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The Laboratory Studies of Sex Recognition in the Blue Crab <u>Callinectes</u> sapidus Rathburn

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Anton Robert Teytaud

Sea Grant Technical Bulletin Number 15

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The Laboratory Studies of Sex Recognition in the Blue Crab Callinectes sapidus Rathbun

Anton Robert Teytaud

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PREFACE

The Sea Grant Colleges Program was created in 1966 to stimulate research, instruction, and extension of knowledge of marine resources of the United States. In 1969 the Sea Grant Program was established at the University of Miami.

The outstanding success of the Land Grant Colleges Program, which in 100 years has brought the United States to its current superior position in agricultural production, was the basis for the Sea Grant concept. This concept has three objectives: to promote excellence in education and training, research, and information services in the University's disciplines that relate to the sea. The successful accomplishment of these objectives will result in material contributions to marine oriented industries and will, in addition, protect and preserve the environment for the enjoyment of all people.

With these objectives, this series of Sea Grant Technical Bulletins is intended to convey useful research information to the marine communities interested in resource development quickly, without the delay involved in formal publication.

While the responsibility for administration of the Sea Grant Program rests with the Department of Commerce, the responsibility for financing the program is shared equally by federal, industrial and University of Miami contributions. The study, The Laboratory Studies of Sex Recognition in the Blue Crab Callinectes sapidus Rathbun, is published as a part of the Sea Grant Program. Graduate research support was provided through a National Defense Education Act Fellowship.

TABLE OF CONTENTS

ACKNOWLEDGMENTS	111
LIST OF TABLES	vi
LIST OF FIGURES	vii
INTRODUCTION.	1
SUMMARY OF THE GENERAL BIOLOGY OF CALLINECTES SAPIDUS	4
SURVEY OF THE LITERATURE ON SEX RECOGNITION IN DECAPODS;	
EACLOSIVE OF THE FAMILY PORTUNIDAE.	9
Olfaction.	10
Contact Chemoreception and Tactile Stimuli	10
Sound	11
Vision	11
SURVEY OF THE LITERATURE ON SEX RECOGNITION IN THE PORTUNIDAE	<u>1</u> 4
MATERIALS AND MAINTENANCE: GENERAL	18
REPRODUCTIVE ETHOGRAM	20
Methods	20
Results.	21
I. Agonistic activities: threat and fighting.	21
(a) Chela lateral display	21
(b) Fighting	22
II. Agonistic activities: defense and armagement	20
(a) Defense reaction	23
(b) Submissive necture	23
	24
(a) (bolo lotorol displayed)	24
	24
b) Paddiing display.	24
(c) Failing-back display.	24
(a) ROCKING display	25
	25
(I) Carrying.	25
(g) Beating	25
(n) Copulation	26

IV. Sea	kual	ac	et:	ivi	it:	Ĺe	s:		fei	na i	le			-				•		٠		۲		,			26
(a)	Che	1a	14	a te	era	1	d ;	ĹS	p14	e y	•	٠		•													26
(b)	Wav	ing	g (dis	зрί	la j	у			-								-									26
(c)	Rocl	kiī	ng	đj	(s)	p1	a y																				26
(d)	Mo 1	tir	ng			•							_														27
(e)	Cop	ula	t:	ior	ລ	•		÷	٠	•			•	•					•			,					27
PRELIMINARY EX	(PER)	IM	EN	TS	01	N 4	SEX	K 1	RE	00	GN	LT:	loł	N		•	•	•		Ŧ	•	•	•		•	•	28
Blinding Ex	(per:	ime	ent	ts																					•	•	28
Olfaction	• •		٠	v	-				•				٠	÷		•	•				•	•	-				29
MODEL EXPERIME	ENTS	•		•	•		v	•	•	•	•		•	•	÷	•	•	•		•	•	•	•	•	٠	•	33
First Serie	≥s .																		•								34
Methods			•																								34
Results					•												•									•	40
Second Seri	ies																				-						42
Methods																	•										42
Results		•	•	٠	•	•	٠	•	•		•	•				•		•	•	•					•	•	44
DISCUSSION .	• •	•	•	•	•	•	•		•	•		•	•		•	•	•	٠		•	•	•		٠			52
SUMMARY	• •		•	•	•	•	۰	٠	•	•	•	-	•	•	•		٠	•	٠		•	•	•	-	•	•	57
LITERATURE CIT	ΈD	•	•	•	•	•	•		•	٠	•			•			•	•		•		•					60

LIST OF TABLES

Table	Page
1. Activity of Males Express	ed as Number of Lines Crossed
per 15 Minutes	••••••• 31
2. Activity of Females Expre per 15 Minutes	ssed as Number of Lines Crossed
3. Responses of Red-Sign Fem per 30 Minutes	ales Expressed as Number of Waves
4. Responses of Red-Sign Fem	ales Expressed as Number of Waves
per 30 Minutes Test Pa	ir (1)
5. Responses of Red-Sign Fem	ales Expressed as Number of Bouts
of Rocking per 30 Minut	es Test Pair (1) 45
6. Responses of Red-Sign Fem	ales Expressed as Total Duration
of Rocking (Seconds) To	est Pair (1) 45
7. Responses of Red-Sign Fem	ales Expressed as Number of Waves
per 30 Minutes Test Par	ir (2)
8. Responses of Red-Sign Femm	ales Expressed as Number of Bouts
of Rocking per 30 Minute	es Test Pair (2) 47
9. Responses of Red-Sign Femm	ales Expressed as Total Duration
of Rocking (Seconds) Te	est Pair (2)
10. Responses of Red-Sign Fem	ales Expressed as Number of Waves
per 30 Minutes Test Pa:	Lr (3)
പി. എട്ടോന്ടേരം വിഷ്ണികളുണ്ടാണ്.	es stressee sentimeesee sortes
Nocking per 30 Minutes	*≁16501Pa1f (8)⊒
12. Responses of Red-Sign Femal	es Expressed as Total Duration
of Rocking (Seconds) Tes	t Pair (3)

LIST OF FIGURES

Figur	*e	Page
1.	Examples of Male and Female Courtship Displays	22
2.	Models 1, 2 and 3, as Seen From the Position of an Animal in the Test Tank	36
3₊	Diagram of the Apparatus Used in the Model Experiments	37
4.	Responses of Red-Sign Females. Median Scores for Number of Waves, Number of Bouts of Rocking, and Total Duration of Rocking (Seconds). In Each Case, the Black Bar Represents Responses to Model 1	51

INTRODUCTION

The blue crab, <u>Callinectes sapidus</u> Rathbun, has long played an important role in the commercial fisheries of the eastern coast of the United States. This marine decapod crustacean is the basis of the largest crab fishery in America, with a total catch in 1969 of 129,900,000 pounds valued at \$12,200,000. Because of the importance of this fishery, an enormous literature exists on population dynamics, growth rates, fishing gears, fishery economics, and general biology and ecology of the blue crab (see Cronin, <u>et al.</u>, 1957, for an extensive bibliography). This species has also been studied to some extent by physiologists. However, as is the case with many commercial species, the ethology of <u>C. sapidus</u> is not well known, and published experimental studies of its behavior are few. This thesis concentrates on the problem of sex recognition during courtship, a subject about which little information is available for most groups of aquatic decapods.

I became interested in the problem of sex recognition in <u>Callinectes</u> after observing, in the field, a male <u>C. sapidus</u> performing an apparent visual display in courting a female. The male stood high on his walking legs, with both chelipeds spread wide apart, and waved his swimming paddles from side to side over the top of his carapace. At intervals he would throw himself backwards and kick up a cloud of sand. I subsequently observed a similar, if not identical, display performed by a male Callinectes marginatus.

The male's display aroused my interest for three reasons:

1) There is no adequate description of the courtship of <u>C. sapidus</u> in the literature. Like many other commercial species, the blue crab has been largely ignored by behavioral scientists.

