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Annual Patterns of Gonadosomatic and Hepatosomatic Indexes and Progesterone-like Substance Levels of Female *Mictyris brevidactylus*

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Jin-Taur Shih (1993) Annual patterns of gonadosomatic and hepatosomatic indexes and progesterone-like substance levels of female *Mictyris brevidactylus. Bull. Inst. Zool., Academia Sinica* **32**(4): 221-228. Female *Mictyris brevidactylus* were collected monthly from the Tanshui mangrove swamp of northern Taiwan between January, 1989 and December, 1991. The gonadosomatic (GSI) and hepatosomatic indexes (HSI) of this crab were then studied. GSI values started increasing in August and reached peak values in November and December. GSI values dropped significantly as soon as the crabs entered their reproductive season (late December to March, egg-carrying). During its nonreproductive months (April to September), *M. brevidactylus* had low GSI values. On the other hand, the peak HSI phase appeared during the nonreproductive months and low HSI values were observed during the crab's reproductive period. Levels of a progesterone-like substance in crab hemolymph varied according to season. Low levels (0.21-0.74 ng/g of w.b.w.) were observed during the nonreproductive months; levels started increasing in September and reached peak phases from October to December (0.77-2.02 ng/g of w.b.w.). These high levels were two to three times those values observed during the nonreproductive months. Egg-carrying crabs showed low levels of progesterone-like substance (0.26-0.80 ng/g of w.b.w.) in their hemolymph. These results suggest that the high GSI values and the peak phase of the hemolymphic progesterone-like substance may indicate physiological readiness for female *M. brevidactylus* to start reproduction. The relationship of GSI, HSI, and the progesterone-like substance levels to crab reproduction is discussed.

Key words: *Mictyris brevidactylus*, GSI, HSI, Hemolymph, Progesterone-like substance

For two reasons, the steroid hormones of crustaceans have only recently been studied: 1) researchers previously were reluctant to use sophisticated methods to study these animals; and 2) many researchers did not believe that crustaceans synthesize sex steroids. However, steroid hormones have indeed been found in the tissues of several crustacean species (Teshima and Kawazawa 1971, Zerbib 1976, Rateua and Zerbib 1978, Sandor 1981, Couch and Hagino 1983, Adiyodi 1985, Skinner 1985, Fingerman 1987, Sasser and Singhas 1988, Shih et al. 1990, Shih and Wang 1993). Shih (1992) also reported that progesterone and estradiol-like substances are detectable in the hemolymph of female *Uca arcuata*; these steroids reach peak levels two to three months before egg-carrying.

The presence of steroid hormones in crustaceans suggests that steroids may play a role in reproduction regulation. For example, progesterone and 17-α-hydroxy-progesterone stimulate yolk protein synthesis in both isopods and penaeid shrimp (Kulkarni et al. 1979, Souty and Picaud 1984). Progesterone and progesterone-like steroid hormones directly effect the development of immature penaied ovaries (Tsukimura and Kamemoto 1988). Quackenbush (1992) reported that in the ovarian fragments of *Penaeus vannamei*, progesterone dramatically stimulates yolk protein synthesis while estradiol has less effect; he also observed that testosterone and ecdysteroids had no effect. Therefore, it is necessary to study the levels of endogenous steroids in crustaceans throughout the year in order to understand the reproductive biology of these animals.

MATERIALS AND METHODS

Female *Mictyris brevidactylus* with carapace widths of 0.5 to 1.05cm were collected monthly from January,1989 to December, 1991 from the Tanshui mangrove swamp of northern Taiwan (Shih et al. 1991). Crabs were taken to the laboratory, rinsed, and wrapped in tissue paper to absorb excess water; specimens were kept frozen until used for experiments.

After thawing, crabs were weighed and wet body weights (w.b.w.) recorded (Table 1). Carapaces were then removed. Ovaries (located on top of the hepatopancreas) were carefully removed and placed on a piece of aluminum foil; each crab's hepatopancreas was similarly removed. The wet weights of these two tissues were recorded, after which GSI and HSI were obtained.

