水産研究・教育機構 機関リポジトリ
FRA Japan Fisheries Research and Education Agency Institutional Repository

Spawning aggregations of checkered snapper (Lutjanus decussatus) and blackspot snapper (L. fulviflamma): seasonality, lunar-phase periodicity and spatial distribution within spawning ground

メタデータ	言語: English 出版者: 公開日: 2024-01-17 キーワード (Ja): キーワード (En): Gonad development; Lunar periodicity; Lutjanus decussatus; Lutjanus fulviflamma; Seasonality; Snapper; Spawning aggregation; Spawning ground 作成者: 名波, 敦 メールアドレス: 所属: 水産研究・教育機構
URL	https://fra.repo.nii.ac.jp/records/2000072

This work is licensed under a Creative Commons Attribution 4.0 International License.

Spawning aggregations of checkered snapper (*Lutjanus decussatus*) and blackspot snapper (*L. fulviflamma*): seasonality, lunar-phase periodicity and spatial distribution within spawning ground

Atsushi Nanami

Yaeyama Field Station, Coastal and Inland Fisheries Ecosystem Division, Environment and Fisheries Applied Techniques Research Department, Japan Fisheries Research and Education Agency, Ishigaki, Okinawa, Japan

ABSTRACT

Snappers (family Lutjanidae) are important fisheries target species and some species are known to form spawning aggregations at particular spawning grounds. The present study investigated the ecological characteristics of fish aggregations of two snapper species (checkered snapper *Lutjanus decussatus* and blackspot snapper *L. fulviflamma*) that form at a particular site. Specifically, the aims were to clarify (1) seasonality and lunar-phase periodicity of fish aggregation formation, (2) fine-scale spatial distribution of fish density (spatial variations of fish density at intervals of several-tens meters) within the aggregation site, (3) size and age frequency distributions of fishes in the aggregation site, (4) gonad development, (5) to compare fish abundance between inside and outside the aggregation site, and (6) to verify that fish aggregations of the two snapper species were spawning aggregation. Underwater observations using a 600 m × 5 m transect revealed that greater fish abundance of *Lutjanus decussatus* was found monthly between May and October, and clear positive peaks in the fish abundance were found only around the last-quarter moon. This lunar-related periodicity in the increase of fish abundance was confirmed by a time-series analysis (correlogram). Within the aggregation site, *L. decussatus* showed a relatively uniform distribution. In contrast, greater fish abundance of *L. fulviflamma* was found monthly between April and October, and clear positive peaks in the fish abundance were found around the last-quarter moon (April, May, June and October) or new moon (July, August and September). This lunar-related periodicity was also confirmed by correlogram. *Lutjanus fulviflamma* showed a relatively clumped distribution within the aggregation site. Most females of the two species in the aggregation site had hydrated eggs, indicating that the two species form aggregations for reproduction. The two species, although occurring simultaneously, are considered to form aggregations of conspecifics only. For *L. decussatus*, average fork length and age of males and females were 229.2 mm and 243.9 mm and 9.4 years and 8.1 years, respectively. For *L. fulviflamma*, average fork length and age of males and females were 233.9 mm and 246.9 mm and 6.8 years and 8.1 years, respectively. Fish abundance inside the aggregation site was 266.8-fold and 141557.1-fold greater than those outside the aggregation site for *L. decussatus* and *L. fulviflamma*, respectively. These results showed that (1) fish aggregation formation

Submitted 27 April 2023
Accepted 8 August 2023
Published 11 September 2023

Corresponding author
Atsushi Nanami,
nanami_atsushi71@fra.go.jp,
nanami@fra.affrc.go.jp

Academic editor
María Ángeles Esteban

Additional Information and Declarations can be found on page 21

DOI 10.7717/peerj.15991

Copyright
2023 Nanami

Distributed under
Creative Commons CC-BY 4.0

OPEN ACCESS

How to cite this article Nanami A. 2023. Spawning aggregations of checkered snapper (*Lutjanus decussatus*) and blackspot snapper (*L. fulviflamma*): seasonality, lunar-phase periodicity and spatial distribution within spawning ground. *PeerJ* **11**:e15991
http://doi.org/10.7717/peerj.15991

of the two snapper species was predictably repeated in particular months and lunar-phase, (2) it was predictably found at the particular site, (3) the fish abundance in the aggregation site markedly exceeded the fish abundance outside the aggregation site, and (4) the two species form aggregations for reproduction. Therefore, it is suggested that the fish aggregations for the two species can be regarded as spawning aggregations.

Subjects Animal Behavior, Aquaculture, Fisheries and Fish Science, Conservation Biology, Ecology, Marine Biology
Keywords Spawning aggregation, Snapper, *Lutjanus decussatus*, *Lutjanus fulviflamma*, Lunar periodicity, Seasonality, Gonad development, Spawning ground

INTRODUCTION

Coral reef fishes show diverse reproductive behaviors (*Thresher, 1984*). Among these diverse fish species, some fish species form aggregations with greater densities during restricted seasons and lunar phases at particular spawning grounds (*Nemeth, 2009*). These fish aggregations are known as spawning aggregations (*Sadovy de Mitcheson & Colin, 2012*). *Domeier (2012)* defined spawning aggregations as conspecific individuals gather at specific sites in a specific period. Over 80 fish species are regarded to form spawning aggregations (*Sadovy de Mitcheson & Colin, 2012*), although it is suggested that the actual number of species would be greater than 160 (*Claydon, 2004*). Two types of spawning aggregations have been reported: resident and transient spawning aggregations (*Domeier & Colin, 1997*). Resident spawning aggregations are characterized by smaller-sized species (*e.g.*, parrotfishes, surgeonfishes and wrasses), shorter migration distance (within several kilometer scale), shorter duration of the spawning event (several hours) and almost daily aggregation. On the other hand, transient spawning aggregations are characterized by larger-sized species (*e.g.*, emperorfishes, groupers and snappers), longer migration distances (scales of several to several-hundreds of kilometers), longer duration of the spawning event (several days to several weeks) and almost monthly or annual aggregations (*Nemeth, 2009*).

Some fish species that form transient spawning aggregations include larger-sized fishery target species such as emperorfishes, groupers and snappers (*Sadovy de Mitcheson & Colin, 2012*). These fish species are mesopredators, which have significant roles in coral reef ecosystems, that control population size of smaller-sized fishes belonging to lower trophic levels (*Graham, Evans & Russ, 2003*). Hence, effective protection of the spawning aggregations for these species is needed (*Beets & Friedlander, 1999*; *Linderman et al., 2000*; *Sala, Ballesteros & Starr, 2001*; *Nemeth, 2005*; *Sadovy & Domeier, 2005*; *Russell, Luckhurst & Lindeman, 2012*), since formation of transient spawning aggregations is spatially and temporally predictable and such spawning aggregations have great vulnerability to fishing (*Samoilys, 1997*; *Rhodes & Tupper, 2008*; *Sadovy de Mitcheson et al., 2008*; *de Mitcheson & Erisman, 2012*; *Chollett et al., 2020*; *Pittman & Heyman, 2020*).

Snappers (family Lutjanidae) are important fishery target species and mesopredators in tropical and sub-tropical waters (*Allen, 1985*; *Polovina & Ralston, 1987*; *Nanami & Shimose, 2013*; *Taylor et al., 2018*; *Amorim et al., 2019*; *Menezes et al., 2022*) and at least 12 species are regarded to form transient spawning aggregations (*Sadovy de Mitcheson & Colin, 2012*). Some ecological characteristics of snappers in terms of spawning aggregations have been studied such as location of spawning ground (*Claro & Lindeman, 2003*; *Heyman & Kjerfve, 2008*; *Sakaue et al., 2016*; *Malafaia, França & Olavo, 2021*), seasonality and lunar-phase periodicity of spawning aggregation formation (*Kadison et al., 2006*; *Sakaue et al., 2016*; *Biggs & Nemeth, 2014*; *Biggs & Nemeth, 2016*; *Cimino et al., 2018*), spawning migration movements (*Farmer & Ault, 2011*; *Feeley et al., 2018*) and spawning behavior (*Carter & Perrine, 1994*; *Heyman et al., 2005*; *Sadovy de Mitcheson, Colin & Sakaue, 2012*).

Two snapper species, checkered snapper *Lutjanus decussatus* (Cuvier, 1828) and blackspot snapper *L. fulviflamma* (Forsskål, 1775), are important fisheries target species in Okinawan coral reefs (*Akita et al., 2016*). *Nanami et al. (2010)* indicated the possibility that *L. decussatus* forms spawning aggregations in this region by clarifying its reproductive biology. The main spawning season was between June and October and clear lunar-synchronized fluctuations in the gonadsomatic index (GSI) of females were found. The highest GSI values were found around the last-quarter moon phase during the main spawning season. However, direct evidence (fish aggregation at a particular site) has not yet been reported. In contrast, *Shimose & Nanami (2015)* showed that the main spawning season of *L. fulviflamma* occurred between April and August. However, lunar-synchronized reproductive activity as well as the possibility of spawning aggregation formation of *L. fulviflamma* have not been examined yet. Although there has been no ecological information from the local communities about spawning aggregations of the two species, fish aggregations of the two snapper species were found in an Okinawan coral reef (Fig. 1, Videos S1 and S2). This finding suggests that the fish aggregation might be a spawning aggregation and should be appropriately protected since these two snapper species are target species of commercial catch and recreational fishing in the region. Thus, understanding precise ecological characteristics of fish aggregations of the two snapper species would be useful for effective management including the necessary spatial scale and duration of spawning ground protection.

The aims of the present study were to clarify ecological aspects of the fish aggregations of *Lutjanus decussatus* and *L. fulviflamma*. Specifically, the aims were to clarify: (1) seasonality and lunar-phase periodicity of fish aggregation formation, (2) fine-scale spatial distribution of fish density (spatial variations of fish density at intervals of several-tens of meters) at the aggregation site, (3) size and age frequency distributions of fishes in the aggregation site, (4) gonad development of fish individuals that were captured in the aggregation site, (5) to compare fish abundance between inside and outside the aggregation site, and (6) to verify that fish aggregations of the two snapper species were spawning aggregation. Since the present study is the first examination about fish aggregation of the two snapper species, the results would contribute to a more comprehensive understanding of ecological aspects about spawning aggregations of snappers in coral reefs.

