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Endocrine Mechanisms in Crayfish, with Emphasis on Reproduction and Neurotransmitter Regulation of Hormone Release[1]

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SYNOPSIS. The neuroendocrine system of crustaceans, crayfish in particular, has been extensively studied. As among vertebrates, a wide variety of processes are hormonally regulated, including reproduction, growth, carbohydrate metabolism and color changes. The sinus gland, a neurohemal organ in the crayfish eyestalk, is the major neuroendocrine center. Herein, crayfish reproductive cycles and their hormonal control, particularly by the gonad-stimulating and gonad-inhibiting hormones, are a major focus. In addition, the aminergic and peptidergic neuroregulators identified in crayfish central nervous organs that either have been or likely may be shown to have neurotransmitter/neuromodulator roles in controlling hormone release are discussed with respect to localization and identified roles.

INTRODUCTION

The endocrine mechanisms and the reproductive system of crayfish have been studied extensively. Hart and Clark (1987) in their bibliography of crayfish "from Aristotle through 1985" list 602 references to the endocrine system, 236 to the physiology of reproduction and 180 to the morphology of the reproductive system. There is some overlap in these numbers in that some of the references to endocrine mechanisms deal with the hormones controlling the gonads. This paper will obviously be concerned with crayfish. But because some of the fundamental relevant discoveries upon which studies with crayfish were based were done with other crustaceans, where appropriate such investigations will be cited.

In this laboratory we have had a long-term interest in the endocrine mechanisms of crustaceans. More recently, we turned our attention to the neuroregulators that control the release of crustacean hormones, and most recently we have become increasingly interested in the reproductive system and seeking ways to facilitate aquaculture of commercially important crustaceans through manipulation of the hormones that regulate the reproductive system. Therefore, this manuscript will integrate three areas of investigation with crayfish that have occupied the interest of the members of this laboratory; namely the endocrine mechanisms, the hormonal regulation of the reproductive system, and the identified roles of neurotransmitters in controlling the release of hormones.

A BRIEF INTRODUCTION TO THE ENDOCRINE SYSTEM OF DECAPOD CRUSTACEANS, AND CRAYFISH IN PARTICULAR

The endocrine system of decapod crustaceans, including crayfish, consists of classical epithelial-type endocrine glands and endocrine structures of neural origin, the neurosecretory cells and neurohemal organs. The neuroendocrine component is of major significance with respect to the number and roles of hormones (neurohormones) produced. In fact, the large majority of crustacean hormones have a neural origin.

The endocrine structures and their secretory products

The medulla terminalis X-organ-sinus gland complex is the major endocrine control center. A sinus gland, a neurohemal organ, lies in each eyestalk of most stalk-eyed crustaceans, including crayfish. This

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gland is the storage and release site for several hormones, including the pigmentary effector hormones, crustacean hyperglycemic hormone, molt-inhibiting hormone, gonad-inhibiting hormone and the neurodepressing hormone.

The postcommissural organs are neurohemal axonal terminals of nerves which emerge from the postesophageal commissure that lies immediately posterior to the esophagus. These organs contain color change hormones.

The pericardial organs are neurohemal organs that lie in the pericardial cavity. They are terminations of axons that arise from thoracic ganglia, and are cardioexcitatory.

The Y-organs are classical non-neural endocrine glands. They are paired and are located in the maxillary segments. These glands are the source of alpha-ecdysone, which is converted peripherally to beta-ecdysone, the molting hormone.

The androgenic gland is also a non-neural endocrine gland. One is attached to each vas deferens. Its hormone is responsible for the differentiation and functioning of the male reproductive system and the secondary sexual characteristics of the males. The testes of crustaceans do not appear to be the source of any hormone; male secondary sexual characteristics are induced by direct action of the androgenic gland hormone on the tissues.

The ovary secretes the ovarian hormone. This hormone induces differentiation of the female secondary sexual characteristics.

The mandibular organ is an enigmatic structure that may be involved in molting and/or reproduction. The paired glands lie close to the Y-organs. The mandibular organs may have a role in stimulating vitellogenesis and/or stimulating ecdysteroid secretion by the Y-organs (Hinsch, 1980; Tamone and Chang, 1993; Laufer *et al.*, 1993).

