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ANNUAL PATTERN OF SEX STEROID-LIKE SUBSTANCE LEVELS IN THE HEMOLYMPH OF FEMALE *UCA ARCUATA*

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Jin-Taur Shih (1992) Annual pattern of sex steroid-like substance levels in the hemolympe of female *Uca arcuata*. *Bull. Inst. Zool., Academia Sinica* **31**(1): 47-56. The gonad somatic index (GSI) and the hepatosomatic index (HSI) of female *Uca arcuata* in the Tanshui mangrove swamp in Taiwan were studied during 1988-1989. Both the GSI and HSI of female *U. arcuata* began to increase in November, and reached peak value from January to March. The peak phase of the GSI had a value which was four times that of the nonreproductive months, while the HSI doubled. After the breeding season in April and May, both GSI and HSI dropped to basal values. Hemolymphs were prepared from female *U. arcuata* collected monthly from August 1987 to July 1989. The sex steroid-like substance in hemolymphs of the female *U. arcuata* were quantified by radioimmunoassays. Both progesterone-like (P_4) and estradiol-like (E_2) substances were detectable in the hemolymphs. High levels of P_4-like and E_2-like substances were found in the January to March samples (1988 and 1989). However, the amount of E_2-like substance in the hemolymph was much less than that of the P_4-like substance. The P_4-likesubstance was also found in the hemolymphs of female crabs of medium size (with carapace width of 1.5-2.0 cm). The content of P_4-like substance in the medium size crabs was two to three times that of the small and large ones. The relation- ship of GSI, HSI, and sex steroid-like substance contents to reproduction of the female *U. arcuata* is discussed.

Key words: *Uca arcuata*, GSI, HSI, Hemolymph, Sex steroid-like substance.

The steroid hormones of crustaceans have been under studied when compared with those of insects. Researchers have been reluctant to use sophisticated methods of analytical biochemistry to study this group of animals, and surgical operations on crustaceans were less successful than those on insects (Adiyodi and Subramonian, 1983). However, Zerbib (1976) and Rateua and Zerbib (1978) reported that the biosynthesis of steroids was found in the follicle cells and oocytes of *Orchestia gammarella*. In one *in vitro* study, Sandor (1981) reported that the ovarian tissue of *Portunus trituberculatus* converted progesterone to 11-deoxycorticosterone. Enzymes involved in the conversion of progesterone to 17α-hydroxyprogesterone, testosterone, and deoxycorticostererone have been isolated from the ovaries of *Portunus trituberculatus* (Teshima and Kawazawa, 1971).

Among sex steroids, estrogen has been found in the American lobster, blue crab, and penaeid shrimp (Donahue, 1940, 1948; Sasser and Singhas, 1988). Ecdysteroids were detected by radioimmunoassay in

the ovaries of *Carcinus maenas* (Lachaise and Hoffmann, 1977). Blanchet *et al.* (1979) reported that the concentration of ecdysteroid in the ovaries and the hemolymphs of *Orchestia gammarella* increased during vitellogenesis, and reached its peak level at the end of vitellogenesis. Recently, progesterone identified by HPLC was found in *Uca arcuata* (Shih *et al.*, 1990).

Crane (1975) stated that the breeding season for *U. arcuata* in South Asia was between April and May. Su and Lue (1984) reported that the breeding season for *U. arcuata* in the Tanshui mangrove swamp in northern Taiwan was in August. In addition, Chang *et al.* (1985) studied the annual pattern of the gonad somatic index (GSI) of *U. arcuata* in the Tanshui mangrove swamp and suspected that this crab had two breeding seasons —one in April, and another in August. Crustaceans have a scheduled breeding season every year; therefore, research on sex steroids and their levels during the year would clarify the breeding season and reproduction mechanisms in this crab. For this report, the GSI, HSI, and hemolymphic sex steroids of the female *U. arcuata* were studied during 1987–1989. The relationships among GSI, HSI, and steroid--like substance contents in reproduction of the female *U. arcuata* will be discussed.