MATERIALS AND METHODS

Main method of this study was field observation. Some fish individuals were caught as samples to examine the gonad development. By placing on ice, these fish individuals

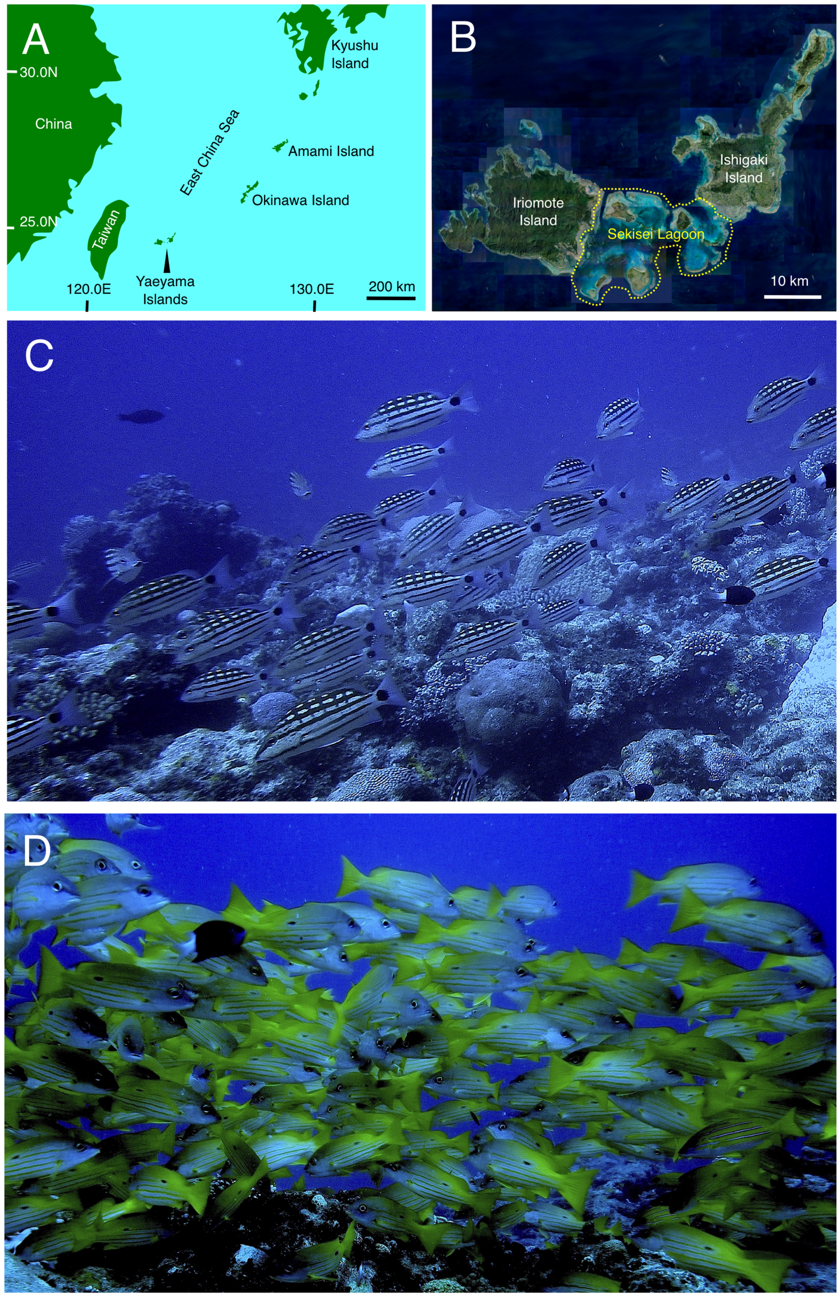

Figure 1 **Study site and fish aggregations of *Lutjanus decussatus* and *L. fulviflamma*.** Location of Yaeyama Islands (A), Sekisei lagoon (B) (enclosed area by a yellow dotted line), fish aggregations of *L. decussatus* (C) and *L. fulviflamma* (D). Aerial photograph in (B) was provided by International Coral Reef Research and Monitoring Center.

Full-size DOI: 10.7717/peerj.15991/fig-1

were immediately killed to minimize pain. Okinawa prefectural government fisheries coordination regulation No. 37 approved the sampling procedure. This regulation permits capture of fishes for scientific purposes on Okinawan coral reefs.

Study site

This study was conducted at Sekisei lagoon in the Yaeyama Islands, Okinawa, located in the southernmost part of Japan (Fig. 1). One fish aggregation site was located in the Sekisei lagoon, although the precise location is not shown. This is because the study site is not protected during the spawning periods of the two snapper species yet and there is concern that showing the precise location might lead to overfishing the fish aggregations of the two snapper species.

Temporal variations in the fish abundance within the aggregation site

To clarify the monthly and weekly variation in the fish abundance at the aggregation site, daytime underwater observations were conducted. After preliminary surveys between September and October 2020, one line transect (600 m $\times$5 m) was set to extend over the whole aggregation site between November 2020 and December 2021. Underwater observations on the transect were conducted using SCUBA. During the main spawning season of the two snapper species (between May and October), weekly observations were conducted (Table 1). The observation days were adjusted to be carried out during each of the four lunar phases whenever possible (new moon, first-quarter moon, full moon and last-quarter moon). When rough sea conditions prevented such adjustments, the observation days were set as several days earlier or later of the four lunar phases (Table 1). During non-spawning seasons (between November 2020 and April 2021, and between November and December 2021), underwater observations were conducted around two lunar phases (last-quarter moon and new moon). This was because: (1) preliminary surveys between September and October 2020 revealed the fish aggregations of the two snapper species were found around the last-quarter moon and new moon and (2) *Nanami et al. (2010)* showed that *Lutjanus decussatus* showed a greater peak of ovary development during the last-quarter moon.

The number of individuals of the two snapper species on the above-mentioned 600 m $\times$5 m line transect was counted per every 1 min. During the observations, a portable GPS receiver, sealed in a waterproof case and attached to a buoy, was towed and the course and distance of the tracks were obtained in accordance with the protocol detailed in *Nanami et al. (2017)*. The water depth range at which the underwater observations were conducted was c.a. 10 m to 15 m.

Analysis of periodicity in the increase of fish abundance in relation to lunar phase

To determine the statistical significance in periodicity of fish increasing at the aggregation site, a time-series analysis (correlogram) was applied. In this procedure, data obtained between May 3 and October 20 was used for the analysis (Table S1), since greater fish abundance at the aggregation site was found in the study period (see RESULTS). In the analysis, 24 time periods were established, in which each time period includes one lunar phase (last-quarter moon, new moon, first-quarter moon and full moon) (Table S1). Correlogram was applied (Fig. S1, Table S2) by using R statistical computing language (function "acf": *R Core Team, 2022*). Statistical significance of auto-correlation coefficient

Table 1 **Date of underwater observations and fish sampling (x: conducted).**

Date	Year	Lunar phase	Underwater Observation	Fish Sampling	Number of samples	
					Lutjanus decussatus	*Lutjanus fulviflamma*
November 8	2020	LQM	x			
November 15	2020	NewM	x			
December 8	2020	LQM	x			
December 14	2020	NewM-1	x			
January 5	2021	LQM-1	x			
January 13	2021	NewM	x			
February 5	2021	LQM	x			
February 13	2021	NewM+1	x			
March 5	2021	LQM-1	x			
March 13	2021	NewM	x			
April 4	2021	LQM	x	x	N.A.	Male = 1, Female = 1
April 11	2021	NewM-1	x			
May 3	2021	LQM-1	x	x	Male = 1, Female = 4	N.A.
May 13	2021	NewM+1	x	x	N.A.	Male = 4, Female = 2
May 22	2021	FQM+2	x			
May 26	2021	FullM	x			
June 1	2021	LQM-1	x	x	Male = 0, Female = 9	Male = 0, Female = 1
June 10	2021	NewM	x	x	Male = 0, Female = 2	Male = 8, Female = 2
June 17	2021	FQM-1	x			
June 25	2021	FullM	x			
July 1	2021	LQM-1	x	x	Male = 5, Female = 10	Male = 3, Female = 6
July 10	2021	NewM	x	x	Male = 5, Female = 2	Male = 6, Female = 9
July 17	2021	FQM	x			
July 31	2021	LQM	x	x	Male = 5, Female = 6	Male = 5, Female = 1
August 9	2021	NewM+1	x	x	Male = 1, Female = 4	Male = 10, Female = 10
August 16	2021	FQM	x			
August 24	2021	FullM+2	x			
August 29	2021	LQM-1	x	x	Male = 0, Female = 9	N.A.
September 7	2021	NewM	x	x	Male = 0, Female = 1	Male = 7, Female = 4
September 16	2021	FQM+2	x			
September 21	2021	FullM	x			
October 2	2021	LQM+3	x	x	Male = 7, Female = 2	Male = 0, Female = 2
October 7	2021	NewM+1	x	x	N.A.	Male = 7, Female = 3
October 15	2021	FQM+2	x			
October 20	2021	FullM	x			
October 29	2021	LQM	x			
November 5	2021	NewM	x			
November 26	2021	LQM-1	x			
December 4	2021	NewM	x			

Notes.

Lunar phases are abbreviated as: LQM, last-quarter moon; NewM, new moon; FQM, firstquarter moon; FullM, full moon; N.A., not available due to low fish density at the aggregation site or logistic constraint.

"+" and "-" mean after and before the lunar phase (*e.g.* "NewM+1" means 1 day after new moon).

was determined by using 95% confidence intervals (CI) as follow:

$$95\%\ \mathrm{CI} = 1.96/\sqrt{(T)}$$

where T is the number of observations.

It can be regarded that the auto-correlation coefficient is significant when the following equation was found:

$$|pk| > 95\%\ \mathrm{CI} = 1.96\sqrt{(T)}$$

where p_k is the value of auto-correlation at k th time lag, and $|p_k|$ is the absolute value of p_k. If $|p_k|$ is greater than the 95% CI, auto-correlation at k th time lag was significant. Since T was 24 in the analysis (Table S1), 95% CI was calculated as $1.96/\sqrt{(24)} = 0.40$. For example, auto-correlation coefficient at 4th time lag (p_4) was over 0.40, the p_4 is a significantly positive value. Namely, the periodic increase in fish abundance was found at the same particular lunar phase.

Since data for July 22 (full moon phase) could not be collected due to a typhoon, the missing value was imputed. In this procedure, other fish abundance data obtained around full moon phase (May 26, June 25, August 24, September 21 and October 20) was averaged and the average value was applied to impute the missing value.

