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Crustacean Vitellogenesis: Its Role in Oocyte Development[1]

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SYNOPSIS. One of the major changes that occurs during the maturation of oocytes is the accumulation of yolk protein, or vitellin (Vn). To better understand how this process is regulated, we characterized the Vn of the ridgeback shrimp, *Sicyonia ingentis* (Penaeoidea). This Vn is a 322 kDa molecule composed of three subunits. Using purified Vn, we developed an anti-Vn antiserum and used it to characterize vitellogenin by Western blot analysis. The antiserum was also used in an ELISA to measure hemolymph levels of vitellogenin. Previous studies suggested the presence of vertebrate-type steroids might stimulate reproductive processes in decapod crustaceans. Treatment of sexually quiescent female shrimp with progesterone, hydroxyprogesterone, and estradiol did not increase hemolymph levels of yolk protein precursor. The absence of a response to these steroids may reflect the presence of other hormones (such as the gonad-inhibiting hormone) that prevent oocyte development. To examine the molecular basis for the regulation of vitellogenesis, ovarian and hepatopancreas expression cDNA libraries were screened using the anti-Vn antiserum. A 2.9 kilobase clone was isolated from both cDNA libraries suggesting that both tissues are sites of vitellogenin synthesis. These molecular tools should be useful for *in vitro* studies of vitellogenin synthesis.

INTRODUCTION

Oogenesis is an energetically expensive reproductive process that can be divided into several phases. The latter phases of oogenesis, periods characterized by the accumulation of yolk proteins in the growing oocytes and by significant increases in oocyte diameter, are referred to as primary and secondary vitellogenesis (Meusy and Charniaux-Cotton, 1984; Meusy and Payen, 1988). Vitellin (Vn) is the common form of yolk stored in oocytes and a nutrient source for developing embryos. In many species, vitellogenin (Vg), the precursor molecule to Vn, is transported through the hemolymph to developing oocytes, where it is sequestered and modified with the addition of polysaccharides and lipids into Vn. Thus, the synthesis of yolk proteins is a good indicator of female reproductive activity in many species. Furthermore, the presence of yolk proteins has been frequently used to study the hormones involved in the control of reproduction.

One organism that seems particularly suitable for such endocrinological studies is the ridgebacked shrimp, *Sicyonia ingentis* (Decapoda: Dendrobranchiata: Penaeoidea). This species is distributed between Isla Maria Madre, Mexico and Monterey Bay, California (Perez-Farfante, 1985) and is found at depths of 55–82 m (Frey, 1971). This shrimp can be obtained from commercial fisheries, and has proven to be easily handled in captivity. Since these shrimps are reproductive between June and November when they broadcast spawn during the phase of the new moon (Anderson *et al.*, 1985; personal observation), they are easy to study during the summer and autumn months. Before examining the effects of various hormones, we first characterized Vn, developed and characterized an anti-Vn antiserum, and used this antiserum to produce an ELISA (enzyme linked immunosorbant assay) to monitor changes in hemolymph levels of yolk protein precursor.

Characterization of vitellogenin and vitellin

Initiation of our investigations required the isolation, purification, and characteriza-

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TABLE 1. *Molecular mass of crustacean vitellin.*

Malacostraca – Decapoda			
Dendrobranchiata:			
Metapenaeus ensis		350 kDa	Qui *et al.*, 1997
Penaeus chinensis	V1	380	Chang *et al.*, 1996
	Vn2	500	Chang *et al.*, 1996
Penaeus japonicus		500	Vazquez-Boucard and Ceccaldi, 1986
Penaeus monodon		540	Quinitio *et al.*, 1990
		492	Chang *et al.*, 1993
Penaeus semisulcatus		283	Browdy *et al.*, 1990
Penaeus vannamei		289	Tom *et al.*, 1992
Sicyonia ingentis		322	Tsukimura *et al.*, 2000
Pleocyemata:			
Carcinus maenas		480	Andrieux and de Frescheville 1992
Emerita asiatica		505	Tirumalai and Subramonian, 1992
Palaemon paucidens		630	Nakagawa *et al.*, 1982
Homarus americanus		360	Tsukimura *et al.*, submitted
Pandalus kessleri		560	Quinitio *et al.*, 1989
Potamon potamios		551	Pateraki and Stratakis, 1997
Branchiopoda			
Triops longicaudatus		376	Riley and Tsukimura, 1998

tion of yolk proteins from *S. ingentis*. Vitellin was isolated from ovarian homogenates through differential centrifugation, and purified from other proteins by differential precipitation with increasing concentrations of saturated ammonium sulfate (Tsukimura *et al.*, 2000). The molecular mass (MM) of *S. ingentis* Vn was found to be 322 kDa ± 3 kDa by gel filtration chromatography. In the Decapoda, the MM of Vn ranges from 283 kDa to over 600 kDa, with the Dendrobranchiata possessing lower Vn MM than the Pleocyemata (Table 1). The Vn MM of *S. ingentis* is consistent within the Dendrobranchiata and the rest of the Decapoda.

Under denaturing conditions on SDS-PAGE, *S. ingentis* Vn separates into three subunits with MM's of 182 kDa, 91 kDa, and 85 kDa (Tsukimura *et al.*, 2000). The range in number of Vn subunits in the Dendrobranchiata and Pleocyemata is 2–8 (Table 2), most of which are between 2–4 subunits. The purified Vn was used to develop an anti-Vn antiserum. Western blot analysis was used to ascertain that the *S. ingentis* anti-Vn antiserum bound only the three Vn bands from oocytes and also cross-reacted with female hemolymph (Fig. 2). No bands were detected in male hemolymph. Most reports of hemolymph yolk proteins in crustaceans have used antisera developed against Vn and found that these proteins did not exist in males or in sexually quiescent females, and thus referred to the protein as Vg. This demonstrated that the Vn and Vg are immunologically related. Caution should be noted in examining results immunological results because lipid and carbohydrate moiety differences can cause proteins to be sufficiently dissimilar that antibody cross-reactivity is diminished or absent (Spaziani *et al.*, 1995). We also used the anti-Vn antiserum to develop an enzyme-linked immunosorbant assay (ELISA) for vitellogenin, with which we determined hemolymph levels of Vg that peaked at about 1 mg/ml during the final stages of oocyte development (Fig. 1). This hemolymph level of Vg is consistent with levels reported for other decapod crustaceans that range from 0.03–10 mg/ml (Table 3).

