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Progesterone-like Substance in Ovary and Hepatopancreas of *Uca vocans borealis*

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Jin-Taur Shih and Su-Shyan Tseng (1999) Progesterone-like substance in ovary and hepatopancreas of *Uca vocans borealis*. *Zoological Studies* **38**(4): 458-465. We study the temporal variation in amount of progesterone-like substance in tissues of the fiddler crab, *Uca vocans borealis*. Both HPLC and radioimmunoassay (RIA) were employed to determine the amount of a progesterone-like substance in extracts of ovary and hepatopancreas. The results show that, during the breeding period (egg-carrying, January-September), the progesterone-like substance concentrations in ovaries and in hepatopancreases of the crabs studied were 13.8-29.3 and 7.7-17.2 ng per gram of tissue, respectively; while the values dropped to 1.0-2.1 and 4.8-6.8 ng per gram of tissue, respectively, during the nonbreeding period (non-egg-carrying, October-December).

Key words: Crab, Ovary, Hepatopancreas, Progesterone-like substance.

The reproductive biology of the crabs *Uca arcuata* and *Mictyris brevidactylus* in the Tanshui mangrove swamp of northern Taiwan has been studied extensively (Shih 1992 1993 1997, Shih and Wang 1993, Shih et al. 1997). Both species ovulate only once a year. The gonadosomatic index (GSI) reaches a maximum during March-April for *U. arcuata* and November-December for *M. brevidactylus*. In addition, a progesterone-like substance was identified in their body fluid by radioimmunoassay (RIA). In both species, high concentrations of progesterone-like substance were found 1 or 2 mo before GSI rose to its maximum.

Although sex steroid-like substances have been located in tissues of crustaceans, the actual functions of these hormones have remained unclear (Lisk 1961, Kanazawa and Teshima 1971, Teshima and Kanazawa 1971, Sandor 1981, Couch and Hagino 1983, Fingerman 1987, Sasser and Singhas 1988 1992, Shih and Wang 1993, Shih 1997). Quackenbush (1992) found that progesterone applied to penaid shrimp ovary fragment stimulates protein synthesis. The steroid hormones in lobster ovary reportedly regulate yolk production (Quackenbush 1994).

A progesterone-like substance was identified in ovary and in hepatopancreas of *U. arcuata* and *M. brevidactylus*. The timing of the detected high levels of progesterone-like substance in ovary and in hepatopancreas of *M. brevidactylus* coincide with that of its maximal GSI (Shih 1997). In tissue extracts of ovary and hepatopancreas of *M. brevidactylus*, tritiated cholesterol can be converted to progesterone-like substance (Shih and Liao 1998). The highest conversion of cholesterol to progesterone-like substance by ovary and hepatopancreas occurred in *M. brevidactylus* collected in August (i.e., somatic growth period), and the conversion capacity began to decline during November-December (high GSI), and diminished completely during January-February (egg-carrying).

In this report, we study the identification and distribution of a progesterone-like substance in fiddler crab, *Uca vocans borealis*, which, unlike *U. arcuata* or *M. brevidactylus*, ovulates every 18-20 d during the breeding period (January to September, Shih and Tseng 1998). During the breeding period, more than 75% of crabs collected were bearing egg. This period lasted for 15 to 20 d. As soon as the eggs were released, the crabs began to breed another

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batch of eggs within 1 to 3 d. However, during the nonbreeding period spanning October until December, no collected female crabs bore any eggs. Thus, it became interesting to find out if endogenous factors, like hormones, may participate in regulating crab reproductive activities. In this work, a progesterone-like substance, identical to that found in both *U. arcuata* and *M. brevidactylus*, was detected in tissues of female *U. vocans borealis*. Their amounts in extracts of ovary and hepatopancreas were determined. The concentration of the progesterone-like substance was found to be higher during the breeding period than during the nonbreeding period.

MATERIALS AND METHODS

Chemicals

Organic solvents used were LC grade from Alps Chemical Company, Taiwan. Authentic steroids were from Sigma (St. Louis, MO, USA).