Fine-scale spatial variations in fish density at the aggregation site

The above-mentioned underwater observations revealed that fish abundance showed positive peaks around last-quarter moon or new moon (see RESULTS). At the two lunar phases, fine-scale spatial variations in fish abundance (variations in fish abundance at intervals of several-tens meters) within the aggregation site were examined. The above-mentioned 600 m × 5 m line transect was divided into 1-minute sub-transects (average distance ± standard deviation = 20.5 m ± 4.1). Then, the number of individuals of the two snapper species and the distance for the 1-minute sub-transect were obtained. From the data, the number of individuals was converted to density (per 20 m × 5 m) for each 1-minute transect. Fish density on the 1-minute sub-transect was individually plotted by bubble plot along the 600 m × 5 m line transect.

The fine-scale spatial variation in fish density per 20 m × 5 m was shown as frequency data (histogram). Then, Kolmogorov–Smirnov test was applied to test the significant difference in fine-scale spatial variation in fish density between the two fish species.

Verification of spawning by ovarian development

Domeier (2012) and *de Mitcheson & Erisman (2012)* have indicated that presence of females with matured eggs in the hydrated stage (hydrated eggs) in aggregation sites is one of the direct evidence that the fish aggregation can be regarded as a spawning aggregation. To clarify whether females have hydrated eggs in the aggregation site, individuals of the two species were caught by spear-gun just after the above-mentioned underwater observations around the last-quarter and new moon (Table 1). In total, 73 and 92 individuals were caught for *L. decussatus* and *L. fulviflamma*, respectively (Table 1). In the laboratory, fork length (FL), whole body weight and gonad weight were measured. The gonadsomatic index

(GSI) was calculated by using the formula:

GSI = Gonad weight (g)/[whole body weight (g) − gonad weight (g)] × 100.

To obtain histological observations, the gonads were preserved in 20% buffered formalin over 48 h and then kept in 70% ethanol baths. Embedded pieces of gonads were sectioned and stained with Mayer's hematoxylin–eosin. Under microscopic observations, developmental stages of ovaries were examined whether the gonads were sufficiently developed for spawning (hydrated eggs). The categorization of ovarian developmental stages followed *Ohta & Ebisawa (2015)* and *Ohta et al. (2017)*. According to the categorization of *Ohta & Ebisawa (2015)*, oocytes with migration nuclear stage, pre-maturation stage and maturation stage were defined as hydrated stage.

Size and age frequency distributions

To determine the size frequency distribution of fishes at the aggregation site, histograms of fork length for the above-mentioned fish samples was plotted. Male and female were separately plotted, and probability density of size frequency was analyzed by R statistical computing language (function "density": *R Core Team, 2022*).

To determine the age frequency distribution of fishes, sagittal otoliths of the above-mentioned fish samples were extracted from each fish, and cleaned in water and dried. Then, one otolith was embedded in epoxy resin and transversely sectioned into 0.5 mm-thick sections using ISOMET low speed saw (Buehler) and attached on a glass slide. The sectioned otoliths were observed under a microscope with reflected light at ×4 magnification, and the number of opaque rings was counted. The procedure of opaque rings count followed *Ohta et al. (2017)*. The number of opaque rings on each otolith was counted twice. If the two counts coincided, the number of rings was used. However, if the two counts did not coincide, the number of opaque rings was counted once more and any two coinciding counts were used. Since *Nanami (2021)* and *Shimose & Nanami (2015)* revealed that each opaque ring was formed annually for the two species, number of opaque rings can be considered as age (year). Age frequency distribution for male and female was separately plotted, and probability density was analyzed by R statistical computing language (function "density": *R Core Team, 2022*).

Mann–Whitney U-test test was applied to determine the significant differences in average fork length and average age between males and females.

Comparison of fish density between inside and outside the aggregation site

Domeier (2012) has proposed the definition of spawning aggregations, indicating that fish abundance in aggregation sites is at least 4-fold greater than that outside the aggregation site. In order to verify the definitions, 65 study sites outside the aggregation site were established in Sekisei Lagoon (Fig. S2) and the number of the two snapper species was counted. Above-mentioned 20-minutes underwater survey with a portable GPS receiver was conducted at each site (details about method was shown in *Nanami (2020)*. Fish abundance per 600 m ×5 m was estimated by using the fish count data and the measured distance. The estimated fish abundance per 600 m ×5 m among the 65 sites were averaged and regarded as average fish abundance outside the aggregation site. Then, the fish abundance per 600 m ×5 m inside the aggregation site was compared with that outside aggregation site.

RESULTS

Temporal variations in the fish abundance and reproductive activity in relation to lunar phase

Greater fish abundance (number of individuals per 600 m ×5 m) of *Lutjanus decussatus* was found between May and October (Fig. 2A), when the water temperature exceeded 25 °C. During the six months, clear peaks in the fish abundance were only found around the last-quarter moon (Fig. 2B). The peak fish abundance ranged from 529 (October) to 1565 (June). Average GSI values ± SD (standard deviation) of females that were caught at the aggregation site was 10.34 ± 4.95 (ranged from 0.42 to 24.89) (Fig. 2C). About 69% of individuals (34 out of 49) had hydrated stage oocytes (Figs. 2C–2E, Table S3).

Greater fish abundance of *L. fulviflamma* was clearly found between April and October (Fig. 3A), when water the temperature exceeded 25 °C. During the seven months, clear peaks in the fish abundance were found around the last-quarter moon in April, May, June and October (Fig. 3B). In contrast, clear peaks were found around the new moon in July, August and September (Fig. 3B). The peak fish abundance ranged from 660 (September) to 6337 (June). Average GSI values ± SD of females were 6.33 ± 2.21 (ranged from 3.44 to 14.51) (Fig. 3C). About 90% of individuals (37 out of 41) had hydrated stage oocytes (Figs. 3C–3E, Table S4).

Periodicity of the increase of fish density

Correlogram of *L. decussatus* revealed that significant positive auto-correlation coefficients were found when time lags were 4 and 8 (Fig. 4A). Correlogram of *L. fulviflamma* revealed that significant positive auto-correlation coefficient was found when time lag was 4 (Fig. 4B).

Fine-scale spatial variations in fish density at the aggregation site

Fine-scale spatial distributions revealed that *L. decussatus* showed relatively uniform distribution at the aggregation site, especially around the last-quarter moon between May and October (Fig. 5). Fish densities per 20 m ×5 m were generally less than 140 individuals in most cases (Fig. 6).

In contrast, greater fish density of *L. fulviflamma* per 20 m ×5 m was found at particular areas (south-eastern side) in the aggregation site (Fig. 7). This tendency was clearly found around last quarter moon in April, May and June, and around new moon in July and August. In some cases, fish density within 20 m ×5 m was over 300 (Fig. 8).

Kolmogorov–Smirnov test revealed that a significant difference in fish density frequency (*i.e.*, patterns in fine-scale spatial distribution) was found between the two fish species from May to October ($p < 0.05$, Table 2).

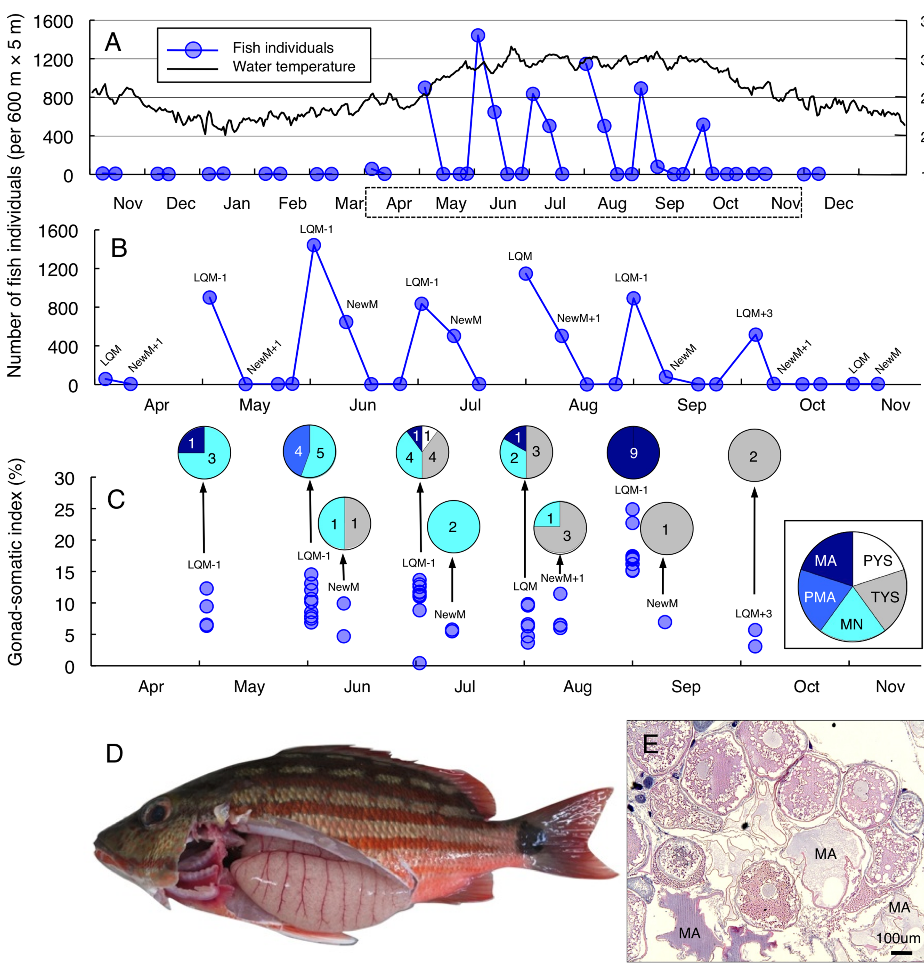

Figure 2 Temporal changes in number of fish individuals and gonad-somatic index for *Lutjanus decussatus*. Temporal changes in number of fish individuals with water temperature (A). Data in months enclosed by a dotted line in (A) are re-plotted in (B), showing lunar-related temporal changes in the number of fish individuals since a greater number of individuals were observed during the study period. Temporal changes in gonad-somatic index between April and October (C), together with oocyte developmental stage (pie charts). Numerals in pie charts indicate the number of females with eggs in each developmental stage. Lunar phases are abbreviated as LQM, last-quarter moon; NewM, New moon. "+" and "-" mean after and before the lunar phase (*e.g.*, "NewM-1" means 1 day before new moon). Oocyte developmental stage was abbreviated as PYS, primary yolk stage; TYS, tertiary yolk stage; MN, migration nuclear stage; PMA, pre-maturation stage; MA, maturation. Fish individual with hydrated egg (D) and cross-section of ovaries having oocytes with maturation stage (E). In (E), MA represents maturation stage of oocytes. Data of water temperature in (A) was provided by International Coral Reef Research and Monitoring Center.