Hormonal regulation of reproduction

Classical eyestalk ablation experiments have demonstrated that malacostracan reproduction is under sinus gland control (Panouse, 1943; Brown and Jones, 1947; Stephens, 1952; Carlisle, 1953; Gomez, 1965). These and more recent studies have been extensively reviewed (Adiyodi, 1985; Fingerman, 1987; Chang, 1992; De Kleijn

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TABLE 2. *Number of vitellin subunits in crustacean yolk proteins.*

		# Subunits	
Pleocyemata:			
Astacidea:	*Homarus americanus*	3	Salares *et al.*, 1979
		3 prs	Tsukimura *et al.*, submitted
	Homarus gammarus	2 prs	Zagalsky 1985
	Ibacus ciliatus	3	Komatsu and Ando, 1992*a*
	Procambarus clarkii	5	Lui and O'Connor, 1976
Brachyura:	*Callinectes sapidus*	4	Lee and Watson, 1995
	Cancer antennarius	3	Puppione *et al.*, 1986
		3	Spaziani *et al.*, 1995
	Carcinus maenas	2+	Andrieux and de Frescheville 1992
	Charybdis feriata	2	Komatsu and Ando, 1992*b*
	Eriocheir japonica	2	Komatsu and Ando, 1992*b*
	Potamon potamios	3	Pateraki and Stratakis, 1997
	Uca pugilator	2	Eastman-Reks and Fingerman, 1985
Caridea:	*Macrobrachium rosenbergii*	2	Lee *et al.*, 1997
		4	Komatsu and Ando, 1992*b*
	Pandalus kessleri	2	Quinitio *et al.*, 1989
Dendrobranchiata:			
Penaeoidea:	*Penaeus chinensis*	5	Chang *et al.*, 1996
	Penaeus monodon	5	Quinitio *et al.*, 1990
		4	Chen and Chen, 1993
		8	Chang *et al.*, 1993
	Penaeus semisulcatus	4	Browdy *et al.*, 1990
		2	Tom *et al.* 1992
		3	Lubzens *et al.*, 1997
	Penaeus vannamei	4	Quackenbush, 1989*a*
		2	Tom *et al.*, 1992
	Sicyonia ingentis	3	Tsukimura *et al.*, 2000

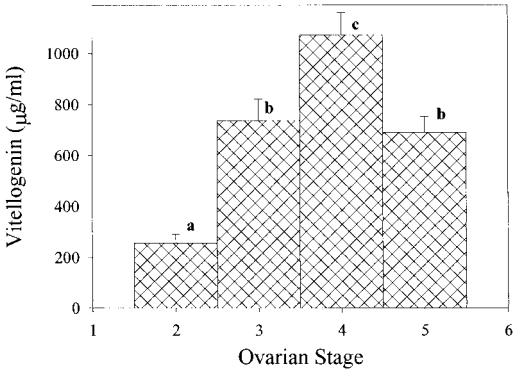

FIG. 1. Yolk protein concentrations by reproductive stage. The female *Sicyonia ingentis* were staged and hemolymph concentrations determined using the ELISA. The Vg levels significantly rise during stage 2–4, where it peaks before significantly decreasing in stage 5, which immediately precedes ovoposition. Means (±SEM) followed by the same letter are not significantly different (1-way ANOVA, Tukey's Test @ $P < 0.05$). From Tsukimura *et al.*, 2000.

and Van Herp, 1995). Briefly, the removal of the sinus gland, a neuralhemal organ that is situated at the peripheral end of the eyestalk allows simple removal by ablation techniques, has demonstrated that a gonad-inhibiting hormone (GIH) exists. This neuropeptide has been also referred to as the ovarian-inhibiting hormone (Klek-Kawinska and Bomirski, 1975; Kulkarni and Nagabhushnam, 1980), and the vitellogenin-inhibiting hormone (Gohar *et al.*, 1984; Soyez *et al.*, 1987; Aguilar *et al.*, 1992). In addition, the sinus gland has been suggested to contain vitellogenin-stimulating compounds (Junéra *et al.*, 1977; Picaud and Souty, 1980; Zerbib and Meusy, 1983; Charniaux-Cotton, 1985).

Eyestalk ablation can also result in molting (see reviews: Adiyodi, 1985; Chang, 1992). Thus, eyestalk ablation stimulates molting in some animals and reproduction in others. This suggests that intermediate factors may be involved in stimulating the

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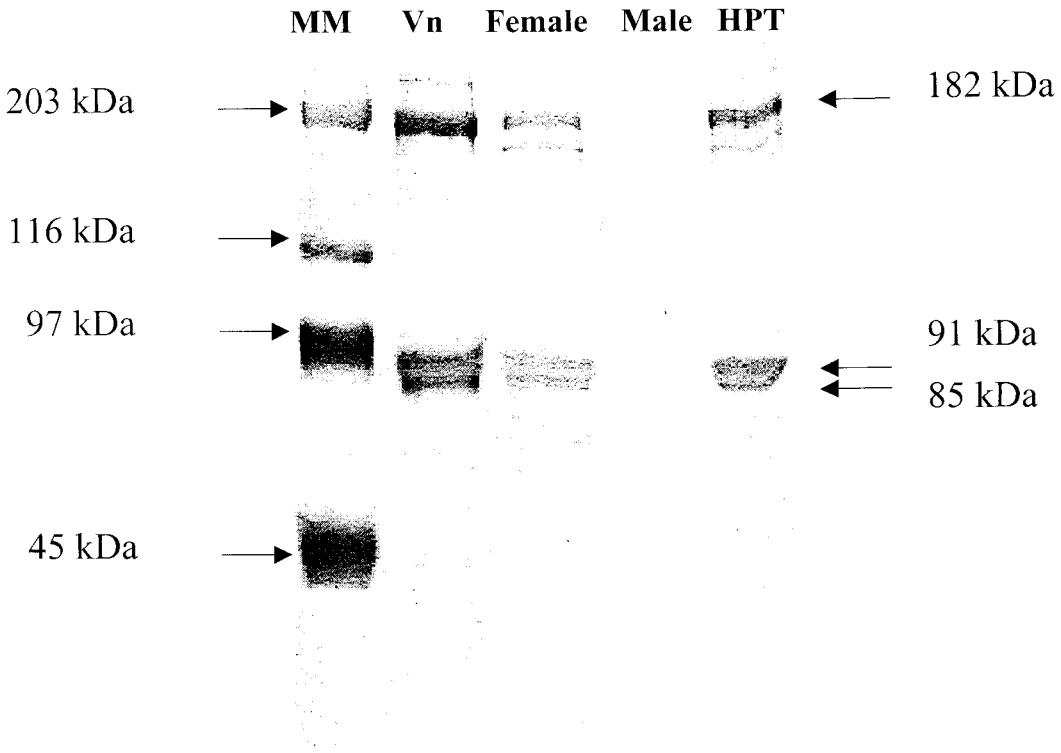