Table 1. Carapace widths and wet body weights of female *Mictyris brevidactylus* used for GSI and HSI studies in 1990-1991[a]

Date of collection	Number of crab		Carapace width (cm)[b]		Wet body weight (g)[b]	
	1990	1991	1990	1991	1990	1991
January	25	27	0.73 ± 0.11	0.81 ± 0.12	0.51 ± 0.13	0.65 ± 0.15
February	26	16	0.64 ± 0.09	0.77 ± 0.15	0.42 ± 0.15	0.60 ± 0.22
March	20	18	0.78 ± 0.13	0.86 ± 0.13	0.59 ± 0.15	0.69 ± 0.14
April	39	23	0.73 ± 0.14	0.77 ± 0.11	0.54 ± 0.13	0.63 ± 0.12
May	17	27	0.63 ± 0.12	0.81 ± 0.12	0.41 ± 0.10	0.66 ± 0.14
June	19	22	0.70 ± 0.14	0.84 ± 0.14	0.49 ± 0.11	0.68 ± 0.15
July	21	22	0.66 ± 0.11	0.80 ± 0.13	0.44 ± 0.12	0.66 ± 0.11
August	23	19	0.74 ± 0.14	0.74 ± 0.10	0.53 ± 0.08	0.55 ± 0.11
September	27	27	0.83 ± 0.15	0.76 ± 0.11	0.66 ± 0.15	0.58 ± 0.12
October	21	26	0.76 ± 0.13	0.75 ± 0.14	0.59 ± 0.15	0.61 ± 0.15
November	32	31	0.86 ± 0.12	0.83 ± 0.15	0.74 ± 0.14	0.66 ± 0.18
December	30	40	0.79 ± 0.10	0.77 ± 0.14	0.63 ± 0.16	0.59 ± 0.15

a. Female crabs (241-489) were collected monthly from the Tanshui mangrove swamp in northern Taiwan between 1990-1991. Forty female crabs (carapace width more than 0.5cm) were randomly chosen for GSI and HSI study.

b. Carapace widths and wet body weights are expressed with mean and standard errors.

Preparation of the crabs' hemolymph was conducted according to procedures described by Shih (1992). Female crabs collected during the same month (usually in groups of 30-50) were crushed; crushed tissues and body fluids were filtered through four layers of cheesecloth, and the filtrate was then centrifuged at 3000 X g at 4°C for 10 min. Supernatant volume was recorded and designated as *M. brevidactylus* hemolymph for this study.

All samples were tested for progesterone (pre-4-one-3,20-dione) content with a Biodata progesterone Maia kit (code 12274, Milano, Italy); progesterone antibody was prepared from rabbit serum. The RIA ranges for the progesterone standard were 0.07-33.00 ng per ml of saline. Any assay with a concentration below the minimum limit was rejected. For each batch of samples (hemolymph of same year crabs), one sample was measured by RIA at 1/2, 1/4, and 1/8 dilution to test for the presence of interfering factors. Dilution curve results did not reveal any contamination. For this study, the concentration of a progesterone-like substance was 0.38-6.67 ng per ml of hemolymph; progesterone detected in the crab hemolymph by RIA is referred to as progesterone-like substance.

RESULTS

Annual *M. brevidactylus* GSI patterns for 1990-1991 are shown in Fig. 1. The high GSI values were observed in November, December, January, and February; during these months, the average GSI value was more than 5.0%, with a maximum level of 7.4%. From March to July GSI values were no more than 2.0%, and at its lowest (April, 1991) was only 0.35%. *M. brevidactylus* GSI started increasing in August and reached values of 2.4-3.6% in October; in November, the observed GSI again reached its peak value for the year.

Annual HSI patterns for 1990-1991 are shown in Fig. 2. From May to September in 1990, HSI values were slightly higher than those for the other months of the year except for November. However, for the same months in 1991, observed HSI had a value which was almost twice as high as the values observed during the rest of the year. June, July, and August HSI had values higher than 3.2%, and reached a peak of 5.8% in