Crayfish have been used extensively in studies of the hormonal control of molting. Because of the existence of an extensive literature on crayfish molting, rivalling if not exceeding that on reproduction, some of the molting studies will be briefly reviewed here. Certainly, the literature on the hormonal control of either molting or reproduction of crayfish exceeds that dealing with any other hormonally regulated function in crayfish. The hormonal control of molting in crustaceans has recently been reviewed by Chang *et al.* (1993), and that of crayfish follows the usual crustacean pattern. The sinus gland is the source of molt-inhibiting hormone. Bilateral eyestalk ablation brings on precocious molting activity (Brown and Cunningham, 1939; Scudamore, 1948; Yamamoto, 1960; Huner and Avault, 1977; Nakatani and Otsu, 1979, 1981). As with other arthropods, ecdysteroids induce molting in crayfish (Stevenson *et al.*, 1979; Keller and Schmid, 1979; Jegla *et al.*, 1983). At least in *Orconectes limosus,* the action of molt-inhibiting hormone appears to be mediated by cGMP, not by cAMP (Sedlmeier and Fenrich, 1993; Von Gliscynski and Sedlmeier, 1993).

REPRODUCTION

Roles of temperature and photoperiod

Several attempts have been made to determine the roles of temperature and photoperiod in the timing of reproductive cycles of crayfish. Stephens (1952), using female *Orconectes virilis,* in a study that extended from September through June, found that crayfish exposed to either normal day-night or a 20-hour photoperiod show cycles of ovarian development of similar frequency whereas in constant darkness no cyclic activity occurs, the ovary undergoing progressive maturation with maximum size being attained in February. These cycles in normal day-night or 20-hour photoperiods involve oocyte maturation processes alternating with yolk resorption and oocyte destruction. Aiken (1969), using the same species, extended these observations. He reported that ovarian development and maturation will occur in warm water (20°C) and darkness, and in cold water (4°C) coupled with a long photoperiod (17 or 18 hr). However, egg laying occurred only if the crayfish were exposed for 4–5 months to the low temperature (4°C) and complete darkness followed by exposure to spring temperature water, at least 12°C. He concluded that while temperature and photoperiod interact to control ovarian maturation, water

temperature controls egg laying. Dubé and Portelance (1992), using *Orconectes limosus,* found that the combination of a water temperature of 10–12°C and darkness induces ovarian maturation and egg laying. Once the ovaries mature, temperature alone stimulates egg laying; photoperiod has no effect. More recently, Portelance and Dubé (1994), disagreeing with Aiken (1969), reported that a 4–5 month period of cold water (7°C) exposure is necessary for ovarian maturation in *Orconectes virilis,* but like Stephens (1952) and Aiken (1969), found that darkness is not essential for this maturation process. Lowe (1961), working in this laboratory, investigated the reproductive cycle of the dwarf crayfish, *Cambarellus shufeldti.* She found two peaks of reproductive activity, the larger occurring in late winter-early spring and a secondary one in June. In *Cambarellus* long photoperiods induce a more rapid cycling of the ovaries, decreased temperature slows ovarian maturation, and elevated temperatures accelerate the process. Perryman (1969), using *Procambarus simulans,* found that crayfish exposed to a 20 hr photoperiod show a more rapid cycling of oocyte maturation and resorption of oocytes than crayfish exposed to a 12 hr photoperiod, as with *Orconectes virilis* (Stephens, 1952) and *Cambarellus shufeldti* (Lowe, 1961). Short days (6 hr photoperiod) and darkness stabilize the ovaries of *Procambarus simulans* in the mature condition, with delayed cycling. In contrast, in *Orconectes nais,* a short daylength (8.5 hr) stimulates ovarian maturation whereas a long daylength (15 hr) does not (Armitage *et al.,* 1973; Rice and Armitage, 1974).

Oogenesis

The development of oocytes of crayfish has been examined by several investigators. Vitellogenesis, yolk synthesis, is still not fully understood in that the site(s) of synthesis of vitellin (yolk protein) in crayfish is not clear. There is evidence, as will be described, that vitellin is synthesized by the crayfish ovary intraoocytically as well as by one or more extraovarian sites.