2) It has long been accepted that sex recognition in the Portunidae is based mainly on tactile and chemical stimuli, and this is thought to be the general rule for most fully aquatic crustacea. Schöne (1968) states, "In general, mechanisms of social communication in aquatic forms [of decapods] seem to be based predominantly on tactile and perhaps chemical sensations, whereas in semi-terrestrial forms visual signs play an important role." This generalization is largely based on the older literature, much of which is inconclusive. In view of the complex and seemingly visually-mediated courtship display of <u>C. sapidus</u>, I felt that a re-investigation of sex recognition in this species would be of interest.

3) I wanted to find out if sex pheromones play a part in the courtship of <u>C. sapidus</u>, as such pheromones have recently been demonstrated (Ryan, 1966) in the closely related genus <u>Portunus</u>.

The specific objectives of this study were as follows: (a) to compile an ethogram (a detailed catalogue of species-typical action patterns) of the reproductive activities of <u>C. sapidus</u>, and (b) to determine experimentally the role of the olfactory, visual, and contact chemical senses in sex recognition, and to estimate the relative importance of each.

2

A possible role of sound or vibrational stimuli in the courtship of <u>C. sapidus</u> was not studied because of time limitations and a lack of appropriate equipment. This was not felt to be a serious omission, since neither the anatomy nor the behavior of this species indicates that a stridulatory mechanism is present. The crabs were never observed to rap on the substrate, nor did they perform any other action during courtship which suggested that auditory signals were being produced.

SUMMARY OF THE GENERAL BIOLOGY OF

CALLINECTES SAPIDUS

Many good reviews of the biology of the blue crab are available. I wish to provide here only a brief survey of the life history, particularly those aspects which are pertinent to the present study. More detailed information can be found in the following papers: (a) Hay (1905), Churchill (1919), Truitt (1939), and Van Engel (1958) for Chesapeake Bay area; (b) Porter (1956) for Delaware; (c) Darnell (1959) for Louisiana; (d) Daugherty (1952), and Pounds (1961) for Texas; (e) Futch (1965) and Tagatz (1968) for Florida.

<u>Callinectes sapidus</u> is a member of the brachyuran family Portunidae, or swimming crabs. It exhibits the morphological characteristics typical of most portunids, such as a transversely fusiform body and a pair of fifth pereiopods modified for swimming. Swimming is accomplished by a rapid sculling motion of the fifth pereiopods, the terminal segments of which are flattened and expanded to form oarlike blades. Swimming is sideways to the right or left, rarely forward or backward; the chela on the leading side is flexed while that on the trailing side is extended.

The blue crab is distributed in the western Atlantic from Nova Scotia to Uraguay (Rathbun, 1930). It is common on the East and Gulf Coasts of the United States from Massachusetts to Texas. Blue crabs are generally found in estuaries and in the ocean on bottoms of mud or fine sand from the shoreline to a depth of about twenty fathoms. They can tolerate a wide range of salinities, and in Florida they are known to penetrate long distances upstream in large rivers.

Because crabs are covered by a hard, calcified exoskeleton, an increase in size can occur only when the exoskeleton is shed in the molting process. In preparation for the molt, a new exoskeleton is formed beneath the old one, and in blue crabs it becomes visible through several parts of the old shell, especially the last two segments of the swimming legs. By observing the color of the new shell in these areas, it is possible to estimate when a crab will molt--a fact of considerable importance to the present study. Van Engel (1958) gives a good description of these so-called "shedding signs" which I quote here in part.

Around the outer edges of [the last two segments of the fifth perciopods] . . . are many fine hairs, called setae, at the base of which there is a thin, dark brown line which represents the outer edge of the hard shell. It is just inside this brown line that the color of the new shell can be observed. The earliest recognized color stage is the 'white rim' which requires the longest time to shed, from one to two weeks. The following stage, 'pink rim', may be expected to shed in three to six days, while the 'red sign' [crab] . . . will shed in one to three days.

<u>Callinectes sapidus</u> exhibits sexual dimorphism in a number of characters. The chelae are larger and heavier in males than in females. The fingers of the chelae are red on both the inner and outer surfaces in females, whereas in large males the fingers are white on the outer surface and blue on the inner, with only the extreme tips being red. Younger males have more red on the fingers than older males. However, the color of the fingers varies widely in both sexes.

Males may be easily distinguished from females (at least by humans) by the shape of the abdomen and by the abdominal appendages. The male's abdomen is shaped like an inverted T after the megalops stage and retains this shape throughout the life of the crab. Males have two pairs of abdominal appendages which are used in copulation; the shorter of the two pairs is usually inserted into the longer pair so that there appears to be but one pair of appendages. The abdomen of young females is triangular in shape and bears four pairs of small, hairless appendages. In mature females the abdomen is broadly rounded, almost semi-circular, and the four pairs of abdominal appendages are large and bear a dense fringe of hairs. Churchill (1919) lists other sexually dimorphic characters of the species, mostly minor differences in measurements.

this is to kill the crab and dissect out the sperm ducts, or to introduce a female that is ready to mate and see if the male responds.

A female which is about to undergo her puberty molt is picked up by a male while she is in the pink rim or early red-sign stage, and is carried around by the male for several days until she molts. The male carries the female by hooking his first walking legs under her carapace. At this time she is right-side-up with the top of her carapace against the underside of the male. The male relaxes his hold on the female as she sheds and hovers over her until she has extricated herself from the exuvia. Shortly after emerging from the old shell, the soft female turns herself over on her back (so that the ventral sides of both male and female are now in contact) and unfolds her abdomen to expose the two genital pores, into which the male inserts his copulatory appendages. Copulation may occur day or night and lasts from five to twelve hours or more. After copulation, the female is again carried right-side-up beneath the male for a period of two or more days, during which time her exoskeleton hardens. Females generally mate only once. Sperm will live in the female's seminal receptacles for at least one year, to be used as often as the female lays eggs (which she may do two or more times). Mating seems to occur year round in Florida (Futch, 1965; Tagatz, 1968; and Teytaud, personal observations).

Mating usually takes place in estuarine waters. The newly adult females then migrate to the ocean to spawn. According to Tagatz (1968), spawning in north Florida waters usually takes place within one to two months after mating in spring and summer; however, if 7

females mate in fall or winter, spawning is delayed until the warmer temperatures of the following spring. Females carry the eggs in a mass known as a "sponge" which is attached to the pleopods beneath the abdominal flap.

Upon hatching, the larvae live in the plankton until the megalops stage, at which time they take up a benthic existence. On transformation to the first crab stage, the young begin migrating from the estuaries to the mating grounds, where they will mature. The maximum life span is thought to be about three and one-half years, but the average is probably little more than a year and a half.

SURVEY OF THE LITERATURE ON SEX RECOGNITION IN DECAPODS; EXCLUSIVE OF THE FAMILY PORTUNIDAE

The problem of sex recognition in decapods has a long history in the literature, but much of the material is anecdotal and unsupported by experimental evidence. Controlled experiments to determine the nature of the factors involved in recognition have seldom been published. In several cases in which such work has been carried out, the design of the experiments leaves them open to other interpretations. The only group that has been well studied in regard to intraspecific sex recognition is the brachyuran family Ocypodidae. in particular those crabs belonging to the genus Uca. The semiterrestrial habits of these crabs and their complex visual displays have made them favorite subjects for behavioral research and good experimental work has been done on sex recognition in various species. The hermit crabs have recently become the object of a great deal of behavioral research; some of these studies also deal with sex recognition. Only scattered observations on sex recognition are available in the remainder of the order Decapoda, and the majority of these were not made within the context of a controlled experimental situation.

Rather than attempting an historical review of the literature on sex recognition, I shall present here a short summary of some relevant studies, grouped for convenience under the various sensory modalities by which information about the sex of the partner is received. A more detailed literature survey of the Portunidae will be given in the next section.