Extraction of steroids

Uca vocans borealis females (carapace width = 2.0–2.5 cm) were collected in the Tanshui mangrove swamp of northern Taiwan. According to Shih and Tseng (1998), during the breeding period (January-September) female crabs collected on the same date were categorized into one of 4 kinds: crabs carrying yellow, brown, black, or no eggs on their pleopods. In the nonbreeding period (October-December), collected crabs carried no eggs. Crabs were rinsed and wiped dry. After being chilled in a refrigerator (4 °C) for 20 min, crabs were weighed. Five to 10 crabs from each category were dissected to remove ovaries and hepatopancreases. Ovaries and hepatopancreases were weighed, pooled, and placed in absolute ethanol (1 g of tissue/30 ml ethanol, and kept in a refrigerator until homogenization).

The extraction of steroids from ovary and hepatopancreas was carried out using a published procedure (Shih 1997). Ovaries or hepatopancreases were homogenized in a mortar and filtered. The filtrate was extracted twice in 10 volumes of methanol and chloroform (1: 2, v/v). The steroids in chloroform were separated from ethanol and methanol by adding enough water to the mixture until the aqueous phase appeared. The chloroform fraction was air dried. This residue was dissolved in 70 ml methanol, mixed with 30 ml $CaCl_2$ (1.0 M), and refrigerated overnight. The precipitate was removed by filtering. The $CaCl_2$ precipitation procedure was performed twice. The steroids were extracted by dichloromethane. To each 40 ml of dichloromethane extract, 8 ml each of water, 0.1 N NaOH, and 0.1 N acetic acid were added and mixed in a separatory funnel. After removing the aqueous phase, the residue in the dichloromethane was evaporated to dryness and designated as the steroid extract used for this study. In order to check the efficiency of the extraction procedure, for each batch of samples, tritiated progesterone was added to 1 sample. The radioactivity of the progesterone was recorded at each step. The efficiencies found in this study were between 61.5% and 68.1%.

High-performance liquid chromatography (HPLC)

The HPLC system used for this study was composed of a Knauer HPLC pump (Type 364, Germany), a sample injector, a Spectral photometor,(Nr/No_o. 731 879), a Chromatocorder 11 (SIC, Japan) and a column of LiChroCART 259-4, RP-C18 (4.0 mm, 244 mm, Merck, Germany).

The gradient elution procedure was carried out according to Huang et al. (1983). The elution was first performed by an isocratic elution with solvent A (water: methanol: acetonitrile: isopropanol, 55: 32: 6.5: 7.5, v/v) for 15 min, followed by a linear gradient built up to 80% of solvent B (water: methanol: n-butanol, 40: 40: 20, v/v) within 35 min. If not otherwise stated, UV absorbance was recorded for all chromatograms at 254 nm; attenuation was set at 32 a.u./cm. The flow rate was 0.5 ml/min. The methanol solvent system used in this study was carried out according to Shih et al. (1990). In this system, except for the solvent being 80% methanol and the flow rate being 0.8 ml/min, other conditions of HPLC were the same as the gradient elution procedure.

For qualitative analysis, steroid standards were: progesterone (pregn-4-one-3,20-dione), estradiol (17-beta-estradiol), 17-alpha-OH-progesterone (4-pregn-17-alpha-ol-3,20-diole), and aldosterone (4-pregnen-18-alpha,11-beta,21-diol-3,20-dione). Steroid extract (1/4 of the extract) of crab was dissolved in 0.8-1.0 ml methanol, filtered through a millipore membrane (0.45 μm) and prepared for HPLC. A sample of 20 μl was injected for each run. For qualitative identification of the progesterone-like substance of steroid extract, a diluted steroid extract sample was prepared for HPLC, because the concentrated sample had a high OD background due to the pigmentation of the tissues (Shih and Liao 1998).

