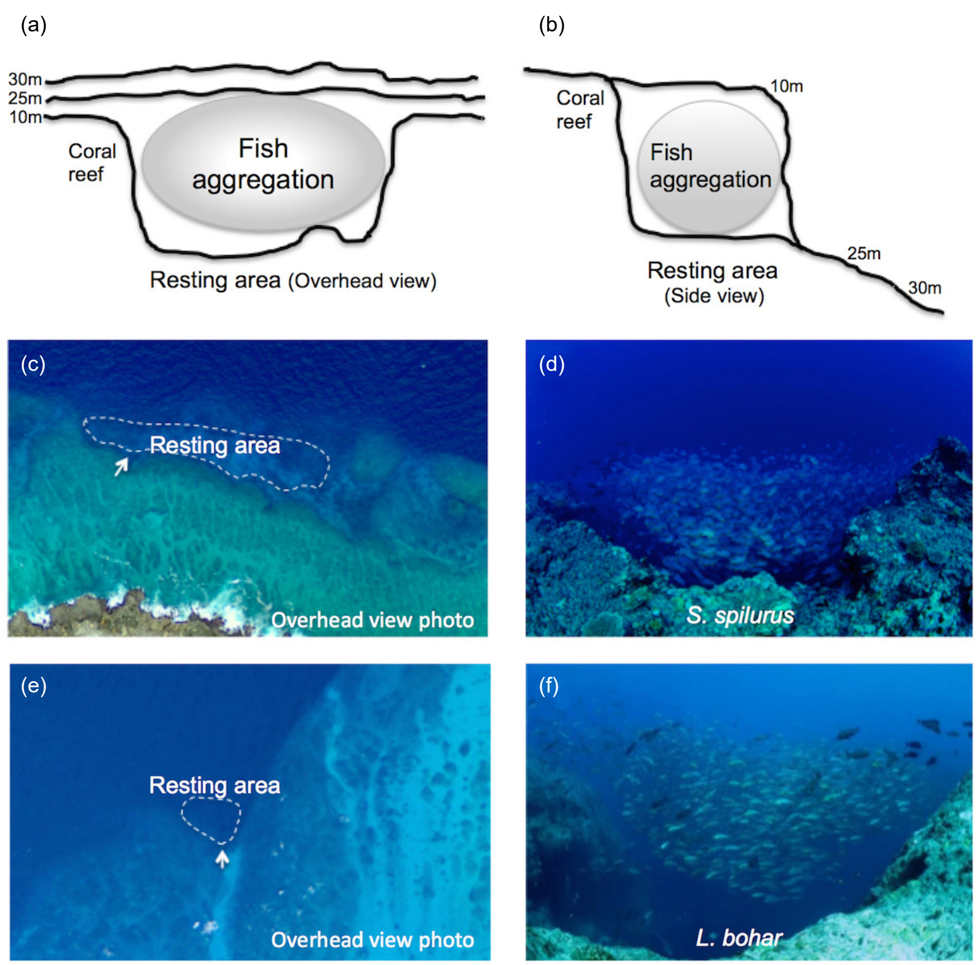

Fig. 4. Resting areas of *Symphorichthys spilurus* and *Lutjanus bohar*. (a) Schematic overview and (b) cross section of resting area. (c) Aerial photo of the resting area of *Ss*. An arrow shows shot direction of, (d) underwater photograph. (e) Aerial photo of resting area of *Lb*. An arrow shows shot direction of, (f) underwater photograph.

when fish spawn, using a simple plankton-net (ø 20 cm, mesh 0.1 mm). Although eggs are not visible, egg collections were possible, because smoke-like sperm was easily observed by divers, thus a diver scoops up the 'smoke' by a plankton net to collect the fertilized eggs.

GSI and Estimation of total egg number in fish

We examined GSI of both species (Tables 1, 2), and the number of eggs in the ovary of sacrificed fish was measured as follows: Fish was dissected to remove the whole ovary, and the weight was measured. From the ovary, 100 ripe eggs were collected and weighed. Total number of eggs ne was estimated by the following equation:

n_e = (Total weight of ovary / weight of 100 eggs) × 100

RESULTS

Size, timing of formation and duration of spawning aggregation

Symphorichthys spilurus

Between 2006 and 2009, and 2014 and 2015 the maximum numbers of fish in the school of spawning aggregation were estimated to be 4,940 ± 5,476 in February, 37,500 ± 9,354 in March and 30,167 ± 13,362 in April (Fig. 5a). A large fluctuation of the data in February is due to luck of emergence of aggregation in the month in certain years when new moon shifted before the middle of the month. They aggregated in the resting area (Fig. 2) of the east reef. Although *S. spilurus* formed a small-scale spawning aggregation in autumn as well, long-term observations were not possible due to strong seasonal wind. The aggregation started right after the full moon, the number of individuals gradually increased, and it became a maximum at around the three quarters of moon. Size of the school reduced gradually after spawning and it dispersed before the new moon (Fig. 5a). We observed a total of 17 periods, 2006 to until 2015. The same aggregation behavior was observed in all the periods and no feeding behavior was observed in the aggregating school.

