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Swimming behaviour and photoresponses of the iridescent copepods, *Sapphirina gastrica* and *Sapphirina opalina* (Copepoda: Poecilostomatoida)

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The swimming behaviour and photoresponses of the iridescent epipelagic copepods, *Sapphirina gastrica* and *S. opalina* were investigated in the laboratory. In continuous dark conditions, both species showed no significant diel variation in their swimming activities. When stimulated with light, they exhibited spiral-swimming in which the males showed a significantly higher speed and frequency of turning than the females. Both sexes of *S. gastrica* and *S. opalina* showed positive phototaxis at intensities higher than 0.05×10^{14} quanta $cm^{-2}s^{-1}$ for light sources of 430 nm and 580 nm. *Sapphirina gastrica* showed increased locomotion over a broad range of wavelengths from 430 nm to 580 nm, while *S. opalina* showed a gradual increase of activity with decreasing wavelength, with the highest value at 430 nm. The photoresponses of these two species suggest that light conditions play an important role in their daytime ascent and in determining the depth distributions that were observed in our previous study. It is suggested that the iridescence and fast spiral-swimming of males, and the species-specific photoresponses of both sexes constitute a putative mate recognition system in the open ocean.

INTRODUCTION

Copepods belonging to the genera *Sapphirina* and *Copilia* are sexually dimorphic in body coloration (Chae & Nishida, 1994). The males have striking iridescence which is generated by the interference of light reflected through the multilayered reflecting platelets in the dorsal integument, while the females are almost transparent. The spectral ranges of the iridescence of males are peculiar to each species and are partly dependent on the thickness of the reflecting platelets of guanine (Chae & Nishida, 1994; Chae et al., 1996; Chae & Nishida, 1999).

Sapphirinid copepods are widely distributed in the epipelagic zone (Boxshall, 1977). The depth distributions of each species in daytime are closely related to the iridescent colours of the males (Chae & Nishida, 1995). Species whose males have yellowish (golden) iridescence occur in shallow layers of the epipelagic zone, while species with blue iridescence are found in deeper layers. The yellowish iridescence of the upper-layer species corresponds to light with longer wavelengths available only in upper layers in the open ocean, while the blue iridescence of deeper species corresponds to the blue light which is the only light available deeper in the water column.

Several species of sapphirinids, especially the species with yellow iridescence, show reverse diel vertical migration; in the daytime, they ascend to near the surface and the depth distributions of conspecific females and males coincide well, whereas they tend to be distributed widely in deeper layers at night (Chae & Nishida, 1995). Iridescence in sapphirinids may provide increasing male visibility against the ambient background (Chae & Nishida, 1999). These observations suggest that male iridescence and the reverse migration of sapphirinids represent parts of a mate-finding mechanism in the open ocean (Chae & Nishida, 1995).

Light is among the major factors that initiates and controls the vertical migration of crustacean zooplankton (Forward, 1988; Busky et al., 1989). Sapphirinids have well-developed lateral eyes which contain two pairs of huge cuticular lenses, and might scan the image (Moray, 1972). However, little is known about the sapphirinid's sensitivities to light spectra or intensity and how this might be related to male iridescence and daytime depth distribution. This paper examines the behavioural response of sapphirinids to different light intensities and spectra. Specific and sexual differences are examined with respect to the endogenous rhythm in darkness, swimming mode, phototaxis, and spectral sensitivity.

MATERIALS AND METHODS

The behavioural responses of *Sapphirina* to differing light intensities and wavelengths were measured in the laboratory. Samples were collected by shallow (0–200 m) oblique plankton tows east of the Philippines during the cruise of the RV 'Hakuho Maru' in June 1994. Experiments were carried out on *Sapphirina gastrica* (Giesbrecht) and *S. opalina* (Dana) whose males iridesce gold and violet, respectively (Chae & Nishida, 1994). The reflection of males of the former species has maxima at wavelengths

longer than 500 nm, while the latter has two maxima, one at about 420 nm which coincide with the reflecting colour and the other at wavelengths longer than 750 nm, which may be related to pigments or a second-order effect of the multilayer system in the integument of the male animals (Chae & Nishida, 1999). Immediately after collection, the copepods were transferred to filtered seawater (Millipore, pore-size 0.45-μm).