MATERIALS AND METHODS

Collection

Female *U. arcuata* with carapace widths of 2.0–2.5 cm were collected in the G-area of the Tanshui mangrove swamp on high spring day (Su and Lue, 1983). Because of this crab's high sensitivity it was necessary to catch them one by one. The crabs were taken back to the laboratory, rinsed, and wrapped with tissue paper to absorb excess water. The crabs were usually kept frozen until ready for use.

Determination of GSI and HSI

After thawing, the carapace of each crab was removed. The H-shaped ovary, located on top of the hepatopancreas, was carefully removed and placed on a piece of aluminum foil; the hepatopancreas was removed in a similar way. The wet weights of the ovary, hepatopancreas, and remains of the crab were recorded, after which the GSI and the HSI were obtained.

Preparation of sample for radioimmunoassay (RIA)

The procedure for preparation of the crab's hemolymph was developed in this laboratory. After removal of ovary and hepatopancreas, the remains of crabs captured during the same month were pooled and crushed. The crushed tissue and fluid were filtered through four layers of cheesecloth, then the filtrate was spun at 3,000×g at 4°C for ten minutes. The supernatant was saved, and its volume recorded. This supernatant was then filtered through a millipore membrane (0.45 μm) and designated as the hemolymph of *U. arcuata* for this study. In order to check if the removal of the ovaries and the hepatopancreases had affected steroid quantities, whole crabs were crushed and put through the same process as stated above.

Most hemolymph samples went through RIA for steroids without further processing. For each batch of samples, one sample was diluted by 1/2, 1/4, and 1/8 for testing of the sex steroids, using RIA to check for contamination. All samples were then tested for progesterone (P_4) and estradiol-17β (E_2) using a Biodata progesterone Maia Kit (Code 12274, Milano, Italy) and a Biodata estradiol-17β Maia Kit (Code 12254, Milano, Italy). The P_4 and E_2 antibodies were prepared from

rabbits. The working ranges of RIA (standards) for P_4 and E_2 were 0.07–33.0 ng/ml of saline and 56–4,500 pg/ml of saline, respectively. Any assay with a concentration below the minimum limits was not used. Cross-reaction of the P_4 antiserum to E_2 was less than 0.01%, and that of the E_2 antiserum to P_4 was less than 0.1%. In this study, the concentration of P_4-like substance was 0.78–3.0 ng/ml of hemolymph, and for the E_2-like substance it was 56–780 pg/ml of hemolymph. Some samples were extracted with ether and put through RIA and EIA for the quantifying and identifying of P_4 and E_2.

A pooled hemolymph sample was extracted with diethyl ether. After the ether evaporated, the residue was dissolved in 0.01 M phosphate buffer saline (PBS, pH 7.40, Yu *et al.*, 1990) containing 0.1% gelatin, then incubated at room temperature (24°C) for one hour. This PBS-gelatin soluble residue was used for the RIA. For a separate fraction of the pooled hemolymph, ^{3}H-progesterone (120,000 cpm) was added and put through the ether extraction procedure until a separate residue was obtained. During extraction, the radioactivity of each step was checked; the efficiency of residue recovery was 69.9%.

RESULTS

The GSI and HSI of the female *U. arcuata* samples taken in 1988–1989 are shown in Table 1. The GSI started to increase from a value of 0.88% in November of 1988 to a peak value of 2.37% by January of 1989; it remained at this level until March, 1989. GSI dropped to 0.57% in May, and remained at that value until July. The peak phase of HSI (4.57%) was found in January and February of 1989. The HSI also dropped in April, reaching a basal value (1.83%) in May.

Female *U. arcuata* hemolymphs were subjected to radioimmunoassays for P_4 and E_2. Results from dilution curve preparations did not show any contamination. In addition, sex steroids were extracted from some hemolymph samples by using ether. From these extracts, P_4 and E_2 were detectable through RIA. For this report, any P_4 or E_2 detected in the

Table 1
Gonad somatic index (GSI) and Hepatosomatic index (HSI) of female *Uca arcuata* collected at Tanshui mangrove swamp during 1988–1989

Date of collection		Number of crabs	GSI[a] (%)	HSI[a] (%)
1988	October	6	0.24±0.08	1.75±0.68
	November	11	0.88±0.41	1.12±0.51
	December	7	1.30±0.45	2.70±0.63
1989	January	4	2.37±1.11	4.57±1.78
	February	3	2.59±1.21	4.05±0.92
	March	4	2.26±1.13	3.83±1.15
	April	5	0.85±0.55	2.56±0.52
	May	6	0.57±0.38	1.83±0.39
	June	3	0.59±0.12	1.77±0.46
	July	5	0.65±0.31	2.18±0.43

a. GSI and HSI were obtained from more than one crab and are expressed as % with mean and standard error.