Lutjanus bohar

Between 2010 and 2015 the number of fish estimated to aggregate in the west reef of the research area (Fig. 2) were 6,917 ± 2,920 in February, 7,417 ± 1200 in March and 5,600 ± 1,140 in April (n = 18) (Fig. 5b). They aggregated exclusively in the resting area of the west reef. The school never exceeded more than 10,000 individuals. The spawning aggregation formed every month throughout the year. Spawning started just after the quarter moon and spawning occurred until a few days before full moon (Fig. 5b). We observed a total of 18 periods from February 2010 until May 2015. The same aggregation behavior was observed in all the periods examined and feeding behavior was observed during the aggregating period.

Table 1. GSI and estimated number of eggs for *S. spilurus*

	TL (mm)	SL (mm)	BW (g)	gland W (g)	GSI	Egg 10_4
Female 1	445	358	1,510	63	4.17	27-26
Female 2	455	363	1,575	67	4.32	28.5-30.5
Male 1	408	323	1,260	16	1.28	-

The fish sampled before 4 days new moon.

Table 2. GSI and estimated number of eggs for *L. bohar*

	TL (mm)	SL (mm)	BW (g)	gland W (g)	GSI	Egg 10_4
Female 1	561	492	3,200	82	2.56	35 - 38
Male 1	665	596	4,900	186	3.80	-

The fish sampled before 4days full moon.

Diel migration

Symphorichthys spilurus

The huge aggregation of *S. spilurus* on the reef performed the following diel migration: In the daytime, loose pre-spawning aggregations occurred at several resting areas located north-eastern part of the spawning site as shown by the yellow zone in figure 2, where tidal currents were much weaker than the spawning ground. The distance from these resting areas to the spawning site was about 1.0 to 1.5 km. The loose aggregations left the resting area before sunset and scattered in the shallow place of south reef (white zone of Fig. 2) of about 10 to 15 m deep. They rested individually, letting themselves drift in the bottom layer. Before dawn, the rested fishes moved to the tip of the reef and aggregated to form a huge school. When the tidal current was strong, exceeding more than two knots (1.0 m/sec), the aggregation formed close to the substrata forming a flat and wide aggregation. Under weaker tidal currents of less than one knot (0.5 m/sec); however, the aggregation became vertical with a narrower expansion. Individuals that completed the spawning at the site swam back to the sheltered resting area one by one. In the earlier stages of the aggregation, the fish remained around the reef wall area and did not move to the tip of the reef, and even if they aggregated at the tip of the reef around at half moon, actual spawning did not occur. They migrated around the spawning site of Peleliu corner as shown by white zone (Fig. 2), about 3 km round trip every day after the beginning of aggregation through the spawning season.

Lutjanus bohar

Daytime, loose pre-spawning aggregations remained in a small resting area located off northwestern area of the spawning site as shown in red in figure 2, where tidal currents were much weaker than that in the protruded point of the spawning ground. The distance from the resting areas to the spawning site was about 300 m. After spawning they remained in the resting area all the day. Every early morning (before dawn) they moved from the resting area to the spawning site, and after spawning they returned to the resting area one by one. All the post-spawning fishes returned to the resting area within one to two hours. Thus the distance of the diel migration for spawning was about 0.6 km/day.

Nuptial coloration and spawning behaviors

The observed nuptial coloration of *S. spilurus* was as follows (Fig. 6). Male: While forming the aggregation, the blue lines became darker, and the dorsal fin and a caudal fin became darker as well. Moreover, the color became much stronger during the courtship behavior; the body coloration became much stronger and was identical with dark body color. Female: Darkening of blue lines on the body, dorsal fin and caudal fin did not occur, while the whole body became pale and brighter which was identical with light body color. Nuptial coloration of

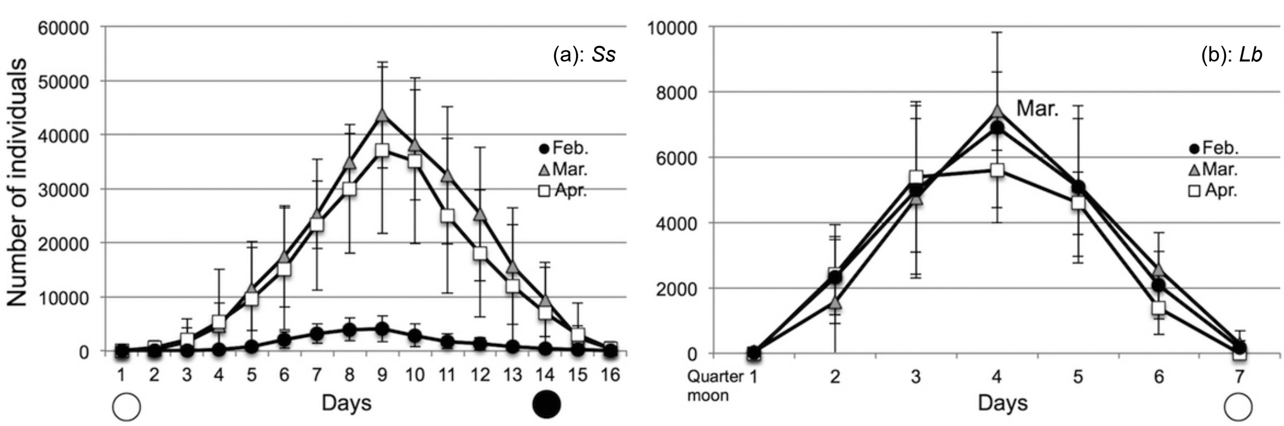

Fig. 5. A comparison of the mode of appearances: (a) *S. spilurus* between 2006 and 2009. The spawning aggregation formed two distinct periods of a year between February to April and September to November. Number of fish observed in the earlier period, between the full (open circle) and new (closed circle) moon are shown. (b) The mode of appearance of *L. bohar* between 2010 and 2013. The aggregation formed every month throughout the year. Representative modes of appearance observed between February and April were shown for direct comparison.