FIG. 2. Western Blot of *Sicyonia ingentis* hemolymph and vitellin. SDS-PAGE separation of yolk proteins demonstrates the presence of three subunits in female hemolymph and purified vitellin that are immunoreactive to the anti-vitellin antiserum. No immunoreactivity is observed with male hemolymph. Vn indicates vitellin, ''Female'' and ''Male'' indicate source of hemolymph, and HPT is a hepatopancreas extract. Molecular mass prestained markers are in the left column (BioRad: myosin, 203 kDa; β-galactosidase, 116 kDa; β-galactosidase, 97 kDa; ovalbumin, 45 kDa). From Tsukimura *et al.*, 2000.

reproductive system. Various tissues, including the brain, thoracic ganglion, and mandibular organ have been examined for substances that stimulate reproductive processes. The thoracic ganglia contain factors that stimulate or accelerate oocyte development (Otsu, 1960; Hinsch and Bennett, 1979; Kulkarni *et al.*, 1981; Sarojini *et al.*, 1982; Takayanagi *et al.*, 1986). Stimulation of sexually quiescent shrimp by extracts of the thoracic ganglion suggests that stimulatory factors exist (Otsu, 1963). Likewise, implantation of brain tissue into the crab, *Paratelphusa hydrodromous*, has been shown to accelerate oocyte development (Gomez, 1965). Brain extracts also enhanced oocyte growth in *Parapenaeopsis hardwickii, Macrobrachium kistnensis* and *Paratya compressa* (Kulkarni *et al.*, 1981; Sarojini *et al.*, 1982; Takayanagi *et al.*, 1986).

Another tissue that may be involved in the regulation of reproduction is the mandibular organ (MO). The histological appearance of the MO led to the suggestion that its activity increases during reproduction (LeRoux, 1974; Hinsch, 1980). Shortly thereafter, lipid based materials, either terpenoid or steroid, were shown to be produced by the MO (Laufer *et al.*, 1987; Couch *et al.*, 1987). Methyl farnesoate (MF), a terpenoid, will be discussed in detail in this volume (see: Borst *et al.*, 2001; Laufer and Biggers, 2001). The role MF in reproduction was originally inferred by correlating oocyte size and MF levels in the hemolymph (Borst *et al.*, 1987; Laufer *et al.*, 1987; and Borst *et al.*, 1995). More ma-

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TABLE 3. *Vitellogenin levels in crustacean hemolymph.*

		Stage	
Pleocyemata:			
Brachyura:			
Cancer antennarius	0.029 mg/ml	Hi*	Spaziani, '88
Callinectes sapidus	4.1	IV	Lee and Puppione, '88
Potamon potamios	10	Hi*	Pateraki and Stratakis, '97
Caridea:			
Macrobrachium rosenbergii	2.5 mg/ml	IV	Chang and Shih, '95
	2.9	III	Lee and Chang, '97
M. nipponense	9	D_0#	Okumura *et al.*, '92
Pandalus kessleri	3.6	Hi*	Quinitio *et al.*, '89
Astacidea:			
Homarus americanus	0.7 mg/ml	IV	Tsukimura *et al.*, submitted
Dendrobranchiata:			
Penaeoidea:			
Penaeus vannamei	0.9 mg/ml	V	Quackenbush, '89b
	7	V	Quinitio *et al.* '94
Penaeus japonicus	1.7	Hi	Yano, '87
Penaeus semisulcatus	0.44	IV	Shafir *et al.*, '92
Sicyonia ingentis	1.04	IV	Tsukimura *et al.*, '00

199: indicates highest amount reported by author(s).
#: data presented in molt stages.

nipulative data has shown mixed results for stimulating oocyte development (Table 4). Incubation of ovarian tissue with MF and the addition of MF to the diet have both been shown to stimulate ovarian development of *Penaeus vannamei* and *Procambarus clarkii* (Tsukimura and Kamemoto, 1991; Laufer *et al.*, 1998). However, no significant effects were detected in *Homarus americanus* and *Macrobrachium rosenbergii* when MF was injected into senescent females with MF (Tsukimura *et al.*, 1993; Wilder *et al.*, 1994). With the half-life of MF less than one hour, it is possible that the incidental presence of MF was insufficient to reinitiate reproduction. Conversely, MF incubations with fully active *T. longicaudatus* ovarian tissue might not be recognized because vitellogenesis was already near maximal capacity (Riley and Tsukimura, 1998).

One hypothesis (Laufer *et al.*, 1987) suggested that MF may act as a juvenile hormone-like compound that, as in insects, maintains juvenile morphology and enhances reproduction in adults. We have recently reported that MF significantly reduced the number of developing oocytes when administered continuously to juvenile tadpole shrimp, *T. longicaudatus* (Linder and Tsukimura, 1999). Our *Triops* data seem to supports Laufer and colleagues (1987) initial ideas that MF may act as a juvenilizing agent in Crustacea.

The ultrastructure of the MO led some investigators to suggest that the MO might

TABLE 4. *Effects of methyl farnesoate on crustacean reproduction.*

~ *Homarus americanus*	Tsukimura *et al.*, '93
~ *Macrobrachium rosenbergii*	Wilder *et al.*, '94
+ *Procambarus clarkii*	Laufer *et al.*, '98
+ *Penaeus vannamei*	Tsukimura and Kamemoto, '91
~ *Triops longicaudatus*	Riley and Tsukimura, '98
−* *Triops longicaudatus*	Linder and Tsukimura, '99

+ Reproductive Stimulation
~ No Effect.
−* Inhibition of Reproduction Following MF Administration During Juvenile Stages.