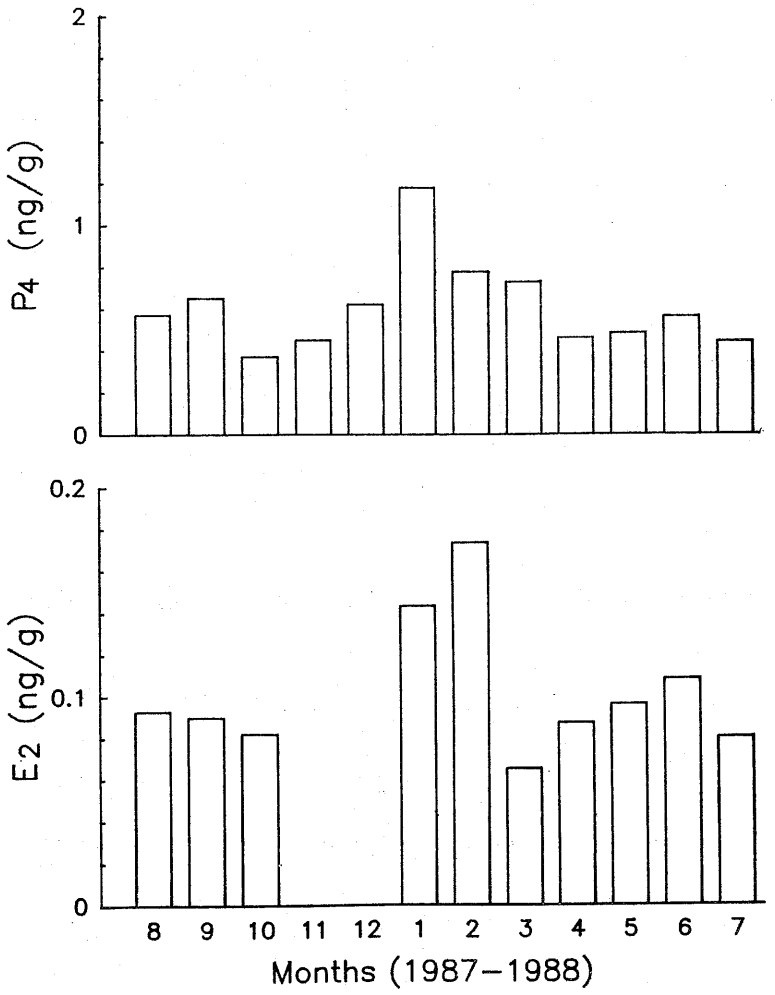

Fig. 1. Levels of sex steroid-like substances in the hemolymphs of female *Uca arcuata* collected during 1987-1988. The upper figure represents the average value of RIA-detected P_4-like substance in the hemolymphs (expressed as *ng* of P_4-like substance per gram of wet body weight of crab). The lower figure is for E_2-like substance. The numbers of crabs used for assaying were 4-18, depending on month.

hemolymph is referred to as P_4-like or E_2-like substances.

The progesterone-like substance and the estradiol-like substance were measured in the hemolymphs of the female *U. arcuata* collected from 1987 to 1988; results are shown in Fig. 1. The progesterone-like substance was detected in all of the hemolymphs; their content ranged between 0.37 ng and 0.65 ng/g of wet body weight (w.b.w.), with the exception that P_4-like substance contents were relatively high during January, February, and March of 1988 (0.78–1.18 ng/g of w.b.w.) The estradiol-like substance was also detected in all samples except for those from November and December, 1987. Results for these months were not available because sample numbers were inadequate for testing. The E_2-like substance contents were 0.06 ng to 0.17 ng/g of w.b.w., much less than those of the P_4-like substance. However, the E_2-like substance was relatively high in content