<u>Olfaction</u>

Many authors have postulated that sex recognition in various species is mediated by olfaction, i.e. the detection of chemical stimuli at a distance. However, in no case has this been demonstrated in a non-portunid decaped by controlled experiments. The receptors for distant chemoreception seem to be located on the antennules (Lockwood, 1967). Authors who have reported this type of recognition are: (a) Edwards (1966) for the cancrid crab <u>Cancer pagurus;</u> (b) Knudsen (1964) and Snow and Nielsen (1966) for <u>Cancer magister;</u> (c) Hughes and Mathiesson (1962) and McLeese (1970) for the lobster <u>Homarus americanus;</u> (d) Hazlett and Winn (1962) for the snapping shrimp <u>Synalpheus hemphylli;</u> (e) Knudsen (1964) for the grapsid crab

Contact Chemoreception and Tactile Stimuli

When recognition depends upon contact between the two partners, both contact chemoreception and touch are usually implicated. Most of the body surface of crustaceans is well supplied with receptors responding to tactile stimuli, while contact chemoreceptors are found on the antennules, mouthparts, and the dactyls of all walking legs and the chelae. Therefore it is extremely difficult in such cases to determine if the animal is using one or the other, or both, of these sensory modalities in sex recognition, and for this reason both are here considered together. Authors who have reported this type of recognition are: (a) Hoglund (1943) for the shrimp <u>Leander squilla</u>; (b) Forster (1951) for <u>Leander serratus</u>; (c) Burkenroad (1947) for the shrimp <u>Palaemonetes vulgaris</u>; (d) Carlisle (1959) for <u>Pandalus</u> borealis.

Sound

Many studies have showed that sounds (or substrate vibrations) are produced and received by numerous species of decapods. According to Frings and Frings (1968), in most cases the communication function of these sounds is questionable or unknown. No clear-cut evidence of their use in sexual signalling is available, with the exception of several species of <u>Uca</u> (Salmon and Atsaides, 1968). However, various authors believe that the sounds might be involved in either territorial or sexual signalling, or both. Some of these authors are: (a) Hazlett and Winn (1962) for snapping shrimp; (b) Linberg (1955) and Moulton (1957) for <u>Panulirus</u> spp.; (c) Salmon and Stout (1962), Salmon and Atsaides (1968), and Crane (1957) for <u>Uca</u> spp.; (d) Linsenmair (1967) for <u>Ocypode saraten</u>.

Vision

Visual recognition of sex has been reported by Bovbjerg (1960) for the crab <u>Pachygrapsus</u> <u>crassipes</u>, by Schöne and Schöne (1963) for the crab <u>Goniopsis</u> <u>cruentata</u>, and by Hazlett (1966) for the hermit crab <u>Coenobita</u> <u>clypeatus</u>.

The best studied crustacean visual displays are those of fiddler crabs. Males have a large claw which is often brightly colored, the females do not. This claw is used by males of most species in territorial (male to male) and sexual (male to female) signalling. The large claw is used in waving or beckoning movements which are extremely stereotyped and species-specific, combined with color changes and "dancing" in some species. As mentioned in the previous section, sound may also be produced, usually at times when visual display would be inappropriate.

This is not the place to review the voluminous literature on Uca, but a few of the more interesting details will be noted. Salmon and Stout (1962) tested Uca pugilator males with models, and found that any model possessing a major chela elicited aggressive behavior, but models and male crabs without a major chela, as well as females. elicited sexual display. Von Hagen (1962) using models, found that he could divide the area around a displaying male Uca tangeri into a series of concentric zones, and the approach of a female through each of these zones produced a distinctive form of display by the male. In the outer zones, the male would court any dark M-shaped object lacking a major chela and moving in a uniform horizontal manner, but inside of a certain radius it was also required to undergo rapid vertical movement. The relative lack of discrimination in U. tangeri males may be compensated by specificity in the female's response to the male's display. The males of each species of Uca have a very characteristic display pattern in regard to the color of the major chela and the type and rate of claw waving, and it is probable that females respond only to males of their own species (Hartnoll, 1969).

Males of two species of <u>Dotilla</u> (Ocypodidae) apparently also use claw waving to attract females (Altevogt, 1957). There is also a difference in coloration between the sexes in this species.

One of the most unusual and interesting visual displays occurs in <u>Ocypode saratan</u>. Males of this species construct a pyramid of sand from which a pathway leads to the spiral mating burrow. It is this pyramid, rather than any feature of behavior or morphology of the male, which functions as the primary courtship stimulus and directs receptive females to the mouth of the burrow (Linsenmair, 1967). The male stridulates only when the female is very close. This is a rare example of the use of a part of the environment by an animal for signalling, instead of signalling with its own body.

13

SURVEY OF THE LITERATURE ON SEX RECOGNITION IN THE PORTUNIDAE

The earliest published account of courtship behavior in the Portunidae seems to be that by J. D. Mitchell (<u>in</u> Rathbun, 1896) for <u>Callinectes sapidus</u>. Mitchell reported that a male, upon encountering a "breeding" female, "... will elevate himself on the tips of his legs, getting as high from the ground as possible, extend his claws to their widest extent, supporting himself with his paddles, and in this position he will strut slowly and pompously in front of her." Verrill (1908) reported that about 1860 L. Aggasiz made similar unpublished observations on <u>C. sapidus</u>.

In 1905, Hay published a summary of what was then known of the life history of <u>C. sapidus</u>, wherein he described the manner in which the male carries the female around prior to copulation, but he made no mention of a courtship display. Hay also believed male <u>C. sapidus</u> could distinguish those females which were about to molt, but he did not suggest a mechanism.

Chidester (1911) conducted experiments on sex discrimination using <u>Cancer irroratus</u> and three species of portunids: <u>Callinectes</u> <u>sapidus</u>, <u>Carcinus maenas</u>, and <u>Ovalipes ocellatus</u>. He tested sex discrimination in males by removing the soft females with which they were copulating and replacing them with hard males, hard mature females, and, in the case of <u>C. sapidus</u> only, females (both hard and soft) of the three other species. Chidester found that the males of all species tested attempted to copulate with any crab of the same species, male or female, hard or soft, which was introduced by the experimenter after removal of the original female partner. However, male <u>C. sapidus</u> would not attempt copulation with females of the other species. From these experiments Chidester concluded that the ability of the male crab to distinguish between the sexes is really a matter of inability to hold and copulate with males, and that sex discrimination depends upon tactual and kinesthetic cues.

Broekhuysen (1937) summarized the literature on sex recognition in decapods to that date, and conducted his own experiments with <u>Carcinus maenas</u>. He reported that male crabs took no notice of newly molted female crabs in the same container until they came into contact, when the females were immediately seized by the males. Broekhuysen concluded from this that there was no evidence for distant chemical attraction of the male by the female in that species. He also pushed males and females, both hard and soft, into contact with male <u>C. maenas</u>. The males tried to copulate with any crab pushed against them. From such experiments, Broekhuysen concluded that males were unable to distinguish hard from soft females, or females from males.

Veillet (1945) experimented with <u>Carcinus maenas</u>, and found that males seemed able to recognize premolt females, apparently by tactile and chemical stimuli on contact. Males tested any crab they came in contact with by pinching it with their chelae, but they would only carry females about to undergo their puberty molt, or those which had just molted and were still soft. He found no evidence for chemical attraction at a distance.

Finally, Ryan (1966), working with Portunus sanguinolentus, noticed that whenever he placed a premolt female in a cage containing several males, the latter began to walk about on the tips of their dactyls with their bodies elevated at maximum height, and extended their chelae. At such times males attempted to pull whatever crab they came into contact with, male or female, into the carrying position. When premolt females were absent, adult males never showed this "search" behavior or attempted to seize premolt males or premolt juvenile females, nor would they show such behavior toward premolt or soft females of two other species of portunids, Thalamita crenata and Podophthalmus vigil. Juvenile males never exhibited this search behavior. Ryan showed that search behavior could be elicited in adult males by siphoning water in which a premolt female had been kept into a tank containing the males. Hard, intermolt females served as controls, and the water in which these females had been kept produced no search behavior in the males. Furthermore, Ryan demonstrated that it was the urine of premolt females which contained the factor responsible for releasing the search behavior.