For quantitative analysis, after 100 ng of proges-

terone standard was cochromatographed with aldosterone, estradiol, and 17-alpha-OH-progesterone by HPLC with a gradient solvent system, 71.1 ng of progesterone (RIA detected) was recovered in the eluate with a retention time of progesterone (71.1% of the applied amount). When a methanol solvent system was used, 86.6% of the progesterone applied to the column was recovered. In this study, 2 solvent systems were used to analyze the identity of a progesterone-like substance in some steroid extracts. Usually a sample of 20 μl of steroid extract was injected for each run. Eluates which had OD_{254} peaks, including the progesterone-like substance, were collected. The eluates between retention times of sex steroid-like substances were also collected at 1- to 2-min intervals. In order to collect enough steroid from HPLC for an RIA assay, 4 to 5 runs were carried out for each steroid extract. All pooled eluates were air dried in preparation for RIA.

For calculation of the content of progesterone-like substance in tissue (Shih 1997), for instance, for 1 ovary sample of August 1995 (crabs carrying brown eggs, ovary weighing 2.71 g and its steroid extraction efficiency being 68.1%), 1/4 of the steroid extract was dissolved in 0.8 ml methanol; 80 μl of this sample was analyzed by HPLC of methanol solvent system; and the progesterone-like substance recovered from the eluate which had a retention time of the progesterone standard was 1.17 ng (RIA detected). For calculation of the progesterone-like substance of ovary, 1.17 ng was multiplied by 4 (1/4 of the extract was used for HPLC), and 10 (80 μl/800 μl), then divided by the efficiency of extraction (0.681), the recovery rate of HPLC (0.866), and the weight of the ovary (2.71 g). The calculated content of progesterone-like substance was 29.3 ng/g of ovary for the sample of August 1995 (Table 3). When the same sample was analyzed by HPLC using a gradient solvent system, the content of progesterone-like substance was 25.3 ng/g of ovary (Table 3).

Preparation of samples for RIA

Steroid extracts (1/4 of each steroid extract) and steroid fractions emerging from HPLC elution were dissolved in 1.0 ml of 0.1 M phosphate buffer saline (PBS, pH 7.4) (Shih and Wang 1993) containing 0.1% gelatin (PBSG). This mixture was incubated in a water bath set at 50 °C for 1 h before the RIA. All samples were tested for progesterone by using an Amerlex-M progesterone RIA kit based on a progesterone antibody prepared from sheep (Johnson and Johnson Clinical Diagnostics, Amersham, UK). The procedure for RIA was: 50-μl aliquots of standard, control, and samples were pipetted into the appropriate tubes; 500 μl tracer (^{125}I-progesterone) was dis-