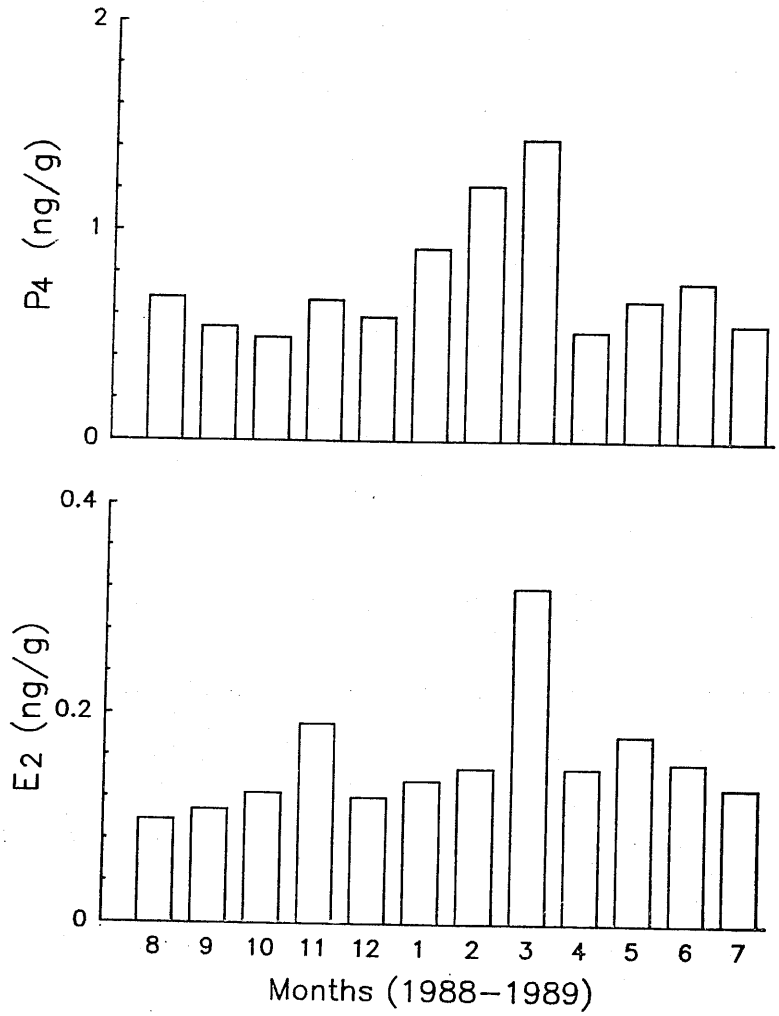

Fig. 2. Levels of sex steroid-like substances in the hemolymphs of female *Uca arcuata* collected during 1988-1989. The upper figure represents the average value of RIA-detected P_4-like substance in the hemolymphs (expressed as *ng* of P_4-like substance per gram of wet body weight of crab). The lower figure represents E_2-like substance. The numbers of crabs used for assaying were 3-12, depending on month.

(0.14-0.17 ng/g of w.b.w.) in January and February of 1988, coinciding with the higher P_4-like substance contents.

Figure 2 shows the annual pattern of the P_4-like and the E_2-like substance contents detected in the hemolymphs of female *U. arcuata* captured during 1988-1989. In fact, the annual patterns shown in Figures 1 and 2 are similar. The P_4-like substance levels started increasing in January (0.92 ng/g of w.b.w.) and reached peak values in March (1.44 ng/g of w.b.w.). The peak values of the E_2-like substance also found between January and March (0.13-0.33 ng/g of w.b.w.).