The presence of an endogenous diel rhythm in activity was first tested in order to examine its possible influence on photobehaviour. To reduce possible artefacts caused by light-adaptation and loss of the rhythm due to time lag from capture to experiment, the specimens for the experiment were collected after sunset then started to test at approximately midnight. Ten live copepods were transferred to a circular aquarium (diameter 7 cm; water depth 7 cm) containing filtered seawater and placed in continuous darkness (water temperature $\sim 20 \pm 2^{\circ}$C). The circular aquarium was used to reduce behavioural disturbance caused by bumping into the wall of the aquarium during swimming. Without any stimulation, in darkness, they showed only occasional swimming movement and sank to the bottom of the aquarium most of the time, falling on their backs without beating the swimming legs (resting phase, see Results). The behaviour of resting and swimming was distinctive, and to measure the locomotory activity, the frequency of bouts of copepod's swimming between the long resting phases was counted for 5 min at four-hour intervals, for 24 h under dim red light. The locomotory index was defined as the frequencies of the bouts of locomotion in 5 min (modified from Hiroki, 1988).

In the following experiments the spectrum of illuminating light was controlled by a monochromator with a halogen lamp as light source (Bausch & Lomb Inc.). The intensity of light was controlled with neutral density filters and measured in the water of experimental aquariums near the walls directed to the light source with a submersible radiometer (QSL-100, Biospherical Instrument Inc.).

Sexual and species-specific differences in the fast spiral swimming, a characteristic behaviour the animals showed when stimulated with light, were observed in a circular aquarium (see Results). The number of times of the spiral swimming between non-spiral swimming with smooth curve was counted for 5 min (times/5 min) in light of 580 nm and 430 nm, which were equivalent to the iridescent colour of males in two species (Chae & Nishida, 1999), with maximal intensities from the monochromator, 1.6×10^{15} quanta $cm^{-2} s^{-1}$ and 0.2×10^{15} quanta $cm^{-2} s^{-1}$, respectively. The swimming behaviour of the male was recorded in a rectangular tank of clear plastic (10 cm long $\times$ 2.5 cm wide $\times$ 4 cm high) with a videotape recorder, and the speed of the copepod's spiral swimming (revolution/sec) was measured. The narrow rectangular tank was used to avoid possible distortion of images recorded and to heighten the possibility of keeping the animals in or at least near the focal range of the camera. When swimming was disturbed or interrupted by the aquarium walls, measurements were excluded.

The phototaxis of dark-adapted animals was measured at six regular intensity steps for each light source (430 and 580 nm) following a method modified from Swift & Forward (1983). The intensities of the light source were controlled by using either single or combined neutral density filters. The animals were placed in a light-tight box for at least 1 h prior to the experiment. To measure phototaxis independently from possible behavioural responses to gravity, a horizontal, clear plastic trough (30 cm long $\times$ 4 cm wide $\times$ 4 cm high) was used. The trough had five chambers of equal size separated by liftable partitions. It was positioned so that the beam of light from the monochromator passed along its long axis. The trough was filled with filtered seawater to a depth of 3 cm for the experiments, and new seawater was always used for each trial. The light was turned off and 8 to 15 (generally 10) individual copepods were introduced into the central section of the trough. The copepods were allowed to adjust to the chamber for 3 min, then the partitions were gently raised. The light stimulus was turned on for 1 min, then the partitions were lowered gently after which the room light was turned on. The number of individuals in each section of the trough was counted. The proportion of positive to negative phototaxis was defined as the number of copepods in the chamber closest to and furthest away from the light source, respectively, divided by the total number in the trough.

The spectral sensitivity was determined indirectly by measuring the locomotory index of the copepods on stimulation with light of two equal quantal intensities at each test wavelength. Five wavelengths were tested (430–630 nm in 50 nm steps) at two differential intensities, approximately 0.24×10^{15} and 0.48×10^{15} quanta $cm^{-2} s^{-1}$. The monochromator used in the experiment has a lower maximum intensity at shorter wavelength, thus 0.24×10^{15} quanta $cm^{-2} s^{-1}$ was the highest intensity that covered the whole range of experimental wavelengths and data at 430 nm in the higher intensity were unavailable. The circular aquarium described above was used. Light was directed horizontally to the upper space of the aquarium (about 6 cm high), and was diffused with white paper placed obliquely across the upper space of the aquarium. Ten copepods were gently introduced into the aquarium and placed in darkness for at least 1 h. The locomotory index was determined by counting the frequency with which any of the individuals moved across a marked horizontal plane (4 cm in height) on the aquarium in 5 min.