Full-size DOI: 10.7717/peerj.15991/fig-2

Size and age frequency distributions

For *L. decussatus*, fork length of most individuals was between 200.0–279.5 mm for both males and females (Figs. 9A, 9B). Average fork length of females (243.9 mm FL ± 21.4 SD: standard deviation) was significantly greater than males (229.2 mm FL ± 19.6 SD: Mann–Whitney U-test, $p < 0.01$). Highest probability of occurrence in fish length was about 210 mm FL for males whereas about 260 mm FL for females. Although average

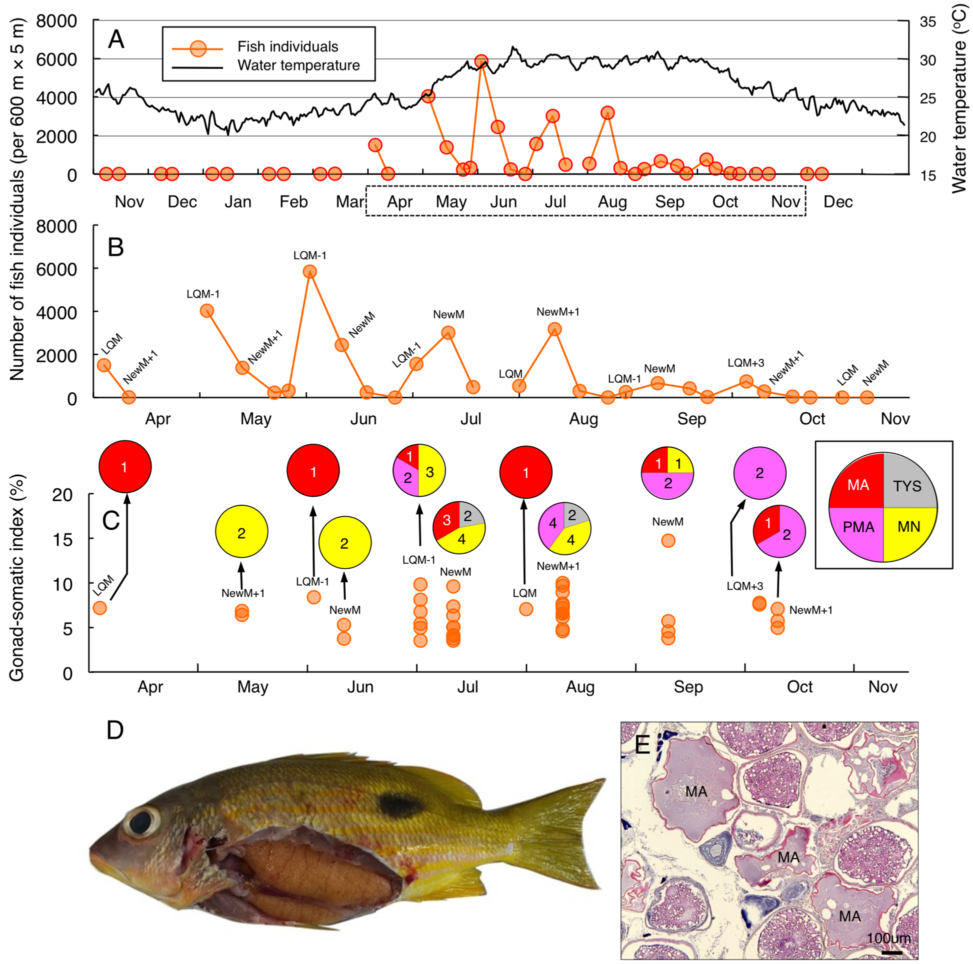

Figure 3 Temporal changes in number of fish individuals and gonad-somatic index for *Lutjanus fulviflamma*. Temporal changes in number of fish individuals with water temperature (A). Data in months enclosed by a dotted line in (A) are re-plotted in (B), showing lunar-related temporal changes in number of fish individuals since greater number of individuals were observed during the study period. Temporal changes in gonad-somatic index between April and October (C), together with oocyte developmental stage (pie charts). Numerals in pie charts indicate the number of females with eggs in each developmental stage. Lunar phases are abbreviated as LQM, last-quarter moon; NewM, New moon. "+" and "-" mean after and before the lunar phase (*e.g.*, "NewM-1" means 1 day before new moon). Oocyte developmental stage was abbreviated as TYS, tertiary yolk stage; MN, migration nuclear stage; PMA, pre-maturation stage; MA, maturation. Fish individual with hydrated egg (D) and cross-section of ovaries having oocytes with maturation stage (E). In (E), MA represents maturation stage of oocytes. Data of water temperature in (A) was provided by International Coral Reef Research and Monitoring Center.

Full-size DOI: 10.7717/peerj.15991/fig-3

age was not significantly different between males (9.4 years ± 5.0 SD) and females (8.1 years ± 4.4 SD: Mann–Whitney U- test, $p = 0.26$), highest probability of occurrence in age was about 6 years for both males and females (Figs. 9C, 9D).

For *L. fulviflamma*, fork length of most individuals was less than 250 mm for males whereas 280 mm for females (Figs. 10A, 10B). Average fork length of females (246.9 mm FL ± 23.0 SD) was significantly greater than males (233.9 mm FL ± 17.8 SD: Mann–Whitney

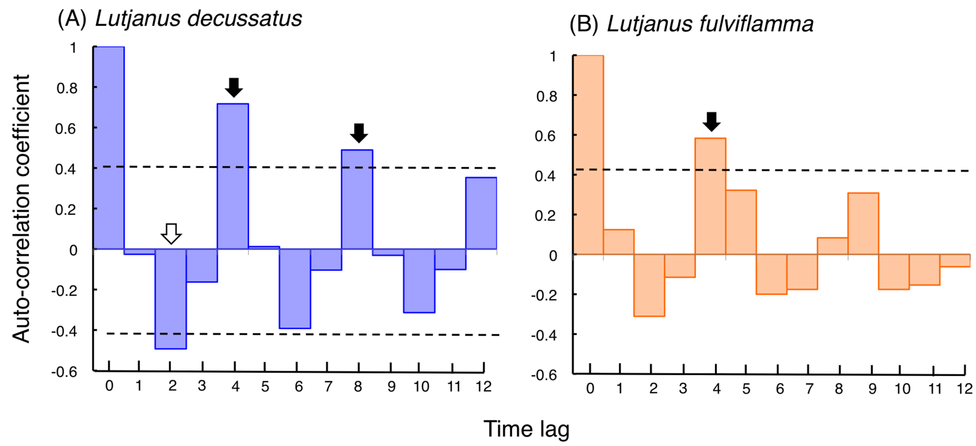

Figure 4 Correlogram showing auto-correlation coefficient with time lag, which examines the periodicity in fish increasing or decreasing at a particular time lag (for time lag, see Tables S1, S2 and Fig. S1). Horizontal dotted line represent 95% confidence interval of the auto-correlation coefficient. Black and white arrows represent significant positive and negative values of auto-correlation coefficient, showing significant periodicity at the time lag.

Full-size DOI: 10.7717/peerj.15991/fig-4

U-test, $p < 0.01$). Probability density revealed that highest probability of occurrence in fish length was about 240 mm FL for males whereas about 260 mm FL for females. Although average age was not significantly different between males (6.8 years ± 3.1 SD) and females (8.1 years ± 4.5 SD: Mann–Whitney U-test, $p = 0.29$), highest probability of occurrence in age was about 7 years and 5 years for males and females, respectively (Figs. 10C, 10D).

Comparison of fish density between inside and outside the aggregation site

Fish abundance per 600 m ×5 m of *L. decussatus* inside the aggregation site was 148.2 to 438.4-fold (average = 266.8-fold) greater than that outside the aggregation site (Table 3).

Fish abundance of *L. fulviflamma* inside the aggregation site was 33000.0 to 316850.0-fold (average = 141557.1-fold) greater than that outside the aggregation site (Table 3).

DISCUSSION

Verification of spawning aggregation of two snapper species

For the two snapper species *Lutjanus decussatus* and *L. fulviflamma*, this study was the first attempt to examine whether fish aggregations at a particular site can be regarded as spawning aggregations. The present study showed: (1) repeated greater fish abundance of the two species and this is predictable in time (particular month and lunar-phase) and space, (2) the fish abundance inside the aggregation site is over 4-fold greater than that outside the aggregation site, and (3) most females inside the aggregation site had hydrated eggs. Thus, the fish aggregations of the two snapper species can be regarded as spawning aggregations.

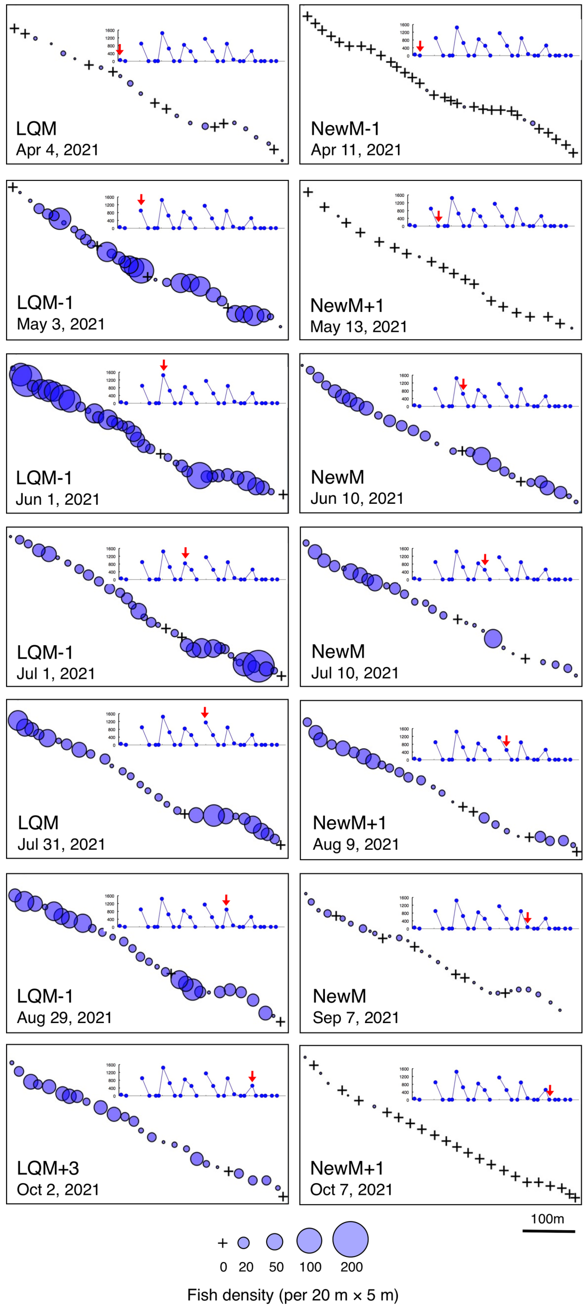

Figure 5 Spatial distribution of *Lutjanus decussatus* on the 600 m × 5 m line transect in the aggregation site. The transect was divided into 1-minute sub-transects. Fish data are shown as bubble plots and each bubble represents the fish density (number of individuals in 20 m × 5 m area) on each 1-minute sub-transect. Cross marks represent no fishes in the sub-transect. Graphs at upper right side show the temporal changes in number of fish individuals (see Fig. 2A) and red arrows represent the day when the data was collected. Lunar phases are abbreviated as LQM, last-quarter moon; NewM, New moon. "+" and "-" mean after and before the lunar phase (*e.g.*, "NewM-1" means 1 day before new moon).