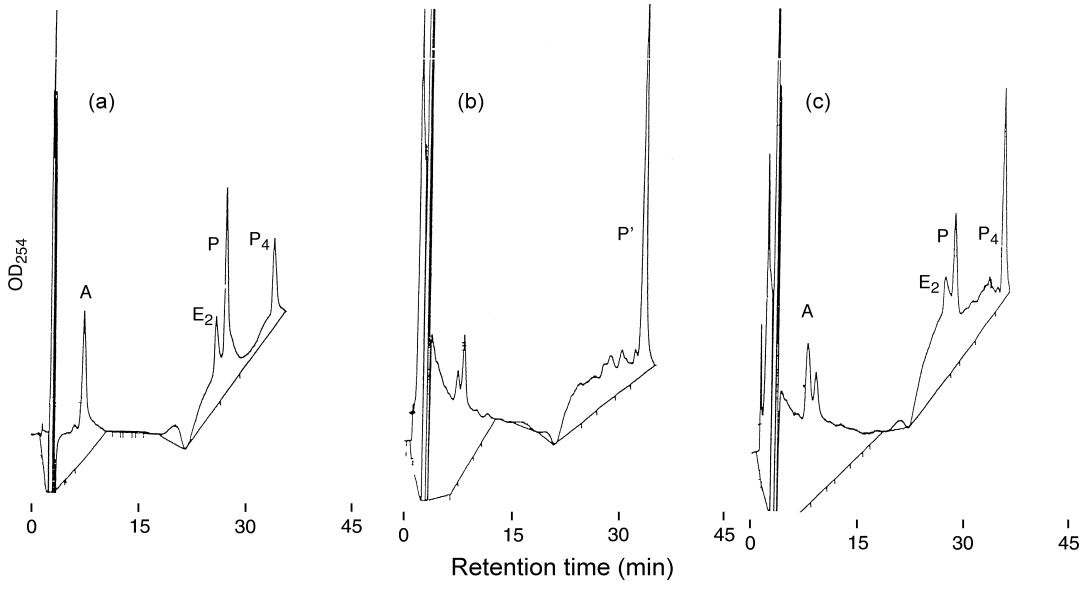

Fig. 1. HPLC chromatograms of steroid standards and ovary steroid extract of *Uca vocans borealis*. (a) Aldosterone (A), 17-beta-estradiol (E_2), 17-alpha-OH-progesterone (P), and progesterone (P_4, at 100 ng each, except for E_2 at 500 ng). The elution was first performed by an isocratic elution with solvent A (water: methanol: acetonitrile: isopropanol, 55: 32: 6.5: 7.5, v/v) for 15 min, followed by a linear gradient elution built up to 80% of solvent B (water: methanol: n-butanol, 40: 40: 20, v/v) within 35 min. UV absorbances were recorded at 254 nm and the attenuation was set at 32 a.u./cm. (b) Steroid extract of ovaries (crab carrying black eggs, August 1995) run on HPLC without steroid standards. P' represents a progesterone-like substance. (c) Sample of (b) run on HPLC with steroid standards.

pensed into the tubes, and radioactivities were counted. Then 500 µl of Amerlex-M antibody suspension was dispensed into all tubes; tubes were vortexed, covered, and incubated at 24 °C for 2 h; tubes were attached to the separator base and left for 15 min; fluid in the tube was decanted and drained for 5 min with blotting; and tubes were counted, and amounts of progesterone-like substance were calculated. The RIA kit had a working range for progesterone of 0.00-40.00 ng/ml of PBSG. In this study the observed concentration of progesterone-like substance was 0.01 to 32.00 ng/ml of PBSG. Any assay with a concentration below 0.01 ng/ml of PBSG was rejected.

RESULTS

Qualitative analysis of progesterone-like substance of crab tissues

As shown in figure 1a, the retention times for steroid standards, aldosterone (A), 17-beta-estradiol (E_2), 17-alpha-OH-progesterone (P), and progesterone (P_4) after gradient elution are 7.56, 27.23, 28.12, and 34.14 min, respectively. The ovary steroid extract from black-egg-carrying *U. vocans borealis* (August 1995) was analyzed by HPLC with the same gradient solvent system, and the results are shown in figure 1b. Some peaks with optical density (OD) were detected during the isocratic elution; a distinct peak (P') with a retention time of 33.90 min appeared in the gradient elution. To identify peak P', a chromatogram was taken for the ovary steroid extract with 4 steroid standards. As shown in figure 1c, P' of ovary steroid extract was identified as having a similar retention time (34.65 min) with progesterone (P_4). Although there were some small OD peaks a few minutes after the gradient elution started, no OD peak corresponding to estradiol (27.00 to 28.00 min) was recorded. When the hepatopancreas and body fluid steroid extracts from black-egg-carrying crabs (August 1995) were analyzed, the P' having a similar retention time as progesterone also appeared (Fig. 2a,b).

As shown in figure 3a, using the methanol solvent system, the retention times of aldosterone, 17-beta-estradiol, 17-alpha-OH-progesterone, and progesterone are 7.06, 10.98, 11.83, and 17.58 min, respectively. Using the same system, the ovary steroid extract from black-egg-carrying crab (August 1995) was analyzed and results are shown in figure 3b. The retention time for P' in figure 3b is 17.52 min. This ovary steroid extract with 4 steroid standards

