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Stargazers (Uranoscopidae) have exceptionally more bile

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Abstract

Stargazers (Uranoscopidae) have a specialized character of having a large volume of bile; our elementary investigation indicates that the stargazers have larger bile volume and body volume ratio than other fishes reported in the present studies. Two hypotheses are proposed to explain why they need so much bile: a large amount of the bile is needed (1) to serve as sex pheromone, and (2) to enhance calcium uptake efficiency in order to build their strong craniums. The bile volumes are not significantly different between the female and male stargazers and this ratio did not show monthly difference. As such, the former hypothesis, i.e., function as a sex pheromone, cannot be supported. The cranium hardness, bone density, and calcium content in stargazers are significantly higher than other studied fishes. Most fish uptake calcium by the gill and the intestine from seawater or food. However, stargazers often hide in the sand and wait for the prey that less water flow through the gills is expected. Therefore, stargazers uptake calcium ions primarily through their intestine instead of the gills.

Key words: Uranoscopidae, bile, sex pheromone, calcium uptake

Introduction

The stargazdae belong to the family Uranoscopidae which is composed of 51 species (including one extinct species) in 8 genera. They live in waters ranging from 8- to 365-meter deep (http://www.fiboni.com/2013/08/stargazer-fish-buries-with-a-preying-gaze/) in the Mediterranean Sea, Atlantic Ocean and Pacific Ocean. They are carnivores; their diet includes fishes, shrimps and crabs (Papouysoglou and Lyndon, 2003; Nelson, 2006). They prefer either to bury under the sandy or muddy bottom, or to stay on the sea floor. When they bury, the eyes on top of the large, hard head and the large superior mouth are the parts not covered by the substrate. Their burying habit, accomplishing their camouflage body coloration and a worm-like appendage attached to its lower lip, make an effective way to ambush and catch the pass-by or attracted preys. Body lengths range from 18 to 90 cm (i.e., the giant stargazer, *Kathetostoma giganteum*).

Three genera and seven stargazer species have been recorded in Taiwan: *Ichthyscopus (I. lebeck), Uranoscopus (U. japonicas, U. bicinctus, U. chinensis, U. oligolepis, U. tosae)* and *Xenocephalus (X. elongates)*. Beside the worm-like appendage mentioned above, some additional characteristics of the soft organs were revealed in all of these species during our gross anatomy works on these stargazer species: (1) the cranium is heavy and hard, (2) the brain is small, leaving surplus unoccupied space within the brain cavity in the cranium (Fig. 1A), and (3) a more or less transparent large gall bladder with light fluid, i.e., bile, were present in these species. (Fig. 1B) Occasionally, a voluminous amount of liquid was found in the posterior section of the intestine where only some well digested food remains were present (Fig. 1C). Unlike most other bony fishes whose gall-bladders are relative small and contain deep greenish bile juice, the exceptional condition of the bile in stargazers had interested us to find out the reason for such large volume of "dilute" bile. Neither this specialization in the amount of bile in the stargazers nor digestion of lipid by bile salts in stargazers have been reported, despite some studies were related to digestive enzymes (i.e., α-amylase, carbohydrases and proteases) in *Uranoscopus scaber* (e.g., Papoutsoglou and Lyndon 2003, 2006).

