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Combined effects of tidal and diurnal cycles on spawning of the puffer, *Takifugu niphobles* (Tetraodontidae)

Kazunori Yamahira
Amakusa Marine Biological Laboratory, Kyushu University, Tomioka, Reihoku-cho, Amakusa, Kumamoto 863–25, Japan

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Synopsis

Spawning in the puffer *Takifugu niphobles* was observed at Tomioka in western Kyushu, Japan, and the factors affecting the spawning time were estimated using a multiple regression analysis. Spawning in this puffer occurred in the intertidal beach during evening rising tides around the full and new moons. Both the time of commencement and ending of a day's spawning were related mainly to the tidal cycle, and occurred later when the high tides occurred later. However, the termination of a day's spawning was also affected by the diurnal cycle; spawning ended earlier when sunset was earlier. Besides, a day's spawning tended to be concentrated within a shorter time span when the interval between the commencement of spawning and the sunset was shorter. Thus the spawning time of the puffer is regulated not only by the tidal but also by the diel cycle, and it is suggested that the adaptive significance of the spawning reactions to both cycles should be examined separately.

Introduction

It has been reported that the patterns of reproduction in many marine fishes are affected by three short cycles: the lunar, tidal, and diurnal. Many ecological causes of the linkages with these cycles have been already discussed (Johannes 1978, Thresher 1984, Gladstone & Westoby 1988, Robertson et al. 1990 for reviews).

As in the grunion (Clark 1925, Walker 1952, 1959) and mummichog (Taylor et al. 1977, 1979), spawning in the intertidal zone has been reported in the puffer, *Takifugu niphobles* (Jordan & Snyder), from many places in Japan (Uno 1955, Katayama et al. 1964, Katayama & Fujita 1967, Nozaki et al. 1976, Kobayashi et al. 1978, Tsutsumi 1978, Tsutsumi et al. 1978, Suzuka & Isogai 1979, Honma & Kitami 1980, Honma et al. 1980, Dotsu et al. 1990). Generally the spawning continues for several days around the new and full moon in the spring and summer. The spawning occurs only during rising tidal phase at dusk each day, thus it seems that the spawning is related to the tidal and diel cycles of the day.

In the earlier works less attention has been paid to the spawning time in each day, to its relationships with the tidal and diurnal cycles of the day. Thus in the present study, I investigate the times of each spawning of the puffer, and detect how the two different cycles: the tidal and diurnal affect the spawning times using a multiple regression analysis.

Material and methods

Study site and material

This study was conducted on a beach in the most inner part of Tomioka Bay, in the western coast of Kyushu, Japan (32°32′ N, 130°2′ E). The beach, which consisted of pebble and gravel, was only 10 m wide along the coast line, and spawning of *T. niphobles* occurred in this zone. The amplitude of tidal fluctuation was about 3.2 m during spring tide. The landward edge of this beach is composed of a concrete wall (290 cm above mean low water spring tide, MLWS), and the beach was exposed for a distance of 18 m seaward from the wall at MLWS.

T. niphobles comes out of water to release their eggs and milt. Before spawning, a number of mature fishes slowly came into the beach along with the rising tide. It was possible to judge whether the spawning would occur a given day or not. After swimming randomly along the beach for a while, males began to chase females. Soon they stranded themselves on the beach with the wave action and flopped back into the wash of the next wave. While this behavior was repeated several times, they suddenly mated splashing in the water and making the seawater around them white by their milt.

Each spawning was conducted by a group of 10–60 individuals of which one was female. When the female laid her eggs on the beach, many males released their milt onto them. Each such group spawning lasted only about 5 seconds. Each day's spawning included repeated group spawnings, involving many individuals and multiple spawnings by the same males.

After the seawater came up to the concrete wall of the landward edge, and there was no exposed area available for the spawning, the spawning was performed under the water; each individual in the group quickly turned round and round. In other words, the puffer can potentially spawn not only at the water egde but also under water.

Field observation and analysis

Field observations were carried out during dusk-evening rising tides in spring from May to August, 1992. When spawning was seen, the time and the number of each mating were recorded.