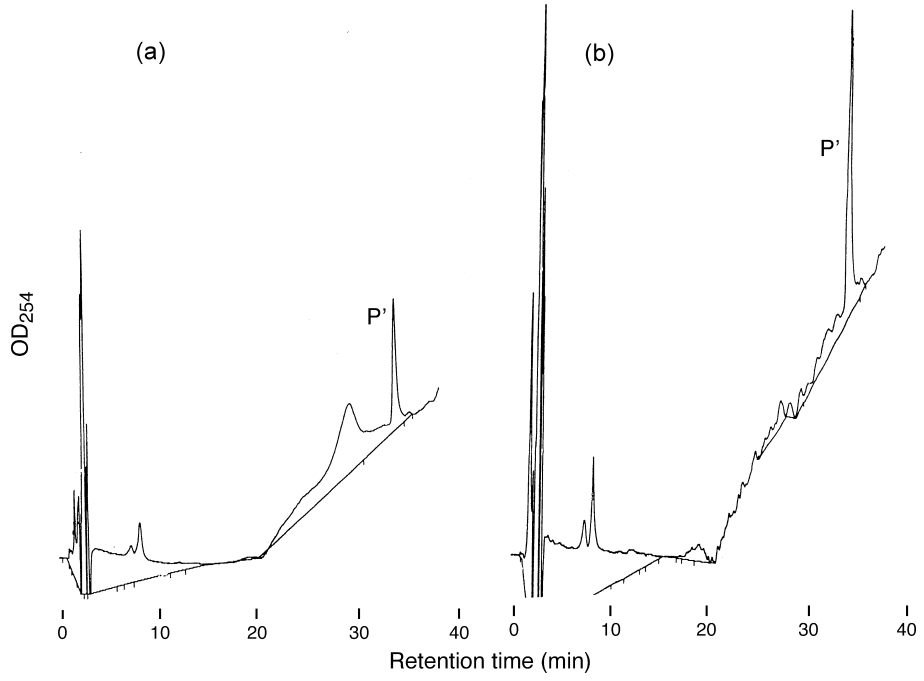

Fig. 2. HPLC chromatograms of hepatopancreas (a) and body fluid (b) steroid extracts from female *Uca vocans borealis* (carrying black eggs, August 1995). P' in (a) and (b) which had similar retention times as progesterone standard (P_4 of Fig. 1a) represents the progesterone-like substance of steroid extracts. The elution procedure was as described in figure 1.

was analyzed, and its chromatogram is shown in figure 3c. Again, P' of ovary steroid extract contained progesterone (17.59 min). Therefore, the P' of figures 1b (gradient elution) and 3b (methanol elution) was tentatively identified as a progesterone-like substance.

Quantitative analysis of progesterone-like substance in crab tissues

When the ovary steroid extract of brown-egg-carrying crabs (August 1995) was analyzed by HPLC with a methanol solvent system, each eluate was collected and analyzed by RIA (Table 1). Fraction no. 9 with a retention time of progesterone contained 1.17 ng of RIA-detected progesterone-like substance. Only a low level of the substance could be detected in other fractions. The same sample was analyzed on HPLC with the gradient solvent system, and 0.83 ng of the substance was collected and identified by RIA in the eluate where progesterone appeared (fraction 17, Table 2). Therefore, the eluate from the ovary extract collected with a progesterone retention time was identified as a progesterone-like substance.

After applying the correction factors (see Materials and Methods), contents of progesterone-like substance of ovary and hepatopancreas of female *U. vocans borealis* during the study period were tabulated (Table 3). During the breeding period, female crabs of August (1995) and April (1996) were analyzed. The concentrations of progesterone-like substance of yellow-, brown-, and black-egg-carrying crabs and those carrying no eggs were 26.6-27.8, 14.2-29.3, 21.6, and 13.8-21.1 ng/g of ovary, respectively, with no significant difference among them ($p > 0.27$). Concentrations of the progesterone-like substance were 7.9-10.8, 7.7-15.2, 11.2-

Table 1. Radioimmunoassays of eluates from HPLC (methanol solvent system) of *Uca vocans borealis* ovary steroid extract of August 1995 sample[a]

Fraction no.	OD_{254}	Retention time (nim)	Progesterone-like substance detected by RIA in eluate (ng)	Steroid standard emerging at this time
1	0.000	0.0-2.5	0.00	
2	0.063	2.5-5.0	0.01	
3	0.006	5.0-6.5	0.00	
4	0.002	6.5-7.5	0.00	A[b]
5	0.001	7.0-10.0	0.00	
6	0.012	10.0-12.0	0.03	E_2, P
7	0.000	12.0-14.0	0.00	
8	0.000	14.0-16.5	0.00	
9	0.028	16.5-17.8	1.17	P_4
10	0.005	17.8-19.0	0.01	
11	0.002	19.0-23.0	0.00	
12	0.008	23.0-27.0	0.02	
13	0.002	27.0-30.0	0.01	

[a]Ovary steroid extract (80 µl, without steroid standards) was analyzed for a progesterone-like substance by HPLC with a methanol solvent system. The elution procedure is described in "Materials and Methods".