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TABLE 5. *Effects of steroids in Dendrobranchiata (Decapoda).*

Progesterone:	
+ *Parapenaeopsis hardwicki*	Kulkarni *et al.*, '79
+ *Metapenaeus ensis*	Yano, '85
+ *Penaeus vannamei*	Quackenbush, '92
~ *Penaeus vannamei*	Tsukimura and Kamemoto, '91
~ *Sicyonia ingentis*	Tsukimura *et al.*, '00
17α-hydroxyprogesterone:	
+ *Parapenaeopsis stylifera*	Nagabushanam *et al.*, '80
+ *Penaeus japonicus*	Yano, '87
+ *Penaeus vannamei*	Tsukimura and Kamemoto, '91
~ *Penaeus esculentus*	Koskela *et al.*, '92
~ *Sicyonia ingentis*	Tsukimura *et al.*, '00
17β-Estradiol:	
~ *Penaeus vannamei*	Tsukimura and Kamemoto, '91
+ *Penaeus vannamei*	Quackenbush, '92
~ *Sicyonia ingentis*	Tsukimura *et al.*, '00

produce steroid compounds (Yudin *et al.*, 1980; Hinsch, 1981). The presence of the vertebrate steroid progesterone was later reported in the MO (Couch *et al.*, 1987). In addition, the presence of the non-ecdysteroid steroid hormones has been reported in other crustacean tissues (Donahue, 1940; Lisk, 1961). Three steroids (17β-estradiol, progesterone, and 17α-hydroxyprogesterone) have been extensively examined for their effects on reproduction and have produced variable results (Table 5). Many of these studies used increases in the Gonad/Somatic Indices or ovoposition as an endpoint that might obscure immediate effects from injected hormones. We have expanded the database on the effects of steroid compounds in *S. ingentis* using the hemolymph levels of yolk proteins. For three consecutive days, sexually quiescent female shrimp were injected with one μg injections of progesterone, 17α-hydroxyprogesterone, or 17β-estradiol. None of the three steroid hormones produced a significant change in hemolymph vitellogenin levels during any of the seven days following the first injection. Using a similar approach, we have also tested these steroids (50 μg) into the American lobster, *H. americanus,* and found them to be ineffective in elevating hemolymph vitellogenin levels (Tsukimura, Waddy and Borst, unpublished data). One explanation for these results it that the endocrine environment of sexually quiescent females, which probably involves high levels of GIH, may have made these animals unresponsive to the steroid compounds. Studies on isolated tissues, physically separated from GIH, would clarify the effects of steroids on vitellogenesis. However, the tissues in which vitellogenin is synthesized has not been clearly demonstrated in many species.

Biological markers to identify endocrine compounds

One difficulty in identifying compounds that stimulate crustacean reproduction is the lack of adequate biological markers for reproduction. As we stated earlier, Vn can easily be purified and characterized. However, a problem with using the presence of yolk proteins as indicators of reproduction is that their presence in tissues does not clearly distinguish between synthesis, storage, or degradation. Indeed, the location of Vg synthesis has produced much controversy. This uncertainty makes difficult the examination of direct hormone effects upon incubated tissues. Previous studies suggest that the site of decapod crustacean vitellogenin synthesis is the ovary, the hepatopancreas or both (Table 6). The primary assay for these reports has been the cross-reaction of anti-Vn antibodies to antigens located in tissues, such as the ovary and hepatopancreas. In addition, recent experiments have examined the incorporation of labeled amino acids into proteins that might co-migrate with native yolk proteins or immunoprecip-

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TABLE 6. *Site of Crustacean Vitellogenin Synthesis.*

Dendrobranchiata:		
Penaeus vannamei	HPT & Ovary	Quackenbush, '92
Penaeus japonicus	Ovary	Yano and Chinzei, '87
Penaeus semisulcatus	HPT & Ovary	Browdy *et al.*, '90 Fainzilber *et al.*, '92 Shafir *et al.*, '92 Khayat *et al.*, '94*b*
Pleocyemata:		
Callinectes sapidus	Ovary	Lee and Watson, '95 Lee and Walker, '95
Carcinus maenas	HPT	Paulus and Laufer, '87
Homarus americanus	HPT and Ovary	Dehn, *et al.*, '83
Libinia emarginata	HPT	Paulus and Laufer, '87
Macrobrachium lanchesteri	HPT	Khoo *et al.*, '90
Macrobrachium rosenbergii	HPT	Sagi *et al.*, '95 Lee and Chang, '97
Procambarus	Ovary	Lui *et al.*, '74 Lui and O'Connor, '76
	HPT	Tuberty and Fingerman, '97
Scylla serrata	HPT	Rani and Subramonium, '97
Uca pugilator	Ovary	Eastman-Reks and Fingerman, '85

itate with anti-Vn antisera. There is no clear division of Vg synthesis sites between Decapoda taxonomic suborders (Dendrobranchiata and Pleocyemata).

Reports suggesting that Vg is synthesized in the ovary extend over a number of taxonomic levels from the Brachyura, Astacidea, Caridea and Penaeoidea (Lui *et al.*, 1974; Yano and Chinzei, 1987; Quackenbush, 1992; Browdy *et al.*, 1990; Fainzibler *et al.*, 1992; Khayat *et al.*, 1994*a*; Lee and Walker, 1995; Lee and Watson, 1995; Sagi *et al.*, 1995). Evidence indicating that Vg synthesis occurs in the hepatopancreas occurs from the same groups (Dehn *et al.*, 1983; Paulus and Laufer, 1987; Khoo *et al.*, 1990; Shafir *et al.*, 1992; Khayat *et al.*, 1994*b*; Sagi *et al.*, 1995; Lee and Chang, 1997; Rani and Subramonium, 1997; Tuberty and Fingerman, 1997). Recent attempts at obtaining Vg-mRNA have thus far been inconclusive. A 1.1 kb mRNA was found to be abundant in both the hepatopancreas and ovary, however the product was never shown to be Vg-mRNA (Khayat *et al.*, 1994*b*). Synthesis of Vg at an extra-ovarian site is corroborated by the discovery of Vg receptors (presumably receptors for endocytosis) in ovarian tissues of lobsters and crayfish (Jugan and Van Herp, 1989). Determining the site of Vg synthesis will permit a more precise examination of chemical mediators that stimulate and enhance Vg production.

To identify the site of Vg synthesis, we have used standard techniques (Chomczynski and Sacchi, 1987) to isolate Vg transcripts from both the ovary and hepatopancreas of *S. ingentis* (Pooyan, 1998; Pooyan *et al.*, 1999). Purity of the total mRNA extraction was significantly increased by performing additional phenol:chloroform extractions (Pooyan, 1998). Reduction in the number of transcripts that were screened was accomplished by synthesizing a 30-mer degenerate probe (biotinylated), which was generated from a ten amino acid residue sequence (N-terminus—**YITEANEVAF**—C-terminus) of the 182 kDa subunit of vitellin. These ten amino acids that produced the least degenerate probe were selected from a longer peptide sequence. The hybrid of transcript and biotinylated-probe were recovered using streptavidin covered magnetic beads. To construct partial cDNA libraries, the first strand was synthesized using AMV reverse transcriptase, and was completed with DNA polymerase I (Young and Davis, 1982). Standard procedures were used to insert the cDNA into an expression vector, λ gt-11 phage. The insertion site was into Lac Z region (β-galactosidase) of the

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phage, which allowed blue/white screening for successful insertions.

