## SOCIAL BEHAVIOR OF ADULT LABORATORY-REARED

CUTTLEFISH, SEPIA PHARAONIS

## THESIS

Presented to the Graduate Council of Southwest Texas State University in Partial Fulfillment of the Requirements

For the Degree

Master of SCIENCE

By

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# COPYRIGHT

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2001

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## ABSTRACT

#### SOCIAL BEHAVIOR OF ADULT LABORATORY-REARED

## **CUTTLEFISH, SEPIA PHARAONIS**

by

## ERIN RENE FOSTER-AITCHISON, B.S. Southwest Texas State University May 2001

### SUPERVISING PROFESSOR: KARI LAVALLI

The cuttlefish *Sepia pharaonis* is widely distributed in the Indo-Pacific from the Red Sea to Japan and Australia, and is commercially important as a food resource. Given the interest in commercially culturing this species, knowledge of its reproductive behavior is most needed. However, studying cuttlefish reproductive behavior is hampered by the lack of anatomical features that distinguish males from females. Thus, behavioral differences and body patterns were examined in 18 captive, socially interacting, adult cuttlefish, *Sepia pharaonis*, over a four-month period to determine if they provided a reliable indicator of sex. Four groups of cuttlefish were identified on the basis of size, display patterns, and mating behavior: large males, average-sized males, small males, and females. Large and average-sized males used the *Intense Zebra Display* 94.7% of the time observed, made flamboyant postures 100% of time observed, and were

highly aggressive towards other males and females. In contrast, small males did not use elaborate patterning (used a muted Zebra Display instead), did not make flamboyant postures, and were not aggressive towards other males or females. They rarely exhibited the Intense Zebra Display (5.3%), but faint stripes were always visible on their fourth arms. Females were usually mottled with very faint stripes visible on their mantle; they completely lacked stripes on their fourth arms. Females never approached males or other females aggressively. Posthumous dissection of all cuttlefish verified that the use of these differences in body patterning and posture provided a highly reliable method of identifying males from females. Subsequent analysis of videotaped data also demonstrated differences in reproductive success between the different size classes of males. While there was a trend for the largest males and smallest males to mate most frequently compared to average-sized males, this trend was not significant. Also, while large males seemed to have longer duration matings compared to small males (mean duration 213 sec vs. 98.5 sec), small sample sizes precluded the use of statistical tests to determine if these differences were significant. Only large and average-sized males interacted; small males were rarely approached, possibly due to their muted body patterns and female-like behaviors. Behavioral transition sequences were calculated for malemale approaches and matings, which demonstrated predictable outcomes for these interactions based on the sequences of the behaviors displayed. Mating behavior in Sepia pharaonis differed from that of S. officinalis in that two male mating strategies were observed and pre-copulatory guarding of females was more common.

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#### **INTRODUCTION**

Some of the most spectacular behavioral displays arise for reproductive purposes. These behaviors are not necessarily favored by natural selection and were first explained by Darwin (1871) in his treatise on sexual selection. Conspicuous secondary sexual traits and behaviors have been intensely studied in insects and birds (as summarized by Andersson, 1994; and Blum & Blum, 1979) to determine their potential fitness value. In contrast, invertebrates other than arthropods have not been well studied until recently with research on cephalopods. Cephalopods have the ability to produce highly complex visual signals deeming them an excellent candidate for investigating sexual displays. However, because of a lack of understanding of the meaning of their body patterns, communication signals have been difficult to assess in this group. In order to fully understand the biology of cephalopods, one must understand their mating systems via their behaviors and body pattern signals. Such is the focus of this study.

An examination of any mating system involves understanding the function of courtship rituals and how those rituals are used. Some courtship rituals are used mainly to identify conspecifics to ensure that both participants mate with appropriate partners. Other rituals involve assessment of potential partners for various qualities and ultimately result in a system of mate choice. Finally, rituals can also play a role in competition, usually between males, for mating opportunities, particularly in situations when females do not come into "estrus" synchronously (Krebs & Davies, 1981). In the case where courtship rituals allow for the assessment of traits of potential partners, traits assessed are usually those that may confer advantages to the partner, the offspring, or both. These

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"assessment" rituals will result in a system of mate choice, which is simply a mechanism used by either males or females that makes it more likely that they will mate selectively with members of the opposite sex (Halliday, 1978; 1983). Such mechanisms can be behavioral or morphological and often lead to a system of sexual selection where female choice exerts a strong evolutionary pressure on the development of the preferred mechanism or trait (Darwin, 1871; Halliday, 1978). Assessment courtship rituals can provide females with different kinds of information about the potential fecundity of a male in the following ways. Courtship feeding provides information about the male's ability to provide food for offspring, and therefore suggests that fecundity may be enhanced with this male. In some cases, it can provide direct evidence of male's quality: some female insects consume the male's spermatophore obtaining not only sperm, but also nutrients (Eberhard, 1996). Parasitized males provide spermatophores with lowered nutritional value and result in reduced fecundity for females (Thornhill, 1976; 1980; Wedell, 1994; Simmons et al. 1994). Courtship within a male's territory demonstrates the ability of the male (and thus his superiority) to retain his territory while still providing himself with sufficient sustenance, and if that territory is larger than needed for survival resources (a superterritory). Courtship vocalizations, dances, or color patterns and changes not only advertise the position of the male to the female, but also advertise the position of the male to potential predators. Males that can continue these displays demonstrate their superiority in avoiding predation, and may also demonstrate their strength, as such displays can be energetically demanding (Clutton-Brock & Albon, 1978; Lambrechts & Dhondt, 1986; Petrie et al., 1991; Wilhelm et al., 1980, 1982). In most cases, these signals (whether visual, vocal, chemical, morphological, etc.) provide a