Kulkarni *et al.* (1991), in this laboratory, extended the earlier observations of Suko (1954) on the stages of oogenesis in *Procambarus clarkii,* by subdividing and describing the process in greater detail. Kulkarni *et al.* (1991) divided the entire ovarian cycle into seven stages. There are two early growth avitellogenic phases followed by three vitellogenic stages. The seven stages were named: (1) oogonial (<10 μm), (2) immature (10–65 μm), (3) avitellogenic (66–160 μm), (4) early vitellogenic (161–245 μm), (5) midvitellogenic (246–455 μm), (6) late vitellogenic (456–980 μm), and (7) postvitellogenic and resorptive. These measurements are oocyte diameters. Stage 1 is a cell multiplication stage. Oocyte maturation occurs during stages 2–6. Stages 4–6 are the yolk granule formation stages, with strongly acidophilic yolk granules accumulating in the cytoplasm. Yolk granules first appear at the periphery and then gradually spread throughout the cytoplasm. Oocyte size increases steadily up to stage 6, but in stage 7 the oocytes appear degenerative and they decrease in size. Van Herp (1992) diagrammed the ultrastructural changes that occur in oocytes of *Orconectes limosus* during vitellogenesis.

Ganion and Kessel (1972), using *Orconectes immunis,* found that tritiated leucine is incorporated into ovarian proteins throughout vitellogenesis. In the oocytes the initial incorporation occurs in the rough endoplasmic reticulum. Ganion and Kessel concluded that little, if any, vitellin has an extraovarian origin in this species. Wolin *et al.* (1973), in contrast, concluded that in *Procambarus clarkii* vitellin is extraoocytic in origin and is taken up by the oocytes primarily through pinocytosis. Such uptake occurs only in vitellogenic (developing) ovaries. On the other hand, Lui *et al.* (1974) and Lui and O'Connor (1976) concluded that the ovary of *Procambarus* sp. is capable of synthesizing vitellin and may not need to incorporate vitellogenin, the vitellin precursor, from the hemolymph, thereby tending to support Ganion and Kessel (1972). Later, Byard and Sigurdson (1979), studying a crayfish that was not named, found that vitellin of the crayfish they were using has two components, one that is taken up by pinocytosis from the hemolymph and one that is synthesized within the oocytes. In the same year, Zerbib (1979) came to

essentially the same conclusion. He reported that vitellin in the oocytes of *Astacus astacus* and *Astacus leptodactylus* is produced in two phases, initially by direct synthesis within the oocytes themselves and later in oogenesis by uptake of vitellogenin produced outside the ovary. More recently, Jugan and Van Herp (1989), using oocytes of *Orconectes limosus,* showed that the oocyte membrane has vitellogenin receptors, presumably used for uptake of vitellogenin from the hemolymph by receptor-mediated endocytosis. Kulkarni *et al.* (1991), using *Procambarus clarkii* also, found that ^{14}C-leucine is incorporated into ovarian proteins throughout vitellogenesis, as Ganion and Kessel (1972) found with tritiated leucine and *Orconectes immunis.* Some of this newly synthesized protein in the ovary of *Procambarus clarkii* might be incorporated into vitellin. Van Herp (1992) using oocytes in various stages of maturation from *Orconectes limosus,* found that pinocytotic incorporation of peroxidase along with the formation of coated pits occurs only during vitellogenesis, being most rapid during the early and middle stages of vitellogenesis.

Hormonal regulation

The endocrine control of crustacean reproduction has been investigated with a wide variety of species. Neurohormones as well as hormones that are products of classical endocrine glands are involved.

The decapod sinus gland is the source of gonad-inhibiting hormone (GIH); eyestalk removal results in precocious gonadal development, as first demonstrated by Panouse (1943, 1944) for *Palaemon serratus.* Although Panouse reported on the ovary only, this inhibitory hormone is also found in males (Otsu, 1963). Panouse's discovery of GIH has now been supported by data from many decapods, including the crayfish *Orconectes immunis* by Brown and Jones (1947), *Orconectes virilis* by Stephens (1952), *Procambarus clarkii* by Kulkarni *et al.* (1991) and *Procambarus bouvieri* by Aguilar *et al.* (1992).

A second decapod reproductive neurohormone, found in the brain and thoracic ganglia, is the gonad-stimulating hormone (Otsu, 1963; Gomez, 1965; Kulkarni *et al.*, 1984, 1991; Eastman-Reks and Fingerman, 1984; Yano, 1992).

Kulkarni *et al.* (1991) incubated ovarian explants of *Procambarus clarkii* with ^{14}C-leucine along with the eyestalk neuroendocrine complex and found leucine incorporation into ovarian protein was inhibited, presumably due to the action of GIH. However, when instead of eyestalk tissue, brains, subesophageal ganglia or thoracic ganglia were used, leucine incorporation increased, presumably due to GSH in these tissues.