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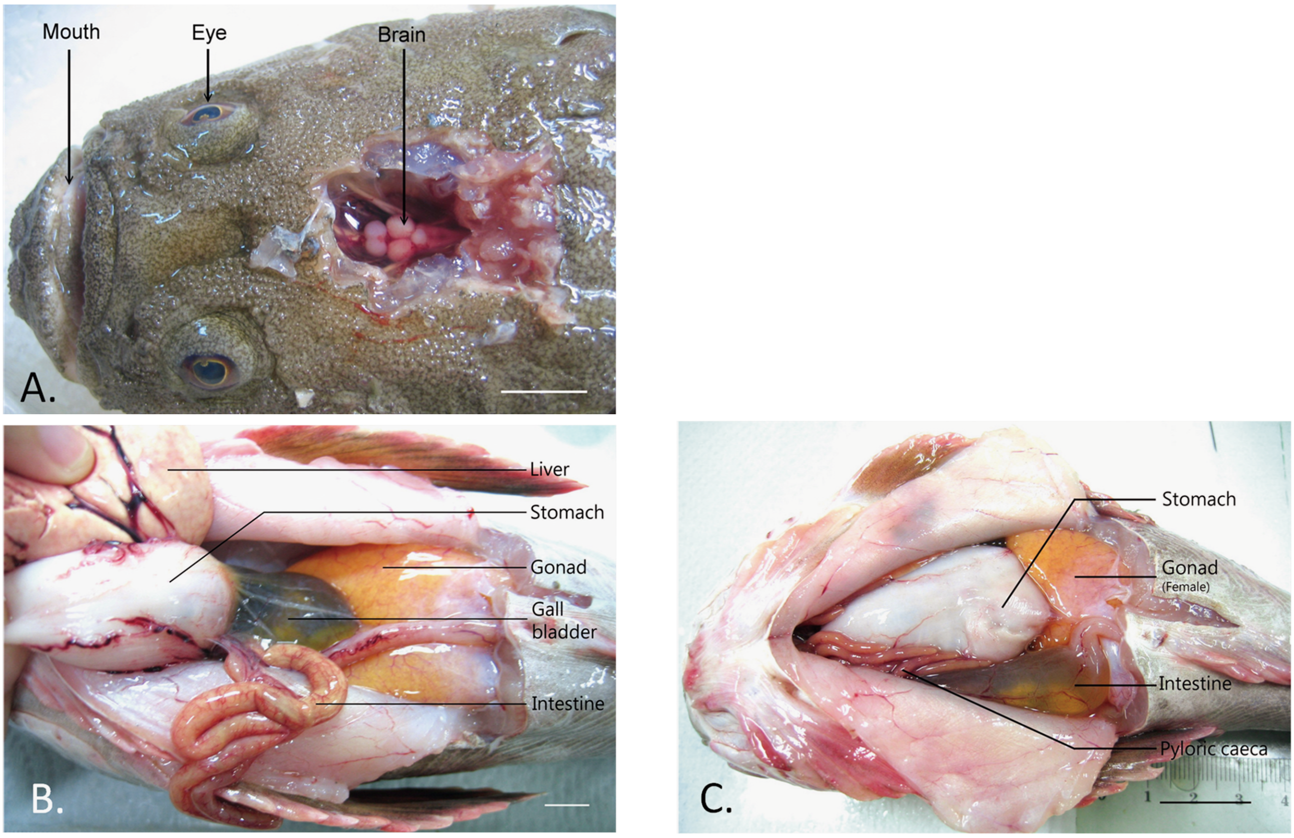

Fig. 1. A. Appearance of a stargazer's brain. Bar = 1 cm; B. Ventral view of the internal organs of a stargazer. Stomach, liver and intestine were moved to the side to show the gall bladder. Bar = 1 cm; **C. Expanded hind intestine of a stargazer.** Bar = 2 cm.

Hypotheses for the needs of more bile:

A. Calcium absorption hypothesis: The main components of bile include bile acids (also called bile salts), phospholipids (mainly phosphatidylcholine), cholesterol, bilirubin, inorganic salts (potassium, sodium and bicarbonate), and very small amounts of copper and other metals (http://www.medscape.com/viewarticle/708713_2.). The role of bile in digestion is for digestion and absorption of fats and fat-soluble vitamins. Waste products like bilirubin are also eliminated from the body by secretion into bile. Bile acts as a surfactant that helps to emulsify fats in food. (http://www.ask.com/question/what-is-the-role-of-bile-in-digestion). In the lack of or reduction in bile the lipid soluble vitamins (including vitamins A, D, E, and K) cannot be easily absorbed through the intestine. Vitamin D helps to retain calcium in the bone. As such, we hypothesize that stargazers require more bile to enhance digestion of lipid, absorption of the fat soluble vitamin D, which helps calcium uptake for bone formation. If this hypothesis holds, we expect the fish with undigested food in the gut have more bile in the gall bladder or in the intestine.

B. Pheromone hypothesis: Despite that a majority of fishes' bile salts will be recollected during the enterohepatic circulation and reused, some bile salts are excreted to the environment along with the feces and urine and was used as pheromone. *Petromyzone marinus* use bile salts as pheromone; males after selecting a suitable place to set up their nests, bile salts were excreted to attract gravid females to the nest for spawning (Li et al. 2002). Zhang et al. (2001) used electro-olfactogram to study the olfactory responses of the Lake char (*Salvelinus namaycush*) to the low concentration of bile salts. These authors inferred that Lake Char showed olfactory response toward low concentration of bile salts and can be an intra-specific communication channel. Similar results have also been reported in *Oncorhynchus mykiss* that olfactory responses toward three types of bile salts were noted (Giaquinto and Hara, 2008). As such, we hypothesize that stargazers require more bile to be excreted to attract mates.

As stargazers used a wait-and-attack predator strategy (Huet et al. 1999, Pietsch 1989), they remain buried in the substrate for a long period of time (activity remain low in most of the time), pheromones are an effective channel for inter-individual communication in spawning season. If this hypothesis holds, we expect the bile volume (1) differs between premature and mature individuals, between mature male and female, and (2) between spawning and nonspawning season (if spawning season exists).

The aims of the present study were to falsify these hypotheses in order to find the possible reason behind the large amount of bile in stargazers.