Full-size DOI: 10.7717/peerj.15991/fig-5

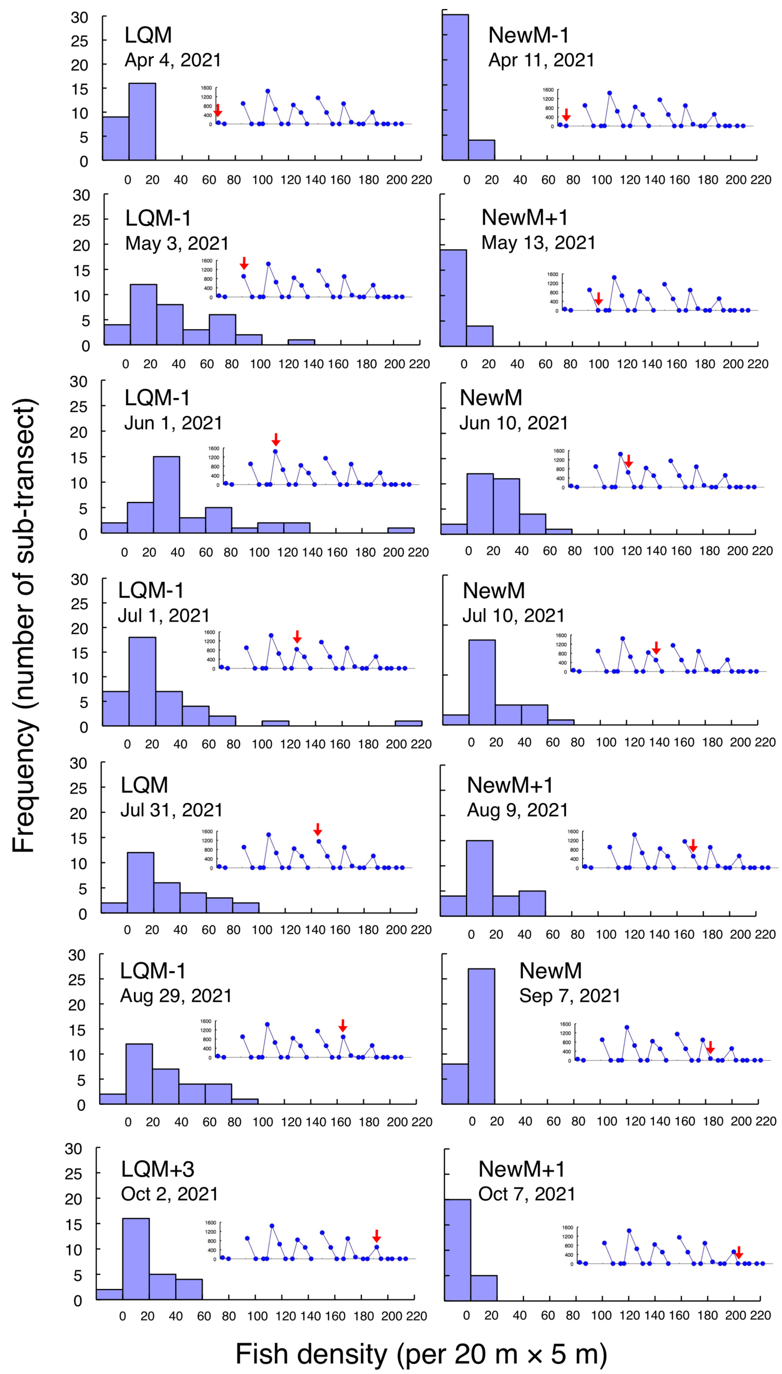

Figure 6 Fish density frequency of *Lutjanus decussatus* for 1-minute sub-transect in the aggregation site. Fish density represents the number of fish individuals in the 20 m × 5 m area. Number of individuals in the 1-minute sub-transect was converted to fish density (20 m × 5 m). Graphs at upper right side show the temporal changes in number of fish individuals (see Fig. 2A) and red arrows represent the day when the data was collected. Lunar phases are abbreviated as LQM, last-quarter moon; NewM, New moon. "+" and "-" mean after and before the lunar phase (*e.g.*, "NewM-1" means 1 day before new moon). For actual spatial distributions, see Fig. 5.

Full-size DOI: 10.7717/peerj.15991/fig-6

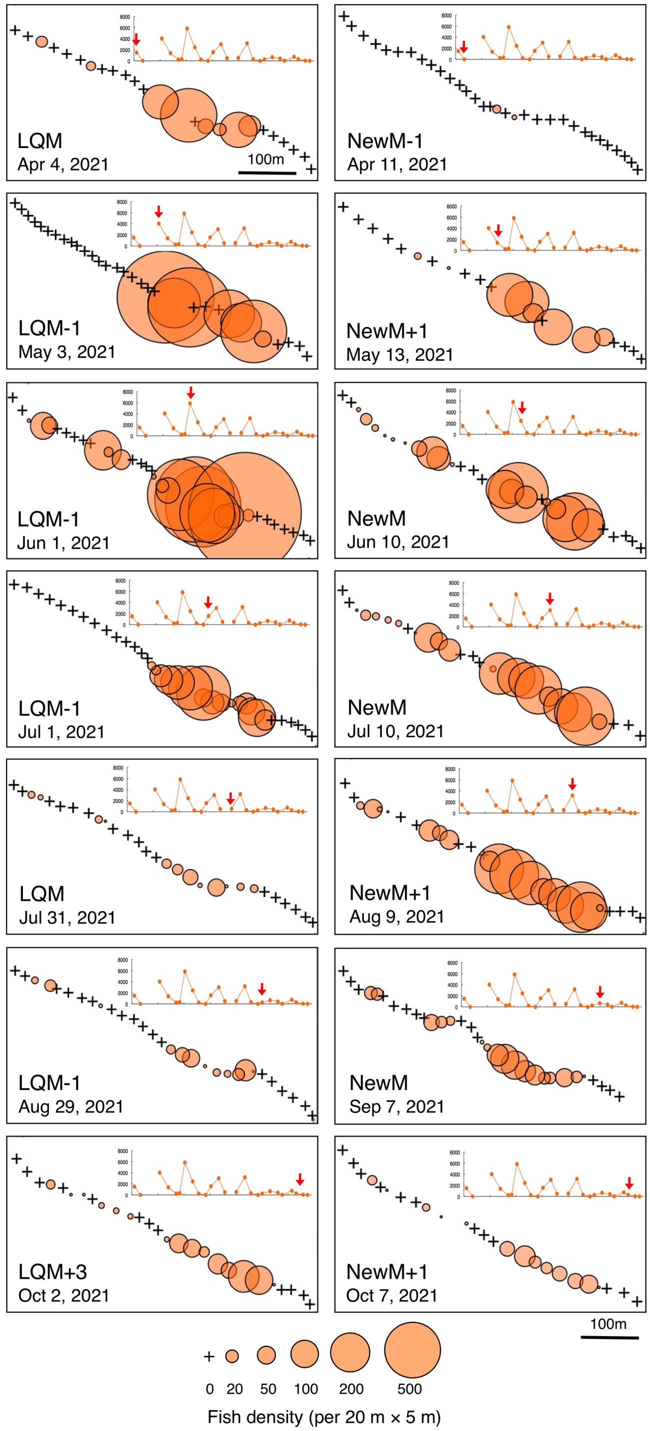

Figure 7 Spatial distribution of *Lutjanus fulviflamma* on the 600 m × 5 m line transect in the aggregation site. The transect was divided into 1-minute sub-transects. Fish data are shown as bubble plots and each bubble represents the fish density (number of individuals in 20 m × 5 m area) on each 1-minute sub-transect. Cross marks represent no fishes in the sub-transect. (continued on next page...)

Full-size DOI: 10.7717/peerj.15991/fig-7

Figure 7 (…continued)
Graphs at upper right side show the temporal changes in number of fish individuals (see Fig. 3A) and red arrows represent the day when the data was collected. Lunar phases are abbreviated as LQM, last-quarter moon; NewM, New moon. "+" and "-" mean after and before the lunar phase (*e.g.*, "NewM-1" means 1 day before new moon).

Spawning season and spawning day

The present study revealed that fish aggregations of the species were found between May and October. *Nanami et al. (2010)* showed similar results from fish samples by commercial catch, i.e., estimated main spawning season of *L. decussatus* was between June and October. Since developed- or matured-oocytes (tertiary yolk stage, migration nuclear stage, pre-maturation stage and maturation stage) were obtained for most female individuals between May and October, it is suggested that the main spawning season of *L. decussatus* can be regarded as between May and October in the study region. Since peaks of fish abundance were found only around the last-quarter moon and the periodicity in the increase of fish abundance during each last-quarter moon phase was significant, it is suggested that spawning occurs around the last-quarter moon.

The present study revealed that spawning aggregations of the species were found and the most females had hydrated eggs between April and October. *Shimose & Nanami (2015)* showed similar results from fish samples by commercial catch, *i.e.*, estimated main spawning season of *L. fulviflamma* is between April and August. Therefore, it is suggested that the spawning season of *L. fulviflamma* can be regarded as between April and October in the study region. The peak fish abundance at the spawning ground was found during the last-quarter moon (in April, May, June and October) and new moon (in July, August and September), suggesting that spawning occurs around the last-quarter moon and new moon. This lunar-related periodicity in the increase of fish abundance at during a particular lunar phase (last-quarter moon or new moon) was also supported by the correlogram in the present study. This evidence for lunar-related spawning is the first finding for *L. fulviflamma*.