Major advances are being made in studying the chemistry of GSH and especially GIH. Soyez *et al.* (1991) published the amino sequence of GIH from the American lobster, *Homarus americanus.* It consists of 77 amino acid residues, with a molecular weight of 9,135 Da and a free N-terminus. This GIH is structurally related to the crustacean hyperglycemic hormone and the molt-inhibiting hormone, the three forming a novel family of neuropeptides. More recently, Aquilar *et al.* (1992) reported the results of their study of GIH from sinus glands of *Procambarus bouvieri.* This GIH, while not yet sequenced, consists of 72–74 amino acid residues, has a molecular weight of 8,388 ± 2 Da and has a blocked N-terminus. While studies of the chemistry of GSH are still in their infancy, Yano (1993) reported that GSH from brains of maturing female shrimp, *Penaeus japonicus,* is a peptide that has a molecular weight of 1,000–2,000 Da.

The discovery of the role of the androgenic gland in the amphipod *Orchestia gammarellus* by Charniaux-Cotton (1954) paved the way for a large number of publications dealing not only with the anatomy but also the function of this gland in a wide variety of crustaceans. This gland controls not only differentiation of the male reproductive system, but also its functioning and the development of the male secondary sexual characteristics. Implantation of androgenic glands into a pubescent or juvenile female *Orchestia* masculinizes the ovaries and secondary sexual characteristics. On the other hand, andrectomy of very young male *Orchestia* and *Macrobrachium rosenbergii* results in the appearance of some oocytes (Charniaux-Cotton, 1964; Nagamine *et al.*,

1980*a, b*). There is no evidence for any hormone from the testes, in spite of attempts to demonstrate such a hormone. For example, an ovary transplanted into an intact or castrated male *Orchestia* transforms into a testis; but an ovary remains normal when transplanted into a male whose androgenic glands have been removed (Charniaux-Cotton, 1962). More recently, masculinization of an immature female *Procambarus clarkii* by androgenic gland implants has been demonstrated by Nagamine and Knight (1987*a*). In females GIH and GSH apparently act directly on the ovaries whereas in males their actions on the testes are indirect, affecting the testes only through a direct action on the androgenic glands. Miyawaki and Taketomi (1978) and Taketomi (1986) described the ultrastructure of the androgenic gland of *Procambarus clarkii.* This gland has a well developed rough endoplasmic reticulum, suggestive of a peptidergic-proteinaceous secretory product. However, the chemical nature of the androgenic gland hormone is still not agreed upon. One group of investigators, using *Carcinus maenas,* has claimed it is lipoidal, suggesting that the hormone is farnesylactone (Berreur-Bonnenfant *et al.,* 1973; Ferezou *et al.,* 1977, 1978; Berreur-Bonnenfant and Lawrence, 1984), whereas other investigators, using *Armadillidium vulgare,* have provided evidence that the hormone is a polypeptide or protein, with molecular weight estimates of 17,000 ± 800 and 18,300 ± 1,000 Da (Katakura *et al.,* 1975, Katakura and Hasegawa, 1983; Hasegawa *et al.,* 1993) and 1,200–8,000 Da (Juchault *et al.,* 1978) having been suggested. The 17,000 and 18,300 Da estimates are based on the fact that these Japanese investigators found two molecular forms of this hormone.

In contrast to the lack of demonstrated endocrine function for the crustacean testis, there is evidence for an ovarian hormone that is responsible for normal development of female secondary sexual characteristics (Nagamine and Knight, 1987*b*). However, the effects of vertebrate reproductive hormones on crustaceans have been observed. Such studies were presumably engendered by reports that crustacean gonads not only possess steroids more usually identified with gonads of vertebrates but also the enzymatic capacity synthesize vertebrate sex steroid hormones (Burns *et al.,* 1984*a*). Donahue (1940, 1948) found estrogens in the ovary of *Panulirus argus* and in eggs of *Homarus americanus*; and Burns *et al.* (1984*b*) showed that the testis of *Homarus americanus* contains testosterone. Human chorionic gonadotropin stimulates both oogenesis in *Crangon crangon* (Bomirski and Klek-Kawinska, 1976) and vitellogenin synthesis by the fat body in *Idotea balthica* (Souty and Picaud, 1984). Similarly, progesterone produces ovarian maturation in the prawn, *Parapenaeopsis hardwicki* (Kulkarni *et al.,* 1979), as do the mammalian gonadotropins, follicle-stimulating hormone and luteinizing hormone, in *Crangon crangon* (Zukowska-Arendarczyk, 1981). Most recently, Ghosh and Ray (1993*a*), using *Macrobrachium rosenbergii,* found that estradiol-17β is in the hemolymph of this prawn and that 17β-hydroxysteroid dehydrogenase activity in the ovaries increases during ovarian maturation. The same investigators (Ghosh and Ray, 1993*b*), with the same prawn, also found that estradiol-17β increases the malate dehydrogenase activity and glucose-6-phosphate dehydrogenase activity in the hepatopancreas.