Materials and Methods

Specimen collection

Specimens of stargazers and other compared species were purchased from local fish markets or from the landed trash fishes in the fishing ports (i.e., Tongkong, Pingtung County, Kezailliao, Kaohsiung County, Wu Che harbor, Tai Chung, and Tai Chi Fishing Port, YeLan County) on the western and northeastern coasts of Taiwan. Fresh specimens were kept in coolers, whereas live stargazer specimens were place in aerated seawater and delivered to the laboratory.

Demersal species in the sandy bottom with similar diet and feeding habit as stargazers were selected for comparison: Pinguipedidae (both Uranoscopidae and Pingupedidae are in the suborder Trachinoide), Platycephalidae, Batrachoididae, and Sciaenidae.

List of species examined

Uranoscopidae: *Ichthyscopus lebeck, Uranoscopus japonicas, Uranoscope chinensis, U. oligolepis, U. tosae , U.bicinctus, Xenocephalus elongates.*
Ariidae: *Arius maculatus;* Batrachodididae: *Allenbatrachus grunniens*; Sciaenidae: *Johnius dussumieri*; Platycephalidae: *Suggrundus meerdervoortii, Rogadius asper*; and Pinguipedidae: *Parapercis sexfasciata.*

Specimen preparation

After the collected specimens were identified to species, their total and standard lengths, body weight, body volume, and bile volumes were measured. Body volume was measured by water displacement method. Bile was sucked into a syringe and its volume was recorded. The bile was put into a 2ml eppendorf and kept in -20℃ freezer for clinical test. Sex of the stargazer specimens was differentiated by external appearance of the gonad.

Items of the bile for clinical measurement and experimental methods

1. Bile juice

A. Identify the gall bladder and bile juice:

Because there is no standard list of items for bile examination, the liquid from the bladder was sent to a clinical laboratory for urinalysis to measure the parameters for urine with addition parameter of bile. Biochemical examination includes: bile salts and bile pigments-bilirubin (a bile pigment produced by breakdown of heme and reduction of biliverdin), and urobilinogen (a colorless compound formed in the intestine by bacterial action on bilirubin and about half is reabsorbed). Physical examination includes: volume, color, reaction (pH), specific gravity, and salinity. The bile salt content in the fluid was estimated using the value from a salinometer. In these chemical components, bilirubin is unique to bile and is not normally found in the urine. Inside the intestine, bacteria break down the bilirubin into urobilinogen; usually about 50% are reabsorbed to the liver through the renal portal vein system while the other half were excrete through the feces. Human urine contains very low amount of urobilinogen (ca. 0.1-1.0 E.U./dl); if a fluid contain more than this amount, it should not be urine. Specific gravity of urine represents the amount of solute in the urine. Normal human adults with adequate fluid intake have their urine specific gravity between 1.016 and 1.022. The normal range of the pH of human urine is between 4.5 and 8.0, whereas that of the bile is between 7.6 and 8.6; pH value can be used to estimate the amount of bile salt. Salinity of the bile was measured using a salinometer. As certain amount of fluid was usually found in the intestine of stargazer, some samples of these fluids were also analyzed to compare with the fluid from the gall bladder. Because at least 3ml of urine is required for urine analysis and urine volumes of other species studied in this paper were lower than this amount, no tests were made in these species. The coloration of fresh “bile” juice was measured by comparing it visually with the CMYK printing coloration table to verify if the juice from the stargazers is bile and to compare the coloration among species.

B. Difference in bile volume among species

The bile volume /body volume ratio for five *Uranoscopus* spp., *Arius maculatus, Allenbatrachus grunniens* and *Johnius dussumieri* were compared and tested with one-way ANOVA, followed by Fisher’s LSD test for inter-specific difference in the bile volume.

C. Relation between bile volume and stomach content

Our observation indicated that when there were solid food in the stomach, the amount of bile in the gall bladder tended to be lower. Twenty stargazers were sorted on the basis of presence/absence of solid food in the stomach and their bile volume/body volume ratios were compared with Student's *t*-test for group difference.

D. Sexual difference in bile volume/body volume ratio

If bile is used as pheromone, usually one of the sexes will excrete a large amount of bile to attract the other sex in the spawning season. As such, seasonal difference in bile volume is expected to exist when the species exhibit distinct spawning season. Because specimen of *Uranoscope chinensis* was not available and number of specimens of each of the other four stargazer species (*Uranoscopus japonicas, U. oligolepis, U. tosae and U.bicinctus*) was not large, all specimens of these four species were pooled and sorted by sex. And the ratios were tested by Student's *t*-test for temporal variation in bile volume.

E. Seasonal difference in bile volume/body volume ratio

If stargazers use bile as a pheromone and the spawning season of *Uranoscopus scaber* in Aegean Sea is between March and September (Coker et al., 2008), it is predicted that the bile volume in one of the sex should show seasonal difference (i.e., high amount in the spawning season). Because male specimens of stargazer were very few, female specimens of the above-mentioned four species were pooled and sorted by month. Data were only available for January, February, March, April, May, July, August, October and November. The ratios were compared using one-way ANOVOA.