L. bohar was less prominent than *S. spilurus*, but male exhibited darker and female showed lighter red nuptial coloration as shown in figure 6.

Spawning

Symphorichthys spilurus

At the spawning site, the aggregation of fully matured *S. spilurus* males and females formed in an ellipsoidal shape close to the bottom around 20 m depth when the current was weak, however, the school moved deeper to around 40 m depth when the current was strong. Dark colored males, *i.e.* fully matured individuals, occurred in the upper layers of the aggregation. The males followed a female and stimulated the flank of the female. At this time, sex ratio was not one to one but males dominated in number. After being stimulated by a male, the female started to swim up and down or right and left within the aggregation, and several males followed. The female kept the forefront position of the group and at a high swimming speed changing the directions quickly and abruptly. Female swam as if trying to shake off the following males. The direction of the rush swimming during the spawning was not constant, on some occasions a female swam horizontally, while other females swam in an arch, or even to obliquely downward, and thus the rush swimming direction was not predictable (Fig. 7a). Right after this behavior, emissions of smoke-like sperm

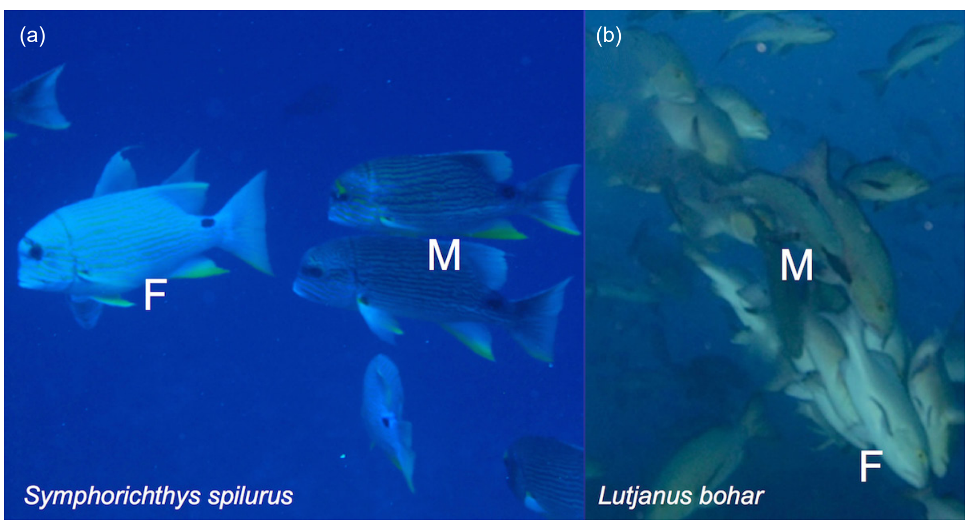

Fig. 6. Nuptial coloration of: (a) *S. spilurus* and (b) *L. bohar*. Each male and female was marked M or F. Male were in the dark color morph, and female were in the light color morph.

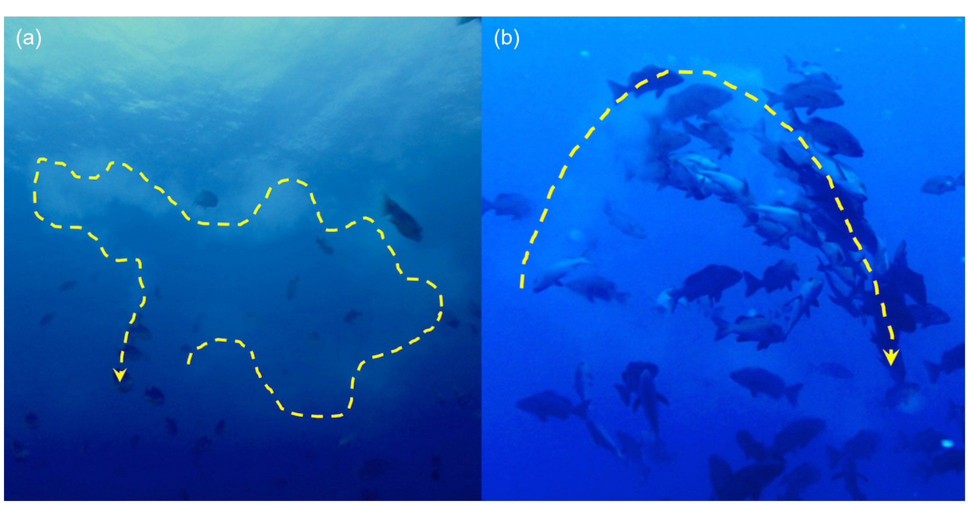

Fig. 7. Photographs of actual spawning of (a) *S. spilurus* and (b) *L. bohar*. Dotted yellow trace indicates a locus of a rushing female individual.