In order to investigate the type and amount of the steroid-like substances in the hemolymph of *U. arcuata* during development, crabs of various sizes were collected, and their hemolymphs subjected to an RIA. Results are shown in Table 2. The hemolymphs of all three sizes of

Table 2
Sex steroid-like substance contents in the hemolymphs of female *Uca arcuata* of various sizes

Date of collection		Size of crabs[a]	Number of crabs	Steroid content[b] (ng/g)	
				P_4-like substance	E_2-like substance
January	1989	Small	22	0.92	0.07
March	1989		6	1.02	0.10
February	1990		13	0.77	0.05
April	1990		6	—	0.12
				0.90±0.10	0.08±0.02
January	1989	Medium	27	1.70	0.11
March	1989		8	3.51	0.09
February	1990		6	3.34	0.06
April	1990		18	—	0.05
				2.85±0.81	0.08±0.02
January	1989	Large	5	0.92	0.13
March	1989		4	1.44	0.16
February	1990		6	1.30	0.10
				1.25±0.23	0.14±0.01

a. Size was defined as: large crabs with carapace widths 2.0-2.5 cm and medium and small crabs with carapace widths of 1.5-2.0 cm and 0.8-1.4 cm, respectively.

b. Steroid content is expressed as ng of steroid-like substance per gram of wet body weight of crab. Within the results of P_4-like and E_2-like substance of each group (small, medium and large sizes), mean and standard error are listed.

crab contained the P_4-like and the E_2-like substances; large and small crabs contained similar concentrations of P_4-like substance (0.90±0.10 and 1.25±0.23 ng/g of w.b.w.). A high level of P_4-like substance was found in the crabs of medium size (2.85±0.81 ng/g of w.b.w.); this value was almost two to three times that found in the small and large crabs. In contrast, the E_2-like substance was found at low levels in the small and medium-sized crabs; large crabs had a relatively high level of E_2-like substance (0.14±0.02 ng/g of w.b.w.).

DISCUSSION

Results of the present study on female *U. arcuata* in the Tanshui mangrove swamp may be summarized as follows: 1) high values of GSI and HSI were found two-to-three months before breeding; 2) P_4-like and E_2-like substances were detected in the hemolymphs; 3) hemolymphs contained more P_4-like substance than E_2-like substance; and, 4) high contents of P_4-like and E_2-like substances were found before the breeding season.

The GSI of Tanshui mangrove swamp *U. arcuata* recorded during 1988-1989 showed a peak phase from January to March of 1989. This high GSI value was four times that found during nonreproductive months. In addition, this high GSI appeared about 2-3 months before breeding began, indicating readiness for reproduction. In crayfish, Lowe (1961) reported that GSI values reached their highest levels one month before or during the egg-carrying period. Koymo (1988), who studied the *Sesarma intermedia* (another mangrove-associated crab)

found that crab had a high GSI (5%) during breeding. Chang *et al.* (1985) reported that Tanshui mangrove swamp *U. arcuata* had two GSI peak phases—one in February and March, and another in June and July. In addition, Su and Lue (1984) stated that the breeding season for *U. arcuata* was in August. In this study, there were no high GSI values uncovered in July or August, and no breeding crabs were caught during that time (Shih, 1990). Therefore, I assert that the reproductive season of *U. arcuata* in this study was between April and May, as stated by Crane (1975).

The hepatopancreas's function as a nutrient center in crustaceans has been reported by many researchers (Adiyodi, 1968; Adiyodi and Adiyodi, 1970; Adiyodi and Adiyodi, 1972; Anilkumar, 1980). The female *U. arcuata* collected for this study in January, February, and March of 1989 had high HSI values which were double those found during the other months. Considering the breeding season for this crab, the large hepatopancreas might, at the right time, function as a nutrient source for oogenesis. In *Sesarma intermedia*, GSI levels increased with increases of HSI, indicating a close relationship between GSI, HSI, and reproduction (Koymo, 1988). After breeding, the HSI and GSI of *U. arcuata* dropped, and both stayed in their basal phases until the following reproductive season.

Both P_4-like and E_2-like substances were detected in the hemolymphs of female *U. arcuata*, but the P_4-like substance had relatively higher concentrations than the E_2-like substance; the E_2-like substance content wasn ever over 0.32 ng/g of w. b.w., while the P_4-like substance contents ranged between 0.37 to 1.44 ng/g of w. b. w. These results confirmed the findings of Shih *et al.* (1990), who reported that progesterone was detectable by HPLC in the whole body extract of female *U. arcuata*, while E_2, testosterone, or cortisol were only occasionally detectable. During the study period (1987–1989), peak values of both P_4-like and E_2-like substances appeared two-to-three months before breeding. The peak level of the P_4-like substance was almost three time that found during the nonreproductive season. Similarly, the E_2-like substance had peak levels coinciding with the P_4-like substance, but the difference in E_2 content between breeding season and the nonreproductive period was not as significant as that of the P_4-like substance.