F. Skull bone density

Several intact *Uranoscopus* sp., *Suggrundus meerdervoortii,* and *Rogadius asper* specimens were sent to the hospital laboratory to measure the skull bone density using the bone densitometer. Data were tested by one-way ANOVA, followed by Fisher's LSD for group comparison.

G. Measuring skull bone thickness and hardness

Thickness of a piece of cleaned skull bone was measured by electronic vernier. The measured bones were dried in oven at 60℃ for 48 hours. It was fixed vertically on a piece of paper by double-sided scotch tape, then placed into a metal mold and coated with epoxy resin mixed with hardener; the mold was left for three days to harden. The solidified mold was removed from the vale and polished by sand paper in increasing coarse size: 80cw、800cw、1200cw、2500cw until the bone was visible. The polished bone plate was glued on a metal platform. The hardness of the plate where the bone was covered was then measured using a nano-indenter. Poissons Ratio was set at 0.33 (Grenoble et al., 1972), the diamond needle was pressed to at least 800nm with a force of 1mN for 5 seconds and withdrew after 10 second. The hardness was calculated by the computer. Hardness of the plate not covering the bone was also measured as a control. 25 points were measured for a bone sample and at least 8 successful points were required from each bone sample (maximum number of successful pointes was 25) for an average value to represent the bone sample. Data for *Uranoscopus* sp. and *Parapercis sexfasciata* was compared using Student's *t*-test.

H. Estimating calcium element content in the skull bone

Skin and muscle attached to the surface of a piece of skull bone were removed under a dissecting microscope. The bone was placed in an eppendorf and dried in a 60℃ oven with the led opened for 48 hours. The dried bone was measured in a 4 digital electronic balance for its weight.

0.0163-0.4070 g of dried bone from each sample was taken and placed in a small beaker. 3ml of 65% nitric acid was added. The beaker was heated on a hot plate to about 105℃ such that the bone was dissolved. After the bone was completely dissolved, milli-Q water was added to a total volume of 10ml (Scancar et al. 2000; Edmonds et al. 1996). The solution was filtered by filter paper (Whatman Grade No. 44 Quantitative Filter Paper, Ashless). Content of calcium element in the filtered solution was measured in an atomic absorption spectrometer. The amount of calcium content was divided by the original weight of the bone dissolved (gram of calcium/ gram of skull bone). Calcium contents for *Uranoscope* sp., *Suggrundus meerdervoortii, Rogadius asper* and *Parapercis sexfasciata* were compared by one-way ANOVA followed by Fisher' s LSD test for inter-group comparison.

Results

Gall bladder

A tube (i.e., the bile duct) connects the bladder containing the liquid to the posterior end of the stomach near the pyloric caeca (Fig. 2). The bladder also connects to the liver via membranes. These features fit the characteristics of the gall bladder.

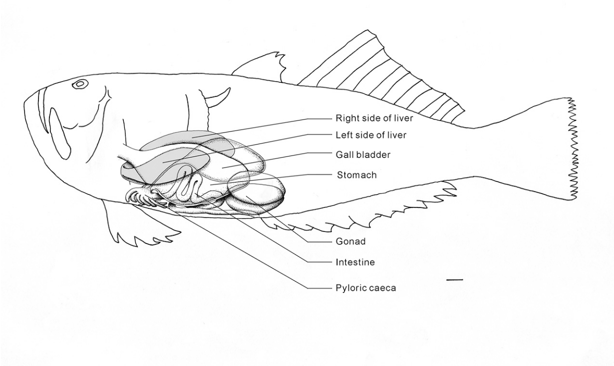

Fig. 2. A side-view sketch for a stargazer's internal organs. Bar = 1cm.

Characteristics of the uranoscopid bile

Five samples of bile and two samples of fluid from the intestine were clinically tested. Presence of bilirubin in uranoscopid bile was positive (value as high as 3 + was also detected; Table 1), whereas it is negative in the fluid from the intestine. Values of urobilinogen in bile and intestinal fluid ranged between 0. 10-1. 00 E. U. /dl and 0. 10 E. U. /dl, respectively. Specific gravity of bile and intestinal fluid ranged between 1.01-1.03and > 1.03, respectively. pH values for bile and intestinal fluid were 5.5-7.5 (acidic), and 8.0-8.5 (alkaline), respectively. Values for salinity of bile and intestinal fluid were > 37‰, and < 27‰, respectively (Table 1).

Table 1. Measurement results for five parameters (bilirubin, urobilinogen, specific gravity, pH, salinity) of bile and intestinal fluid.