reliable measure of the male's superiority either as a sperm donor (assessing fertility) or as a parent (assessing fecundity) (Andersson, 1994; Zahavi & Zahavi, 1997). While females are usually the more "choosier" sex, males can also exhibit mate choice. Some males can assess whether females have previously mated (affecting their paternity) from sequences within the courtship dances (Erickson & Zenome, 1976). In some insects, males can determine the fecundity of females by comparing weights and will choose to mate only with the heaviest females. Males choose the most fecund females to insure an increase in the number of offspring. For example, a male freshwater isopod carries a female between his legs until she is receptive to mating. Larger females become receptive more quickly and produce more eggs than smaller females maximizing the males reproductive success with preference towards larger females (water louse, *Asellus aquaticus*: Manning, 1975).

Female choice is more prominent in nature because females typically invest more resources into fewer, large gametes than do males (Bateman, 1948). Greater investment in fewer gametes results in females being more selective in choice of mates (Andersson, 1994). Because males produce many small gametes, they typically have a greater reproductive potential than do females (Andersson, 1994), leading to conflict (Trivers, 1972). Issues of sexual conflict include choice of mate, food provisioning for the zygote, and care for the young (Krebs & Davies, 1981). Conflict is apparent because female reproductive success can be achieved by a single copulation and males maximize success by increasing the number of females in which he fertilizes (Krebs & Davies, 1981). By males maximizing their reproductive output, competition for the limiting resource—females—occurs, resulting in intrasexual selection, or mate competition (Halliday, 1983). Mate competition has influenced the evolution of different mating strategies (or systems). Four basic types of mating systems are recognized: monogamy, polygyny, polyandry, and promiscuity.

Monogamy is a mating system in which a male and female pair and remain bonded throughout some period of time, whether that be the breeding season or beyond. In some species, the bond lasts for life. Monogamy is found in communally breeding birds, but rarely found in other vertebrates (Andersson, 1994), except mammals (e.g., the rock-haunting possum: Runcie, 2000; Malagasy jumping rat: Sommer, 2000). It is typified by a system of parental care involving one or both parents. In species such as the volcano mouse, *Neotodon alstoni*, both male and female take part in all parental duties (Luis et al., 2000). In other species, such as laughing kookaburras, males and females divide the duties (Legge & Cockburn, 2000).

In contrast to monogamy, polygyny is a system in which one male mates with many females in a breeding season. This system can include resource-defense polygyny, where a male defends a resource that many females will need. Such resources may include food, shelter, egg-laying sites, or nesting sites. Other males are precluded from using the defended resources, and thus are prevented from obtaining matings (Vehrencamp & Bradbury, 1984). Resource-defense polygyny is seen in blue gouramis (*Trichogaster trichopterus*: Hollis, 1999), giraffes (*Giraffa camelopardalis*: van der Jeugd & Prins, 2000), bees (*Indicator xanthonotus*: Cronin & Sherman, 1977), and some birds (*Tematodytes palustris*: Verner, 1964; *Agelaius phoeniceus*: Holm, 1973). Another type of polygyny is female-defense polygyny and results in the formation of harems. Males defend several females from other males rather than resources such as food and territory (Emlen & Oring, 1977). Female-defense polygyny is found in various species including Zoraptera insects (*Zorotypus gurneyi*: Choe, 1994), the Caribbean rosy razorfish (*Xyrichtys martinicensis*: Victor, 1987), blackbirds (*Montezuma oropendola*: Webster & Robinson, 1999), and the Atlantic walrus (*Odebenus rosmarus rosmarus*: Sjare & Stirling, 1996).

Polygyny can also lead to lekking. In a lek system, males defend territories but do not defend additional resources or mates. Females choose to mate with one or a few males in such territories. These territories are called "display territories" where they attract the attention of approaching females (Vehrencamp & Bradbury, 1984). The only known benefit the female receives from the sexual transaction is the donation of sperm and it is unclear why females choose to mate with a particular male or males within or between a lek (Andersson, 1994). It is hypothesized that females choose the central or most dominant male in the lek but this has yet to be verified. Parental care, if provided, is carried out by the female alone. Like most other mating systems, most research on lek mating has been focused on birds (e.g. black grouse, *Tetrao tetrix*: Karvonen et al., 2000; great snipe, Gallinago media: Sæther et al., 2000; Guianan cock-of-the-rock, Rupicola rupicola: Trail & Adams, 1989). Other representatives displaying lekking include insects (Mediterranean fruit fly, Ceratitus capitata: Shelly, 2000), fish (bluehead wrasse, Thalassoma bifasciatum: Warner, 1984; 1990), and mammals (Uganda kob antelope, Kobus kob thomasi: Buechner & Roth, 1974; fallow deer, Dama dama: Clutton-Brock et al., 1988).