were observed. Although the eggs are not visible, we were able to collect fertilized eggs after the spawning using the smoke-like sperms as marker. This mode of spawning was characterized by highly unpredictable swimming pattern of female fish and has hitherto not been reported in other reef fishes. In some cases a female escaped form the pursuing males as a result of its so quick motion, but in that case, the female did not release the eggs. The male fishes that could follow the quick-moving female spawned successfully. The number of the successful male can be one or few and the fish(es) release sperm at the same time. After the spawning, fish returned to the lower layers of the aggregation, and then gradually left the aggregation. Typically, spawning duration on a day of *L. bohar* was around 30-60 min, while that of *S. spilurus* lasted for 1-5h. Three females caught by spear fishing (average size of 45 cm SL, 2.2 kg BW), carried between 27 to 29 thousand eggs with a 0.8 mm in diameter.

Lutjanus bohar

The spawning aggregate of *L. bohar* formed early in the morning before dawn. The actual spawning took place within groups composed of one female with several males occurring in the upper peripheral part of the aggregation (Fig. 7b). Once a female made an upward rush, several males followed the female, and spawning took place at the apex of the rushing group.

Lutjanus bohar shared the same spawning site with *S. spilurus* but its sub-aggregating site was on the opposite side of the reef to that of *S. spilurus* (Fig. 2). The number of fish in the aggregation was several thousands and the aggregation duration was shorter than that of *S. spilurus*. The spawning season of *L. bohar* was not limited to spring and autumn but occurred almost throughout the whole year.

Characteristics of fertilized eggs

The size of fertilized eggs of *S. spilurus* was 0.80 ± 0.01 mm in diameter, single oil globule and pelagic. That of *L. bohar* was slightly larger 0.82 ± 0.01 mm in diameter, single oil globule and pelagic also.

Spawning duration and interval

We observed the spawning duration of *S. spilurus* over seven years. Data collected by direct observations (diving) and interviews with other divers were gathered to summarize all-daytime fish behaviors for eight days as shown in figure 8. The spawning started 6 days before every new moon. Core spawning lasted about one hour while sporadic spawning occurred prior to and after the

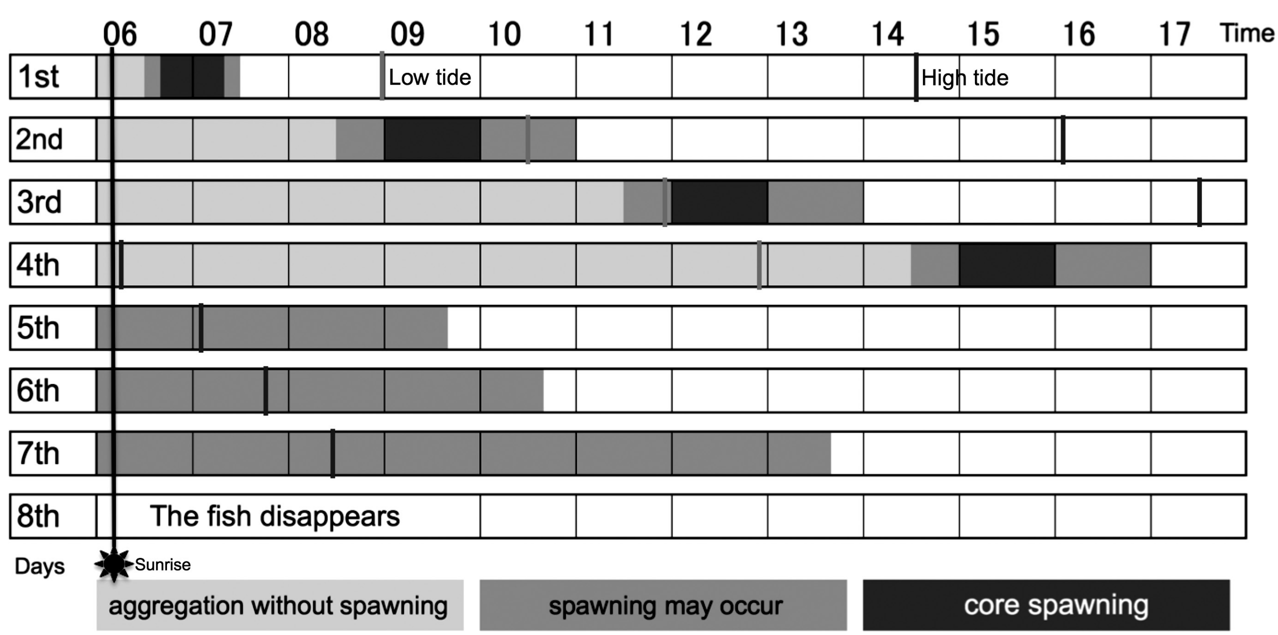

Fig. 8. Spawning profiles for *S. spilurus*. The spawning profiles (onset and duration of sporadic and core spawning) for *S. spilurus* acquired from direct observation and interview to other divers are summarized. Short lines in dark gray and gray indicate high and low tide, respectively.