RESULTS

Generally, the copepods were active immediately after capture. Without any stimulation, in darkness, after transfer into filtered seawater, they showed only occasional swimming and sank to the bottom of the aquarium most of

Table 1. *The frequency of spiral swimming (times/5 min) of both sexes of* Sapphirina gastrica *and* S. opalina *(mean ± standard deviation) in 5 min.*

	580 nm		430 nm	
	female	male	female	male
S. gastrica	4.2 (±3.3) N=5	33.0 (±11.4) N=6	3.5 (±3.4) N=4	11.2 (±5.5) N=4
S. opalina	2.0 (±1.7) N=5	19.0 (±5.7) N=3	1.0 (±1.4) N=5	19.0 (±9.8) N=5

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Table 2. *The speed of spiral swimming (revolution/sec) of males of* Sapphirina gastrica *and* S. opalina *(mean ± standard deviation).*

S. gastrica		*S. opalina*	
580 nm	430 nm	580 nm	430 nm
7.4 (±1.4) N=7	6.0 (±1.7) N=3	3.5 (±1.9) N=6	3.3 (±0.5) N=4

N, number of animals observed. 1–4 shots of video-recordings were collected and measured for each animal.

the time, falling on their backs without beating the swimming legs. They also occasionally showed slow (below 10 body lengths/sec approximately) horizontal movements, maintaining the dorsal side-down position. When the animals swam, they showed various, but generally higher speed with a smooth curve in any direction. When stimulated by light, they showed much higher frequencies of fast swimming and, in the case of males, the fast swimming was often followed by extremely fast spiral swimming which was conspicuously distinctive with non-spiral swimming. In many cases, they started from slow moving before the fast swimming or spiral swimming. Spiral swimming was a characteristic behaviour of males, even though females also occasionally showed the same swimming mode. The males of *Sapphirina* exhibit remarkably frequent spiral swimming (Table 1). The number of revolutions per sec of the spiral swimming in the males of the two species was also markedly different (Table 2).

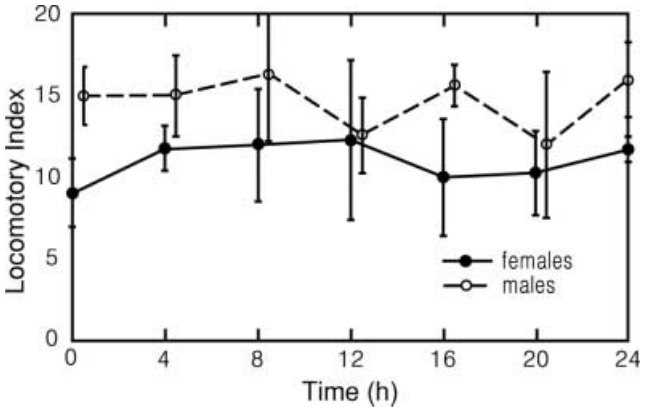

Figure 1. Locomotory index (mean standard error) of *Sapphirina gastrica* in continuous darkness.

In dark conditions, there was no significant variation in the locomotory index in *Sapphirina gastrica* during the whole day (Figure 1, Mann–Whitney U test, $P > 0.05$). For *Sapphirina opalina*, the data were not available, because too few copepods were collected for the experiment.

Both sexes of *Sapphirina gastrica* showed a larger proportion of positive phototaxis than negative phototaxis in most series of light stimuli (Figure 2). Exceptions were in the females at intensities lower than 0.05×10^{15} quanta $cm^{-2}\,s^{-1}$ and in the males at 0.025×10^{15} quanta $cm^{-2}\,s^{-1}$, all at 430 nm. Effects of light intensity on the level of positive phototaxis were apparent in females. Females showed a sharp decrease in positive phototaxis from 0.4×10^{15} to 0.2×10^{15} quanta $cm^{-2}\,s^{-1}$ at 580 nm, but the percentage of positive phototaxis was still higher than negative. No significant difference in percentage of positive and negative phototaxis was observed below 0.05×10^{15} quanta $cm^{-2}\,s^{-1}$. Both sexes recorded higher mean values of positive responses at 580 nm than at 430 nm for the same intensity levels from 0.05×10^{15} to 0.2×10^{15} quanta $cm^{-2}\,s^{-1}$. The males of *S. opalina* also showed positive phototaxis in most series of light stimuli with exceptions at levels lower than 0.05×10^{15} quanta $cm^{-2}\,s^{-1}$ (Figure 3). It showed a moderate increase in positive phototaxis with increasing intensity and higher positive response at 430 nm than at 580 nm at the same intensity.