[b]Abbreviations A, E_2, P, and P_4 represent aldosterone, 17-beta-estradiol, 17-alpha-OH-progesterone, and progesterone, respectively.

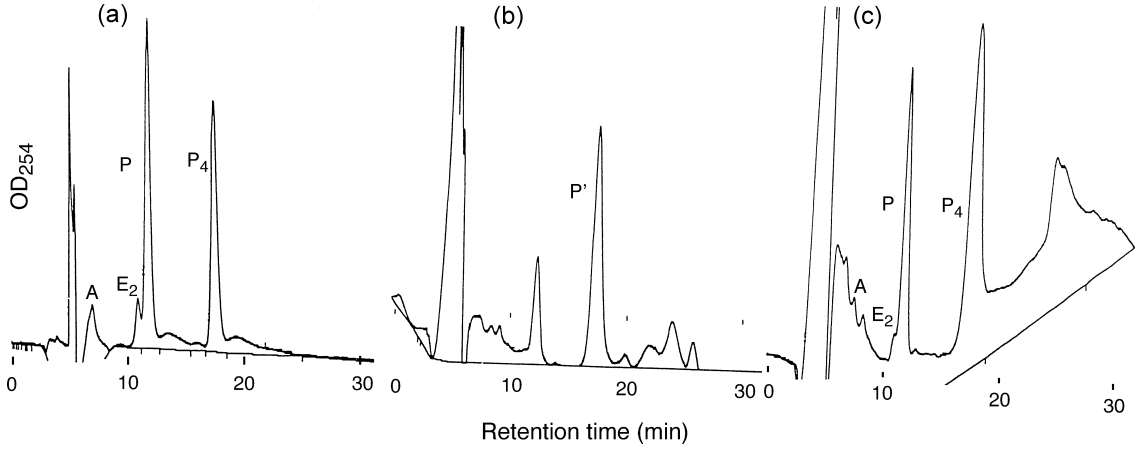

Fig. 3. HPLC chromatograms of steroid standards and ovary steroid extract of *Uca vocans borealis*. (a) Aldosterone (A), 17-beta-estradiol (E_2), 17-alpha-OH-progesterone (P), and progesterone (P_4, at 100 ng each, except for E_2 at 500 ng). The solvent was 80% methanol. The flow rate was 0.8 ml/min. UV absorbances were recorded at 254 nm, and the attenuation was set at 32 a.u./cm. (b) Steroid extract of ovary (crabs carrying black eggs, August 1995) runs on HPLC without steroid standards. P' represents a progesterone-like substance. (c) Sample of (b) runs on HPLC with steroid standards.

17.2, and 8.8-14.1 ng/g of hepatopancreas, respectively, with no significant difference among them ($p > 0.54$). However, both yellow- and brown-egg-carrying crabs contained more progesterone-like substance in ovary than in hepatopancreas ($p < 0.03$), while there was no significant difference ($p > 0.06$) in either black-egg-carrying or non-egg-carrying crabs.

During the nonbreeding period of November and December (1995), concentrations of progesterone-like substance in ovary and hepatopancreas were 1.0-2.1, and 4.8-6.8 ng/g of tissue, respectively. However, their concentrations are relatively low compared with those during the breeding period.

DISCUSSION

Female *Uca vocans borealis* has distinct annual breeding and nonbreeding periods (Shih and Tseng 1998). Each reproductive cycle lasts for 18-20 d during the breeding period. After ovulation, the eggs are retained on pleopods for 15-20 d. Ovulation in female crabs is asynchronized, and the colors of carried eggs correspond to different stages of egg development. They may range from yellow of the earliest embryo to brown and, finally, black which is the peak phase of the cycle. Crabs carrying yellow eggs had the lowest GSI value (0.15), while those carrying brown or black eggs contained higher values at 0.20, and 0.33, respectively.