core spawning. On the second day, the spawning time shifted about 90 minutes later from that of the first day, and the spawning duration expanded to one and half hours. The third day, spawning took place about 90 to 120 minutes later than on the second day (spawning occurred in the early afternoon). Similar time shift was observed on the fourth day. On day five, however, spawning took place after dawn and the mode of spawning was sporadic. On the last day of spawning in the new moon period, sporadic spawning lasted from dawn to early afternoon. Thus, the whole spawning activity lasted for one week on every new moon. On some occasions, the actual spawning did not take place on the last day. The tendency of overall behavior was consistent throughout the years of 2009 to 2015.

The spawning duration tendency of *L. bohar* was also observed for six years as shown in figure 9. The first spawning takes place two days before the full moon when the aggregation started about 1.5 hour before sunrise and the fish spawned frequently at around sunrise for about half an hour. On the second day, the aggregation started at almost the same time with the first day, but the core spawning time was delayed by about half an hour. On the third day, the aggregation started again the same time with the first day, but the onset of core spawning was delayed by about an hour. The duration of the core spawning was one hour. On the fourth day, no spawning behaviors were observed. This tendency was also consistent throughout the years of 2010 to 2015.

Water temperature in spawning site

Fluctuation of water temperature at the spawning site between March 3rd and April 18th, was recorded in 2015. The water temperature of the spawning site changed between 21.4 and 29°C)(n = 3033, average 27.7 ± 0.81°C in the spawning season (Fig. 10a). The water temperature, recorded when spawning of fish actually was observed, changed between 25.0 and 28.7°C (n = 15, average 27.2 ± 1.23°C) for *S. spilurus*, and between 24.2 and 28.6°C (n = 15, average 27.5 ± 1.22 for *L. bohar*. No significance between spawning and the grand average was found in both species (Fig. 11a). When waters from deeper layers upwelled into the site, it resulted in a large decrease in temperature to 22 or even 21°C). Spawning behaviors in both species, however, were not affected even by such a large fluctuation of water temperature. Of note, on the day when the water temperature went down below 21°C. *S. spilurus* showed the same spawning behavior. These data suggested that there is no clear correlation between the temperature and the behavior of the fish within the fluctuations observed in the present study. Larger fluctuations in temperatures, however, may occur because of “unusual” climate factors such as El Nino, continuous observations are necessary to draw conclusions as to the temperature dependence of the spawning behavior.

Current velocity in spawning site

Fluctuation of the current velocity at the spawning site between March 3 and April 18,

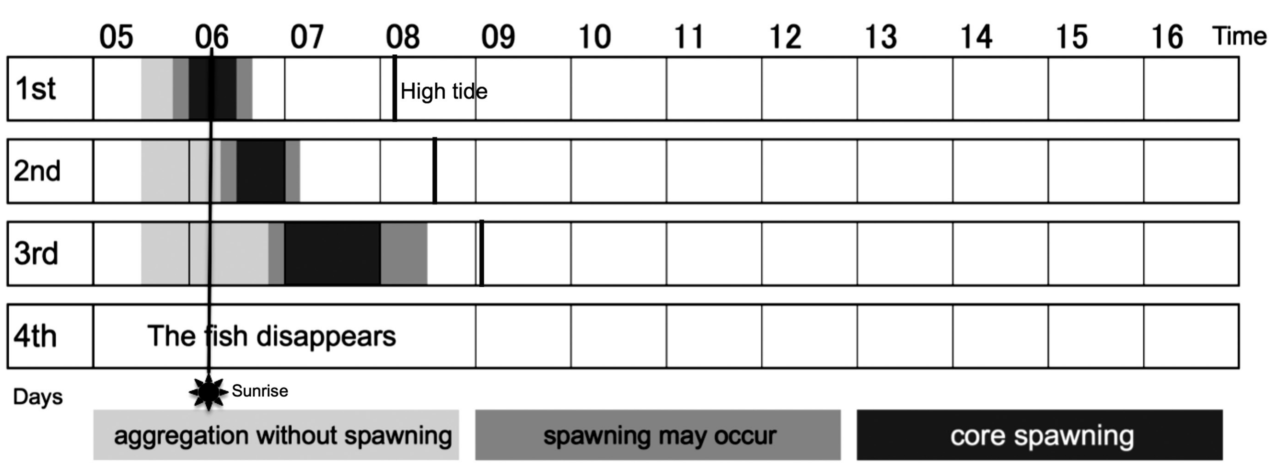

Fig. 9. Spawning profiles for *L. bohar*. The spawning profiles (onset and duration of sporadic and core spawning) for *L. bohar* acquired from direct observation and interview to other divers are summarized. Short gray lines indicate high tide.