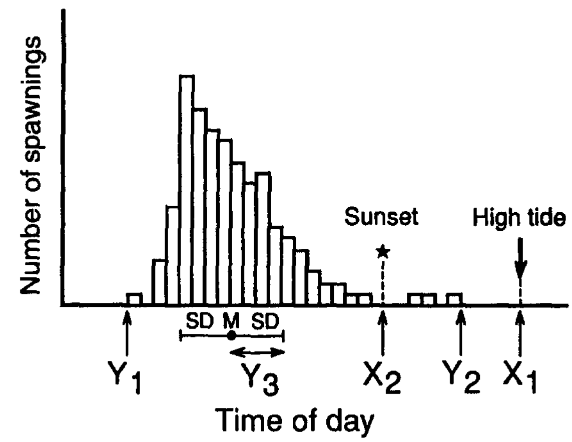

Fig. 1. Example of sequential distribution of spawnings of *Takifugu niphobles*. Variables used for the multiple regression analysis are shown; X_1 = high tide time, X_2 = sunset time, Y_1 = commencement time of spawning, Y_2 = ending time of spawning, Y_3 = standard deviation of spawning times.

To analyze the effects of both tidal and diurnal cycles on spawning time of the puffer, the high tide time (X_1) and the sunset time (X_2) were taken as independent variables (Fig. 1). These values were collected from the tidal table. As indicators of spawning time, the commencement time, the ending time, and the standard deviation (the degree of dispersion) of spawning times in each day were selected, and regarded as dependent variables (Y_i). The proportion of the variance in each Y_i explained by the independent variables (X_i) was estimated using a stepwise multiple regression analysis.

In the present analysis, since X_1 and X_2 are independent of each other, the square of standard regression coefficient represents the percentage of variance explained by each independent variable. Forward stepwise selection with a probability of $F_{in} = F_{out} = 2$ (stepwise regression parameters) to enter or remove was used to identify those variables contributing the most variance to the overall model. Subsequent multiple regression was run repeatedly until all variables with statistically insignificant regression coefficients have been identified.