Number	Species	Bilirubin (+/-)	Urobilinogen (E.U./dl)	Specific gravity	pH	Salinity (‰)
Bile1	*U. chinensis*	+	0.10	1.02	6.5	47
Bile2	*U. chinensis*	+	1.00	1.03	5.5	37
Bile3	*U. chinensis*	2+	1.00	1.02	6.5	39
Bile4	*U. chinensis*	+	0.10	1.02	7.0	40
Bile5	*U. chinensis*	3+	1.00	1.01	7.5	43
Intestine4	*U. chinensis*	-	0.10	>1.03	8.5	26
Intestine5	*U. chinensis*	-	0.10	>1.03	8.0	27

Color of the bile of nine uranoscopid species was characterized by matching it with the CMYK color model. C, M, Y, and BL represent cyan, magenta (red), yellow and black, respectively. Numbers in parentheses are %. Results of comparison show that color of the uranoscopid species are: *U. tosae* (Y_{0-100} + BL_{0-100} (30, 10; Y_{0-100} + BL_{0-100} (30, 10); C_{0-100}+ Y_{0-100} (0, 40); C_{0-100}+ Y_{0-100} (0, 60); C_{0-100}+ Y_{0-100} (0, 60)), *U.bicinctus* (C_{0-100}+ Y_{0-100} (10, 70); C_{0-100}+ Y_{0-100} (20, 80)), *U. oligolepis* (C_{0-100} + Y_{0-100} (0, 20)), and *Uranoscopus japonicas* (C_{0-100}+ Y_{0-100} (0, 40))。 Their color ranged from light yellow to deep yellow or even to green. They differ from most other species in which the color is deep green.

Difference in bile volume among uranoscopids and other species compared

Significant correlation was shown between bile volume and body volume ratio (P < 0.01) (Fig. 3). Bile volume/body volume ratio among the 7 uranoscopid species differed (ANOVA, P = 0.0343). The average value of the uranoscopid species was twice as much as the other compared species. As such, all data from the uranoscopid species were pooled together to represent the stargazers.

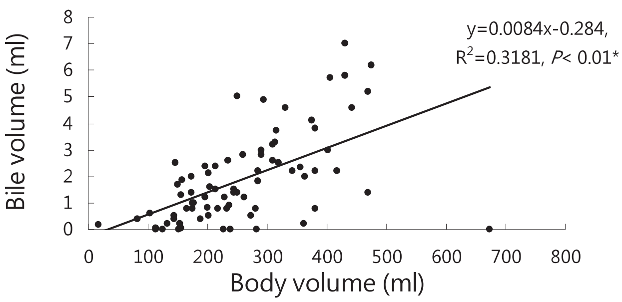

Fig. 3. Correlation of bile volume and body volume.

In the 89 uranoscopid specimens, the maximum bile volume occurred in a 430-ml *U. tosae* specimen (7 ml of bile). 8.6% of the 89 specimens had no bile in the gall bladder.

The bile volume/body volume ratios of the studied species were: Uranoscopid species 0.688 ± 0.474% (n = 76), *Arius maculatus* 0. 198 ± 0. 094% (n = 13), *Johnius dussumieri* 0.025 ± 7.47E-03% (n = 6), and *Allenbatrachus grunniens*0.207 ± 0.089% (n = 4). The ratio (or bile volume) in the four families differed (ANOVA test, $P < 0.01$). Bile volume in uranoscopids was higher than the species in the other three families, whereas no significant difference was found among the other three families (Fisher's LSD comparison among groups).

Fisher's LSD test showed significant species difference in the following groups: (*Uranoscopus japonicas* and *Ichthyscopus lebeck*), (*U. oligolepis* and *U. bicinctus*), (*Uranoscope chinensis* and *U. oligolepis*), and (*Uranoscope chinensis* and *U. bicinctus*). The ratio of *Xenocephalus elongates* and *U. tosae* did not significantly differ from that of other five species. Both *Uranoscopus japonicas* and *Uranoscope chinensis* differed from *U. oligolepis,* and *U. bicinctus.* On the other hands, the first two species and the last two species were more similar to each other in this ratio.

Relation between bile volume and condition of stomach content

When the stomach contained solid food, the bile volume/body volume ratio has an average of 0.2729 ± 0.2532% (n = 9); the ratio became 0.8146 ± 0.2934% (n = 11) when the stomachs were contained no solid food (Fig. 4); the ratio was lower when there was solid food in the stomach ($P < 0.05$) implying that the bile was used to digest the food.