The cDNA libraries were screened using standard protocols (Sambrook *et al.*, 1989). We used the *S. ingentis* anti-Vn antiserum, which we developed earlier, to determine which of the positive colonies contained the Vg sequence. We found a 2.9 kilobase clone in both the hepatopancreas and ovary cDNA libraries, which suggests that both tissues are responsible for synthesizing Vg (Pooyan, 1998). These data are similar to that found in *Penaeus vannamei* using incorporation of label-amino acids (Quackenbush, 1989*a*). Future studies will be conducted to determine when Vg of these two tissues is expressed and if differential expression occurs during the reproductive cycles. We are currently in the process of determining the gene sequence of Vg and will compare the sequences between the hepatopancreas and ovary to determine their similarity. Obtaining the gene sequence will allow us to develop probes that will assist us in the assessment of hormonal control of vitellogenesis in *S. ingentis* and provide a model system with which other hormones can be tested in other crustaceans.

SUMMARY

Determining the regulation of crustacean vitellogenesis has been a difficult and protracted task. The increase in the number descriptions of vitellogenin and vitellin has increased the ability to comprehend the differences that exist in decapod crustaceans. Using *S. ingentis,* we have characterized the Vn as a 322 kDa molecule that separates into three subunits. Against the purified Vn, we developed an anti-Vn antiserum that we used to develop an ELISA to detect hemolymph levels of Vg. Injections of progesterone, hydroxyprogesterone and estradiol did not increase hemolymph levels of yolk proteins in sexually quiescent shrimp. A likely reason for this result is that gonad-inhibiting hormone may prevent any oocyte development.

The introduction of molecular techniques will augment the ability to study the regulation of yolk protein synthesis. As a start, through screening cDNA libraries, we have identified a 2.9 kilobase clone both the ovary and hepatopancreas as sites of Vg synthesis. We are currently obtaining a sequence for these clones, with which we will be able to screen a number of hormones in vitro to determine the efficacy on the stimulation of Vg transcription.

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REFERENCES

Adiyodi, R. G. 1985. Reproduction and its control. *In* D. E. Bliss and L. H. Mantel (eds.), *Integument, pigments, and hormonal processes. Biology of Crustacea, Vol. 9,* pp. 147–215. Academic Press, Inc. Orlando, Fla.

Aguilar, M. B., L. S. Quackenbush, D. T. Hunt, J. Shabanowitz, and A. Huberman. 1992. Identification, purification and initial characterization of the vitellogenesis-inhibiting hormone from the Mexican crayfish *Procambarus bouveri* (Ortmann). Comp. Biochem. Physiol. 102B:491–498.

Anderson, S. L., W. H. Clark, and E. S. Chang. 1985. Multiple spawning and molt synchrony in a free spawning shrimp (*Sicyonia ingentis*: Penaeoidea). Biol. Bull. 168:377–394.

Andrieux, N. and J. de Frescheville. 1992. Caratérization de la vitelline secondaire chez le Crustacé Brachyoure Carcinus maenas. C.R. Acad. Sci. Paris, Ser. III 314:227–230.

Borst, D. W., H. Laufer, M. Landau, E. S. Chang, W. A. Hertz, F. C. Baker, and D. A. Schooley. 1987. Methyl farnesoate and its role in crustacean reproduction and development. Insect Biochem. 17: 1123–1127.

Borst, D. W., B. Tsukimura, and M. Frinsko. 1995. Methyl farnesoate levels in the crayfish *Orconectes virilis.* In *Freshwater crayfish VIII,* pp. 462–