These observations of Ryan's are important in that they constitute the first well-controlled experimental demonstration of a chemical sex-attractant, or pheromone, in any decapod crustacean. Karlson and Butenandt (1959) have defined pheromones as "... substances that are secreted by an animal to the outside and cause a specific reaction in a receiving individual of the same species ..., for example, a definite behavior or a developmental process." In other words, pheromones are intraspecific chemical signals, and they function either by evoking immediate behavioral responses (releaser effects) or by means of endocrine-mediated effects (primer effects) that alter the physiology and subsequent behavior of the receiver. In addition to sex attractants, other classes of pheromones are known which function as: alarm substances, individual and group recognition signals, trail markers, aggregating or dispersing stimuli, territorial markers, and triggering stimuli for long-term physiological changes. None of these latter classes of pheromones has been demonstrated in any decapod crustacean to date.

MATERIALS AND MAINTENANCE: GENERAL

Most of the animals used in the present investigation were purchased from commercial trap fishermen operating in the Card Sound area on the east coast of South Florida. I personally collected a few crabs near Black Point on Biscayne Bay, Florida. Only prepubertymolt females, mature females, and mature males were used in the experiments.

All crabs were maintained in the laboratory at the Rosenstiel School of Marine and Atmospheric Sciences of the University of Miami. The crabs were kept in large water tables (180 cm X 90 cm X 25 cm) provided with sand and artificial seaweed for cover, and supplied with running seawater from Biscayne Bay. The water tables were filled to a depth of 18 cm.

Each water table was equipped with one 60 watt incandescent lamp, hung 38 cm above the water surface. These lamps were connected to an automatic timer which regulated the length of the photoperiod. During the period of preliminary observation of behavior, the timer was reset every two weeks to conform to the natural photoperiod. During the experimental phase of the work all animals were maintained on a schedule of 12 hours of light (0600 hours to 1800 hours) and 12 hours of darkness.

All animals were fed to satiety once every two days. Food usually consisted of fish scraps.

Mature males used in experiments were generally kept in the laboratory no longer than two weeks. Some animals used only as sources of scent were kept longer than this. Immature females were kept until they underwent the puberty molt.

During the experimental phase of the study, males, mature females, and immature females were maintained in separate water tables. All crabs were marked with identifying numbers on the back of the carapace by means of a waterproof felt-tip marker.

REPRODUCTIVE ETHOGRAM

Methods

Before an experimental analysis of courtship behavior could be undertaken, it was necessary to obtain an idea of the range of this type of behavior in <u>C. sapidus</u>. The method I used was to make a catalogue of the species-typical action patterns compiled from many hours of observation in the laboratory. Such a descriptive catalogue of action patterns is termed an <u>ethogram</u>.

Several early attempts to observe behavior in the field met with little success because of the poor underwater visibility in the estuarine areas where the blue crab is most abundant; I therefore decided that field observation was not a feasible approach. I found, however, that these crabs readily performed courtship and mating activities in the laboratory water tables and even in 20 gallon aquaria; I was thus able to carry out most of the work in the laboratory. Observations in the field were made as chance permitted.

Over a period of approximately nine months, observations were made on courtship and copulatory behavior as well as aggressive interactions. Observation periods covered both day and night and different temperature conditions in summer, autumn, and winter. Films made with a Kodak K-100 16 mm movie camera were used to supplement written descriptions of the action patterns. During observation and filming, an opaque plastic blind was used to shield the observer from view.

Results

The term <u>display</u>, as used below, is reserved for those action patterns which satisfy the following three criteria (Wright, 1968):

(a) The motor pattern is not directly functional in feeding, digging, insemination, or other non-communicatory activities.

(b) The occurrence of the motor pattern in time and space is such that it could usually be sensed by other animals and thus perform a communicatory function.

(c) The motor pattern is more than an unmodified incomplete movement from a directly functional motor pattern.

These three criteria arbitrarily exclude the communicative role of such things as variations in walking speed and body height (taken by themselves) from consideration.

The following catalogue of motor patterns constitutes a reproductive ethogram for <u>Callinectes sapidus</u>; not included are such activities as feeding, burrowing in the substrate, etc., which are not of interest in the context of the present investigation. As in many other species of crabs (Schöne, 1968), certain motor patterns are common to both agonistic and sexual activities; therefore both of these categories are included in the ethogram.

I. Agonistic activities: threat and fighting

(a) <u>Chela lateral display</u>.--The chelae are adducted so that, at the highest intensity of the display, they form almost a straight line with the meri of the chelipeds, which are also held laterally (see Fig. 1A). The fingers of the chelae are usually held open. This <u>display is usually given with the brim highly reduct similar</u> substrate. At lower intensities of the display the chelae are held pointing more or less anteriorly, but there seems to be no basis for

FIGURE 1

Examples of male and female courtship displays

A. Male performing the chela lateral and paddling displays. Note how the body is elevated to maximum height on stiffly extended walking legs. The swimming paddles are waved from side to side over the carapace, and the chelae are fully adducted.

B. Female performing the waving display while backing toward a male. Only one of the chelae is shown in motion, but both may be used.





A



dividing the display into discrete subtypes as Wright (1968a) has done for other species of crabs. I reject "<u>lateral merus</u>", Wright's (1968a) term for the same display, since in this species the meri are held laterally most of the time.

At times, only one chela may be extended. A crab giving the display usually faces another crab; this orientation exhibits the bright blue and white colors on the palms and distal ends of the meri in both sexes, and the bright red color of the fingers in females.

The chela lateral display is shown by males and females in both aggressive and sexual contexts.

(b) Fighting. -- Callinectes sapidus lacks the ritualized

<u>fighting.bakeminr.jupicslocfsome.ordermrack_lkevevevedesops</u> pouch. sexes engage in "wild fights" (Schöne, 1968), i.e. encounters which are relatively unstereotyped and consist of an irregular exchange of beats. The chelae are very powerful and fights often result in the loss of one or more appendages by one of the contestants.

II. Agonistic activities: defense and appeasement

(a) <u>Defense reaction</u>.--The meri of the chelipeds are held forward at about a 45 degree angle and the chelae are held in front of the mouthparts with the fingers open and pointing medially. Sometimes the fingers are interlocked. This reaction is generally seen as a response to aggressive thrusts of the chelae or to body contact by another crab. The chelae may be used to push the offending crab away. This pattern is shown by both sexes. (b) <u>Submissive posture</u>.--The chelae are fully flexed and held close against the mouthparts with the fingers closed, and the body is held very low (the abdomen may be in contact with the substrate). Motion, if any, is slow and creeping. This posture is seen in both sexes. It is adopted by: (a) individuals which have gotten the worst of an agonistic encounter, (b) females which are being carried by males, (c) crabs which have a tendency to flee.

III. Sexual activities: males

(a) <u>Chela lateral display</u>.--This display is given in sexual contexts as well as in agonism (see description under I. [a] above). The body is usually highly elevated. Males give this display to both males and females.

(b) <u>Paddling display</u>.--Both swimming paddles of the male are raised vertically over the carapace and turned so that the flat side of the blade faces anteriorly; they are then waved vigorously from seems best to regard them as three separate displays for the present. The falling-back display is given to both males and females.

(d) <u>Rocking display</u>.--The male rocks his body from side to side. It is typically seen in combination with the chela lateral display, and is given to both males and females.

(e) <u>Grab</u>.--The male seizes another crab with his chelae, holding it by any part of the body. The behavior preceding the grab is highly variable.

(f) <u>Carrying</u>.--Once the male has grabbed another crab he attempts to pull it under him in the carrying position. The male uses the first one or two pairs of walking legs to hold the other crab (which is right-side-up) against his ventral side. Males carry a female about in this way until she molts. The male's chelipeds are then held apart as in the chela lateral display, except that their tips are allowed to rest on the ground when the crabs are undisturbed. This action pattern is also performed after copulation.

Males may attempt to carry both males and females, but usually succeed only with premolt females or adult females which have recently molted. Other crabs struggle violently until they are released.

(g) <u>Beating</u>.--Upon being grabbed and placed in the carrying position by a male, a premolt female always exhibits a great deal of cheliped-waving. Males invariably react to such waving by beating on the female's chelae with their own chelipeds, until the female becomes quiescent and keeps her chelae flexed against her mouthparts in the submissive posture. (h) <u>Copulation</u>.--Copulation begins shortly after the female has molted, and is performed with the male uppermost and the female beneath him, ventral side to ventral side. Females usually turn themselves over when they are ready to copulate, but the males sometimes assist them. However, if a male grabs a soft mature female he will immediately turn her on her back and begin copulating. The copulatory appendages of the male are a pair of long, thin pleopods which he inserts in the genital pores on the sternum of the female.