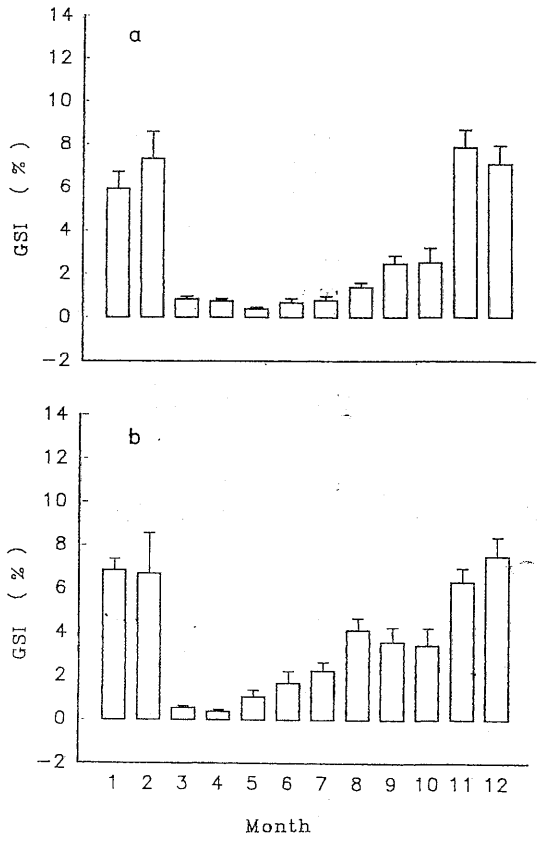

Fig. 1. Annual GSI patterns of female *Mictyris brevidactylus* collected at Tanshui mangrove swamp in northern Taiwan in 1990(a) and 1991(b). The height of each column represents the mean GSI value for that month. Each bar represents standard error. Numbers, carapace widths, and wet body weights of crabs used for the GSI study are listed in Table 1. January and February crabs (1990 and 1991) were not carrying eggs.

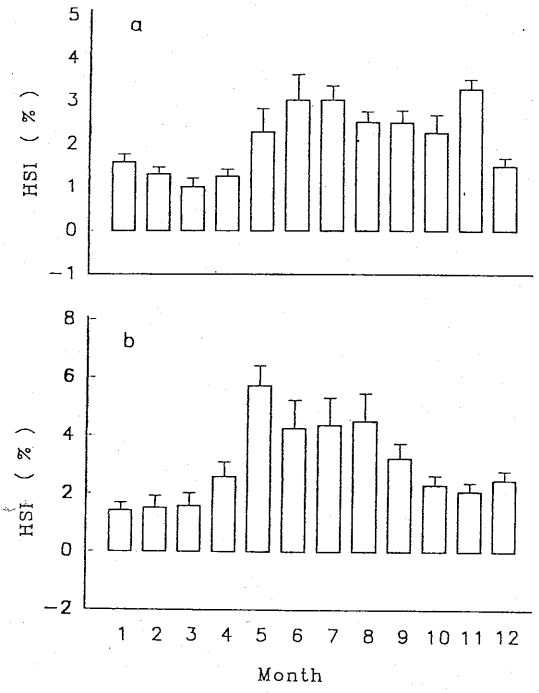

Fig. 2. Annual HSI patterns of female *M. brevidactylus* collected at Tanshui mangrove swamp in northern Taiwan in 1990(a) and 1991(b). The height of each column represents the mean HSI value for that month. Each bar represents standard error. Numbers, carapace widths, and wet body weights of crabs used for the HSI study are listed in Table 1. January and February crabs (1990 and 1991) were not carrying eggs.

May. In contrast, the observed HSI during the winter months remained low, never going beyond 2.5%.

A progesterone-like substance was detected in all collected hemolymph; with contents ranging between 0.21 ng and 2.02 ng per gram of wet body weight (Fig. 3). Hemolymph prepared from crabs collected between January and August contained lower levels of progesterone-like substance (less than 0.8 ng/g of w.b.w.). Levels started increasing in September and reached peak levels in October (1991), November (1990), and December (1989). These peak levels ranged between 1.57-2.02 ng/g of w.b.w. which were almost two to three times those values measured during the other months.