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474. Louisiana State University Printing Office, Baton Rouge, LA.
Borst, D. W., J. Ogan, B. Tsukimura, T. Claerhout, and K. C. Holford. 2001. Regulation of the crustacean mandibular organ. Am. Zool. 41:430–441.
Browdy, C. L., M. Fainzilber, M. Tom, Y. Loya, and E. Lubzens. 1990. Vitellin synthesis in relation to oogenesis in in vitro-incubated ovaries of *Penaeus semisulcatus* (Crustacea, Decapoda, Penaeidae). J. Exp. Zool. 255:205–215.
Brown, F. A. and G. M. Jones. 1947. Hormonal inhibition of ovarian growth in the crayfish, *Cambarus.* Anat. Rec. 99:657.
Carlisle, D. B. 1953. Studies on *Lysmata seticaudata* Risso (Crustacea: Decapoda) V. The ovarian inhibiting hormone and the hormonal inhibition of sex-reversal. Pubbl. Staz. Zool. Napoli 24:355–372.
Chang, C., F. Lee, and Y. Huang. 1993. Purification and characterization of vitellin from the mature ovaries of prawn, *Penaeus monodon.* Comp. Biochem. Physiol. 105B:409–414.
Chang, C.-F., S.-R. Jeng, M.-N. Lin, and Y.-Y. Tin. 1996. Purification and characterization of vitellin from the mature ovaries of prawn, *Penaeus chinensis.* Invert. Reprod. Dev. 29:87–93.
Chang, C.-F. and T.-W. Shih. 1995. Reproductive cycle of ovarian development and vitellogenin profiles in the freshwater prawns, *Macrobrachium rosenbergii*. Invert. Reprod. Dev. 27:11–20.
Chang, E. S. 1992. Endocrinology. *In* A.W. Fast and L. J. Lester (eds.), *Marine shrimp culture: Principles and practices*, pp. 53–91. Elvsevier Science Publishers, Amsterdam.
Charniaux-Cotton, H. 1985. Vitellogenesis and its control in malacostracan Crustacea. Amer. Zool. 25(1):197–206.
Chen, C. C. and S. N. Chen. 1993. Isolation and partial characterization of vitellin from the egg of the giant tiger prawn, *Penaeus monodon.* Comp. Biochem. Physiol. 106B:141–146.
Chomczynski, P. and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 161:156–159.
Couch, E. F., N. Hagino, and J. W. Lee. 1987. Changes in estradiol and progesterone immunoreactivity in tissues of the lobster, *Homarus americanus,* with developing and immature ovaries. Comp. Biochem. Physiol. 87A:765–770.
Dehn, P. F., D. E. Aiken, and S. L. Waddy. 1983. Aspects of vitellogenesis in the lobster *Homarus americanus.* Can. Tech. Rep. Fish. Aquat. Sci.#1161, pp. 24.
De Kleijn, D. V. P. and F. Van Herp. 1995. Molecular biology of neurohormone precursors in the eyestalk of Crustacea. Comp. Biochem. Physiol. 112B:573–579.
Donahue, J. K. 1940. Occurrence of estrogens in the ovaries of certain marine invertebrates. Endocrinology 27:149–152.
Eastman-Reks, S. B. and M. Fingerman. 1985. In vitro synthesis of vitellin by the ovary of the fiddler crab *Uca pugilator*. J. Exp. Zool. 233:111–116.
Fainzilber, M., M. Tom, S. Shafir, S. W. Applebaum, and E. Lubzens. 1992. Is there extraovarian synthesis of vitellogenin in *Penaeus* shrimp? Biol. Bull. 183:233–241.
Fingerman, M. 1987. The endocrine mechanisms of crustaceans. J. Crustacean Biol. 7:1–24.
Frey, H. W. 1971. California's living marine resources and their utilization. Calif. Dep. Fish Game Resour. Agency, pp. 148.
Gohar, M., C. Souty-Grosset, G. Martin, and P. Juchault. 1984. Mise en évidence d'une inhibition de la synthèse de la vitellogénine par un facteur neurohumoral (V.I.H.) chez le Crustacé Isopode terrestre *Porcellio dilatatus* Brandt. C.R. Acad. Sc. Paris, Ser. III 299:785–787.
Gomez, R. 1965. Acceleration of development of gonads by implantations of brain in the crab *Paratelphusa hydrodromous.* Naturwiss. 52:216.
Hinsch, G. W. 1980. Effect of mandibular organ implants upon the spider crab ovary. Trans. Amer. Micros. Soc. 99(3):317–322.
Hinsch, G. W. 1981. The mandibular organ of the female spider crab, *Libinia emarginata,* in immature, mature, and ovigerous crabs. J. Morph. 168: 181–187.
Hinsch, G. W. and D. C. Bennett. 1979. Vitellogenesis stimulated by thoracic ganglion implants into destalked immature spider crabs, *Libinia emarginata.* Tissue and Cell 11(2):345–351.
Jugan, P. and F. Van Herp. 1989. Introductory study of an oocyte membrane protein that specifically binds vitellogenin in the crayfish *Orconectes limosus.* Invert. Reprod. Develop., 16:149–154.
Junéra, H., M. Martin, A. Solari, and J.-J. Meusy. 1977. Détermination du poids moléculare de la vitellogénine et des lipovitellines d'*Orchestia gammarella,* Crustacé Amphipode. C.R. Acad. Sc. Paris 285:909–912.
Khayat, M., E. Lubzens, A. Tietz, and B. Funkenstein. 1994*a*. Cell-free synthesis of vitellin in the shrimp *Penaeus semisulcatus* (de Haan). Gen. Comp. Endo. 93:205–213.
Khayat, M., E. Lubzens, A. Tietz, and B. Funkenstein. 1994*b*. Are vitellin and vitellogenin coded by one gene in the marine shrimp *Penaeus semisulcatus*? J. Mol. Endo. 12:251–254.
Khoo, H. W., P. Y. Goh, and P. H. Tong. 1990. Possible extra-ovarian sites of yolk protein synthesis in the riceland shrimp, *Macrobrachium lanchesteri* (De Man). Occ. Papers Singapore Inst. Biol. 7:1–7.
Klek-Kawinska, E. and A. Bomirski. 1975. Ovary-inhibiting hormone activity in shrimp (*Crangon crangon*) eyestalks during the annual reproductive cycle. Gen Comp. Endocr. 25:9–13.
Komatsu, M. and S. Ando. 1992*a*. A novel low-density lipoprotein with large amounts of phospholipid found in the egg yolk of Crustacea sand crayfish *Ibacus ciliatus*: Its function as vitellogenin-grading proteinase. Biochem. Biophys. Res. Comm. 186:498–502.
Komatsu, M. and S. Ando. 1992*b*. Isolation of crustacean egg yolk lipoproteins by differential density gradient ultracentrifugation. Comp. Biochem. Physiol. 103B:363–368.

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Koskela, R. W., J. G. Greenwood, and P. C. Rothlisberg. 1992. The influence of prostaglandin E_2 and the steroid hormones, 17α-hydroxyprogesterone and 17β-estradiol on moulting and ovarian development in the tiger prawn, *Penaeus esculentus* Haswell, 1879 (Crustacea: Decapoda). Comp. Biochem. Physiol. 101A:295–299.

Kulkarni, G. K., R. Nagabhushanam, and P. K. Joshi. 1979. Effect of progesterone on ovarian maturation in a marine penaeid prawn *Parapenaeopsis hardwickii* (Miers, 1878). Ind. J. Exp. Biol. 17(9): 986–987.

Kulkarni, G. K. and R. Nagabhushanam. 1980. Role of ovary-inhibiting hormone from eyestalks of marine penaeid prawns (*Parapenaeopsis hardwickii*) during ovarian developmental cycle. Aquaculture 19:13–19.

Kulkarni, G. K., R. Nagabhushanam, and P. K. Joshi. 1981. Neuroendocrine regulation of reproduction on the marine female prawn, *Parapenaeopsis hardwickii* (Miers). Ind. J. Mar. Sci. 10:350–352.

Laufer, H., D. W. Borst, F. C. Baker, C. Carrasco, M. Sinkus, C. C. Reuter, L. W. Tsai, and D. A. Schooley. 1987. Identification of a juvenile hormone-like compound in a crustacean. Science 235:202–205.

Laufer, H., W. J. Biggers, and J. S. B. Ahl. 1998. Stimulation of ovarian maturation in the crayfish *Procambarus clarkii* by methyl farnesoate. Gen. Comp. Endocr. 111:113–118.

Laufer, H. and W. J. Biggers. 2001. Unifying concepts learned from methyl farnesoate for invertebrate reproduction and post-embryonic development. Amer. Zool. 41:442–457.

Lee, C.-Y. and R. D. Watson. 1995. In vitro study of vitellogenesis in the blue crab (*Callinectes sapidus*): Site and control of vitellin synthesis. J. Exp. Zool. 271:364–372.

Lee, F.-Y. and C.-F. Chang. 1997. The concentrations of vitellogenin (vitellin) and protein in hemolymph, ovary and hepatopancreas in different ovarian stages of the freshwater prawn, *Macrobrachium rosenbergii*. Comp. Biochem. Physiol. 117A:433–439.

Lee, F.-Y., T.-W. Shih, and C.-F. Chang. 1997. Isolation and characterization of the female-specific protein (vitellogenin) in mature female hemolymph of the freshwater prawn, *Macrobrachium rosenbergii*: Comparison with ovarian vitellin. Gen. Comp. Endocrinol. 108:406–415.