In polyandry, a female mates with many males in one breeding season. Males normally render parental care alone. Polyandry is rare, but is found in such birds as the American jacana (*Jacana spinosa*), and the northern phalarope (*Phalaropus lobatus*) (Emlen & Oring, 1977), and in the tobacco budworm moth, *Heliothis virescens* (Lamunyon, 2000).

Promiscuity is a system (or lack thereof) in which both males and females mate multiply with different individuals (Krebs & Davies, 1981). Parental care is exhibited from either sex. Some have referred to this system as a mixture of polygyny and polyandry, or polygynandry, and usually occurs within species that are predominantly monogamous, polygynous, or polyandrous (Krebs & Davies, 1981; Andersson, 1994).

Although the systems described above represent almost all types of mating systems observed in nature, they are not exhaustive. It should also be understood that multiple mating systems may exist within a species as well as vary across species (Emlen & Oring, 1977), due to differing ecological conditions and operational sex ratios (Davies & Lundberg, 1984). For example, in those mating systems where certain males are excluded from mating with females, alternative male reproductive strategies exist (Sinervo & Lively, 1996). "Sneaker" or "satellite" males are males that receive extrapair copulations (EPCs) with females that are already paired with other males. Evidence of such copulations have been well documented in what were perceived as monogamous birds (review in McKinney et al., 1984; Lank et al., 1989; Birkhead & Møller, 1992). "Satellite males" have been witnessed in several species of frogs (Rana spp.) where subordinate males 'steal' matings as females make their way to the territories of dominant males (Wells, 1977). In some salmon species large males act more aggressively towards females while the smaller males attempt to "sneak" matings while exhibiting female-like behavior (Gross, 1985; 1991).

Recently, researchers observed 'sneaker male' copulations in squid (Hanlon, Smale & Sauer, unpubl. data as cited in Hanlon & Messenger, 1996). Female squid are thought to mate any male with which they pair temporarily. They have been observed to accept matings with "sneaker males" while paired with another male with no resistance (Hanlon & Messenger, 1996). The lack of male-male aggression may be due to "sneaker males" being smaller and more similar in appearance to females (Hanlon, Smale & Sauer, unpubl. data as cited in Hanlon & Messenger, 1996). The mean durations of such sneak matings lasted six seconds, compared to 16 seconds in pair-bonded matings (Hanlon, Smale & Sauer, unpubl. data as cited in Hanlon & Messenger, 1996).

Although alternative strategies have been observed in squid, squid or other cephalopod mating systems are not well known. On the whole, it is believed that monogamy does not exist within any cephalopod group (Hanlon & Messenger, 1996). Current evidence indicates that males mate promiscuously and females are also promiscuous or exercise what Hanlon & Messenger (1996) have termed 'simultaneous polyandry'. In 'simultaneous polyandry' females temporarily pair and mate with several males during the same breeding season. Some have also speculated a "lek-like" system in cuttlefish (Corner & Moore, 1980; Hanlon & Messenger, 1996) but more fieldwork is needed. The task of determining a mating system has been difficult because mating systems are generally assessed by the degree of parental care (Emlen & Oring, 1977) and the dispersion of females in a population (Davies, 1991). Unfortunately, due to the lack of fieldwork, little is known of the role of either of these factors in determining the mating system of cephalopods. To better explore possible mating systems and behavior, cephalopod biology and reproductive behavior will be discussed here in detail.

#### **Biology of Cephalopods**

The Class Cephalopoda includes the chambered nautilus, octopus, cuttlefish and squid. There are approximately 700 extant species worldwide, occupying both shallow and deep waters. Over 10,000 species of this class are found in the fossil record (Hanlon & Messenger, 1996; Boyle, 1983). The modern forms, which include everything except nautilids, first appeared in the Devonian period (Boyle, 1983), and are collectively called Coleoids. They are considered the most highly evolved marine invertebrates in that they possess the largest brains (Wells, 1962), well-organized, vertebrate-like eyes (Dees, 1961), and giant axons (up to 1 mm in diameter) (Hanlon & Messenger, 1996). In addition to these advancements over the typical molluscan form, coleoids have also modified the molluscan radula into a beak-like mouth, surrounded by eight arms (and two tentacles in squid and cuttlefish; see Fig. 1) that bear hundreds of suckers. The presence of paired tentacles, as well as eight arms, places squid and cuttlefish in a functional grouping, called decapods.

Coleoids are carnivores that prey by sight or touch, and actively pursue prey into holes and crevices. Typical prey includes small crustaceans, fish, gastropods and bivalves, and other cephalopods (Hixon, 1983; Summers, 1983; Worms, 1983; O'Dor, 1983; Hanlon, 1983; Van Heukelem, 1983; Hartwick, 1983; Mangold, 1983). Cephalopods are very mobile and either move quickly or subtly in order to capture prey. Fast movement is accomplished via jet propulsion where water is drawn into the mantle cavity and forced out through the siphon. Octopuses and cuttlefish can also crawl over substrates and bury within them (Hanlon & Messenger, 1996).