Since western Atlantic snapper species (*Lutjanus cyanopterus* (Cuvier, 1828) and *L. jocu* (Bloch & Schneider, 1801)) spawned for a period of four to seven consecutive days (*Heyman & Kjerfve, 2008*), the two snapper species in this study might spawn during several consecutive days around last-quarter moon and new moon. Intensive daily observations around the two lunar phases would clarify more precise ecological aspects about aggregation formation of the two snapper species.

Water temperature is one of the main factors for gonad development and reproduction of marine fishes (*Wang et al., 2010*). The present study revealed that reproductive activity of the two snapper species is likely to occur when water temperature is over 25 °C. In addition, decline of temperature (beginning of October) might be one of the factors leading to reduced ovarian development. This trend is consistent with cubera snapper (*Lutjanus cyanopterus*) in the Caribbean (*Heyman et al., 2005*; *Motta et al., 2022*), showing that spawning aggregation of *L. cyanopterus* related to increasing water temperature in the summer.

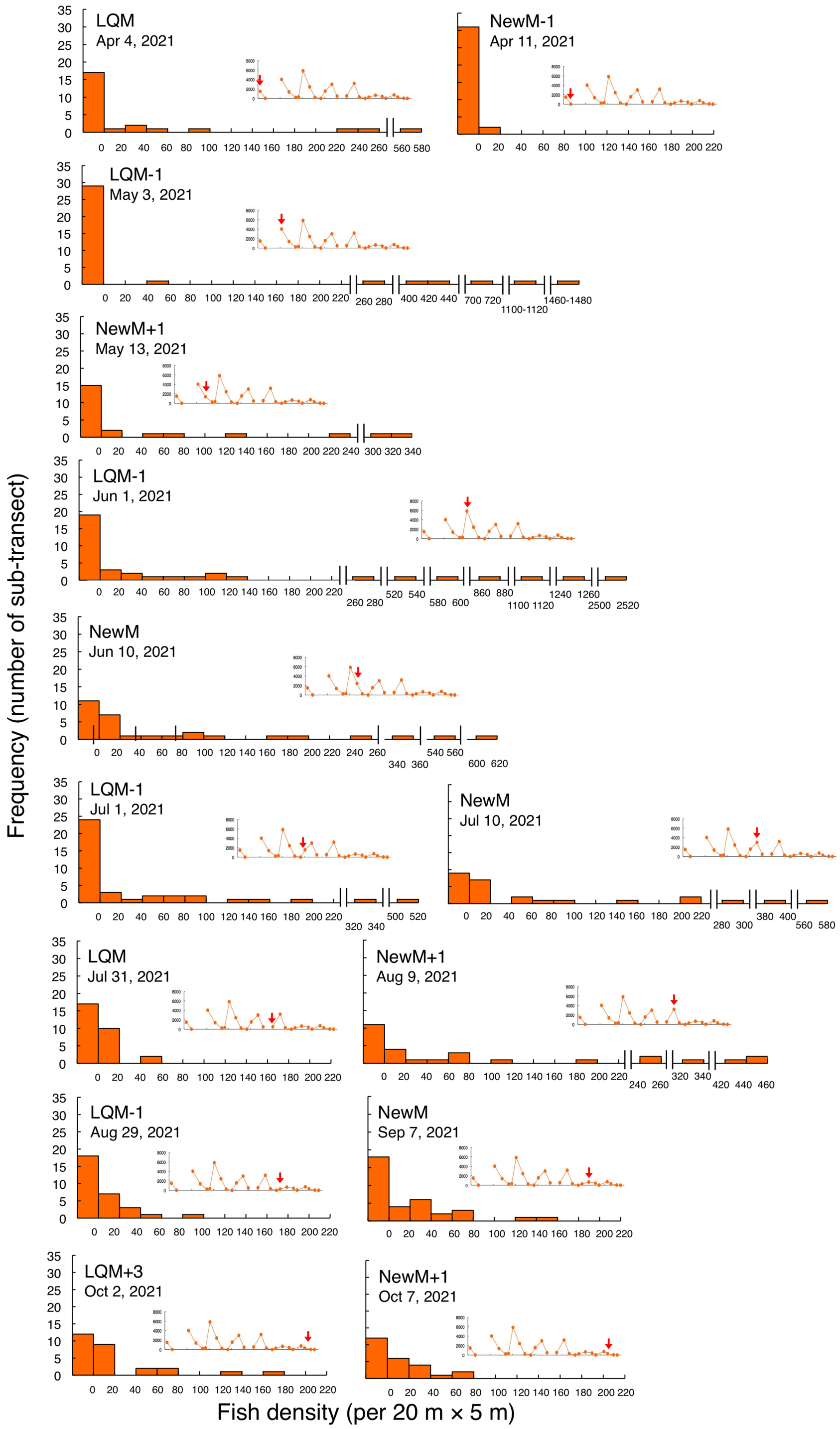

Figure 8 Fish density frequency of *Lutjanus decussatus* for 1-minute sub-transect in the aggregation site. Fish density represents the number of fish individuals in the 20 m × 5 m area. Number of individuals at 1-minute sub-transect was converted to fish density (20 m × 5 m). Graphs at upper right side show the temporal changes in number of fish individuals (see Fig. 3A) and red arrows represent the day when the data was collected. Lunar phases are abbreviated as LQM: last-quarter moon; NewM: New moon. "+" and "-" mean after and before the lunar phase (*e.g.*, "NewM-1" means 1 day before new moon). For actual spatial distributions, see Fig. 7.

Full-size DOI: 10.7717/peerj.15991/fig-8

Table 2 **Results of Kolmogorov-Smirnov test to test the significant difference in fish density (number of fish in 20 m ×5 m area) frequency at aggregation site between Lutjanus decussatus and *L. fulviflamma*.**

Date	Lunar phase	*p*-value	*n* (*L. decussatus*)	*n* (*L. fulviflamma*)
April 4	LQM	0.155	25	25
April 11	NewM-1	>0.999	33	33
May 3	LQM-1	<0.001	36	36
May 13	NewM+1	0.124	23	23
June 1	LQM-1	<0.001	37	37
June 10	New M	0.035	30	30
July 1	LQM-1	0.001	40	40
July 10	NewM	0.056	28	28
July 31	LQM	<0.001	29	29
August 9	NewM+1	0.012	28	28
August 29	LQM-1	<0.001	30	30
September 7	NewM	0.007	35	35
October 2	LQM+3	0.004	27	27
October 7	NewM+1	0.037	25	25

Notes.

Lunar phases are abbreviated as: LQM, last-quarter moon; NewM, new moon.
"+" and "-" mean after and before the lunar phase (*e.g.* "NewM+1" means 1 day after new moon).

Fine-scale spatial variation in fish density within spawning ground

Some previous studies have shown fine-scale spatial variations of fish density at intervals of several-tens meters within spawning grounds for groupers (*e.g.*, Colin, 2012; Nanami et al., 2017; Sadovy de Mitcheson et al., 2020). These studies have shown species-specific spatial variations among multiple grouper species and each species showed species-specific core sites, in which a very high density was found in a limited area within the spawning ground (Nanami et al., 2017; Sadovy de Mitcheson et al., 2020). Spatial variations of fish density at intervals of 250 m was also observed for two snapper species (Biggs & Nemeth, 2016), showing fine scale movement of two snapper species (*L. cyanopterus* and *Lutjanus jocu*) within the aggregation site in relation to spawning time.

The present study revealed a significant difference in the fine-scale spatial distributions in fish density between the two snapper species. Although the reasons why the two species showed species-specific spatial variations within the spawning ground remain unknown, this might be related with preference to a particular environmental condition or mating behavior.

Size and age frequency of fish aggregation

The present study showed that the maximum fork length of fish individuals at the spawning ground was 304 mm and 295.5 mm for *L. decussatus* and *L. fulviflamma*, respectively. In contrast, previous studies revealed that maximum fork length was 316.5 mm and 347.0 mm for *Lutjanus decussatus* and *L. fulviflamma*, respectively (Shimose & Nanami, 2015; Nanami, 2021). Since average fork length of fish individuals at the spawning ground was

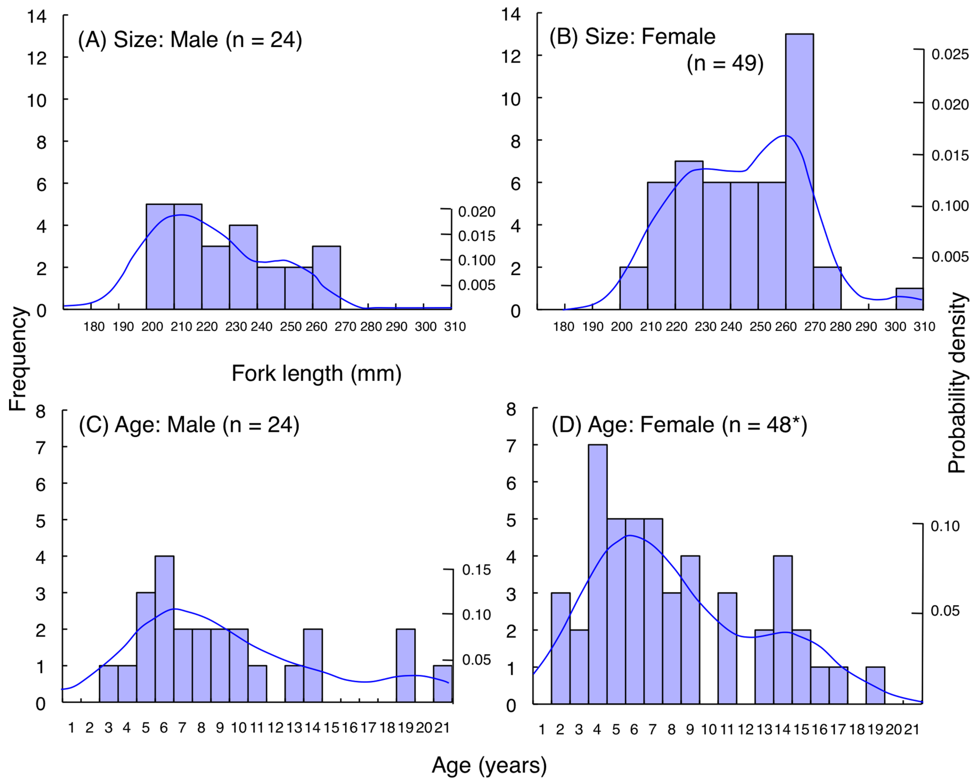

Figure 9 (A–D) Size and age frequency of *Lutjanus decussatus* individuals that were captured at the aggregation site. Solid lines represent the probability density function. *: Among the 49 individuals in (B) age of one individual could not be identified due to difficulty in counting of number of opaque rings on otolith. Thus, sample size was 48 in (D).