The crabs studied were divided into 4 groups based on the colors of their eggs or absence of eggs, and the progesterone-like substance in ovary and hepatopancreas which we identified and analyzed (Table 3). We found no significant difference in concentrations in ovaries during the reproductive cycles among all 4 groups. In contrast, *M. brevidactylus* ovulates only once a year, and concentrations of progesterone-like substance in its ovary and hepatopancreas were minimal during the nonbreeding period (May to August) and rose gradually to maximum immediately before ovulation (December) (Shih 1997).

There were no significant differences in concentrations of progesterone-like substance in hepatopancreas among the groups studied. In crabs carrying yellow or brown eggs, however, the concentration of progesterone-like substance was lower in hepatopancreas than in ovary; while there was no difference in both tissues for crabs carrying black eggs or no eggs. The significance of this variation in these 2 tissues requires further study.

The progesterone-like substance was found in a concentration of 13.8-29.3 ng/g of ovary, which is comparative higher than that in *U. arcuata* (3.42 ng/g) (Shih and Wang 1993), but far less than that in *M. brevidactylus* (408.0 – 1791 ng/g) (Shih 1997). A similar trend was discovered in hepatopancreas of *U. vocans borealis* (7.9-15.2 ng/g), *U. arcuata* (0.53 ng/g), and *M. brevidactylus* (263.0 – 6638.0 ng/g).

During the nonbreeding period, female *U. vocans borealis* not carrying eggs retained GSI values (0.10-0.20) that were nearly half that at the peak phase, and low concentrations of progesterone-like substance were detected in both ovary and hepatopancreas. We determined their hepatosomatic index, and there was no difference between the samples studied during breeding and nonbreeding periods (Tseng 1996, Shih and Tseng 1998). Thus, crabs remained in apparent good health during the nonbreeding period.

The progesterone-like substance was also identified (Fig. 2b) in body fluid (hemolymph) of female *U. vocans borealis*. There was no difference in concentrations among groups collected during the breeding period (unpubl. data). We have reported before that the progesterone-like substance in body fluid rose to

Table 2. Radioimmunoassays of eluates from HPLC (gradient solvent system) of *Uca vocans borealis* ovary steroid extract of August 1995 sample[a]

Fraction no.	OD_{254}	Retention time (nim)	Progesterone-like substance detected by RIA in eluate (ng)	Steroid standard emerging at this time
1	> 0.500	0.0-2.4	0.03	
2	0.009	2.4-5.7	0.00	
3	0.012	5.7-7.5	0.04	A[b]
4	0.000	7.5-9.0	0.00	
5	0.000	9.0-11.0	0.00	
6	0.000	11.0-14.0	0.00	
7	0.000	16.0-19.0	0.00	
8	0.000	19.0-22.0	0.00	
9	0.000	22.0-24.0	0.00	
10	0.003	24.0-25.5	0.00	
11	0.004	25.5-27.2	0.00	
12	0.010	27.2-28.1	0.03	E_2
13	0.011	28.1-29.5	0.02	P
14	0.012	29.5-32.0	0.00	
15	0.012	32.0-33.0	0.00	
16	0.012	33.0-33.5	0.04	
17	0.054	33.5-35.0	0.83	P_4
18	0.012	35.0-40.0	0.00	

[a]Ovary steroid extract (80 µl, without steroid standards) was analyzed for a progesterone-like substance by HPLC with a gradient solvent system. The elution procedure is described in "Materials and Methods".

[b]Abbreviations A, E_2, P, and P_4 represent aldosterone, 17-beta-estradiol, 17-alpha-OH-progesterone, and progesterone, respectively.

a maximum 1 to 2 mo before ovulation in both *U. arcuata* and *M. brevidactylus* (Shih 1992 1997). Females of *U. vocans borealis* have a shorter reproductive cycle, however, progesterone-like substance concentration variations may not properly reflect changes in their body fluid.