Fig 1 Diagram of a cuttlefish

. Cephalopods have the ability to change color and appearance in seconds and do so frequently (Wells, 1962). These changes in appearance result from changes in color patterns, textural differences, and postural components. One or a combination of these changes makes up what are known as body patterns (Hanlon & Messenger, 1996). Chromatophores are pigment cells that are found just beneath the epidermis, over the entire body (Hanlon & Messenger, 1996) and are responsible for changes in color patterns. Each pigment cell possesses a different color, and when expanded, the color is visible through the skin. Chromatophores are under neuromuscular control, which allows for rapid color changes (Hanlon & Messenger, 1996). When the chromatophore is retracted, the pale whiteness of the skin is revealed. Color patterns are made up of a combination of retracted or expanded chromatophores (Boycott, 1958) working in concert with iridophores that enhance reflection (Hanlon & Messenger, 1996). Different body patterns can provide signals for attack, willingness to mate, mode of feeding, or gregarious behavior (Moynihan, 1985), although none are particularly well understood or described at this time. In addition, the ability to rapidly change body patterns previously has made it very difficult to identify individual cephalopods to the species level, as well as to determine their sex.

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Coleoids have both internal and external fertilization. In all groups, females have large, yolky eggs, while males store sperm in complex spermatophores that are passed to the female during mating. Parental care is rarely provided by either sex after fertilization and egg laying are accomplished (Hanlon & Messenger, 1996). Often this is due to the high mortality of the mating population following egg laying. Octopuses are the only group to typically provide parental care, and this is accomplished at the expense of the female which starves to death while continuously aerating her cluster of hundreds of tiny, rice-shaped eggs (Gabe, 1975; Hanlon, 1983; Mangold, 1983; Hanlon & Messenger, 1996). Cephalopod embryos exhibit direct development, hatching into miniature adult forms, with voracious appetites. The juveniles grow rapidly, reaching maturity within the same year, and most have an average life span of one year. There are exceptions, however, such as the spineless cuttlefish that only lives a few months (Nabhitabhata, 1997), or some octopuses that live as long as 4 to 5 years (*Octopus* spp.: Boyle, 1983). In longer-lived species, breeding and spawning can occur seasonally (Hanlon, 1983).

While the general life cycle of each group of cephalopods is known to some degree, many gaps remain for individual species.

#### **Reproductive behavior**

In coleoid cephalopods, both males and females are promiscuous (Hanlon & Messenger, 1996). Nonetheless, particular groups (octopus, squid, cuttlefish) vary in their level of sociality and thus in their level of promiscuity (Hanlon & Messenger, 1996). Octopuses are usually solitary, squid are highly social exhibiting shoaling behavior, while cuttlefish are thought to be somewhere in between, living part of their lives in isolation and part in loose aggregations (Corner & Moore, 1980). Sexual dimorphism, as far as external factors are concerned, is also varied among cephalopods. Hanlon & Messenger (1996) distinguish between sexes via differences of chromatophore expression, presence of enlarged suckers, gonadal shape, or color and, in a few genera, the presence of photophores. There are also slight differences in the anatomy of reproductive organs among the groups--octopod and decapod males have modified one of their arms into a

hectocotylus, which they use to transfer spermatophores into the mantle cavities of females (Dees, 1961). Internally, female cephalopods have seminal receptacles in different regions of their bodies; the position of which varies between species. Because of the differences in sociality and anatomy, there are also differences in courtship and mating behavior, which are summarized below for each type of coleoid.

#### **Octopuses**

Adult octopus range in size from 30 g (*O. joubini*: Forsythe, 1981) to more than 25 kg (*O. dofleini*: Hartwick, 1983). In some octopuses, breeding and spawning occur seasonally. For example, *Octopus dofleini* has a mating season between October and April; females then lay and brood their eggs between January and April, whereupon they die (Hanlon, 1983). Some species, such as *Octopus cyanea*, have no seasonal spawning; instead, they appear to spawn year-round (Van Heukelem, 1983).

Octopuses are primarily solitary animals, and when they encounter conspecifics of the opposite sex, there is little to no courtship. For example, Cheng & Caldwell (2000) found that the blue-ringed octopus made contact with an introduced conspecific within 3-4 minutes, regardless of its sex. In some species, the approaching male displays his suckers (Packard, 1961) or spreads its arms to expand the web of skin in between ("web spreading") to a female to identify himself as a male (Hanlon & Messenger, 1996). In other species, males mount the conspecific and insert their hectocotylus arm before determining its sex (Cheng & Caldwell, 2000). After proper identification of the sexes, there are two basic mating tactics. In the first, the male mounts the female's mantle and inserts his hectocotylus into the mantle opening, while pumping about 50 spermatophores into the oviduct (Hanlon & Messenger, 1996). In the second method, the male does not mount the female, but instead extends the hectocotylus to insert spermatophores while maintaining some distance from the female. According to Hanlon & Messenger's (1996) account of octopus mating behavior, out of 16 species studied, 7 species demonstrated the mounting technique, 5 species demonstrated the distance technique, and 4 species were observed using both techniques.

Exceptions to these mating patterns exist. Although it is not thought to be common, there have been observations of female mating with more than one male simultaneously (Wood, 1963; Voight, 1991; Ambrose, unpublished data as cited by Hanlon & Messenger, 1996). In addition, an unidentified species exhibited pair bonding and distinct display patterns on the hectocotylized arm (Rodaniche, 1991).

Mating durations vary greatly among species of octopus, but on the whole are longer than in any other coleoid group. Duration ranges from one minute to six hours (Hanlon & Messenger, 1996). At the end of mating, which is usually terminated by the female, the pair separates. Males do not guard their mates.