Full-size DOI: 10.7717/peerj.15991/fig-9

less than 250 mm for both species, most of the fish individuals forming the spawning aggregations were relatively small sized individuals.

A similar trend was also found for age frequency. Maximum age was respectively 24 and 23 for *Lutjanus decussatus* and *L. fulviflamma* (*Shimose & Nanami, 2015*; *Nanami, 2021*), whereas maximum age of fish individuals at the spawning ground was respectively 21 and 17 for *L. decussatus* and *L. fulviflamma*. Since average age of fish individuals at the spawning ground was less than 10 for both species, most of the fish individuals that form the spawning aggregations were relatively young individuals.

CONCLUSIONS

Results of the present study revealed that fish aggregations of the two snapper species (*Lutjanus decussatus* and *L. fulviflamma*) can be regarded as spawning aggregations due to their spatially and temporally predictable formation of fish aggregation as well as the presence of hydrated eggs in females within the aggregation site. In the Caribbean and Palau, spawning aggregations for some species of snappers have been previously found by local communities (*Hamilton, Sadovy de Mitcheson & Aguilar-Perera, 2012*). Since there is almost no local ecological information about the spawning aggregations of *L. decussatus*

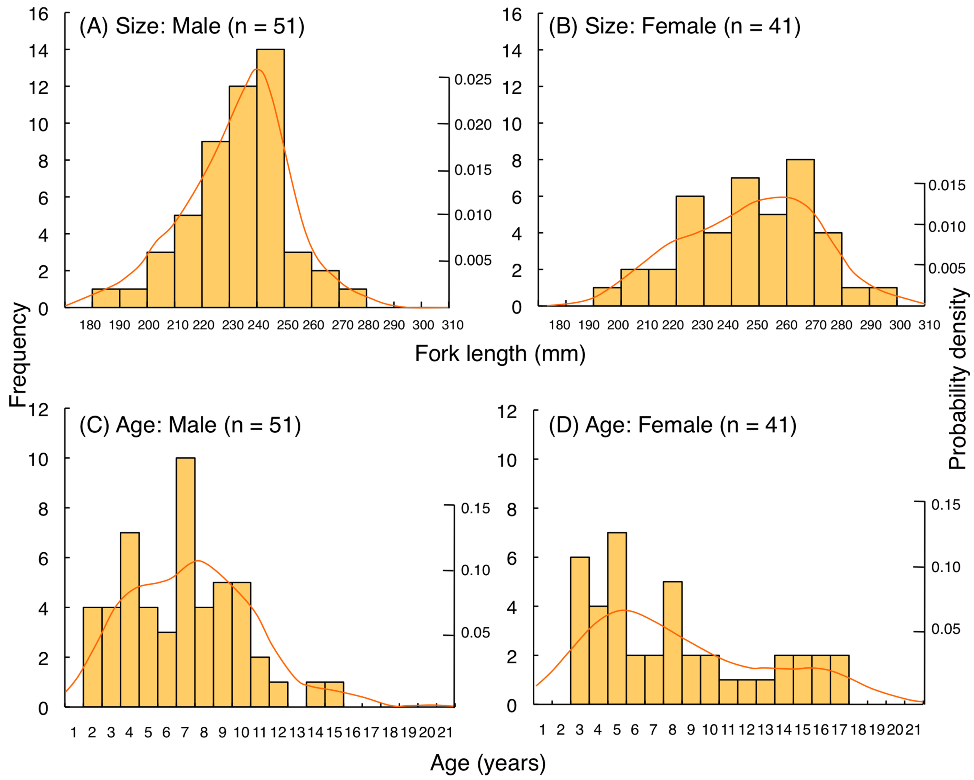

Figure 10 **(A–D) Size and age frequency of *Lutjanus fulviflamma* individuals that were captured at the aggregation site.** Solid lines represent the probability density function.
Full-size DOI: 10.7717/peerj.15991/fig-10

and *L. fulviflamma*, the present study is probably the first finding of a spawning aggregation of the two snapper species. As the two species are major fishery target species in the study region, the results of the present study should be applied to consider when and where a marine protected area should be established for effective protection of the spawning aggregation of the two species. Namely, the aggregation site of the two snapper species should be protected during their main spawning season (between April and October) and the protected area should completely cover the fish aggregations.

ACKNOWLEDGEMENTS

I express my grateful thanks to M. Sunagawa and S. Sunagawa for piloting the research boat YAEYAMA, K. Aramoto, M. Yoshida, F. Nakamura and K. Ohishi for their assistance in the field, K. Nema for assistance in laboratory works and the staff of Yaeyama Field Station, Fisheries Technology Institute for support during the present study. I would like to thank Dr. Norman for English language editing. Providing of aerial photograph and data of water temperature from International Coral Reef Research and Monitoring Center and constructive comments from K. Mansor and two anonymous reviewers were much appreciated. The present study complies with current laws in Japan.

Table 3 Comparison about number of fish individuals per 600 m ×5 m of two snapper species between inside and outside aggregation site. Average number of fish individuals outside the aggregation site were obtained from underwater observations at 65 sites (Fig. S2).

	Inside	aggregation	site	Outside aggregation site	
	Month	Lunar phase	Number of fish individuals	Number of fish individuals	Inside/ Outside
Lutjanus decussatus	May 3	LQM-1	903	3.57	252.9
	June 1	LQM-1	1565	3.57	438.4
	July 1	LQM-1	877	3.57	245.7
	July 31	LQM	939	3.57	263.0
	August 29	LQM-1	901	3.57	252.4
	October 2	LQM+3	529	3.57	148.2
	Average		952.33	3.57	266.8
Lutjanus fulviflamma	April 4	LQM	1521	0.02	76050.0
	May 3	LQM-1	4040	0.02	202000.0
	June 1	LQM-1	6337	0.02	316850.0
	July 10	NewM	3000	0.02	150000.0
	August 9	NewM+1	3495	0.02	174750.0
	September 7	NewM	660	0.02	33000.0
	October 2	LQM+3	765	0.02	38250.0
	Average		2831.14	0.02	141557.1

Notes.
Lunar phases are abbreviated as: LQM, last-quarter moon; NewM, new moon.
"+" and "-" mean after and before the lunar phase (*e.g.* "NewM+1" means 1 day after new moon).

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This study was supported by the Environment Research and Technology Development Fund (S-15-3(4): JPMEERF16S11513) of the Ministry of the Environment, Japan. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the author:
Environment Research and Technology Development Fund of the Ministry of the Environment, Japan: S-15-3(4): JPMEERF16S11513.

Competing Interests

The author declares that there are no competing interests.

Author Contributions

- Atsushi Nanami conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

Animal Ethics

The following information was supplied relating to ethical approvals (i.e., approving body and any reference numbers):

Fisheries coordination regulation no. 37 by Okinawa Prefectural Government.

This study mainly involved observing free-living fishes in their natural habitat. Individuals that were caught by spearing for sampling were immediately killed by placing them on ice to minimize pain. The sampling procedure was approved by the Okinawa Prefectural Government in compliance with the fisheries coordination regulation no. 37, which permits the capture of marine organisms on Okinawan coral reefs for scientific purposes.

A copy of the fisheries coodination regulation is attached as a Supplementary File.

In addition, English translation for regulation No. 37 is also attached as a Supplementary File.

Data Deposition

The following information was supplied regarding data availability:

The raw measurements are available in the Supplementary File. The location of the study site is confidential.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/peerj.15991#supplemental-information.

REFERENCES

Akita Y, Ohta I, Ebisawa A, Uehara M. 2016. Estimation of the fish catches of coastal species of the Yaeyama Islands. *Fauna Ryukyuana* **3**:113–127.

Allen GR. 1985. FAO species catalogue. Vol 6: snappers of the world. In: *An annotated and illustrated catalogue of lutjanid species known to date*. Rome: FAO, 208.

Amorim P, Sousa P, Jardim E, Menezes GM. 2019. Sustainability status of data-limited fisheries: global challenges for snapper and grouper. *Frontiers in Marine Science* **6**:654 DOI 10.3389/fmars.2019.00654.

Beets J, Friedlander A. 1999. Evaluation of a conservation strategy: a spawning aggregation closure for red hind, Epinephelus guttatus, in the U.S. Virgin Islands. *Environmental Biology of Fishes* **55**:91–98 DOI 10.1023/A:1007404421518.

Biggs C, Nemeth RS. 2014. Timing, size, and duration of a dog (*Lutjanus jocu*) and cubera snapper (*Lutjanus cyanopterus*) spawning aggregation in the U.S. Virgin Islands. *Proceedings of the 67Th Gulf and Caribbean Fisheries Institute* **67**:240–245.

Biggs CR, Nemeth RS. 2016. Spatial and temporal movement patterns of two snapper species at a multi-species spawning aggregation. *Marine Ecology Progress Series* **558**:129–142 DOI 10.3354/meps11846.

Carter J, Perrine D. 1994. A spawning aggregation of dog snapper, Lutjanus jocu (Pisces: Lutjanidae) in Belize, central America. *Bulletin of Marine Science* **55**:228–234.

Chollett I, Priest M, Fulton S, Heyman WD. 2020. Should we protect extirpated fish spawning aggregation site? *Biological Conservation* **241**:108395 DOI 10.1016/j.biocon.2019.108395.

Cimino M, Colin P, Schramek T, Lindfield S, Domeier M, Terrill E. 2018. Oceanographic, acoustic, and remote approaches reveal the spatio-temporal dynamics of blackfin snapper at an aggregation site in Palau. *Marine Ecology Progress Series* **601**:185–201 DOI 10.3354/meps12651.

Claro R, Lindeman KC. 2003. Spawning aggregation sites of snappers and grouper species (Lutjanidae and Serranidae) on the insular shelf of Cuba. *Gulf and Caribbean Research* **14**:91–106.

Claydon J. 2004. Spawning aggregations of coral reef fishes: characteristics, hypotheses, threats and management. *Oceanography and Marine Biology: An Annual Review* **42**:265–302.