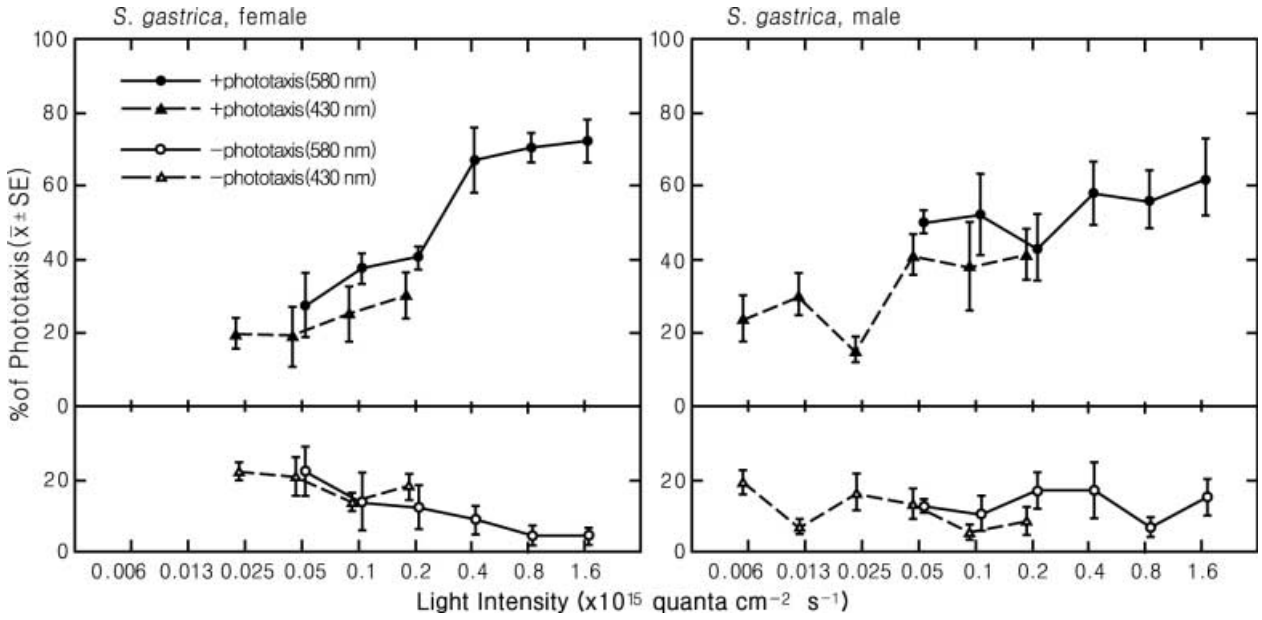

Figure 2. Phototaxis of dark-adapted *Sapphirina gastrica* (mean standard error). Measurements were carried out at 580 nm (circle with solid line) and 430 nm (triangle with dotted line) of various intensities. Closed circle and triangle represent positive phototaxis, and open circle and triangle represent negative phototaxis.

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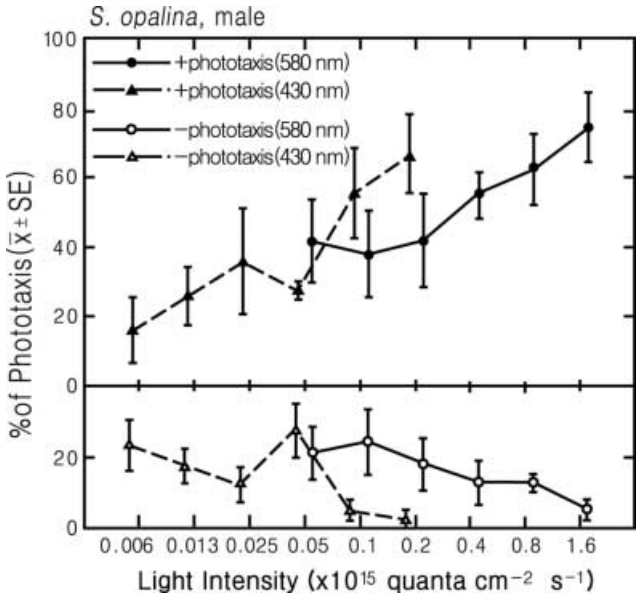

Figure 3. Phototaxis of dark-adapted *Sapphirina opalina* (mean standard error). Measurements were carried out at 580 nm and 430 nm. Key as in Figure 2.