Fertilization is internal and occurs as eggs pass through the oviduct into the oviducal gland (Hanlon & Messenger, 1996). Females typically deposit their eggs in dens. Females continuously guard, aerate, and clean their eggs following egg laying, and rarely eat or leave their den during this time. By the time the eggs hatch, females generally die due to starvation (O'Dor & Wells, 1978).

The eggs take 50-80 days to hatch; hatchlings reach maturity within 4 1/2 months to a year. Size and age of maturity depend mostly on availability of nutrients and temperature. Some species have planktonic young with rapid growth rates—they can

reach full size at 10 months. In others, the young are benthic, living in shallow water, while feeding on crustaceans, fish, gastropods, bivalves and other octopods. Life span for octopus ranges 10-17 months in smaller species to 3-5 years in larger species, and may have a temperature-dependent component (Hanlon, 1983).

#### Squid

Squid have lifespans ranging from 1-3 years and are terminal spawners (Worms, 1983). At adulthood, they range in size from approximately 5 mm (mantle length, ML) in the Thai pygmy squid (Nabhitabhata, 1998), to over 6600 mm ML in *Architeuthis* (Cousteau & Diolé, 1973). They live in groups of only a few individuals up to thousands of individuals (Boal & Gonzalez, 1998). Their gregarious nature, as opposed to the solitary octopods, allows for very complex courtship, mating, and agonistic behaviors (Hanlon & Messenger, 1996). In some species, social hierarchies are present and seem to be permanent (Arnold & Williams-Arnold, 1977).

Most species of squid exhibit moderate sexual dimorphism, including accentuated gonads that can be seen through the mantle (*Loligo plei*: Hanlon, 1981), patterns and displays that are sex specific (*Loligo* spp.: Hanlon et al., 1994), and differences in the size of the individuals and shape of the posterior end of their mantle (*Sepioteuthis sepioidea*: Arnold, 1965). Males show specific sexual display patterns to all conspecifics such as *Accentuated Testis* and *Lateral Flame* (See Table 1. for a description of patterns) that signal their intention to mate; if the conspecific fails to respond, it is an indication that the individual is female. The signaling male then approaches the female (Hanlon & Messenger, 1996); this behavior then elicits the interest of other males. Small subgroups

Pattern or	Description	Behavior	Species	Reference
Behavior		(male, female)		
Accentuated Testis	light or dark coloration over the area of the testis	dısplay maleness (m)	Loligo vulgaris L. plei	Hanlon et al., 1994 Hanlon & Messenger, 1996
Lateral Flame	lateral mantle streaks	dısplay maleness (m)	Lolıgo pleı L. forbesı	Hanlon & Messenger, 1996 Hanlon, 1988
Mutual Rocking	a male and female rocking back and forth together	courtship (m, f)	Sepioteuthis sepioidea	Arnold, 1965; Moynıhan & Rodanıche, 1982; Hanlon & Forsythe (unpubl. data)
Pied Display	exaggerated silver- white at times with dark coloration	females repel males; or attract attention of other males (f)	Sepioteuthis sepioidea	Moynıhan & Rodanıche, 1982; Hanlon & Forsythe (unpubl. data)
Zebra Spread	black and white transverse stripes on dorsal mantle with arms spread	agonistic (m)	Sepioteuthis sepioidea	Arnold, 1965; Moynıhan & Rodanıche, 1982; Hanlon & Forsythe (unpubl. data)
Lateral Silver	bright silver shown unilaterally to a male	agonistic (m)	Sepioteuthis sepioidea	Arnold, 1965; Moynıhan & Rodaniche, 1982
Intense Zebra	black and white transverse stripes on dorsal mantle and fourth arms, arms spread	agonistic (m)	Sepia officinalis S. latimanus S. pharaonis	Holmes, 1940; Hanlon & Messenger, 1988 Corner & Moore, 1980 pers. obs.
Precopulatory Pattern	pale arms, textured mantle, and gray body	courtship (f)	Sepia latımanus	Corner & Moore, 1980
Mottle Pattern	uniform dark brown markings on mantle and arms	mating or resting (m, f)	Sepia officinalis S latimanus	Hanlon & Messenger, 1988 Corner & Moore, 1980
Acute Disruptive	white bar on head, white square on center of mantle, white triangle on posterior mantle, dark otherwise	advances of males unwanted by female (f)	Sepia officinalis S latimanus	Hanlon & Messenger, 1988 Corner & Moore, 1980

Table 1. Body pattern descriptions that have been discussed within the text

form thereafter, which usually consist of one female and several males. One male, usually the largest, swims closest to the female and tries segregating her from the subgroup. If the female is willing, the pair swims back and forth together in the same direction in a behavior that is termed *Mutual Rocking*. The male then approaches the female, which typically flees while he pursues her. At this time, the female may show the *Pied Display*, which, according to Moynihan & Rodaniche (1982), is an indication to the male that he should flee.