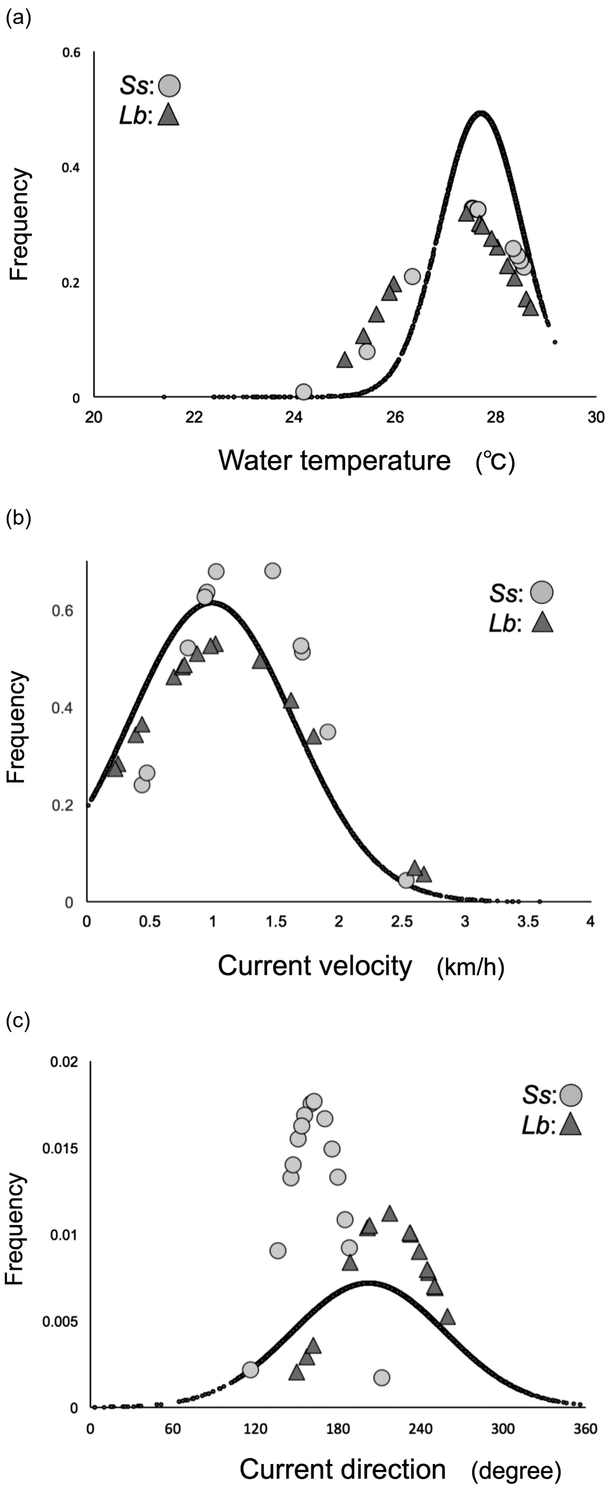

Fig. 10. Normal distributions of: (a) Ambient water temperature (bold line), that at the time *S. spilurus* (circles), and *L. bohar* (triangles) spawn. (b) Current velocity of spawning site (bold line), that at the time *S. spilurus* (circles), and *L. bohar* (triangles) spawn, (c) Current direction of spawning site (bold line), that at the time *S. spilurus* (circles), and *L. bohar* (triangles) spawn. Above data were recorded between February 2 and April 18, 2015.

2015 is shown in figure 10b. The average current velocity at the spawning site was 0.99 ± 0.65 km/h (n = 3033). The minimum and maximum value changed between 0.01 and 3.59 km/h in the spawning season. The current velocity of the spawning day of *S. spilurus* changed between 0.2 and 2.7 km/h, average velocity was 1.1 ± 0.75 km/h (n = 15) (Fig. 10b). The current velocity of the spawning day of *L. bohar* changed between 0.44 and 2.53 km/h, average velocity was 1.25 ± 0.54 km/h (n = 15) (Fig. 10b). Together, the spawning behaviors in both species, were not affected even by such an extreme fluctuation of current velocity (Fig. 11b). Of important note, however, some current, even weak ones, was needed for spawning for both species (See below).

Current direction in spawning site.

Current directions in spawning site were recorded between March 3 and April 18, 2015. The current direction of the spawning site changed between 3.0° and 355.9° (average 202.2° ± 55.6, n = 3033) (Fig. 10c). *S. spilurus* spawned under the condition when the mean current direction across the spawning site was between 116° and 211° with an average value of 162.7° ± 22.6 (n = 15, p = 0.0059 against the grand average) (Figs. 10c, 11c). *L. bohar*, on the other hand, spawned when

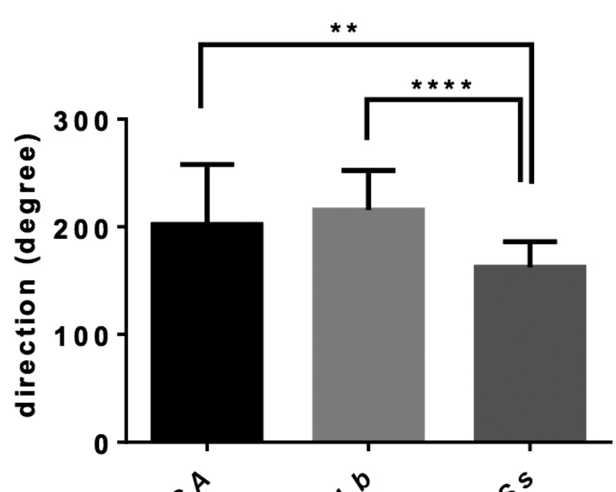

Fig. 11. Current directions (mean ± SD) for: grand average (*GA*) of all data points (n = 3033), when *L. bohar* (*Lb.*), and *S. spilurus* (*Ss.*) spawn (n = 15, for each). **p < 0.005, ****p < 0.001, unpaired t-test.