Mandibular organs secrete methyl farnesoate, which can be converted to juvenile hormone (Laufer *et al.,* 1985; Borst *et al.,* 1985). Inasmuch as juvenile hormones are gonadotropic in many female insects, juvenile hormone-like compounds have been tested on female crustaceans, but do not promote vitellogenesis (Payen and Costlow, 1977; Hinsch, 1981). In *Rhithropanopeus* these analogs even produce the opposite effect, having a chemosterilizing action (Payen and Costlow, 1977). Spaziani *et al.* (1991) reported that prostaglandin $F_{2\alpha}$ can be synthesized from arachidonic acid by ovarian homogenates of *Procambarus paeninsulanis,* and suggested that prostaglandins may have a role in contolling vitellogenesis.

ROLES OF NEUROREGULATORS IN CRAYFISH HORMONE RELEASE

Identification and localization

By use of various techniques several neuroregulators that may have roles in regula-

tory hormone release have been identified and localized in crayfish neurons. Elofsson *et al.* (1966), using the Hillarp-Falck fluorescence method observed green fluorescent neurons, indicative by this method of catecholamines, and yellow fluorescent neurons, indicative of 5-hydroxytryptamine (5-HT, serotonin), in the protocerebrum, medulla externa, medulla interna and ventral nerve cord of *Astacus astacus*. The yellow fluorescent neurons were sparse compared with the relatively more abundant green fluorescing ones. Later, Elofsson *et al.* (1977*a, b*), using another crayfish, *Pacifastacus leniusculus,* observed not only green fluorescence in both the lamina ganglionaris and medulla externa of the eyestalk, but also such axons were seen running from the lamina ganglionaris to the medulla externa. Later, Elofsson (1983), using anti-5-HT antibodies and immunocytochemical techniques (the indirect immunofluorescence method and the peroxidase-antiperoxidase method) found immunoreactive neurons in the medulla externa, medulla interna, medulla terminalis and brain of *Pacifastacus leniusculus*. Both Elofsson (1983) and more recently Johansson (1991) have observed that in the brain of *Pacifastacus leniusculus* there are two sizes of neurons that exhibit 5-HT-like immunoreactivity. Johansson called them "small" and "giant." Such 5-HT localization studies with the aid of anti-5-HT antibodies have also been performed with other crayfish, namely *Orconectes virilis* by Sandeman and Sandeman (1987), *Cherax destructor* by Sandeman *et al.* (1988) and *Procambarus clarkii* by Fujii and Takeda (1988), Aréchiga *et al.* (1990) and Real and Czternasty (1990). Sandeman and Sandeman (1990) found that 5-HT-like immunoreactivity first appears in neurons in the brain of *Cherax destructor* when 75% of embryonic development is complete, and only during the first adult stage do all the 5-HT-containing neurons finally appear.

Elofsson *et al.* (1968) extended their earlier study of catecholamine-like fluorescence, and found that nerve fibers from the terminal abdominal ganglion of *Astacus astacus* that innervate the hindgut show a green fluorescence, but none showed a yellow fluorescence. Microspectrophotofluorometry gave evidence that this green fluorescence is due at least in part to norepinephrine, but the possibility that dopamine is also present could not be ruled out. Later, Elofsson and Klemm (1972), again with the Falck-Hillarp technique, found green fluorescing neurons in the optic ganglia of *Astacus astacus*. With the same technique Myhrberg *et al.* (1979) showed that dopamine is taken up by optic ganglia and the brain of *Pacifastacus leniusculus*. More recently with an anti-dopamine antibody Elekes *et al.* (1988) showed the presence of dopaminergic neurons in the last abdominal ganglion, in the intestinal nerve and in axons in the hindgut musculature of *Orconectes limosus*; and confirmed the presence of dopamine by high performance liquid chromatography (HPLC). Also, Mercier *et al.* (1991) by HPLC showed dopamine is present in the intestinal nerve of *Procambarus clarkii*.