On the other hand, Hanlon & Forsythe (unpublished data as cited in Hanlon & Messenger, 1996) observed that *Pied Display* attracted the attention of the other males in the subgroup, which resulted in agonistic behavior among males. In these agonistic encounters, males position themselves such that one male is below another male. They display patterns (including Zebra Spread Display and Lateral Silver Display) to one another as the encounter intensifies. A videotaped account in Little Cayman Island showed that the lower individual was always the male that had approached the female, and 43 out of 45 times, the lower male also won the encounter (Hanlon & Forsythe unpublished data as cited in Hanlon & Messenger, 1996). After such male-male encounters, the winner typically reinitiates contact with the female. During pairing, the male usually exhibits a Lateral Silver Display in such a manner that other males could see but the female could not. After the pair is somewhat isolated, the male takes up a parallel position to the female and places a spermatophore swiftly onto her head or her arms with his hectocotylus. If the spermatophore is dropped or is not attached to the female, then the male continues this same action until it is transferred successfully. A pulsating chromatophore pattern was observed during copulation (Moynihan &

Rodaniche, 1982). Post-copulatory mate guarding was also observed in this species both in the laboratory (Arnold, 1965) and in the field (Moynihan & Rodaniche, 1982). These observations stem mostly from the oval squid, *Sepioteuthis sepioidea* which has been studied extensively both in the field and the laboratory and which forms shoals of 10-30 individuals (Arnold, 1965; Moynihan & Rodaniche, 1982; Boal & Gonzalez, 1998; Hanlon & Messenger, 1996; Hanlon & Forsythe, unpublished data as cited in Hanlon & Messenger, 1996). Similar behavioral patterns are exhibited by *Sepioteuthis lessoniana*, but they do not appear to exhibit post-copulatory mate guarding (Boal & Gonzalez, 1998).

The tropical arrow squid, *Loligo plei*, has also been studied both in the field and the laboratory (Hanlon & Messenger, 1996; DiMarco & Hanlon, 1997). This species differs from *Sepioteuthis sepioidea* in that one male establishes and maintains dominance in the shoal. The dominant male exhibits agonistic behavior towards other males, consumes the majority of available food, and isolates several females away from the shoal (Hanlon et al., 1983). This species not only mates in the parallel position, but also in a head-to-head position. Another difference between the two species is the placement of the spermatophore, which here is dependent on the position of mating. When the parallel position is used, the male places the spermatophore near the oviduct in the mantle cavity. The spermatophore is placed near or in the seminal receptacle when the head-tohead position is used. The copulation only lasts five to ten seconds and post-copulatory mate guarding is common.

According to Hanlon & Messenger's (1996) account of squid mating behavior, out of 15 species studied, 5 species have been observed mating in the parallel position, 4 species have been observed mating in the head-to-head position, and 6 have been observed mating by both techniques. Another tactic observed in several squid species is that used by a 'sneaker male'. This male usually comes in and mates very quickly with a female, usually while her temporarily paired male is occupied in displaying to another male during an agonistic encounter (Hanlon & Messenger, 1996). The sneaker male places the spermatophore in the arms of the female. Because the females can store sperm for some time, the possibility for sperm competition exists within this group (Hanlon & Messenger, 1996). It has been suggested that females may then manipulate the spermataphore after mating to different areas of sperm storage (Hanlon et al., 1999). Recent DNA fingerprinting of embryos has demonstrated that multiple males have fertilized the eggs comprising one egg finger (Hanlon, pers. comm.), suggesting that females may have adopted the evolutionarily stable strategy of producing offspring from multiple males (Hanlon & Messenger, 1996).

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Fertilization in squid is external and eggs are most often laid in large communal masses. Squid, for the most part, migrate seasonally nearshore to spawning grounds where both mating and egg lying occurs (Fields, 1965). Males usually accompany females to the egg laying areas to guard her from last minute matings. Females lay eggs in egg capsules or fingers, with an average of 212 eggs/capsule (Okutani & McGowan, 1969) at depths of 3 to 180 m (Hixon, 1981). Squid die shortly after the spawning period without any parental care of eggs or young.

Eggs usually hatch somewhere between 10 and 35 days. When the hatchlings emerge they can immediately swim, feed, and ink. They start out with an energy reserve of yolk that lasts them between 2-4 days (Fields, 1965). Hatchlings use their tentacles to capture prey, mostly calanoid copepods and fish larvae. As they grow, their diet shifts to larger crustaceans and juvenile fish. As they mature, they migrate offshore to feeding grounds. Between 10-13 months they become sexually mature and then migrate back to inshore spawning grounds.

### Cuttlefish

Cuttlefish are set apart from other cephalopods in the shape of their body and their mechanism of neutral buoyancy. They have a broader, flattened body and possess a structure—the cuttlebone--that evolved from the outer shells of other molluscs. This structure has gas-filled chambers that enable them to remain buoyant without exerting excessive amounts of energy (Hanlon & Messenger, 1996). There are about 100 known species of cuttlefish, but primarily research has been focused on one species, *Sepia officinalis*. This species has been studied carefully in the laboratory and it's behavior thoroughly described by both Tinbergen (1939) and Adamo & Hanlon (1996) in that context. Fewer observations have been made in the field.

Cuttlefish apparently migrate offshore to feed and grow and then return to shallow, inshore sites (of depths 30-40 m) as sexually mature adults (Boletsky, 1983). Cuttlefish species reach sexual maturity at a large range of sizes—for example, *S. orbignyana* reaches maturity at a small size of approximately 40 mm ML (Boletsky, 1988), and *S. apama* reaches maturity at a larger size of approximately 1000 mm ML (Foale & Norman, 2000). Other species display a range of sizes at which maturity is reached: some males have been found to be mature at less than 60 mm ML, while others of the same species were found to still be immature at sizes larger than 100 mm ML (Boletsky, 1979).