the mean current direction ranging from 150.1 and 259.4° with an average of 215.7° ± 35.5 (n = 15) (Fig. 10c). No significant difference was evident between the grand average and the average value (Fig. 11c). It is interesting to note however, the distribution of the current direction when *L. bohar* spawned (Fig. 10c) is narrower than that of average, suggesting that *L. bohar* actually has a "preferred" direction.

These data illustrated that the spawning behavior of *S. spilurus* is influenced largely by the current direction. The difference in the current direction between the two species is about 60° and the direction of transportation of fertilized eggs was estimated to be SES in *S. spilurus* and WSW in *L. bohar* (Fig. 12).

Although the rhythmicity and the regular daily spawning for both species as shown in figures 8 and 9 were consistent, no spawning occurred at the expected time when the current was minimal (less than 0.23 km/h) in both species. Probability of such a slow tide was about 2.7% from the normal distribution curve.

DISCUSSION

Spawning aggregations have been formerly categorized into two types: resident and transient aggregations (Domeier and Colin 1997). Although the formation of aggregations has been documented for both *L. bohar* and *S. spilurus*, the type was not defined due to the lack of sufficient information of their spawning behavior. In the present study, the behaviors of these fishes were documented in detail enabling the "categorization" based on the above definition. The former list of Domeier and Colin (1997) is comprised of behavioral criteria to discriminate "resident" and "transient" aggregations, these criteria include: frequency of formation, annual reproductive effort, migration distance, duration of aggregation, body size of aggregating species, trophic level, mode of spawning, and areas of spawning (Domeier and Colin 1997) as summarized in table 3. Although many of those criteria are qualitative, the spawning behaviors for *L. bohar* and *S. spilurus* observed in the present study were adapted into the table and we found that the behaviors of *S. spilurus* fall into "transient" while these for *L. bohar* were in between "resident" and "transient" (Table 3). These results support recent interpretation of "resident-transient" dichotomy where "intermediates", as represented by *L. bohar* exist (Sadovy and Colin 2011).

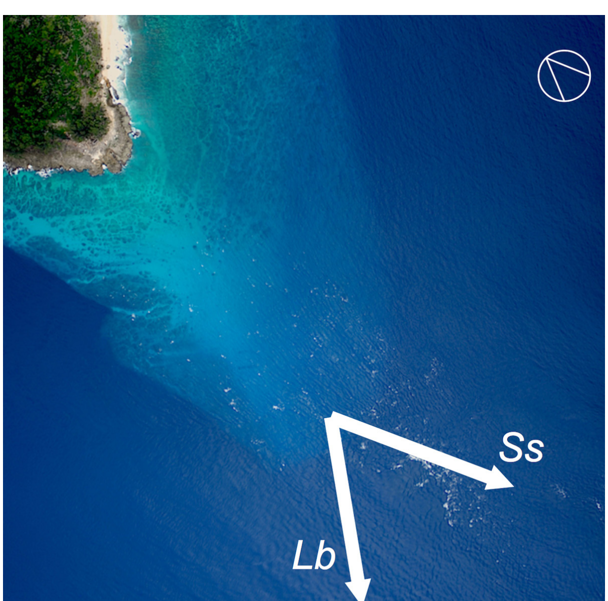

Fig. 12. Averaged current directions for the spawning days of *L. bohar* (*Lb*), and *S. spilurus* (*Ss*).

In the present study, we observed behaviors of *L. bohar* and *S. spilurus* in detail employing novel and quantitative measures and found the following characteristics: First, although the two species shared the same spawning site at dawn, the resting sites during the daytime were spatially separate from the spawning site. In the case of *S. spilurus*, the resting site is located more than 1000 m east of the spawning site whereas that for *L. bohar* is located about 300 m north of the spawning site. These spatial separations thus result in a diel migration from the resting site to the spawning site throughout the spawning season. Daily migrations are reported for *Lutjanus cyanopterus* from Belize (Heyman et al. 2005), and *Lutjanus jocu* from Cuban waters (Claro and Lindeman 2003) but in both studies, the detailed mode of migration was not clarified. Second, the "spawning rush", a rapid burst of swimming that triggers the release of gametes, was observed in both species, but the manner differed clearly between *L. bohar* and *S. spilurus*. This difference can be related to the way of selection. For example, females of *L. bohar* swim at a rather normal speed so that most males can follow and succeed to fertilize its eggs. In contrast, females of *S. spilurus* swam so quickly that slow males were unable to follow, and thus, only faster males seemed to succeed in fertilization. These behaviors, we propose here to call as “shake off behavior” recorded for the first time among the family Lutjanidae, can be useful factors to further categorize the fish behaviors in the spawning aggregates. A video record of the behavior is provided in the supplementary material. Lastly, we found in the present study that *L. bohar* is regarded as a year-round spawner, like *Rhomboplites aurorubens* of the West Indies, reported by Heileman and Phillip (1999), another example among the family Lutjanidae was *L. jocu* from Cuban waters (Claro and Lindeman 2003). The spawning activity occurred in relation to the lunar phase both in *S. spilurus* and *L. bohar*. The synchronization of spawning activity with moon phase is common in a number of other lutjanids, including *Lutjanus cyanopterus* in the Virgin Islands (Kadison et al. 2006), *L. jocu* in western equatorial Atlantic (Carter and Perrine 1994; Krajewski and Bonaldo 2005), *Lutjanus argentimaculatus* in northeastern Queensland (Russel and McDougall 2008). Detailed observations in the present study distinguished marked differences in the pattern even within the same family of fishes.