Lee, R. F. and D. L. Puppione. 1988. Lipoproteins I and II from the hemolymph of the blue crab *Callinectes sapidus*: Lipoprotein II associated with vitellogenesis. J. Exp. Zool. 248:278–289.

Lee, R. F. and A. Walker. 1995. Lipovitellin and lipid droplet accumulation in oocytes during ovarian maturation in the blue crab, *Callinectes sapidus*. J. Exp. Zool. 271:401–412.

LeRoux, A. 1974. Mise au point a propos de la distinction entre l'organe Y et l'organe mandibulaire chez les Crustaces eucarides. C.R. Acad. Sc. Paris, Ser. D 278:1261–1264.

Linder, C. J. and B. Tsukimura. 1999. Ovarian development inhibition by methyl farnesoate in the tadpole shrimp, *Triops longicaudatus*. Amer. Zool. 3: 20A.

Lisk, R. D. 1961. Estradiol-17β in the eggs of the American lobster, *Homarus americanus*. Can. J. Biochem. Physiol. 39:659–662.

Lubzens, E., T. Ravid, M. Khayat, N. Daube, and A. Tietz. 1997. Isolation and characterization of the high-density lipoproteins from the hemolymph and ovary of the penaeid shrimp *Penaeus semisulcatus* (de Haan): Apolipiproteins and lipids. J. Exp. Zool. 278:339–348.

Lui, C. W. and J. D. O'Connor. 1976. Biosynthesis of lipovitellin by the crustacean ovary. II. Characterization of and in vitro incorporation of amino acids into the purified subunits. J. Exp. Zool. 195: 41–52.

Lui, C. W., B. A. Sage, and J. D. O'Conner. 1974. Biosynthesis of lipovitellin by the crustacean ovary. J. Exp. Zool. 188:289–296.

Meusy, J. J. and H. Charniaux-Cotton. 1984. Endocrine control of vitellogenesis in Malacostraca crustaceans. *In* W. Engels (ed.), *Advances in invertebrate reproduction 3*, pp. 231–242. Elsevier Science Publ., Amsterdam.

Meusy, J. J. and G. G. Payen. 1988. Female reproduction in malacostracan Crustacea. Zool. Sci. 5:217–265.

Nagabhushanam, R., P. K. Joshi, and G. K. Kulkarni. 1980. Induced spawning in prawn *Parapenaeopsis stylifera* (H.Milne-Edwards) using a steroid hormone 17 Hydroxy-progesterone. Ind. J. Mar. Sci. 9(3):227.

Nakagawa, H., A. Salam, and S. Kasahara. 1982. Female-specific lipoprotein level in hemolymph during egg formation in freshwater shrimp. Bull. Jap. Soc. Sci. Fish. 48(8):1073–1080.

Okumura, T., C.-H. Han, Y. Suzuki, K. Aida, and I. Hanyu. 1992. Changes in hemolymph vitellogenin and ecdysteroid levels during the reproductive and non-reproductive molt cycles in the freshwater prawn *Macrobrachium nipponense*. Zool. Sci. 9: 37–45.

Otsu, T. 1960. Precocious development of the ovaries in the crab, *Potamon dehaani*, following implantation of the thoracic ganglion. Annot. Zool. Japon. 33(2):90–96.

Otsu, T. 1963. Bihormonal control of sexual cycle in the freshwater crab, *Potoman dehaani*. Embryologia 8:1–20.

Panouse, J. 1943. Influence de l'ablation du pédoncle oculaire sur la croissance de l'ovaire chez la crevette *Leander serratus*. C.R. Hebd. Seanc. Acad. Sci., Paris 217:553–555.

Pateraki, L. E. and E. Stratakis. 1997. Characterization of vitellogenin and vitellin from the land crab *Potamon potamios*: Identification of a precursor polypeptide in the molecule. J. Exp. Zool. 279: 597–608.

Paulus, J. E. and H. Laufer. 1987. Vitellogenocytes in the hepatopancreas of *Carcinus maenas* and *Libinia emarginata* (Decapoda Brachyura). Int. J. Invert. Reprod. 11:29–44.

Perez-Farfante, I. 1985. The rock shrimp genus *Si-*

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cyonia (Crustacea: Decapoda: Penaeoidea) in the Eastern Pacific. Fish. Bull. 83:1–79.

Picaud, J. L. and C. Souty. 1980. Démonstration par immunoautoradiographie de la synthése de la vitellogénine par le tissu adipeux de *Porcellio dilatatus* Brandt (Crustace', Isopode). C.R. Acad. Sc. Paris, Ser. D 290:1019–1021.

Pooyan, R. 1998. Determination of the site of vitellogenin synthesis in the ridgeback shrimp, *Sicyonia ingentis.* Master's Thesis, Department of Biology, California State University, Fresno.

Pooyan, R., K. C. Holford, and B. Tsukimura. 1999. Determination of the site of vitellogenin synthesis in the ridgeback shrimp, *Sicyonia ingentis.* Amer. Zool. 38:187A.

Puppione, D. L., D. F. Jensen, and J. D. O'Connor. 1986. Physicochemical study of rock crab lipoproteins. Biochem. Biophys. Acta 875:563–568.

Quackenbush, L. S. 1989*a*. Vitellogenesis in the shrimp, *Penaeus vannamei*: *In vitro* studies of the isolated hepatopancreas and ovary. Comp. Biochem. Physiol. 94B:253–261.

Quackenbush, L. S. 1989*b*. Yolk protein production in the marine shrimp *Penaeus vannamei.* J. Crustacean Biol. 9:509–516.

Quackenbush, L. S. 1992. Yolk synthesis in the marine shrimp, *Penaeus vannamei.* Comp. Biochem. Physiol. 103A:711–714.

Qui, Y. W., T. B. Ng, and K. H. Chu. 1997. Purification and characterization of vitellin from ovaries of the shrimp *Metapenaeus ensis* (Crustacea: Decapoda: Penaeidae). Invert. Reprod. Dev. 31:217–223.

Quinitio, E. T., A. Hara, and K. Yamaguchi. 1989. Identification and characterization of vitellin in a hermaphrodite shrimp, *Pandalus kessleri.* Comp. Biochem. Physiol. 94B:445–451.

Quinitio, E. T., A. Hara, K. Yamaguchi, and A. Fuji. 1990. Isolation and characterization of vitellin from the ovary of *Penaeus monodon.* Invert. Reprod. Dev. 17:221–227.