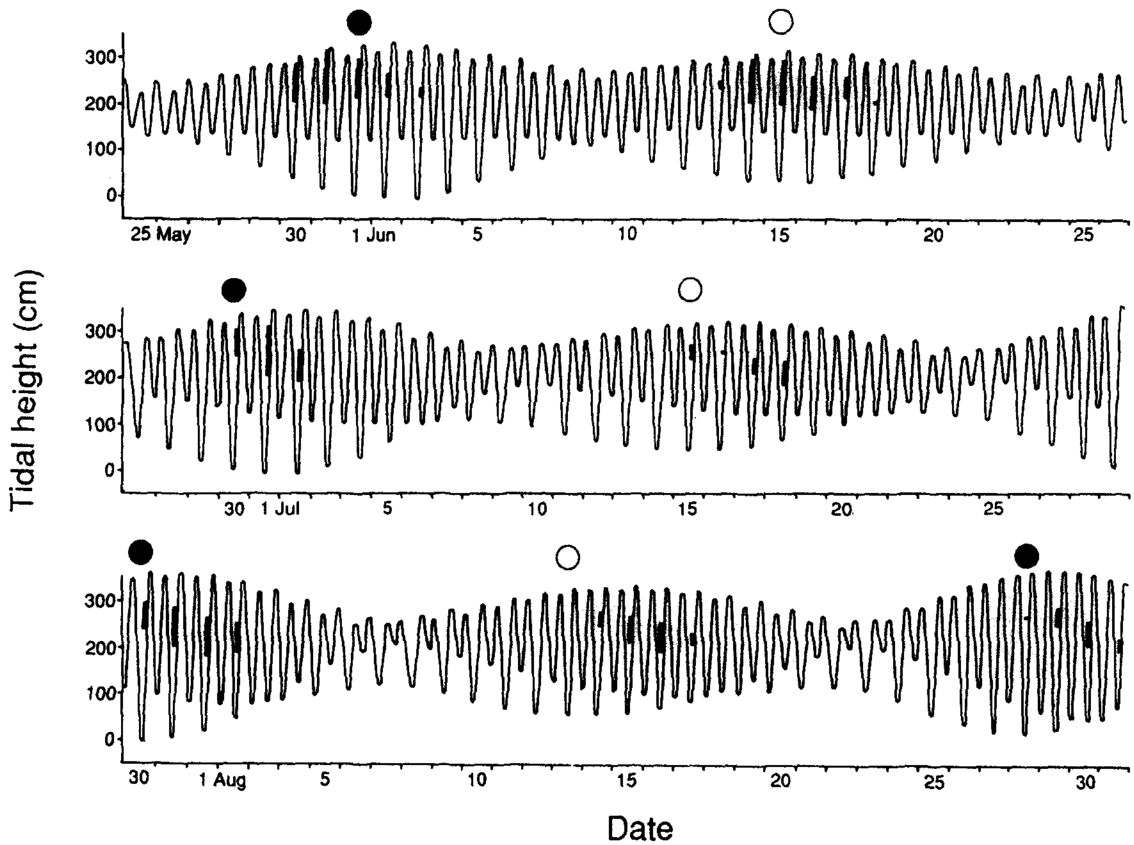

Fig. 2. Relationship between tidal phase and spawning of *Takifugu niphobles*. Black bars show occurrence of spawning. ● = new moon, ○ = full moon.

Results

Timing of spawning

At the study site, spawning of the puffer was observed from May to August, 1992. The timing of spawning was synchronized with the semi-lunar cycle; spawning occurred only during several days of spring tide (Fig. 2). I call this spawning period around each new or full moon the 'spawning series'. As the spawning season progressed, days on which spawning occurred tended to shift later in each spring tide (Figs 2, 3). In the first and second spawning series (May-June), spawning started 2 days before the full or new moon day, and each series lasted 5–6 days. During the later spawning season (July-August), each spawning series started from the day of or 1 day after the lunar syzygies, and continued for 3–4 days. Spawning commenced in the later rising tide phase of dusk-evening and ended by the time of high tide. That is, the spawning was performed in the upper intertidal zone.

In every spawning series the total number of matings per day was highest on the first 1–2 days after each series started, and the modes of the histograms shifted later day by day. The mating peaks in each day were reached 20–30 minutes after the commencement of spawning. Although the standard deviation of spawning times varied day by day, the spawning tended to be concentrated in a shorter time span as the spawning season progressed (Fig. 3).

Analysis of factors regulating spawning time

The relative contributions of tidal and diurnal cycle to each feature of the spawning time were evaluated by a multiple regression analysis (Table 1). The commencement of spawning was determined only