Colin PL. 2012. Studying and monitoring aggregation species. In: Sadovy de Mitcheson Y, Colin PL, eds. *Reef fish spawning aggregations: biology, research and management. Fish and fisheries series*, vol. 35. Berlin: Springer Verlag, 285–329.

de Mitcheson YS, Erisman B. 2012. Fishery and biological implications of fishing spawning aggregations, and the social and economic importance of aggregating fishes. In: Sadovy de Mitcheson Y, Colin PL, eds. *Reef fish spawning aggregations: biology, research and management. Fish and fisheries series*, vol. 35. Berlin: Springer Verlag, 225–284.

Domeier ML. 2012. Revisiting spawning aggregations: definitions and challenges. In: Sadovy de Mitcheson Y, Colin PL, eds. *Reef fish spawning aggregations: biology, research and management. Fish and fisheries series*, vol. 35. Berlin: Springer Verlag, 1–20.

Domeier ML, Colin PL. 1997. Tropical reef fish spawning aggregations: defined and reviewed. *Bulletin of Marine Science* **60**:698–726.

Farmer NA, Ault JS. 2011. Grouper and snapper movements and habitat use in Dry Tortugas, Florida. *Marine Ecology Progress Series* **433**:169–184 DOI 10.3354/meps09198.

Feeley MW, Morley D, Acosta A, Barbera P, Hunt J, Switzer T, Burton M. 2018. Spawning migration movements of mutton snapper in Tortugas, Florida: spatial dynamics within a marine reserve network. *Fisheries Research* **204**:209–223 DOI 10.1016/j.fishres.2018.02.020.

Graham NAJ, Evans RD, Russ GR. 2003. The effects of marine reserve protection on the trophic relationships of reef fishes on the Great Barrier Reef. *Environmental Conservation* **30**:200–208 DOI 10.1017/S0376892903000195.

Hamilton R, Sadovy de Mitcheson Y, Aguilar-Perera A. 2012. The role of local ecological knowledge in the conservation and management of reef fish spawning aggregations. In: Sadovy de Mitcheson Y, Colin PL, eds. *Reef fish spawning aggregations: biology, research and management*. *Fish and fisheries series*, vol. 35. Berlin: Springer Verlag, 331–369.

Heyman WD, Kjerfve B. 2008. Characterization of transient multi-species reef fish spawning aggregations at Gladden Spit, Belize. *Bulletin of Marine Science* **83**:531–551.

Heyman WD, Kjerfve B, Graham RT, Rhodes KL, Garbutt L. 2005. Spawning aggregations of *Lutjanus cyanopterus* (Cuvier) on the Belize Barrier Reef over a 6 years period. *Journal of Fish Biology* **67**:83–101 DOI 10.1111/j.0022-1112.2005.00714.x.

Kadison E, Nemeth RS, Herzlieb S, Blondeau J. 2006. Temporal and spatial dynamics of *Lutjanus cyanopterus* (Pisces: Lutjanidae) and *L. jocu* spawning aggregations in the United States Virgin Islands. *Revista de Biologia Tropical* **54**:69–78.

Linderman KC, Pugliese RP, Waugh GT, Ault JS. 2000. Developmental patterns within multispecies reef fishery: management applications for essential fish habitats and protected areas. *Bulletin of Marine Science* **66**:929–956.

Malafaia PN, França AR, Olavo G. 2021. Spawning aggregation sites of the cubera snapper, *Lutjanus cyanopterus*, on the continental shelf of Bahia state, Northeastern Brazil. *Fisheries Research* **242**:106037 DOI 10.1016/j.fishres.2021.106037.

Menezes R, Giglio VJ, Albuquerque CQ, Rosa RS. 2022. A review of the dog snapper (*Lutjanus jocu*) along the Brazilian Province: distributional records, ecology, fisheries and conservation. *Ocean and Coastal Management* **220**:106094 DOI 10.1016/j.ocecoaman.2022.106094.

Motta F, Freitas MO, Rolim FA, Abilhoa V, Filho GHP. 2022. Direct evidence of a spawning aggregation of cubera snapper (*Lutjanus cyanopterus*) in southeastern Brazil and its management implications. *Fisheries Research* **252**:106339 DOI 10.1016/j.fishres.2022.106339.

Nanami A. 2020. Spatial distribution and feeding substrate of butterflyfishes (family Chaetodontidae) on an Okinawan coral reef. *PeerJ* **8**:e9666 DOI 10.7717/peerj.9666.

Nanami A. 2021. Age, growth, and length–weight relationship of the checkered snapper *Lutjanus decussatus* in an Okinawan coral reef. *Galaxea, Journal of Coral Reef Studies* **23**:1–6.

Nanami A, Okuzawa K, Yamada H, Suzuki N, Aonuma Y. 2010. Reproductive activity in the checkered snapper, Lutjanus decussatus, off Ishigaki Island, Okinawa. *Ichthyological Research* **57**:314–318 DOI 10.1007/s10228-010-0155-5.

Nanami A, Sato T, Kawabata Y, Okuyama J. 2017. Spawning aggregation of white-streaked grouper *Epinephelus ongus*: spatial distribution and annual variation in the fish density within a spawning ground. *PeerJ* **5**:e3000 DOI 10.7717/peerj.3000.

Nanami A, Shimose T. 2013. Interspecific difference in prey items in relation to morphological characteristics among four lutjanid species (*Lutjanus decussatus*, L. fulviflamma, L. fulvus and *L. gibbus*). *Environmental Biology of Fishes* **96**:591–602 DOI 10.1007/s10641-012-0049-7.

Nemeth RS. 2005. Population characteristics of a recovering US Virgin Islands red hind spawning aggregation following protection. *Marine Ecology Progress Series* **286**:81–97 DOI 10.3354/meps286081.

Nemeth RS. 2009. Dynamics of reef fish and decapod crustacean spawning aggregations: underlying mechanisms, habitat linkages, and trophic interactions. In: Nagelkerken I, ed. *Ecological connectivity among tropical coastal ecosystems*. Dordrecht: Springer, 73–134.

Ohta I, Akita Y, Uehara M, Ebisawa A. 2017. Age-based demography and reproductive biology of three *Epinephelus* groupers, E. polyphekadion, *E. tauvina*, and *E. howlandi* (Serranidae), inhabiting coral reefs in Okinawa. *Environmental Biology of Fishes* **100**:1451–1467 DOI 10.1007/s10641-017-0655-5.

Ohta I, Ebisawa A. 2015. Reproductive biology and spawning aggregation fishing of the white-streaked grouper, Epinephelus ongus, associated with seasonal and lunar cycles. *Environmental Biology of Fishes* **98**:1555–1570 DOI 10.1007/s10641-015-0382-8.

Pittman S, Heyman WD. 2020. Life below water: fish spawning aggregations as bright spots for a sustainable ocean. *Conservation Letters* **13**:e12722.

Polovina JJ, Ralston S. 1987. *Tropical snappers and groupers: biology and fisheries management*. Boulder: Westview Press.

R Core Team. 2022. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. *Available at https:www.R-project.org/*.

Rhodes KL, Tupper MH. 2008. The vulnerability of reproductively active squaretail coral grouper (*Plectropomus areolatus*) to fishing. *Fisheries Bulletin* **106**:194–203.

Russell MW, Luckhurst BE, Lindeman KC. 2012. Management of spawning aggregations. In: Sadovy de Mitcheson Y, Colin PL, eds. *Reef fish spawning aggregations: biology, research and management. Fish and fisheries series*, vol. 35. Dordrecht: Springer Verlag, 371–404.

Sadovy de Mitcheson Y, Colin PL. 2012. *Reef fish spawning aggregations: biology, research and management. Fish and fisheries series*, vol. 35. Berlin: Springer Verlag.

Sadovy de Mitcheson Y, Colin PL, Lindfield SJ, Bukurrou A. 2020. A decade of monitoring an Indo-Pacific grouper spawning aggregation: benefits of protection and importance of survey design. *Frontiers in Marine Science* **7**:571878 DOI 10.3389/fmars.2020.571878.

Sadovy de Mitcheson Y, Colin PL, Sakaue J. 2012. Lutjanus fulvus—blacktail snapper with notes on other species of Atlantic and Indo-Pacific snappers (Lutjanidae). In: Sadovy de Mitcheson Y, Colin PL, eds. *Reef fish spawning aggregations: biology, research and management. Fish and fisheries series*, vol. 35. Berlin: Springer Verlag, 458–468.

Sadovy de Mitcheson Y, Cornish A, Domeier M, Colin PL, Russell M, Lindeman KC. 2008. A global baseline for spawning aggregations of reef fishes. *Conservation Biology* **22**:1233–1244 DOI 10.1111/j.1523-1739.2008.01020.x.

Sadovy Y, Domeier M. 2005. Are aggregation-fisheries sustainable? Reef fish fisheries as a case study. *Coral Reefs* **24**:254–262 DOI 10.1007/s00338-005-0474-6.

Sakaue J, Akino H, Endo M, Ida H, Asahida T. 2016. Temporal and spatial site sharing during spawning in snappers *Symphorichthys spilurus* and *Lutjanus bohar* (Pisces: Perciformes: Lutjanidae) in waters around Peleliu Island, Palau. *Zoological Studies* **55**:44.

Sala E, Ballesteros E, Starr RM. 2001. Rapid decline of Nassau grouper spawning aggregations in Belize: fishery management and conservation needs. *Fisheries* **26**:23–30.

Samoilys MA. 1997. Periodicity of spawning aggregations of coral trout *Plectropomus leopardus* (Pisces: Serranidae) on the northern Great Barrier Reef. *Marine Ecology Progress Series* **160**:149–159 DOI 10.3354/meps160149.

Shimose T, Nanami A. 2015. Age, growth, and reproduction of blackspot snapper *Lutjanus fulviflammus* (Forsskål 1775) around Yaeyama Islands, southern Japan, between 2010 and 2014. *Journal of Applied Ichthyology* **31**:1056–1063 DOI 10.1111/jai.12894.

Taylor BM, Oyafuso ZS, Pardee CB, Ochavillo D, Newman SJ. 2018. Comparative demography of commercially-harvested snappers and an emperor from American Samoa. *PeerJ* **6**:e5069 DOI 10.7717/peerj.5069.

Thresher RE. 1984. *Reproduction in reef fishes.* Neptune City, New Jersey: T.F.H. Publications.

Wang N, Teletchea F, Kestemont P, Milla S, Fontaine P. 2010. Photothermal control of the reproductive cycle in temperate fishes. *Reviews in Aquaculture* **2**:209–222 DOI 10.1111/j.1753-5131.2010.01037.x.