A progesterone-like substance was detected in tissues of 3 mangrove crab species. Concentrations of hemolymphic progesterone-like substance of *U. arcuata* and *M. brevidactylus* were low and reached an apex before ovulation. Similar fluctuations of progesterone-like substance concentrations in ovary and hepatopancreas were found in these 2 species studied. In this report, we found that the progesterone-like substance concentrations in ovary and hepatopancreas of female *U. vocans borealis* were low in the nonbreeding period and rose to relatively higher concentrations during the breeding period. These results indicate that progesterone-like substance may play a role in the reproduction of these crabs. However, there are still 2 questions: (1) Is this progesterone-like substance synthesized endogenously? (2) Does this hormone have any function in reproduction of *U. vocans borealis*? In vitro experiments were carried out in which tritiated cholesterol was used as a precursor for sex steroid in crab tissues of female *M. brevidactylus* and *U. vocans borealis*. Among labeled steroids, a progesterone-like substance was identified with HPLC analysis (Shih and Liao 1998, unpubl. data). However, more quantitative studies are needed to support the possibility that *U. vocans borealis* can synthesize sex steroid endogenously. Further experiments have to be conducted to elucidate the function of progesterone

Table 3. Contents of progesterone-like substance in steroid extracts of ovary and hepatopancreas of *Uca vocans borealis* during 1995-1996[a]

Category of crab[b]	Content of progesterone-like substance (ng/g of tissue)	
	Ovary	Hepatopancreas
Breeding period:		
crabs carrying yellow eggs		
August 1995[c]	26.6 ± 2.5 (2)[d]	7.9 ± 0.9 (2)
April 1996[c]	27.8	10.8
crabs carrying brown eggs		
August 1995 (I)[c]	21.4	7.7
August 1995 (II)[c]	25.3	11.1
August 1995 (II)[e]	29.3	15.2
April 1996[c]	14.2	10.0
crabs carrying black eggs		
August 1995[c]	21.6 ± 1.9 (2)	11.2 ± 1.4 (2)
April 1996[c]	—[f]	17.2
crabs carryig no eggs		
August 1995 (I)[c]	9.7	8.8
August 1995 (II)[c]	13.8	13.0
August 1995 (II)[e]	18.1	9.1
April 1996[e]	21.1	14.1
Nonbreeding period		
November 1995[e]	2.1	6.8
December 1995[c]	1.0	4.8

[a]The identity of progesterone-like substance was determined, and the content of progesterone-like substance of tissues was calculated as described in “Materials and Methods”.

[b]According to Shih and Tseng (1998), more than 75.0% of collected females of *Uca vocans borealis* were egg-carrying during the breeding period (January-September). Crabs collected on the same date were categorized into 4 kinds: crabs carrying yellow, brown, black, or no eggs. In the nonbreeding period (October-December), non-egg-carrying females were collected.

[c]Samples (ovary and hepatopancreas) were analyzed by HPLC with a gradient solvent system. (I) and (II) were 2 steroid extract samples of August 1995.

[d]The number in parenthesis represent 2 steroid extracts prepared and assayed by HPLC with a gradient solvent system. Results are expressed as mean and standard deviations.

[e]Samples (ovary and hepatopancreas) were analyzed by HPLC with a methanol solvent system.

[f] Not determined.

in reproduction of this crab.

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北方呼喚招潮蟹(*Uca vocans borealis*)卵巢與肝胰臟中之類助孕酮

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成熟雌性北方呼喚招潮蟹卵巢和肝胰臟的膽固醇類，經生化方法萃取後，分別由高效能色層分析儀(HPLC)及放射免疫測定法(RIA)的鑑定，得知兩種組織中均有類助孕酮。此蟹在生殖期內（抱卵，1~9月）卵巢中類助孕酮的含量是 13.8~29.3 ng/g of ovary。肝胰臟之含量是 7.7~17.2 ng/g of hepatopancreas。在非生殖期內（不抱卵，10~12月），卵巢和肝胰臟中類助孕酮的含量均比生殖期少，其含量各為 1.0~2.1ng/g of ovary 及 4.8~6.8 ng/g of hepatopancreas。報告中對此蟹的生殖生理有所討論。

關鍵詞：蟹，卵巢，肝胰臟，類助孕酮。

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