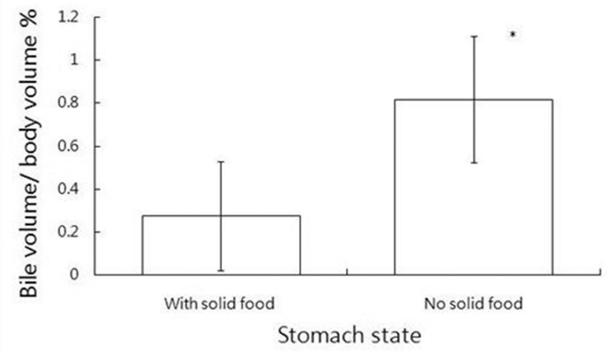

Fig. 4. Mean value of bile volume/ body volume percentage with solid food in stomach and with No solid food in the stomach. Vertical bars represent mean ± SD. *Significant difference ($P < 0.01$).

Sexual difference in bile volume

The average bile volume/body volume ratio for uranoscopid females was 0.804 ± 0.430% (n = 41), and 0.591 ± 0.305% (n = 12)for males. No significant difference was found between sex ($P = 0.116$).

Monthly variation in bile volume

No significant difference was found in the monthly data on the bile volume /body volume ratio for uranoscopid females ($P = 0.2836$; Fig. 5 and Table 2).

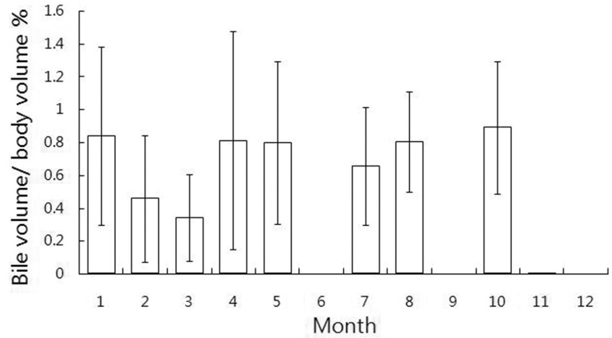

Fig. 5. Mean values of bile volume /body volume percentage in female *Uranoscopus* spp. for each month. No difference between months ($P = 0.283$). Vertical bars reported mean ± SD. No data in June, September, and December.

Table 2. Statistical results for monthly difference in bile volume/ body volume percentage among *Uranoscopus* spp.

(a) ANOVA table

Source	df	SS	MS	F	*P*
Month	8	1.839	0.230	1.285	0.2836
Residual	34	6.082	0.179		

(b) Mean table

Source	N	Mean	SD	SE
January	2	0.841	0.543	0.348
February	5	0.458	0.386	0.172
March	3	0.340	0.263	0.152
April	4	0.811	0.665	0.332
May	4	0.795	0.495	0.247
July	5	0.657	0.359	0.161
August	5	0.802	0.305	0.136
October	14	0.890	0.403	0.108
November	1	1.484E-5	-	-

Skull bone density

Average skull bone density for *U. bicinctus* was 0.464 ± 0.0610 g/cm^2 (n = 6); *Suggrundus meerdervoortii* 0.126 ± 0.0171 g/cm^2 (n = 6); *Rogasdius asper* 0.096 ± 0.0623 g/cm^2 (n = 5). ANOVA test showed significant difference existed among these three species($P < 0.01$); Fisher's LSD test for inter-group difference indicates skull density for *U. bicinctusas* significantly higher than *S. meerdervoortii and R. asper* (Table 3).

Table 3. Statistical results for the differences in bone density among *Uranoscopus bicinctus, Suggrundus meerdervoortii,* and *Rogadius asper.* *Means significant difference ($P < 0.01$).

(a) ANOVA table

Source	df	SS	MS	F	P
Species	2	0.48411	0.24205	95.08	< 0.0001*
Residual	14	0.03564	0.00254		

(b) Mean table

Source	N	Mean	SD	SE
Uranoscopus bicinctus	6	0.465	0.061	0.025
Suggrundus meerdervoortii	6	0.127	0.017	0.007
Rogadius asper	5	0.096	0.062	0.028

(c) Fisher's LSD table

Source	Mean Diff.	Crit. Diff.	P
Uranoscopus bicinctus, Suggrundus meerdervoortii	0.338	0.062	< 0.0001*
Uranoscopus bicinctus, Rogadius asper	0.369	0.066	< 0.0001*
Suggrundus meerdervoortii, Rogadius asper	0.030	0.066	0.3351

Skull thickness and hardness

The average skull bone thickness for *Uranoscopus* spp. was 1.65 ± 0.15 mm (n = 5) and *Parapercis sexfasciata* 0.76 ± 0.09 mm (n = 5); the bone was significantly thicker in the *Uranoscopus* spp.

The average skull-bone hardness for *Uranoscopus* spp. was 0.4961 ± 0.2440 GPa (n = 11) and *Parapercis sexfasciata* 0.3206 ± 0.2130 GPa (n = 8). Student's *t*-test showed the difference almost reached statistical significance (p = 0.06).