The only field study of cuttlefish aggregation and mating behavior was on a species found near Guam, S. latimanus (Corner & Moore, 1980). As with squids, social interaction within the aggregation makes for complex courtship and agonistic encounters. In S. latimanus, three or four lone males were observed to aggregate near a coral head that was prime location for egg laying. When females emerged from deeper waters to lay previously fertilized eggs, on most occasions, the males approached rapidly, with the largest male most often reaching the female first. Males participating in agonistic behavior display an Intense Zebra Display. Males normally spread their arms forward or towards one another; smaller males generally terminate the encounter by fleeing. In some cases, these encounters escalate to physical contact in which males approach each other in a parallel position with the arms spread, providing an opportunity to bite. The male that wins the contest approaches the female, which then responds with a *Precopulatory Pattern.* The male rubs the tips of his arms along the dorsal surface of the female's mantle. If the female rejects the advances, she displays an *Acute Disruptive Pattern.* In response to this, the male usually jets back and approaches the female again until the female responds positively by opening her arms to mate. The couple then aligns themselves into a head-to-head position and mates for 0.5-1.5 min. While mating, a Mottle Pattern is displayed by both unless another male approaches. In that case, the mating male displays an Intense Zebra Pattern. Females of this species were observed mating before and after egg laying.

Mating behavior of S. officinalis has been studied by Tinbergen (1939) and more recently by Adamo & Hanlon (1996) and Hanlon et al. (1999). Males approach conspecifics while presenting an Intense Zebra Pattern. If the same pattern is returned with the extension of the fourth arm, then the conspecific is presumed to be male; if the fourth arm is not extended or it is extended without an Intense Zebra Pattern the individual is presumed to be female by the approaching male. Typically when a male returns an Intense Zebra Display with his fourth arm projecting outwards, an agonistic contest follows. In an agonistic encounter, the males align in a parallel position (facing the same direction or in different directions) and rotate around each other for up to 10 minutes. If one of the males does not then retreat, the interaction escalates to pushing against one another with arms while intensifying the Zebra Display. On some occasions, encounters escalate to the point where one male attacks and bites the other (Adamo & Hanlon, 1996). The loser retreats some distance and discontinues the Intense Zebra *Display.* The other male then approaches the female and swims parallel to her while displaying an Intense Zebra Display. He continues by hovering beside or above her while gently rubbing his arms across the dorsal side of her mantle, head, and arms. Tinbergen noticed hovering prior to the *Head to Head* position; this, however, is perceived as courtship and not pre-copulatory guarding. More recent behavioral observations suggest that there is little to no pre-copulatory guarding (Boal, 1996; Hanlon et al., 1999). Following an approach, the male grasps the female in the head-to-head position and flushes water into her buccal cavity (Hanlon et al., 1999). This action apparently flushes out the sperm of previous matings with other males. The male then places spermatophores in her seminal receptacle (inside her mantle cavity). Matings like

this typically last an average of 2-5 min, but can last up to 20 min. Mate guarding has been infrequently witnessed after copulation, but the pairings were only temporary and seem to focus on egg laying (Hanlon & Messenger, 1996). Egg laying bouts are intermixed with subsequent mating, but not necessarily with the same male. Sometimes previous losers reinitiate agonistic encounters in attempts to obtain matings (Hanlon & Messenger, 1988).

Although the male in *S. officinalis* initiates mating and courtship, it may be more accurate to state that mate choice is left to females (Hanlon & Messenger, 1996). Some speculate that females use chemical cues to choose their mates, rather than elaborate displays and the outcome of male contests (Boal, 1996; 1997). This has yet to be determined and further investigation is needed (Boal & Golden, 1998). From the males perspective, females are viewed as a resource, but individual females are not fought for. For example, when two *S. latimanus* pairs were approached by a third male, the males that were paired with females left them to engage in a contest with the approaching male. At the end of the encounter, the same two males returned to the females but changed partners (Corner & Moore, 1980).

In cuttlefish, fertilization is external. Eggs are fertilized in the arm bundle after the seminal receptacles have been emptied and the eggs have been released from the oviducts (Hanlon & Messenger, 1996). Viable sperm can be stored by females for long periods of time – up to five months (witnessed by Boletsky, as cited by Hanlon et al., 1999). Egg laying in cuttlefish starts between the ages of 6.6 and 12.6 months (Forsythe et al., 1994). Females lay eggs one by one attaching each to a fixed object such as a crevice or ledge of a coral (Hanlon & Messenger, 1996). One way the eggs can be protected from predators is to lay the eggs in crevices that certain fish cannot reach. This behavior is observed in *Sepia pharaonis* (Gutsal, 1989). Another way the female protects her eggs is to coat them with ink (Boycott, 1958) or with a sticky substance that collects sand (Natsukari & Tashiro, 1991). Aside from these methods, no other parental care has been observed of eggs or young. Like squid, some species of cuttlefish lay eggs in communal masses. It is believed that females die shortly after egg laying, although in the laboratory, spawning can last up to several months.