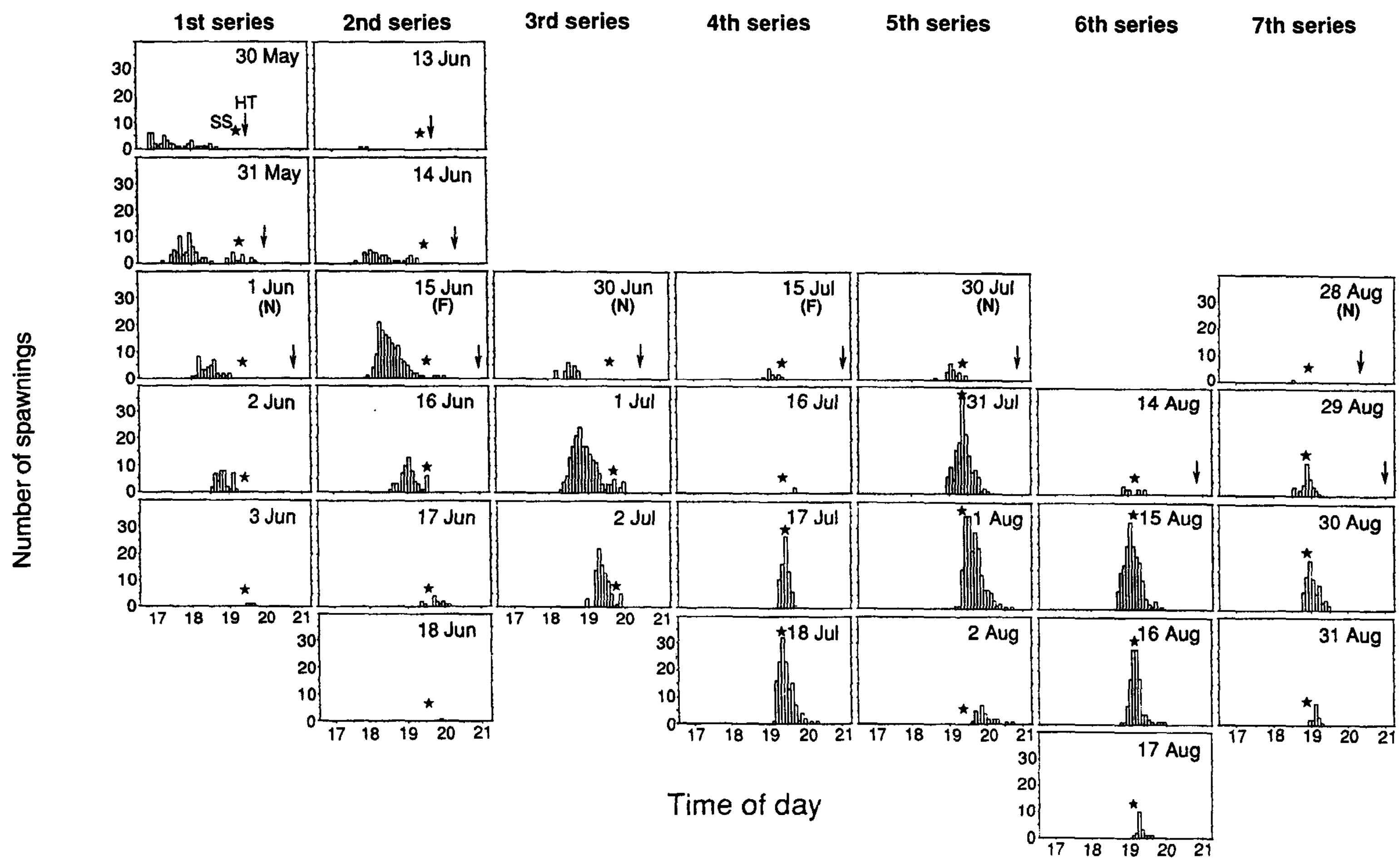

Fig. 3. Mating frequencies of *Takifugu niphobles* in 5 minutes. Arrows and stars indicate the high tide (HT) and sunset times (SS), respectively. (N) = new moon, (F) = full moon.

Table 1. Stepwise multiple regression analysis to determine the percentage of variance in each feature of spawning explained by the tidal and diurnal cycles. The values are the square of standard regression coefficient. (+) and (–) indicate that the independent variables had a positive or negative effect on the dependent variables, respectively. NS indicates that no statistically significant association exists between the relevant variables.

Independent variables	Dependent variables					
	Commencement (Y1)		End (Y_2)		Degree of dispersion (Y_3)	
X_1: High tide time (Tidal cycle)	0.80768 (+)		0.57780 (+)		0.24990 (–)	
X_2: Sunset time (Diurnal cycle)	NS		0.03731 (+)		NS	
ANOVA summary						
Source	d.f.	MS	d.f.	MS	d.f.	MS
Regression	1	42980.10	2	11361.10	1	546.52
Residual	28	365.50	27	561.39	28	58.59
F	117.5920		20.2373		9.3281	
p	$\ll 0.01$		$\ll 0.01$		$\ll 0.01$	
Multiple R^2	0.807681		0.59985		0.249895	

by the high tide time, and its contribution to the commencement was estimated as 80.8%. The later the high tide time was, the later the spawning commenced (see also Fig. 3). The end of spawning was also determined mainly by the high tide time (57.8%), and the later the high tide was, the later the end of spawning occurred (see also Fig. 3). However, the time of sunset also played a part to determine the end of spawning (3.7%), and the spawning tended to finish earlier when the sunset was earlier. The degree of dispersion of spawning time (SD) was determined only by high tide time, though its contribution was relatively small (25.0%). When the high tide was later, the degree of dispersion was usually small, that is, matings were concentrated in a shorter time.