Since there is a lack of information in the literature concerning P_4 and the E_2 levels in the hemolymphs of crustaceans during their reproductive period, the exact physiological functions of these hormones are still unknown. However, results from the present study indicated a possible relationship between the steroids and reproduction. First, the medium-sized crabs held large amounts of P_4-like substance in their hemolymphs. It has been suspected that the higher concentration of P_4 is required for sex differentiation; in fact, it has been reported that the ovaries and ovarian hormones (unspecified) enhanced gonad growth and the development of secondary sex characteristics in some crustaceans (Kleinhalz and Keller, 1979; Nagamine and Knight, 1981). Second, the hemolymphs prepared from egg-carrying crabs contained 1.0 ng of P_4-like substance per gram of w. b. w., while crabs analyzed a few days after the release of their eggs only had 0.14 ng/g of w. b. w.—one-fifth the level of their counterparts (unpublished results).

It has been reported that estradiol has been found in the hemolymphs and ovaries of some crustaceans (Donahue, 1940, 1948; Zerbib, 1976; Rateau and Zerbib, 1978; Sasser and Singhas, 1988). In addition, the follicle-stimulating hor-

mone (FSH), as well as the human chorionic gonadotropin (HCG), have both been found to enhance ovarian growth in crustaceans (Bomirski and Klek-Kawinska, 1976; Zukowska-Arendarczyk, 1981; Sarojiri *et al.*, 1985). The luteinizing hormone (LH) was also reported to trigger oogonia to undergo meiosis in the ovaries of sand shrimp (Zukowska-Arendarczyk, 1981). These findings suggest that ovarian growth or oogenesis might have relationships to or are affected by both steroid and gonadotropic hormones.

Sandor (1981) reported on a detailed biosynthetic pathway of the sex steroids in the arthropoda. That pathway, indicated P_4, E_2, testosterone, and other intermediate substances existed. However, at the present time, the location of sex steroid biosynthesis in decapods is not yet known for certain. Further analysis of the endocrinal tissues (such as ovary, hepatopancreas and adipose tissue) is required. Purification of sex steroids from the ovaries and hepatopancreases of female *U. arcuata* are underway. It is expected that more accurate estimates of sex steroids in this crab will provide clues to understanding its mechanisms of reproduction.

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弧邊招潮蟹 (*Uca arcuata*) 體液內性類固醇含量的全年型態

史 金 森

於 1987 至 1989 年間，按月採集臺灣省淡水紅樹林內的雌性弧邊招潮蟹，研究其生殖生理現象。從生殖腺重比值（GSI）及肝胰重比值（HSI）結果得知其生殖腺及肝胰臟重量自 1988 年 11 月開始增加，於次年一至三月間（生殖期前二至三個月）達到高峰，此時的 GSI 及 HSI 值各爲其他月份的四倍及二倍，但於生殖期（四至五月）後則下降，並與非生殖期間之值接近。弧邊招潮蟹的體液經放射免疫分析法的測定，發現體液中含有類助孕酮和類雌二醇。全年中體液內類助孕酮和類雌二醇的含量因月份而異，在元月至三月間（1988 及 1989），體液中此二激素的含量較多，其他月份則含量較少。生殖期間或非生殖期間，體液內類助孕酮的含量均較類雌二醇爲高。中型蟹（背甲寬爲 1.5–2.0 cm）體液中的類助孕酮含量甚高，爲大型或小型蟹（背甲寬各爲 2.0–2.5 cm 及 0.8–1.4 cm）的二至三倍。以上結果顯示於十一月間，淡水紅樹林雌性弧邊招潮蟹的卵巢和肝胰臟重量開始增加，卵巢在二至三月間成熟，同時體液內類助孕酮和類雌二醇的含量增多，可能是這些因素促成此蟹在四至五月間進行繁殖，報告中對弧邊招潮蟹的生殖生理有所討論。