5-HT was found by spectrophotofluorometry in the central nervous system of *Orconectes virilis* by Welsh and Moorhead (1960). Later, 5-HT, dopamine, octopamine and norepinephrine were found by HPLC in the central nervous system of *Pacifastacus leniusculus* by Elofsson *et al.* (1982) and Laxmyr (1984). More recently, in central nervous organs of *Procambarus clarkii,* 5-HT (Kulkarni and Fingerman, 1992*a*) and norepinephrine (Fingerman and Kulkarni, 1993) have been identified. β-endorphin-like material and β-endorphin degrading enzymes have been identified in the hepatopancreas of *Procambarus clarkii* by Hara *et al.* (1985) and Watabe *et al.* (1985).

Identified roles

5-HT produces hyperglycemia in intact *Orconectes limosus* but not in eyestalkless specimens (Keller and Beyer, 1968). Presumably, this action of 5-HT is effected by stimulating release of the crustacean hyperglycemic hormone from the sinus gland. Similarly, 5-HT produces hyperglycemia in intact *Astacus leptodactylus* (Strolenberg and Van Herp, 1977). The effect of 5-HT on color changes of the dwarf crayfish, *Cambarellus shufeldti,* has been determined by Rao and Fingerman (1975). 5-HT produced dispersion of the pigment in the erythrop-

hores, presumably by stimulating release of the red pigment-dispersing hormone.

The red pigment-concentrating hormone and red pigment-dispersing hormone are widely distributed in the central nervous system of *Cambarellus shufeldti* (Fingerman, 1957). Landau *et al.* (1989) found that the red pigment-concentrating hormone stimulates the mandibular organ of *Procambarus clarkii* to synthesize methyl farnesoate, and this synthesis is inhibited by the pigment-dispersing hormone. Multiple roles of these hormones, including functioning as specific neurotransmitters, can explain their widespread distribution throughout the central nervous system.

The sinus gland contains a neurodepressing hormone (NDH) (Aréchiga *et al.*, 1974, 1977). NDH depresses both sensory and motor functions. When applied to isolated neurons and ganglia NDH reduces neuronal responsiveness and spontaneous activity. Using the isolated eyestalk neuroendocrine complex from crayfish, *Procambarus clarkii* and *Procambarus bouvieri,* Aréchiga *et al.* (1985) found that GABA inhibits NDH release, whereas 5-HT stimulates its release.

Van Deijnen *et al.* (1985) did an immunocytochemical study of the optic ganglia of *Astacus leptodactylus* with antisera raised against peptides isolated from invertebrates and vertebrates. Cell bodies and/or axons reacted to anti-FMRFamide, anti-α-melanocyte stimulating hormone, anti-vasotocin, anti-gastrin, anti-cholecytokinin, anti-oxytocin, anti-secretin, anti-glucagon and anti-glucose dependent insulinotropic peptide antibodies.

In this laboratory we continue to have an ongoing interest in identifying the neuroregulators that control release of crustacean neurohormones. With respect to control of the gonads we have found with the fiddler crab, *Uca pugilator* (Richardson *et al.*, 1991; Kulkarni and Fingerman, 1992*b*), and *Procambarus clarkii* (Kulkarni *et al.*, 1992) that 5-HT stimulates ovarian maturation. With respect to the crayfish study, females were administered 15 μg/g body weight of norepinephrine, dopamine, octopamine or 5-HT on days 1, 5 and 10 and were sacrificed on day 15. Crayfish given 5-HT showed significant increases in ovarian index (30.5%) and oocyte size (34.0%) over the concurrent controls, while norepinephrine, dopamine and octopamine did not significantly affect either the ovarian index or oocyte size. But when ovarian explants were incubated *in vitro* with 5-HT added to the incubation medium, no significant change in the level of incorporation of ^{14}C-leucine into ovarian proteins occurred. This lack of effect is consistent with our hypothesis that the stimulatory action of 5-HT *in vivo* is an indirect one, presumably by stimulating GSH release.

Most recently in our laboratory (Sarojini *et al.*, 1993) we have shown with male *Uca pugilator* that *in vivo* 5-HT also stimulates testicular maturation. Like 5-HT, the 5-HT releaser, fenfluramine, and the 5-HT potentiator, fluoxetine, induced testicular maturation also, but the 5-HT receptor blocker LY53857 did not.

Although major advances have been made in our knowledge of the environmental and endogenous factors that regulate the crustacean reproductive system, there are still major gaps in our basic knowledge. In the foreseeable future, the basic information that continues to be gathered will facilitate the aquaculture of commercially important crustacean species.

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