The locomotory indices in both sexes of *Sapphirina gastrica* were significantly higher at wavelengths of 430 nm to 580 nm than at 630 nm (Kruskal–Wallis test, $P<0.05$), but a relatively lower value at 430 nm in the female (Figure 4). Both sexes of *S. opalina* showed a gradual increase of locomotory index with decreasing wavelength, with the highest values at 430 nm or 480 nm (Kruskal–Wallis test, $P<0.05$). At all wavelengths, higher locomotory responses were recorded at higher intensities (Wilcoxon test, $P<0.05$).

DISCUSSION

In some crustacean zooplankton, diel vertical migration may be the result of an endogenous circadian rhythm of an alternation of active and passive phases of the animals. Hiroki (1988) observed marked diel changes in the locomotory activity of *Themisto japonica* in continuous darkness and the changes were consistent with the vertical migration patterns of the animals in the field. However, present results suggest that an endogenous rhythm may play only a marginal role, if any, in the vertical migration of *Sapphirina gastrica* (Figure 1).

From the intensity-dependent positive response of sapphirinids (Figures 2&3), it appears that high intensity of light in daytime leads to the reverse vertical migration. In the laboratory experiments, positive phototaxis to higher intensities and negative phototaxis to lower intensities, or positive phototaxis over a wide range of light intensities is often found in zooplanktonic organisms which show vertical migration of night ascent (Stearns & Forward, 1984a; Forward, 1988 for review). However, Stearns & Forward (1984b) and Forward (1988) showed that these results were laboratory artefacts caused by using a narrow directional beam as a light source. Under natural angular light distributions, positive phototaxis is uncommon in studies where organisms undergo diel vertical migration of night ascent (Forward, 1988). Buskey et al. (1989) found that *Pleuromamma gracilis* and *P. xiphias*, which live in the oceanic ecosystem and undergo night-ascent migration of 200 to >500 m, displayed negative phototaxis consistently in the laboratory. They stated the artefacts of a narrow directional beam are limited to the coastal and estuarine zooplankton which may remain near the bottom during the day where light still penetrates. Many sapphirinids ascend in the daytime and descend at night within the euphotic zone in