IV. Sexual activities: females

(a) <u>Chela lateral display</u>.--As described under I. (a) above.
The chela lateral display is distinguished from the following motor
pattern IV. (b) by being a <u>prolonged</u> extension of one or both chelae.

(b) <u>Waving display</u>.--This display consists of a series of <u>relatively rapid</u> lateral extensions and flexions of one or both chelae, with the fingers open. A female may either face a male while performing the display, or turn her back toward him as in Fig. 1B. A highly motivated female will approach a male, then turn around and try to back under him, waving as she does so. The waving pattern is only seen in premolt females during courtship.

(c) <u>Rocking display</u>.--Premolt and recently molted females sometimes rock the body from side to side during courtship, as described for the male under III. (d). In the female, this action may be combined with the chela lateral and waving displays. (d) <u>Molting</u>.--The female molts while being loosely cradled in the male's walking legs, extricating herself from the exuvia without the help of the male. After she has molted, the female is picked up by the male and held for a short while in the carrying position before copulation begins.

(e) <u>Copulation</u>.--Shortly after molting the soft female begins to turn herself over on her back beneath the male. The method by which she does so is variable, and she may be assisted by the male. Once she is turned over on her back the female opens her abdominal flap, thus permitting the male to insert his copulatory appendeges.
PRELIMINARY EXPERIMENTS ON SEX RECOGNITION

Blinding Experiments

To determine whether vision is essential for pairing, I blinded mature male crabs by covering their eyestalks with opaque black plastic tape and allowed them contact with premolt females. The following is an account of a typical experiment with a blinded male. Experiments were conducted in a wooden tank (approximately 61 cm X 61 cm X 30 cm) with a slow circulation of seawater.

I introduced a red-sign female to the male's tank after allowing the male 24 hours to adjust to the tank and his blinded condition. In moving about the tank, the male came into contact with the female, whereupon he immediately grabbed her and placed her in the carrying position. The female offered no resistance.

I then separated the above pair and removed the female from the tank, replacing her with the molted exoskeleton of another female of similar size. I pushed the exoskeleton into contact with the male, but he made no attempt to grab it; he pushed it away with his chelae instead. This was repeated three times, with the same results. The male also made no attempt to grab the wooden handle of a dipnet when I gently touched him with it.

I next placed a small male in the tank, after removing the exoskeleton. I pushed this male into contact with the blinded male, whereupon the blinded male grabbed the other and tried to place him in the carrying position. The smaller male resisted violently and escaped. The blinded male then began paddling vigorously, and continued paddling after I removed the smaller male.

This series of tests was repeated four times, using different crabs, with essentially the same results. The blinded males reacted to contact with another crab of <u>either</u> sex by attempting to pull it into the carrying position, but would not respond in this way to contact with the cast exoskeleton of a premolt female, or to contact with inanimate objects such as the handle of a dipnet. In no case did any male show a sexual response prior to touching another crab.

Five different red-sign females were blinded with tape and tested in the same tank described above. After allowing a blinded female time to adjust to her condition, a blinded male crab was introduced to the tank. Typically, no display was given by either crab until, in moving about the tank, they came into contact with each other; then the male would grab the female and tuck her into the carrying position. Occasionally the male's grab would miss its target, but the females maintained contact and made no attempt to escape. I separated each pair after the initial contact and allowed them to find each other again. Four of the males assumed a chela lateral display immediately after separation, and two of them also began paddling. Two females gave intermittent rocking and chela lateral displays as they moved about the tank after separation; the others gave no display.

Olfaction

To test the reaction of unblinded males and premolt females to an olfactory stimulus in the absence of visual and tactile cues from other

crabs, I devised the following procedure. The crab to be tested was placed in a circular plastic tub, approximately 80 cm in diameter and 20 cm deep, filled with seawater to a depth of 12 cm. The floor of the tub was divided into quadrants by painted lines. The tub was drained and flushed with fresh seawater between tests. Seawater from two identical plastic scent buckets could be siphoned into the tub by the experimenter. An opaque plastic screen with narrow eye-slits shielded the observer from view.

A test using this apparatus was conducted in the following manner. The crab to be tested was placed in the tub one hour before the start of a test. In tests with male crabs, a single red-sign female was placed in one of the plastic buckets for two and one-half hours to serve as a source of scent, and was then removed before beginning the test. Another bucket contained unscented seawater as a control. When testing premolt females, the source of scent was a mature male. To begin a test, "unscented" water from one bucket was run into the tub (flow lasted about two minutes). The activity of the test animal, measured by the number of lines it crossed in moving about the tub, was recorded during a fifteen minute period designated as the "control period". Then the "scented" water from the other bucket was run into the tub, and observations were made for another fifteen minutes, designated as the "experimental period".

The eight males tested in this manner showed no significant differences in behavior between the control and experimental periods. A two-tailed Wilcoxon Matched-Pairs Signed-Ranks Test (Siegel, 1956) was used to analyze the results. The scores for these tests are given in Table 1.

Male	No scent	Female scent	Row Sums
l	13	16	29
2	11	о	11
3	14	2	16
4	l	2	3
5	0	2	2
6	3	4	7
7	15	14	29
8	2	0	2
Colum	n —	······································	
sums	59	40	99

TABLE 1.--Activity of males expressed as number of lines crossed per 15 minutes*

*Two-tailed Wilcoxon test. T=14.5, not significant at $\propto =0.05$.

One of the females used as a source of scent underwent her puberty molt in the scent bucket, but the test male showed no noticable change in behavior when the water from this bucket was siphoned into the tub. That the males were not simply unresponsive to females was shown by introducing a red-sign female to the test tub after the end of an experiment. In the four cases in which this was done, the male always seized the female and pulled her into the carrying position shortly after she was introduced. In three cases the female made the initial approach, in the other case the male approached the female and grabbed her. Nine different red-sign females were tested in the same apparatus used in the male tests. As in the case of male crabs, there was no statistically significant difference in activity between control and experimental periods. No female gave a display of any kind during a test sequence. The data for the female tests are shown in Table 2.

Female	No scent	Male scent	Row sums
1	18	17	35
2	3	12	15
3	8	12	20
4	10	6	16
5	0	2	2
6	0	6	6
7	2	15	17
8	5	o	5
9	3	13	16
Column		<u> </u>	—
sums	49	83	132

TABLE 2.--Activity of females expressed as number of lines crossed per 15 minutes*

*Two-tailed Wilcoxon test. T=8, not significant at $\propto =0.05$.

Under the experimental conditions, an olfactory stimulus alone does not release display behavior in either sex, nor does it cause any significant change in the level of activity of males or females.

MODEL EXPERIMENTS

Once a male has grabbed a red-sign female, the subsequent behavior of both individuals is quite stereotyped and predictable. Immediately following the grab the male exhibits a brief bout of paddling and tucks the female under him in the carrying position. If necessary, he first turns her around so that both crabs face the same way. Once in the carrying position the female invariably begins waving rapidly, and the male reacts by beating on her chelae with his own until she adopts the submissive position with fully flexed chelae. The male then adopts a relaxed chela lateral position with the tips of his chelae resting on the substrate, a position he maintains until the female is ready to molt.

However, as I compiled observations for the ethogram, it became obvious that the behavior leading up to the grabbing of a premolt female by the male was not so easily predictable. For example, some males simply grabbed a female with no prior display at all, while others first gave a chela lateral display, and still others carried out the entire repertoire of chela lateral, paddling, falling-back, and rocking before grabbing the female. The female's responses were also variable. Therefore, it was not clear how the actions of one crab were affecting the behavior of the other. 33

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One approach commonly used by investigators confronted with this situation is to test males and females separately by presenting them with various standard stimuli. The experimenter chooses stimulus situations which he believes have signal value and presents them one at a time and in a uniform manner, then records the responses of the subject. By providing a constant stimulus situation, one is able to reduce the variation in the responses of the test animal. By using different stimulus situations, the experimenter can discover which stimuli are important in releasing the behavior patterns of interest. I decided to use this method to investigate the role of visual and olfactory stimuli in courtship.