Small crabs (carapace widths below 0.5cm) had small amounts of progesterone-

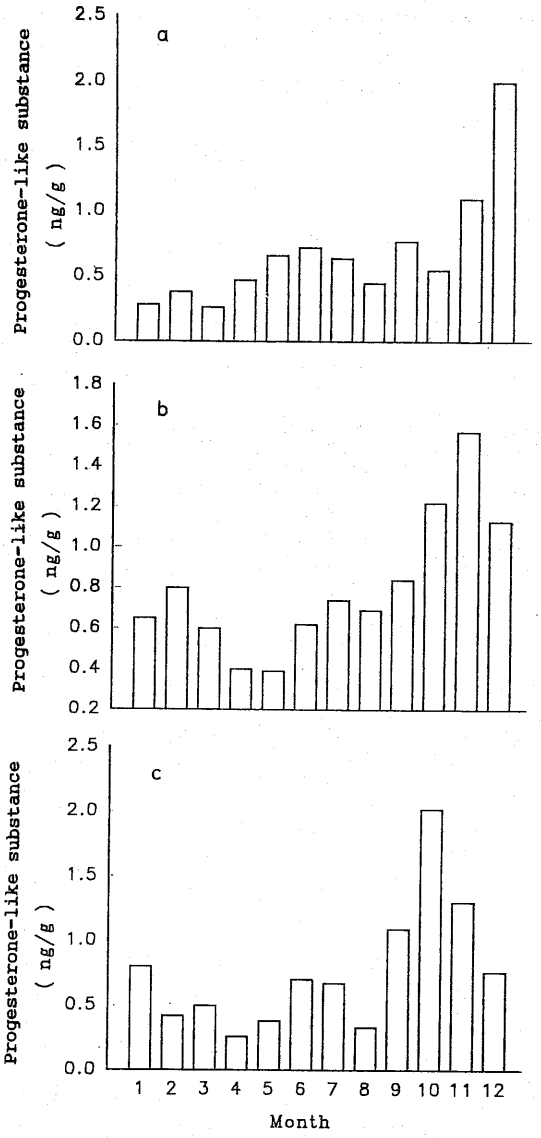

Fig. 3. Levels of progesterone-like substance in the hemolymph of female *M. brevidactylus* collected in 1989(a), 1990(b), and 1991(c). The height of each column represents the average value of RIA-detected progesterone-like substance in crab hemolymph for that month (expressed as ng of progesterone-like substance per gram of wet body weight). The numbers of crabs used for assays ranged between 30-115, depending on month.

Table 2. GSI, HSI, and progesterone-like substance contents of second generation *Mictyris brevidactylus*[a]

Date of collection	GSI (%)	HSI (%)	Progesterone-like substance (ng/g)[b]
1990			
July	—[c]	—	0.20(121)
1991			
August	0.47 ± 0.15(20)[d]	3.35 ± 1.40(20)	0.18(79)
September	0.52 ± 0.27(21)	2.20 ± 0.72(21)	0.16(88)
October	0.46 ± 0.07(23)	1.40 ± 0.60(23)	0.14(50)
November	1.21 ± 0.15(25)	1.55 ± 0.45(25)	0.21(55)
December	4.84 ± 2.35(16)[e]	2.83 ± 0.46(16)	0.49(50)

a. Second generation crabs had carapace widths of less than 0.50 cm.
b. Progesterone-like substance content represents the average value of RIA-detected progesterone-like substance in each hemolymph (expressed as ng of progesterone-like substance per gram of wet body weight).
c. Not determined.
d. The numbers in parentheses represent the number of crabs used for that study. GSI and HSI are expressed as % with mean and standard error.
e. The carapace widths of second generation crabs collected in December, 1991 had a range of 0.40-0.65 cm.

like substance (0.16-0.21 ng/g of w.b.w.) from July to November, 1991; by the time these crabs had become medium or large crabs (carapace widths above 0.5 cm) in December, their hemolymph contained a high level of progesterone-like substance (0.49 ng/g of w.b.w.) (Table 2). The GSI of small crabs ranged between 0.46% and 1.21% during August-November,1991; these values were only one-sixth the GSI of the large crabs (Fig. 1 and Table 2). The GSI value reached 4.84% in December—the same as large crabs. The HSI of small crabs from August to December, 1991 ranged between 1.40% and 3.35%, which were equal to values recorded for large crabs (Fig. 1 and Table 2).

DISCUSSION

The results of this study on female *M. brevidactylus* may be summarized as follows: 1) a progesterone-like substance was detected in the crab hemolymph; 2) high levels of progesterone-like substance and high GSI values were detected one to two months before the crab's egg-carrying stage; and 3) high HSI values were found during the crab's nonreproductive months.

In Taiwan, *M. brevidactylus* has only one breeding season; egg-carrying females were first found in late December, and more than 80% of all female crabs collected from mid-February to mid-March were found to carry eggs. Since late April, no egg-carrying crabs were found until the next reproductive season (Huang 1991, Shih et al. 1991).

Though peak levels of progesterone-like substance did not appear during the same month for each of the three years of this study, they did appear one to two months before the egg-carrying stage. In addition, peak levels were almost two to three times those observed during the crab's nonreproductive months. After comparing the annual pattern of progesterone-like substance levels with the GSI pattern, it is clear that the peak

level of sex steroid either appeared one month before or coincided with the peak GSI values.

Similar results were reported for *Uca arcuata* by Shih (1992); he found that both progesterone and estradiol-like substances reached peak levels one to two months before *U. arcuata*'s egg-carrying stage. It therefore appears reasonable to suggest that sex steroids may serve a physiological function in crustacean reproduction; they may enhance the ovaries growth (or oocytes), or promote the timely maturation of crustacean oocytes (Quackenbush 1986 1989, Fingerman 1987).