Calcium content in skull bone

The average amount of calcium element in the skull bone of *Uranoscopus* spp. was 253.64 ± 26.85 mg/g of bone (n = 11), platycephalids 222.99 ± 37.43 mg (n = 7), and *Parapercis sexfasciata* 217.33 ± 27.59 mg (n = 10). ANOVA test showed significant difference existed among groups (P < 0.05). Fisher's LSD test for inter-group difference showed *Uranoscopus* spp. was significantly higher than platycephalids and Parapercis sexfasciata (Table 4).

Table 4. The differences of calcium content in cranium among Uranoscopidae, Pinguipedidae, and Platycephalidae. *Means significant difference ($P < 0.01$).

(a) ANOVA table

Source	df	SS	MS	F	*P*
Family	2	7841.989	3920.995	4.363	0.0237*
Residual	25	22.468.792	898.752		

(b) Means table

Source	N	Mean	SD	SE
Pinguipedidae	10	217.3318	27.590	8.725
Platycephalidae	7	222.9967	37.433	14.148
Uranoscopidae	11	253.6411	26.852	8.096

(c) Fisher's LSD Table

Source	Mean Diff.	Crit. Diff.	*P*
Pinguipedidae, Platycephalidae	-5.665	30.427	0.7046
Pinguipedidae, Uranoscopidae	-36.309	26.978	0.0104*
Platycephalidae, Uranoscopidae	-30.644	29.852	0.0446*

Discussion

Bony fish bile has a pH value ranged from 5 to 7 (McKay, 1929). The pH value of the *Uranoscopus* bile was also acidic, whereas that of their intestine juice was weak alkaline. It is a reasonable condition that the acidic bile drained into the intestine and neutralizes the pH of the alkaline intestine to form a weaker alkaline environment. In a pH balanced human body, urine is slightly acid in the morning, (pH = 6.5 - 7.0) because no food or soft drink are consumed while sleeping and generally becoming more alkaline (pH = 7.5 - 8.0) by the evening (http://www.chemcraft.net/acidph2.html). Bilirubin is not present in the urine of healthy human individuals; these characteristics of urine dislike that of the *Uroscopus* spp. suggesting that the fluid in the *Uranoscopus* spp. bladder is not urine.

Some of the bile salts were reabsorbed in the intestine to be reused, while some are excreted into the feces. A certain amount of fluid was found in the hind part of the intestine of some *Uranoscopus* specimens. As the salinity of the intestinal fluid was much lower (and < 27‰) than that of the bile (> 37‰), it is possible that the salts in the fluid have already been reabsorbed at the anterior section of the intestine.

Studies suggest that bile salts play key role both in digestion and pheromone (Li et al. 2002; Zhang et al. 2001; Monte et al. 2009). Bile salt composition might differ among fish species (Hofmann et al. 2010). Further detailed analyses to identify the types of bile salts in *Uranoscopus* bile are important to clearly understand the function of their voluminous bile.

The fish bile was generally yellow, light greenish yellow, cyan, or blue black. Bile of cyclostomes, elasmobranchs, and teleosts contains a considerable amount of biliverdin (a green bile pigment) and bilirubin (a yellowish bile pigment) conjugates with lesser amounts of unconjugated bilirubin in certain species. Certain elasmobranch and teleost species have been reported to have primarily biliverdin or bilirubin. While bile of most aquatic species contains appreciable amounts of both pigments, the bile of most avian and reptilian on the other hand contains mainly biliverdin. Color of the bile may be affected by the amount of these bile pigments in the bile. On the basis of the present data set, correlation between bile volume and bile coloration did not exist. Individual variations in bile volume and amount of bilirubin may be rather high.

The reason for the inter-species variation in the bile volume /body volume ratio mentioned above may be related to hardness of their skull bone or other unknown factors which remain to be found.

Calcium ions are in surplus supply in seawater. The main calcium source in fishes is directly from the swallowed water or food in the intestine and gill (Flik & Verbost 1993; Boroughs et al. 1957; Kurita et al. 2008; Hossain & Furuichi 1998; Hossain & Furuichi 2000). If a large amount of calcium is needed for building an exceptionally hard skeletal system, fish need a more efficient process to take up calcium from these sources (water and food). As vitamin D is required for transition of calcium ions through the cell membrane into the cell and fish are not capable in producing this vitamin on their own (Lock et al. 2010), bile juice is needed to assist the fish to breakdown the fat from the ingested food to get the vitamin D in the fat (Lengemann & Dobbins 1958; Monte et al. 2009; Webling & Holdsqorth 1966).

When there were foods within the stargazer's stomach, significantly less bile juice was presented in the gall bladder suggesting that the bile juice had been drained into the intestine. This condition also suggests that the large volume of bile juice is for digestive function to enhance the breakdown the lipid.