Initially, I used films of a displaying crab as a standard stimulus. A 16 mm color film loop of a display was projected at natural size on a sheet of rear projection screen, separated from the side wall of the test tank by cardboard spacers. At the same time water from a scent bucket was siphoned into the test tank. I found, however, that neither male nor female crabs would give any response to the image on the screen, possibly because the projection speed of 24 frames/second which was used may not have been fast enough to achieve flicker fusion for the crabs. I abandoned this apparatus and began using models, which were more successful in eliciting responses.

First Series

Methods

I constructed the models used in all the following experiments by gluing the appendages of cast exoskeletons or dried formalinpreserved specimens into the desired positions. The appendages of all

models were rigidly fixed. The models were then painted with watercolor paints to resemble living crabs as closely as possible, and the painted surfaces were fixed with a clear acrylic plastic spray (Krylon). The entire surface of each model was given two coats of a clear plastic compound (Varathane) which waterproofed and strengthened it. The models were mounted on a piece of stiff wire passing through the carapace. Three different models, numbered for convenience 1, 2 and 3, were used in most of the tests (see Fig. 2). Model 1 was a male in a non-displaying attitude, with flexed chelae and lowered swim paddles, body only slightly raised on semi-flexed legs. Model 2 was a male in sexual display, with swim paddles raised over the carapace, both chelae extended laterally, and the body raised high on fully extended legs. Model 3 was a mature female in a non-displaying attitude like Model 1, but with bright red fingers on the chelae. All three models were of similar size, with carapace widths of 133 mm, 140 mm and 130 mm for Models 1, 2 and 3 respectively. Two other models, 4 and 5, were used in the male tests only.

The test apparatus consisted of a pair of 57-liter aquaria (61 cm X 31 cm X 31 cm) positioned one in front of the other (see Fig. 3) and separated by a removable opaque screen. Both aquaria were filled with 29 liters of seawater to a level of 16 cm. The model tank was closer to the observer and contained the model being used as a stimulus. The other aquarium, the test tank, contained the crab being tested. For convenience, two identical set-ups were used.

The model in use was suspended from the runner of a curtain rod of the traverse type, equipped with a drawstring by means of

FIGURE 2

Models 1, 2 and 3, as seen from the position of an animal in the test tank

A. Model 1: a non-displaying male.

B. Model 2: a male performing the chela lateral and paddling displays. Note the apparent increase in size.

C. Model 3: a non-displaying female. The fingers of the chelae are red.

FIGURE 2



A



В



С

FIGURE 3

Diagram of the apparatus used in the model experiments

- A. Model tank
- B. Test tank
- C. Removable screen
- D. Drawstring
- E. Opaque blind
- F. Scent bucket
- G. Seawater inflow
- H. Standpipe and drain



FIGURE 3

which the model could be moved from one side of the model tank to the other. An opaque blind, provided with eye-slits, hid the observer from the animal in the test tank. The test tank was equipped with a siphon tube leading to a scent bucket and a seawater inflow which allowed the tank to be flushed after every test. A standpipe maintained the water at a constant level in the test tank regardless of any water added from the scent bucket. Lighting was provided by the four 60 watt flourescent lamps in the ceiling of the laboratory plus a 200 watt incandescent lemp with a 30 cm reflector hung one meter above the model tank.

A test using this apparatus was conducted in the following manner. An animal was introduced to the test tank at least one hour before a test was begun. The animal used as a source of scent was placed in a scent bucket, containing 7.6 liters of seawater, for two and one-half hours and then removed. A test was begun by removing the opaque screen between the two tanks and simultaneously starting water flow from the scent bucket into the test tank. Flow lasted about two minutes, until the scent bucket was emptied.

The models were moved, slowly and in a uniform manner, from one end of the model tank to the other, at the start and at twominute intervals throughout the test. The responses of the test animal to the model were recorded by the observer, using a hand tally

counter and a tally sheet.

tank was drained and then flushed with seawater for 15 minutes. Temperatures in the test tank ranged from 22 to 29 degrees C throughout

ion the water temperature in the test tank was taken, and

Each test lasted 30 minutes: at its

this series. All testing was carried out between 0900 hours and 1800 hours. A minimum interval of three hours was observed between successive tests with any one individual.

The first series of tests using this apparatus was designed as a pilot study to determine the feasibility of further model experiments. The objective of this series was to find answers to three questions: (a) Is there a significant difference in the way blue crabs, male or female, react to the various stimulus situations, or do they react in the same way to all the stimulus situations? (b) How does the presence or absence of olfactory cues affect the way in which males and females react to the models? (c) Do redsign females react differently to the various stimulus situations than white-sign females?

Three models were used in testing white-sign and red-sign premolt females: Model 1 (non-displaying male); Model 2 (male in paddling display); and Model 3 (non-displaying mature female). Each model was presented both with the appropriate scent and with no scent at all--a total of six different stimulus situations. In those cases in which no scent was used, unscented seawater was siphoned into the test tank as a control.

Each female was subjected to all six stimulus situations. On several occasions a red-sign female molted before completion of the six tests. These particular females were then dropped from the analysis. The sequence of stimulus situations was different for each female tested. The response measured was the number of waves per 30-minute test period.

Five models were used in the male tests. In addition to Models 1, 2 and 3, Model 4 (premolt female in waving display) and Model 5 (non-displaying premolt female) were used. Each model was presented both with and without the appropriate scent giving a total of ten stimulus situations to which each male was subjected. As in the female tests, the sequence of stimulus situations was systematically varied. The number of attempted grabs, number of falling-back responses, number of chela lateral responses, and the number of bouts of paddling were the responses measured in male tests.

Results

None of the male crabs tested gave a measurable response to any of the models. After five individuals had been exposed to the full range of stimulus situations with no results, further testing of males was abandoned. Apparently the stimulus situations used were not adequate to elicit responses from males.

The red-sign females, however, responded well. The scores of the six individuals I was able to test under the full range of conditions are given in Table 3.

As shown in Table 3, by far the greatest number of waving responses were elicited by those stimulus situations in which a male model was presented together with male scent. Also, with the single exception of female number 4 (which appeared to have an unusually high level of internal motivation), no red-sign female responded in any way to the combination of Model 3 plus mature female scent. This strongly suggested that red-sign females are capable of discriminating between males and mature females.

	Model 1 (non-disp. male)		Model 1 Model 2 (non-disp. male) (disp. male)		Mod (non-dis		
Female	Male scent	No scent	Male scen	No t scent	Female scent	No scent	Row sums
1	l	0	2	0	0	0	3
2	51	0	0	6	0	0	27
3	12	ο	0	ο	o	0	12
4	53	ο	54	0	39	0	146
5	2	0	4	ο	o	8	14
6	3	0	23	Q	o	0	26
Col. sums	92	0	83	6	39	8	228

TABLE 3.--Responses of red-sign females expressed as number of waves per 30 minutes

In practically all cases, red-sign females ignored the models when no scent was added to the test tank. A visual stimulus alone usually does not release courtahip behavior, at least under the experimental conditions.

In marked contrast to the red-sign females, none of the six white-sign females tested in the apparatus gave any response to the stimulus situations. They simply ignored the models as they moved around the test tank. This was interpreted to mean that at the whitesign stage, females have such a low level of internal motivation that all stimulus situations tested were inadequate to release a response.

Second Series

Methods

The first series of model experiments gave valuable information about the mechanism of sex recognition in premolt females, but it suffered from two serious shortcomings. First, the experimental design did not permit statistical comparisons to be made between the various pairs of stimulus situations (e.g. Model 1 plus male scent vs. Model 3 plus female scent). The repeated use of a two-sample test on such data would not be valid, since such a procedure would capitalize on chance by increasing the alpha level. Second, because each female had to be subjected to all six stimulus situations, a full test sequence for any given female took three days to complete, and the data was wasted if the female molted before completion of the sequence. Since it can be assumed that the female's motivational level increases as the time of molting draws nearer, the difference in response scores between a given pair of stimulus situations would probably be greater if they were presented, for example, on the first and third days rather than both on the same day. This tended to increase the variability of the data.