It should be noted that the annual HSI pattern differs from the GSI and progesterone-like substance patterns. The HSI showed peak values during the nonreproductive months and minimum levels during the reproductive season. This result differs from those of two other mangrove-associated crabs, *Sesarma intermedia* (Kyomo 1988) and *Uca arcuata* (Shih 1992); both of these species had peak HSI values during their reproductive seasons. It has also been reported that the HSI of male *M. brevidactylus* has higher values during nonreproductive months than during reproductive months (Shih and Chang 1991).

Crabs of two sizes were collected between late July and November in 1990-1991. Large crabs (designated as first generation crabs) had carapace widths larger than 0.5 cm and thick shells of light blue color. Small crabs (designated as second generation crabs) had carapace widths of less than 0.5 cm and thin shells of purple color (Nakasone and Akamine 1981, Huang 1991, Shih et al. 1991). GSI and progesterone-like substance levels in second generation crabs were low during August-November when compared with those observed in large crabs. Second generation crabs became medium or large crabs in late December, and thereafter had similar values as first generation crabs. Crabs began to carry eggs as soon as they contained high levels of GSI and progesterone-like substance. More than 70% of second generation female crabs (some with carapace widths of even less than 0.4 cm) were carrying eggs by mid-March.

It still has not been proven that crustaceans synthesize sex steroids. However, sex steroids, ecdysteroids, or enzymes involved in the conversion of sex steroid precursors have been found in various crustaceans (Teshima and Kawazawa 1971, Zerbib 1976, Lachaise and Hoffmann 1977, Rateua and Zerbib 1978, Blanchet et al. 1979, Sandor 1981, Couch and Hagino 1983, Adiyodi 1985, Skinner 1985, Fingerman 1987, Sasser and Singhas 1988, Shih et al. 1990, Shih 1992, Shih and Wang 1993). The author studied the ethanol extracts of ovaries, hepatopancreas, and hemolymph of female *Uca arcuata*; a progesterone-like substance was detected via high pressure liquid chromatography and RIA in all three tissues (Shih and Wang 1993). Quackenbush (1992) reported that progesterone and estradiol stimulate yolk protein synthesis in the ovarian fragments of *Penaeus vannamei*. Therefore, it appears that crustaceans do have the ability to synthesize sex steroids which may affect reproduction.

Presently, the location of sex steroid biosynthesis in decapods is unknown; further analysis of endocrine tissues such as ovary, adipose tissue, and hepatopancreas is required. The purification of sex steroids from the ovaries, hepatopancreas, and hemolymph of female *M. brevidactylus* is being currently conducted. Quantitative studies of sex steroids and seasonal changes of sex steroid levels in *M. brevidactylus* will provide a more complete understanding of the crab's reproductive mechanisms.

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雌性短趾和尚蟹(*Mictyris brevidactylus*)性腺、肝胰臟體重值及類助孕酮含量的全年型態

史金熹

本報告的目的是研究雌性短趾和尚蟹的生殖生物學，作者自1989年1月至1991年12月，按月採集台灣淡水紅樹林的雌性短趾和尚蟹，從性腺體重值(GSI)全年的型態得知雌蟹卵巢重量自8月開始增加，於11至12月達到高峰後，即進入生殖期（孵卵，12月至次年3月），孵卵雌蟹的GSI隨即下降，雌蟹於非生殖期間(4月至9月)，其GSI是全年的最低值。肝胰臟體重值(HSI)全年的型態與GSI不同，其最高值出現於非生殖期，而最低值出現於生殖期。雌蟹體液經放射免疫分析法的測定，發現體液中含有類助孕酮。連續三年測定的結果，顯示全年中體液內類助孕酮的含量因月份而異。在非生殖期時，其含量較低(0.21－0.74 ng/g of w.b.w.)，自9月開始上升，於10至12月間達到高峰(0.77－2.02 ng/g of w.b.w.)，其含量是非生殖期的二至三倍。雌蟹孵卵後，體液內類助孕酮的含量即下降(0.26－0.80 ng/g of w.b.w.)。以上結果顯示於10至12月間，雌性短趾和尚蟹卵巢重量和體液內類助孕酮含量均達到全年高峰，促成雌蟹於生殖期進行繁殖，報告中對此蟹的生殖生理有所討論。