The two species studied here spawned two weeks apart, *i.e.* just before new moon and just before full moon. Interestingly, our data indicate that the important determinants for *S. spilurus* to spawn are not only the lunar phase but also the current direction (Fig. 5). Both *S. spilurus* and *L. bohar* seem to require some current to spawn because if the current stopped (less than 0.22 km/h) at the expected spawning time, spawning behaviors were not observed till the current resumed (Fig. 10b). *S. spilurus*, however, almost “selectively” spawned when the current was bound for the southeast to south-southeast (Fig. 12). These peculiar differences may explain above speculation for the time shift in spawning. It can thus be postulated that the spawned eggs will be transferred to the offshore to far south. In the case of *L. bohar*, it preferred to spawn when the current direction was in between 150.1 and 259.4° (an average of 215.7°), same as average current direction in the site.

It may therefore be speculated that the spawning behavior of *S. spilurus* observed in the present study results in distribution of the larvae over a wide range, while that for *L. bohar* results in a more short-range distribution of the larvae. This might be, at least in part, supported by the fact that the adult fishes of *S. spilurus* were hardly found in the sea and market except for the spawning seasons. To assess the fate of fertilized eggs after spawning, detailed study on the ocean currents is required. Because the eggs of *L. bohar* float, it must be under influence of the surface current. However, the current around Peleliu are highly complex including a presence of strong upwelling and downwelling current, determination of egg distribution is not an easy task. Acquisition of basic knowledge of the ocean and tidal currents in the area using floating data logger followed by

Table 3. Criteria used to categorize the resident and transient aggregation formerly, and that was adapted to the present observation

Characteristics	Resident aggregations	*Ss*	*Lb*	Transient aggregations	*Ss*	*Lb*
Frequency	occur regulary; often daily		○	occur infrequently; at specific times of the year	○	
Portion of reproductive effort	single aggregation constitutes minor portion of annual reproductive effort			single aggregation constitutes major portion of annual reproductive effort	○	○
Migration distance	migrate relatively short distance		○	migrate relatively large distance	○	
Duration of aggregation	aggregation is ephemeral, lasting only a few hours or less			aggregation persists for several days	○	○
General size of aggregation species	species are relatively small to medium sized representatives of their respective families			species are relatively large sized representatives of their respective families	○	○
Type of food habits	species feed low on the food chain			species feed high on the food chain	○	○
Modes of spawning	only group spawning has been observed		○	group and pair spawning have been observed	○	
Areas of spawning	known to spawn outside of aggregation			not known to spawn outside aggregation	○	○

modeling study would be of the most accessible solutions to the problem.

Taken together, the present study illustrates differences in the spawning behaviors of two Lutjanidae fish species by observing the spawning aggregation using various factors including former criteria as shown in table 1, and newly introduced behavioral and environmental profiles such as diel migration, swimming modes in the spawning, water temperature, current velocity and direction. Moreover to the best of our knowledge, the present study is the first example that estimated the number of individual fishes in the aggregation. The counting method employed in this study is simple and thus potentially useful to estimate the size of aggregations in future investigations. We found that the current direction was especially useful to discriminate the spawning profiles of the two fish species. Because fates of the eggs rely mainly on the current in the spawning site, the current direction largely dictates the ecological and embryological features affecting the eggs. Perhaps, Peleliu corner is ideal, both geographically and oceanographically, for the fishes to accomplish their reproductive purposes. Our preliminary observations suggested a marked difference in egg development between the two species. We thus hypothesize that the behavioral difference observed here stems to the physiological and ecological differences between the fishes. An investigation to test this concept is now in progress, and the results will be published elsewhere.

We conclude that, as demonstrated by the present study, analysis and categorization of spawning aggregation should be conducted by using diverse behavioral profiles in addition to the former ones. We believe continuous observations of the spawning aggregates on key fish species in Palauan water will accumulate useful data for resource predictions that in turn result in effective protection of the fish species for sustainable use of the gifted nature of Palau.

At last, there are marine conservation areas designated by Palau conservation society in Palau, and they designated 82.7% of Exclusive Economic Zone as the marine sanctuary. However, our research area has not been accepted in the designation of a marine reserve still now. Although there is no fishing pressure of local people or by foreign fishermen for the moment, in consideration of protection of the future marine environment, we would like to propose constitution of conservation area through this manuscript.

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