Quinitio, E. T., A. Hara, K. Yamaguchi, and A. Fuji. 1994. Changes in the steroid hormone and vitellogenin levels during the gametogenic cycle of the giant tiger shrimp, *Penaeus monodon.* Comp. Biochem. Physiol. 109C:21–26.

Rani, K. and T. Subramonium. 1997. Vitellogenesis in the mud crab *Scylla serrata*—an in vivo isotope study. J. Crustacean Biol. 17:659–665.

Riley, L. G. and B. Tsukimura. 1998. Yolk protein synthesis in the riceland tadpole shrimp, *Triops longicaudatus,* measured by in vitro incorporation of 3H-Leucine. J. Exp. Zool. 182:238–247.

Sagi, A., Y. Soroka, E. Snir, O. Chomsky, J. Calderon, and Y. Milner. 1995. Ovarian protein synthesis in the prawn *Macrobrachium rosenbergii*: Does ovarian vitellin synthesis exist? Invert. Reprod. Develop. 27:41–47.

Salares, V. R., N. M. Young, H. J. Bernstein, and P. R. Carey. 1979. Mechanisms of spectral shifts in lobster carotenoproteins. The resonance raman spectra of ovoverdin and the crustacyanins. Biochim. Biophys. Acta, 576:176–191.

Sambrook, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular cloning. A laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Sarojini, R., M. S. Mirajkar, and R. Nagabhushanam. 1983. Bihormonal control of oogenesis in the freshwater prawn, *Macrobrachium kistnensis*. Acta Physiol. Hung. 61(1):5–10.

Shafir, S., M. Ovadia, and M. Tom. 1992. *In vivo* incorporation of labeled methionine into proteins, vitellogenin, and vitellin in females of the penaeid shrimp *Penaeus semisulcatus* de Haan. Biol. Bull. 183:242–247.

Soyez, D., J. E. Van Deijnen, and M. Martin. 1987. Isolation and characterization of a vitellogenesis-inhibiting factor from sinus glands of the lobster, *Homarus americanus.* J. Exp. Zool. 244:479–484.

Spaziani, E. 1988. Serum high density lipoprotein in the crab, *Cancer antennarius* Stimpson: II. Annual cycles. J. Exp. Zool. 246:315–318.

Spaziani, E., W. L. Wang, and L. A. Novy. 1995. Serum high-density lipoproteins in the crab *Cancer antennarius*—IV. Electrophoretic and immunological analysis of apolipoproteins and a question of female specific lipoproteins. Comp. Biochem. Physiol. 111B:265–276.

Stephens, G. J. 1952. Mechanisms regulating the reproductive cycle in the crayfish, *Cambarus.* I. The female cycle. Physiol. Zool. 25(1):70–85.

Takayanagi, H., Y. Yamamoto, and N. Takeda. 1986. An ovary-stimulating factor in the shrimp, *Paratya compressa.* J. Exp. Zool. 240:203–209.

Tirumalai, R. and T. Subramoniam. 1992. Purification and characterization of vitellogenin and lipovitellins of the sand crab *Emerita asiatica*: Molecular aspects of crab yolk proteins. Mol. Reprod. Dev. 33:16–26.

Tom, M., M. Fingerman, T. K. Hayes, V. Johnson, B. Kerner, and E. Lubzens. 1992. A comparative study of the ovarian proteins from two penaeid shrimps, *Penaeus semisulcatus* de Haan and *Penaeus vannamei* (Boone). Comp. Biochem. Physiol. 102B:483–490.

Tsukimura, B. and F. I. Kamemoto. 1991. In vitro stimulation of oocytes by presumptive mandibular organ secretions in the shrimp, *Penaeus vannamei*. Aquaculture 92:59–66.

Tsukimura, B., F. I. Kamemoto, and D. W. Borst. 1993. Cyclic nucleotide regulation of methyl farnesoate synthesis by the mandibular organ of the lobster *Homarus americanus.* J. Exp. Zool. 265:427–431.

Tsukimura, B., J. S. Bender, and C. J. Linder. 2000. Developmental aspects of gonadal regulation in the ridgeback shrimp, *Sicyonia ingentis.* Comp. Biochem. Physiol. 127A:215–224.

Tsukimura, B., S. L. Waddy, J. M. Vogel, C. J. Linder, and D. W. Borst. (Submitted) Characterization and quantification of yolk proteins in the lobster, *Homarus americanus.*

Tuberty, S. and M. Fingerman. 1997. Extra-ovarian yolk protein production in the red swamp crayfish, *Procambarus clarkii.* Amer. Zool. 37:191A.

Vasquez-Boucard, C., H. J. Ceccaldi, Y. Benyamin, and C. Roustan. 1986. Identification, purification et caractérisation de la lipovitelline chez un Crus-

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tacé Decapode Natantia *Penaeus japonicus* (Bate). J. Exp. Mar. Biol. Ecol. 97:37–50.

Wilder, M. N., T. Okumura, Y. Suzuki, N. Fusetani, and K. Aida. 1994. Vitellogenin production induced by eyestalk ablation in juvenile giant freshwater prawn *Macrobrachium rosenbergii* and trial methyl farnesoate administration. Zool. Sci. 11: 45–53.

Yano, I. 1985. Induced ovarian maturation and spawning in greasyback shrimp, *Metapenaeus ensis*, by progesterone. Aquaculture 47:223–229.

Yano, I. 1987. Effect of 17a-hydroxyprogesterone on vitellogenin secretion in Kuruma prawn, *Penaeus japonicus.* Aquaculture 61:49–57.

Yano, I. and Y. Chinzei. 1987. Ovary is the site of vitellogenin synthesis in Kuruma prawn, *Penaeus japonicus.* Comp. Biochem. Physiol. 86B:213–218.

Young, R. A. and R. W. Davis. 1982. Efficient isolation of genes by using antibody probes. Proc. Natl. Acad. Sci. U.S.A. 80:1194–1198.

Yudin, A. I., R. A. Diener, W. H. Clark, and E. S. Chang. 1980. Mandibular gland of the blue crab, *Callinectes sapidus.* Biol. Bull. 159:760–772.

Zagalsky, P. F. 1985. A study of the astaxanthin lipovitellin ovoverdin isolated from the ovaries of the lobster *Homarus gammarus.* Comp. Biochem. Physiol. 89b:213–218.

Zerbib, C. and J. J. Meusy. 1983. Electron microscopic observations of the subepidermal fat-body changes following ovariectomy in *Orchestia gammarellus* (Pallas) (Crustacea: Amphipoda). Int. J. Invert. Reprod. 6:123–127.

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