Another possible reason for the large volume of bile is related to the feeding behavior of the stargazers. They feed by a wait-and-prey strategy; they can also put the captured prey size (body length) up to 1/2 of its own body length. To digestive this voluminous food in a short time, much more bile is needed. Sea catfish is a species with relatively large volume of bile as that of the stargazer. They differ from the latter in having a smaller mouth and the ingested foods are smaller than the gazed width (Lin, pers. comm.); they feed on shrimps and polychaetes (Mazlan et al. 2008). Relative volume and coloration (deep yellow to light green) of bile in stargazers and toadfish are approximate; their feed habits are similar indicating these resemblances in bile might be a result of fulfilling ecological needs.

The four skull-bone parameter (bone density, thickness, hardness and calcium content) and bile volume in Uranoscopid were highest among the studied groups. These parameters may be interrelated. Despite that correlation does not always due to a cause-result relation, it is likely that the voluminous bile in stargazers is for building a stronger skull through depositing more calcium in the bone.

As volumes of bile juice in stargazer, sea catfish, and toadfishes are approximate, cranium-bone hardness, density, and calcium content of the last two species remain to be analyzed for knowing if these bone parameters are correlated. Characteristics of other bones besides the skull were not analyzed in this study. Calcium content in muscles and Vitamin D content in the liver remain to be analyzed to interpret the function of this voluminous bile in stargazers.

For humans, ultraviolet light is needed by the skin for transforming 7-dehydrocholesterol into previtamin D3, which will be further converted to vitamin D3. Once vitamin D3 is formed, it will be metabolized in the liver and in the kidney to vitamin D. Availability of proper amount of ultraviolet B protons from the sun light influence the cutaneous production of this vitamin (Holick, 2004).

The stargazers bury and hide themselves in sand, keeping only their eyes and mouth observable. Unlike most species of fish that swallow water in through their mouths and expel them through the gills to breathe, the stargazer, living between 8-400 m, breathes through its nostrils.

However, do fish rely on sun light to produce vitamin D? Fishes beneath the photic zone are exposed only to very low or no sun light. Despite shallow water fishes can benefit from UV light, they get vitamin D from animal food and store it in their livers. Fish actually are a source of dietary vitamin D for other marine carnivores(animals.mom.me/animals-receive-vitamin-d-sun-11493.html).

Stargazers have fewer pyloric caeca and shorter intestine, suggesting that they are carnivores; they acquire their energy mainly from the proteins and fat in their food (Papoutsoglou & Lyndon 2003; Papoutsoglou & Lyndon 2006). Stargazers were found to have large volume of bile in this study, which can be applied for food digestion to obtain more energy. However, as stargazers are not very active, how do the surplus energy used. It is possible that they are used for reproduction. This possibility remained to be explored. Unfortunately, bile volume did not increase in the spawning season, making the possibility less likely.

Some fishes were reported using bile as pheromone. Unfortunately, no information about their bile volume and their bile chemical characteristics are available in literature. The information should shed light on finding the reason for the voluminous bile in stargazers

Combining the biochemical parameters and coloration, the stargazer' s bile is a dilute solution. In case the bile components were somehow released to the seawater, it must be quickly further diluted, making its function as pheromone much less likely. This function might be possible if their bile contains high content of bile salt and other specific chemicals which did not easily diluted in seawater. Hamdani & Døving (2007) pointed out that bile salts can attached to other materials that can serve as a sign for marking the substrate or water mass and used by migrating lamprey and salmon to trace and home the river where they grow up. Whether stargazers also apply their bile for such purpose is unknown. Urinalysis should be made for the stargazers for its bile salt content.

Despite that no significant differences were found in bile volume between males and females, the hypothesis of the bile serves as pheromone cannot be rejected on this basis, it is because that both sexes may use it as a chemical signal. However, lacking of seasonal difference make this possibly much unlikely; the volume is expected to increase in the spawning season. It can stand if stargazers spawn year round.

An additional possibility is that stargazers use their bile to attract preys. No matter it is used for attracting mates or preys, the bile has to be released to the water through the substrates when the stargazers are burrow in sand; it seems an inconvenient way of sending out the signal. Some behavioral experiments may help finding the results.

The specimens used in this study were approximate in body length. More small-size specimens will bring insight to the hypotheses—their bile volumes are expected to be even larger bile volume if it was mainly required for cranium hardness, but smaller if it was for pheromone.

Brittain *et al.* (1989) analyzed the bili-protein in the serum of the spotted stargazer, *Genyagnus monopterygius* and found the biliverdin is tightly linked with the protein, giving the blood a blue-green color. Biliverdin is a production after the hemes were broken down. The burrowed spotted stargazer was considered using the biliverdin in the blood serum carry out gas exchange as they pass through the capillaries on the skin and avoid relay too much process in the gills. However, the blood of the *Uranoscopus, Ichthyoscopus,* and *Xenocephalus* species analyzed in the present study are red. Whether their blood contains biliverdin to carry out the role remained to be analyzed.

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