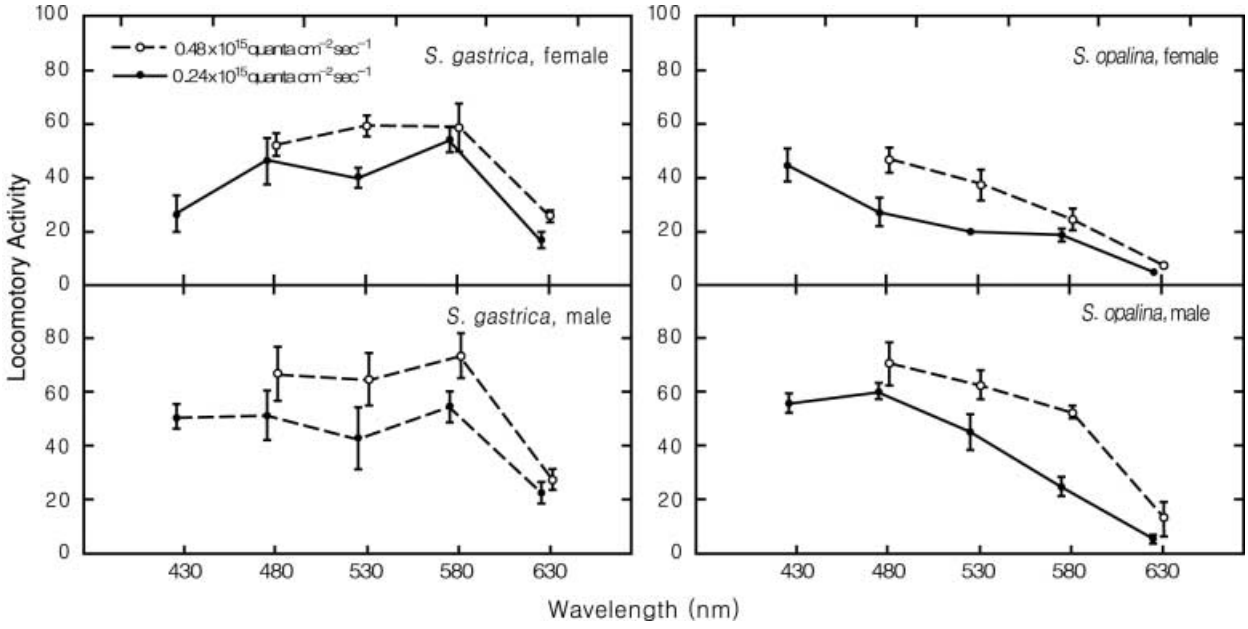

Figure 4. The locomotory index of *Sapphirina gastrica* and *S. opalina* (mean standard error) on stimulation with the light of various spectra in two equal quantal intensities (open circle with dotted line: 0.24×10^{15} quanta cm^{-2} s^{-1} and closed circle with solid line: 0.48×10^{15} quanta cm^{-2} s^{-1}).

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oceanic waters where the bottom barrier against the vertical migration is non-existent (Chae & Nishida, 1995).

At the same intensity (from 0.05×10^{15} to 0.2×10^{15} quanta $cm^{-2}\,s^{-1}$), the two species showed different responses to the wavelengths (Figures 2&3). In Figure 4, the locomotory activities of *S. gastrica* of females and males are high from 480 nm to 580 nm and from 430 nm to 580 nm, respectively. Thus, *S. gastrica* seems to have a broad spectral sensitivity. However, *S. opalina* showed peaks of activity in short wavelengths of 430 nm and 480 nm which declined at longer wavelengths. The estuarine copepod *Acartia tonsa* showed nearly uniform sensitivity from 453 nm to 620 nm (Stearns & Forward, 1984a), while *Pleuromamma gracilis* and *P. xiphias*, oceanic species, have maximum sensitivities near 430 nm (Buskey et al., 1989). These support the suggestion of Forward & Cronin (1979) that if a zooplankter's photoreceptors are primarily used during vertical migration, then its visual-system characteristics should be matched to the spectral distribution of light in the individual's environment ('Sensory Hypothesis', Levine & MacNichol, 1982). The absence of an endogenous rhythm in locomotory activities in the dark and the spectral sensitivity of sapphirinids also support this hypothesis. *Sapphirina gastrica* is distributed at shallow depths in the ocean where ambient light has a comparatively wide range of wavelengths (Chae & Nishida, 1995). The exact depth distribution of *S. opalina* in the daytime is unknown because of their rare occurrence in the samples. However, *S. opalina* was found only below 50 m in the daytime during our previous study (Chae & Nishida, 1995), even though the number of specimens found was small (personal observation). If the results of the previous study that sapphirinids which iridesce blue (shorter wavelength, Chae & Nishida, 1999) are distributed in deeper layers (Chae & Nishida, 1995) can be applied to this species, the above-mentioned hypothesis may also be applicable.

The frequent spiral-swimming in males of *Sapphirina* showed another aspect of adaptive significance of their iridescence. Since the iridescence is caused by the interference of light, the change of angle of the body changes the wavelength of the iridescence (Chae & Nishida, 1994; 1999). Theoretically, the larger the angle of incidence, the shorter the wavelength of light the sapphirinids reflect. If the angle increases beyond a critical point, the iridescence disappears (Huxley, 1968). Thus, *S. gastrica* will reflect yellow to blue light, whereas *S. opalina* will reflect only blue light. Actual observations, made when they are swimming under white light, shows that golden-reflection predominates in *S. gastrica*, followed by sudden blue reflection, and *S. opalina* showed relatively weak, blue reflection and yellow colour, the latter being probably caused by transmission of light from the ventral side of body. At the depth where the species are distributed, the fast spiral-swimming of *S. gastrica* would be seen as a golden-brilliance with intermittent blue-sparkling and *S. opalina* as a blue-brilliance the pulse of which is slower than that of *S. gastrica* (see Table 2). If the role of the iridescence is mate finding, especially by the females (Chae & Nishida, 1995), the actual sensing by females of their mating partners may also include the males' swimming modes as well as its colour.

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