Since there was a strong positive relationship between the high tide time and the commencement of spawning (Table 1), it was expected that the degree of dispersion of spawning time would be determined by the commencement of each day. Besides, the interval between the commencement of spawning and the sunset became shorter when the spawning started later, and vice versa. Thus, this interval would also be a critical factor to determine the dispersion of spawning time. Thus using a linear regression analysis, I evaluated their contributions to the variance in the degree of dispersion of spawning time (Table 2). It showed that a larger part of the variance in the degree of the dispersion was explained by the commencement (53.9%) and the interval between commencement and sunset (57.4%). The spawning tended to be concentrated in a shorter time span when the spawning commenced later, that is, the interval between commencement and sunset was shorter.

Discussion

Takifugu niphobles in this study site spawned with semi-lunar cycle, and spawning occurred only in the

Table 2. Linear regression analysis to determine the percentage of variance in the degree of dispersion of spawning time explained by the commencement (upper) and the interval between the commencement and the sunset (lower). (+) and (–) indicate that the independent variable had a positive or negative effect on the dependent variable, respectively. $p \ll 0.001$ for all regression.

Independent variables	r^2	F	n
Commencement of spawning	0.539439 (–)	32.79540	30
Interval between commencement and sunset	0.573629 (+)	37.67050	30

rising tide phases. The multiple regression analysis revealed that both tidal and diurnal cycles affected the timing of spawning in the puffer.

First, the start and end of spawning was determined by the tidal cycle (time of high tide). Consequently, the spawning was performed in the upper intertidal zone. In the case of this study site, the puffer spawned within 6 m from the shoreline. However, zones where the spawning occurred were slightly different between spawning series, because the tidal amplitude of the site was different between spring tides. This suggests that the spawning was regulated only by the time, not by the absolute tidal height of the site.

Second, the degree of dispersion of spawning time in each day was determined by the interval between the commencement of spawning and the sunset. In addition, the end of spawning was affected a little (3.7%) by the diurnal cycle (time of sunset), and the spawning tended to terminate earlier when the sunset was earlier. As the spawning season progressed, days on which spawning occurred tended to shift toward the later period of the new and full moon days, thus time of high tide, that is, commencement of spawning also shifted later progressively. On the other hand, progressive shift of sunset was relatively smaller (although sunset shifts slightly earlier). The intervals between the commencement of spawning and the sunset became shorter progressively. This is why the spawning of the later season was concentrated in a shorter duration.

The above facts suggest that the spawning time of *T. niphobles* was determined not only by the tidal cycle but also by the diurnal one. The fish which come into the spawning beach in synchrony with the tidal cycle, would tend to finish their spawning by the time when it becomes dark.

The possible releasers of spawning behavior in this puffer have been suggested: (1) stimulus by wave action (Uno 1955, Nozaki et al. 1976, Syzuka & Isogai 1979), (2) sudden egg release by one female (Suzuka & Isogai 1979), (3) bitting each other (Uno 1955, Suzuka & Isogai 1979), and (4) joining other spawning groups (Suzuka & Isogai 1979, Honma & Kitami 1980). If the puffer rely on the visual sense (3rd and 4th factors of the above), the decline of sunlight would inhibit their reproductive behaviors.

Although the spawning days of the puffer were highly synchronized with the semi-lunar cycle, this rhythmicity would be caused by a temporal combination of the tidal cycle and the diurnal one, which, in turn, produces the semi-lunar cycle (i.e. spring tides are days when later high tides are at early evening, and this combination is a usual phenomenon around this site). Moreover, these two cycles: the tidal and diurnal would affect the spawning of this puffer in different ways: (1) reaction to the tidal cycle makes it possible that the puffer spawns in the upper intertidal beach, and (2) reaction to the diurnal cycle makes it possible that the puffer spawns at dusk. The adaptive significance of these two aspects should be examined separately.

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