To answer these questions, I devised a second series of model experiments, utilizing a different experimental design. Using the same three models as in the first series, I measured the responses of red-sign females to the following pairs of stimulus situations: (a) Model 1 (non-displaying male) plus male scent vs. Model 3 (nondisplaying female) plus female scent, (b) Model 1 (non-displaying male) plus male scent vs. Model 3 (non-displaying female) plus male scent, (c) Model 1 (non-displaying male) plus male scent vs. Model 2 (displaying male) plus male scent. Since each pair of stimulus situations was independent of the others, it was not necessary that each female be tested on all three pairs. The majority of females molted after completion of one or two pairs of tests; only four of the fifteen females used completed all six tests.

The order of presentation of test pairs was randomized for each female by drawing lots; then the order of the stimulus situations within a pair was determined by flipping a coin. The responses measured were number of waves, number of bouts of rocking, and total duration of rocking (in seconds). Scores were recorded with a hand tally counter, a tally sheet, and a stopwatch. Except for the experimental design, the testing procedure and the apparatus were identical to that used in the first experimental series.

During the entire second series of model experiments the water temperature in the test tank varied between 23 and 27 degrees C.

A one-tailed Wilcoxon Matched-Pairs Signed-Ranks Test (Siegel, 1956) was used to analyze the results. A one-tailed statistical test is more powerful than the corresponding two-tailed test, but it

requires that the alternative hypothesis predict the direction of the difference in advance of the test. The alternative hypothesis for each test pair was as follows: (a) test pair (1)--response to Model DIUS TEMALE Scent Built Company of Montel States and Strates and Strat (b) test pair (2)--response to Model 1 plus male scent is greater than to Model 3 plus male scent; (c) test p air (3)--response to Model 2 plus male scent is greater than to Mo lel 1 plus male scent. For each test pair the null hypothesis was th at there was no difference in response between the two stimul is situations. Results Nine replications of test pair (1) wer run. The data for waves, bouts of rocking and total duration of rocking are given in

ie) it	Model 3 (non-disp. female) plus female scent	Row sums	Female	Model 1 (non-disp. ma plus male sce
	21. 9 48 28 8 15 44 2 12	30 62 67 107 42 55 140 119 159	1 2 3 4 5 6 7 8 9	9 53 19 79 34 40 96 117 147
	187	 781	Column sums	 594

Tables 4, 5 and 6, respectively.

est. T=5, significant at CA =0.05.

*One-tailed Wilcoxon

Female	Model 1 (non-disp. male) plus male scent	Model 3 (non-disp. female) plus female scent	Row sums
1 2 3 4 5 6 7 8 9 2 0 20 20 20 20 20 20 20 20 20 20 20 20	0 2 10 0 14 31 5 0 0 0	0 4 17 9 0 20 1 0 0	0 6 27 9 14 51 6 0 0

TABLE 5.--Responses of red-sign females expressed as number of bouts of rocking per 30 minutes* Test pair (1)

*One-tailed Wilcoxon test. T=8, not significant at 0.=0.05.

TABLE 6.--Responses of red-sign females expressed as total duration of rocking (seconds)* Test pair (1)

Female	Model 1 (non-disp. male) plus male scent	Model 3 (non-disp. female) plus female scent	Row sums
1 2 3 4 5 6 7 8 9	0 5 23 0 21 132 3 0 0	0 8 26 55 0 112 4 0 0	0 13 49 55 21 244 7 0 0
Colum sums	184	205	389

*One-tailed Wilcoxon test. T=9, not significant at 0.05.

The females reacted much more strongly to Model 1 plus male scent than they did to Model 3 plus female scent, as measured by the waving response. The other two measures (number of bouts of rocking and total duration of rocking) did not show this preference. This inconsistency between the data in Table 4 and that in Tables 5 and 6 is due primarily to two factors: (a) three females (2, 4 and 7) which showed a greater response to Model 1 as measured by the vertex

Female	Model 1 (non-disp. male) plus male scent	Model 3 (not-disp. female) plus male scent	Row sums
1 2 3 4 5 6 7 8 9	32 9 29 56 1 7 29 120 30	47 0 19 59 19 65 6 44 91	79 9 48 115 20 72 35 164 121
Colum sums	a 313	350	663

TABLE 7.--Responses of red-sign females expressed as number of waves per 30 minutes* Test pair (2)

*One-tailed Wilcoxon test. T=20, not significant at CX =0.05.

TABLE 8.--Responses of red-sign females expressed as number of bouts of rocking per 30 minutes* Test pair (2)

Female	Model 1 (non-disp. male) plus male scent	Model 3 (non-disp. female) plus male scent	Row sums
1 2 3 4 5 6 7 8 9	5 0 27 6 0 4 16 2 0	0 0 9 3 19 6 15 5 0	5 0 36 9 19 10 31 7 0
Column sums	60	57	 7117

*One-tailed Wilcoxon test. T=12.5, not significant at \propto =0.05.

1			
Female	Model 1 (non-disp. male) plus male scent	Model 3 (non-disp. female) plus male scent	Row sums
1 2 3 4 5 6 7 8 9	6 0 117 10 0 11 99 4 0	0 94 4 45 12 142 9 0	6 0 211 14 45 23 241 13 0
Colu sum	mn 18 247	306	 553

TABLE 9.--Responses of red-sign females expressed as total duration of rocking (seconds)* Test pair (2)

*One-tailed Wilcoxon test. T=12, not significant at & =0.05.

Tables 10, 11 and 12 present the results for the ten replications of test pair (3).

All three measures (number of waves, number of bouts of rocking, and total duration of rocking) show that a model of a displaying male in combination with male scent elicits a greater response from red-sign females than a non-displaying male model plus male scent.

Female	Model 1 (non-disp. male) plus male scent	Model 2 (displaying male) plus male scent	Row suns
1 2 3 4 5 6 7 8 9 10 Colum sums	14 18 14 35 4 0 59 32 107 13 13 m 296	13 66 29 98 22 4 92 27 87 29 467	27 84 43 133 26 4 151 59 194 42 763

TABLE 10.--Responses of red-sign females expressed as number of waves per 30 minutes* Test pair (3)

*One-tailed Wilcoxon test. T=11, significant at 🕵 =0.05.

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TABLE	11Respons	es of	red-s:	ign	females	expressed	86	number	of	bouts
	of r	ocking	g per 🤅	30 n	inutes*	Test pair	: (;	3)		

Female	Model 1 (non-disp. male) plus male scent	Model 2 (displaying male) plus male scent	Row sums
1 2 3 4 5 6 7 8 9 10	1 8 11 9 14 22 6 4 3 3	8 1 37 20 25 50 5 7 6 10	9 9 48 29 39 72 11 11 9 13
Colum sums	n —— 81.	169	250

*One-tailed Wilcoxon test. T=6, significant at $\propto =0.05$.

Female	Model 1 (non-disp. male) plus male scent	Model 2 (displaying male) plus male scent	Row sums
12345678910 Co	58 4 8 2 25 11 38 18 11	169 2 50 5 12 1 56 87 18 30	227 6 58 13 14 26 67 125 36 41
£	sums 183	430	613

TABLE 12.--Responses of red-sign females expressed as total duration of rocking (seconds)* Test pair (3)

*One-tailed Wilcoxon test. T=8, significant at 🛇 =0.05.

Figure 4 on the next page is a graphical comparison of median scores in each of the three test pairs.

FIGURE 4

Responses of red-sign females. Median scores for number of waves, number of bouts of rocking, and total duration of rocking (seconds). In each case, the black bar represents responses to Model 1.

A. Test pair 1. Model 1 (non-displaying male) plus male scent vs. Model 3 (non-displaying female) plus female scent.

B. Test pair 2. Model 1 (non-displaying male) plus male scent vs. Model 3 (non-displaying female) plus male scent.

C. Test pair 3. Model 1 (non-displaying male) plus male scent vs. Model 2 (displaying male) plus male scent.



FIGURE 4





B

С

DISCUSSION

Little quantitative data on the sex recognition system of male Callinectes sapidus was obtained by the present study, since I was not able to induce males to respond to the models. However, various observations appear to support Chidester's (1911) argument that males cannot discriminate between conspecific males and females. In the experiments on blinded males, those individuals with taped eyes grabbed other males just as readily as females. Males in the experiments on olfaction showed no reaction to water in which premolt females had been kept. Also, unblinded, sexually motivated males in water tables often grabbed any crab that came close, regardless of sex or molting stage. These observations are not conclusive evidence, but they make it seem unlikely that males can recognize the sex of a conspecific by olfactory, contact chemical, or tactile cues. Visual cues may possibly be used by males to effect a discrimination -- the existence of female sexual displays would appear to be a priori evidence for such a conjecture. Alternatively, the rocking and waving displays of females (which involve rapid movements of the appendages or the entire body) may merely act to lower the threshold of the male's grabbing response and prevent habituation, especially when coupled with movement toward the male. Blue crabs of both sexes seem to habituate very rapidly to non-moving objects, including live crabs and models. In the model experiments any

movement of a model immediately increased the frequency of the female's response to it.

Published data on the spectral sensitivity of <u>C. sapidus</u> (Goldsmith and Fernandez, 1968) show that maximum sensitivity occurs at 505 nannometers (blue-green region), and that only a single visual pigment is present. Color vision therefore seems unlikely in this species. This does not necessarily mean that differences in coloration between the sexes have no signal value, since intensity differences might still be sensed. The fact that the chelae are waved by the female during courtship is very suggestive, since the fingers of the chelae are so obviously different in color (at least to human eyes) from those of the male.

Female crabs, at least in the red-sign premolt stage, do have a well-developed ability to discriminate between adult males and females, as the present study has demonstrated. This would certainly be adaptive, since a recently molted female is defenseless owing to her soft condition and would be prey to any crab other than an adult male.

It seems clear that red-sign females recognize males primarily by olfactory cues. When male models were presented without scent, they were usually ignored; the same models together with male scent elicited a sexual response from the females. When female models were presented together with male scent they elicited a response equal to that elicited by male models. When the same female models were presented with female scent they elicited a much lower level of response. Visual cues appear to play a subordinate, but important, role in females. That is, females will not give a sexual display if they are exposed to male scent alone; they also require a visual stimulus in addition to male odor to release the display. The visual stimulus can be relatively unspecific (either a male or a female model) as long as male scent is present in the water. Motion is evidently also important, for females always gave a greater response to a moving model than to one which was at rest. Females also showed a significantly higher level of response to a model of a displaying male, compared to a non-displaying model.

In his work on <u>Portunus sanguinolentus</u>, Ryan (1966) found that the female's sex pheromone was released in the urine; sealing the excretory pores with paraffin blocked the release of the pheromone. To see if the urine of male <u>C. sapidus</u> was the source of their attractiveness to females, I made several attempts in the course of this study to seal the excretory pores of males before placing them in the scent buckets. Unfortunately, I was never able to obtain a satisfactory seal, although a variety of sealing agents were tried. The nature and site of production of the male attractant still remain to be investigated.

The female's response to male odor and male sexual displays probably compensates for any lack of discrimination on the part of the male. It would be interesting to carry out tests on the ability of male and female <u>C. sapidus</u> to discriminate between conspecifics and members of closely related species such as <u>C. marginatus</u>. Interspecific discrimination in females may depend on olfactory cues, since the visual

courtship displays of males of both <u>C. marginatus</u> and <u>C. sapidus</u> are very similar (Teytaud, personal observations). Comparative studies of the occurrence and taxonomic distribution of courtship displays throughout the Portunidae would be of value, especially as the habitats of various species range from the intertidal (e.g. <u>Carcinus</u>) to very deep water (e.g. <u>Bathynectes</u>).

When compared with semi-terrestrial forms such as grapsids and ocypodids, the courtship behavior of the blue crab does not appear to be highly "formalized" (in the sense of Schöne [1968], a "formalized" encounter consists of a "... more or less fixed set of patterns following certain rules.") In the present study I observed a relatively wide variation in the sequence of action patterns during a courtship encounter (see p. 33), and in the orientation of the display with respect to the partner. Wright (1968b) and others have cautioned that such variation may be a laboratory artifact--this needs to be checked in field studies of <u>C. sepidus</u>.

Sexual behavior in the natural environment has not been well studied in most fully aquatic brachyurans. When such work is carried out, it may well be found that communication by visual displays has a much larger role in the interactions of these animals than most authors currently assume, at least over short distances. The rocking, paddling, and falling-back displays of male <u>C. sapidus</u> and (possibly) the waving and rocking displays of females seem to be part of a visual communication system which is at least as complex as those described for many shoreliving forms. It may still be true that chemical communication plays a larger part in fully aquatic species than in semi-terrestrial ones, but it should be noted that the possible existence of air-borne pheromones in semi-terrestrial brachyura has not been experimentally investigated to date (Hartnoll, 1969).

Female blue crabs, if they are not mated at the time of their puberty molt, retain their sexual receptivity for some time. They are able to copulate even though their exoskeletons are quite hard. I observed several cases of this; one female in particular courted and copulated with a male two and one-half weeks after molting. If unmated, mature females are kept together in the absence of males, some individuals will occasionally exhibit pseudo-male behavior, picking up other females and attempting to hold them in the carrying position. However, this action differs from that of the male in that the chela lateral display which invariably accompanies carrying by males is not given in this context by females.

Finally, the results of this study are supported by the observations of Dr. Richard Jachowski (personal communication) among the Chesapeake Bay watermen. He reports that crab fishermen in that area occasionally bait their pots with live males to attract "peelers" (red-sign females), and some people catch females with a male on a string. However, female peelers generally make poor bait for catching males.

SUMMARY

1. Both sexes of the blue crab <u>Callinectes sapidus</u> Rathbun (Decapoda: Portunidae) perform relatively complex courtship displays not commonly observed in other fully aquatic brachyurans. This paper reports the results of behavioral studies conducted by the author to determine the function of these displays in intraspecific sex recognition.

2. The courtship displays of <u>C. sapidus</u> have not been described in detail in the literature. Therefore, a preliminary step in the present study was the compilation of a reproductive ethogram (i.e. a descriptive catalogue of species-typical action patterns) for both sexes. Most of the observations of courtship activities were carried out under laboratory conditions.

of adult males splaying) were he appropriate the test apparatus separately in a specially designed apparatus. Models and females in various postures (displaying and non-di used as a visual stimulus. Water in which a crab of t sex and molting stage had been kept was siphoned into to provide an olfactory stimulus. of the data for these immature females showed a significantly greater intensity of response to certain test situations, namely those in which a visual stimulus (male or female model) was combined with male odor. The level of response was much lower in those test situations involving either olfactory or visual stimuli alone, or visual stimuli in combination with odor from mature females. Male models in display postures elicited significantly greater responses than non-displaying models, when both were presented with male odor.

5. White-sign, prepuberty-molt females did not respond in any way when exposed to the identical stimulus situations as the red-sign females. This was interpreted to mean that white-sign females have such a low level of internal motivation that none of the stimulus situations tested were adequate to release a response.

6. Mature males gave no measurable responses when tested in the apparatus. Further testing of males was discontinued.

7. The model experiments showed that prepuberty-molt red-sign females are able to discriminate between adult males and females. Olfactory stimuli appear to be of primary importance in effecting the discrimination. Vision plays a subordinate, but important, role. No conclusions could be reached regarding male crabs, due to their failure to respond in the test apparatus. Various observations under different conditions suggested that males are not capable of discriminating between the sexes, but this remains to be demonstrated experimentally.

8. Females of <u>Callinectes</u> <u>sapidus</u> normally mate only at the time of their puberty molt. The ability of females to recognize males

is adaptive, since females are very vulnerable when molting and would be killed and eaten by any conspecific other than a mature male.

9. A summary of the general biology of the blue crab is given, emphasizing those aspects pertinent to the present study. The literature on sex recognition in the family Portunidae is reviewed, and a less detailed account of the literature on non-portunid decapods is also presented.

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63