Hatching usually occurs between 40-45 or 80-90 days after laying, depending on temperature (Boletsky, 1983). Cuttlefish display direct development and can capture prey such as small mysids immediately upon hatching. They also have the ability to ink at this time.

Because octopus, squid, and cuttlefish represent important sources of protein in many cultures and sustain fisheries around the world, it is important to better understand their reproductive biology to maintain healthy fisheries over long periods of time. However, due to their complex behaviors and body patterns, they have not proven to be easy subjects for study. This study focuses on the Indonesian cuttlefish, *Sepia pharaonis*, which sustain fisheries off the coasts of the People's Democratic Republic of Yemen (Aoyama & Nguyen, 1989), India (Silas et al., 1985), and in the Suez Canal (Gabr et el., 1998). They are fished primarily by trawlers and little is currently known about their population numbers. Information on their reproduction would be of great use for further management if fisheries for them are to remain sustainable. Most information about the distribution, growth rate, and sex ratio of *S. pharaonis* in the wild has been obtained by fisherman (Silas et al. 1985). One study examined body measurements and stomach contents (Gutsal, 1989), while another provided histological determinants of maturity and fecundity (Gabr et al., 1998). It is unclear if *S. pharaonis* travel alone, in groups, or in pairs. It is also not known if they have more than one mating partner in their natural habitat. Given its fishery value and the interest in commercially culturing this species, knowledge of this cuttlefish's reproductive behavior is most needed, particularly that concentrating on mating strategies and natural sex ratios. Behavioral research on *S. pharaonis* would not only be helpful to fisheries managers but also to researchers in the field (Hanlon, 1988).

Some information exists on mate choice in *S. officinalis*, yet nothing is known about *S. pharaonis*. Such information will be useful for comparisons between these two species which are both considered important for aquaculture purposes. The purpose of this study is to learn more about the biology of *Sepia pharaonis*; specific objectives include: 1) learning how to identify individuals, particularly to distinguish between males and females; 2) determining if a dominance hierarchy exists among a captive group of males; 3) determining if large male reproductive behavior differs from that of small male reproductive behavior; 4) determining relationships between displaying patterns and specific behaviors; and 5) determining the mating sequence from pair formation through copulation and/or post-copulatory guarding (if it exists).

#### **MATERIALS AND METHODS**

Sepia pharaonis eggs were obtained from Thailand and delivered to the National Resource Center for Cephalopods, University of Texas Medical Branch in Galveston on 3 May 1998 where they were cultured to adulthood. The offspring (F1 generation) which matured from these eggs were the subjects of this observational study, as well as the subsequent (F2) generation individuals. Specifications for rearing, maintenance, and culture have previously been described for *Sepia officinalis* (Forsythe et al., 1994; Oestmann et al., 1997) and were identical for *S. pharaonis*, with the exception of using slightly higher water temperatures in the holding tanks (25-26°C). The cuttlefish were housed in an indoor rectangular, opaque tank (6 m long x 3.6 m wide x 1.5 m deep). A light: dark regime of 12:12, using overhead fluorescent lighting, was maintained throughout the observations. The animals were fed *ad libitum* 2-3 times daily on a diet of live fish and crustaceans, when available, or frozen shrimp.

## **Description of the Subject**

Sepia pharaonis (Ehrenberg) is widely distributed from the Indo-Pacific to Japan and reaches far south to Australia; it also occurs in the Red Sea (Gabr et al., 1998). At maturity, individuals range in size depending on geographical distribution. In the Suez Canal, male average size at first maturity is 61 mm (ML), while female size is 122 mm ML (Gabr et al., 1998). On the coast of India, size at first maturity increases from east to west, with females ranging from 120-160 mm and males ranging from 119-154 mm (Silas et al., 1985). Spawning periods also differ with distribution, occurring between February and April in India (Silas et al., 1985) or March to June in the Suez Canal (Gabr

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et al., 1998). Reproductive behavior is present throughout the latter two-thirds of their life cycle, approximately at 6-9 months (Gabr et al., 1998).

In this study, F1 generation cuttlefish were observed late in the mating and egg laying period from 24 May 1999 to 29 July 1999 (see shaded areas on Table 2). At this time, the cuttlefish had been displaying reproductive behavior for nearly 3 months and were nearing the end of their life (which is approximately 9-11 months). Three hours of video was obtained on intraspecific interactions of this generation. However, no mating behavior was video-recorded for the F1 generation. Three hours of video was later obtained during mating of the F2 generation on 29 January 1999.

Generation Hatching Mating Egg Laving Eggs (first observed) (first observed) 5/15/98 9/98 - 3/99Parental 5/3/98 - 5/59810/18/98 (shipped from Thailand) F1 10/18/98 11/4/98 3/21/99-7/26/99 6/10/99 F2 6/10/99 7/5/99 1/15/00 - 5/001/2/00

Table 2. Timeline for the subjects observed, highlighted regions are during observation periods.

#### Identification of Individual Cuttlefish

The F1 generation cuttlefish were identifiable by their unique striping of the mantle (Boal, 1996). The area of the mantle on the dorsal side that projects between the eyes was the focus for identification (Fig. 1). During the Identification and Initial Observation Period (Table 3) photographs were taken of each individual from the top in order to see the projected area of the mantle. After the film was processed, the photographs were studied until the markings on the area could be identified and differentiated by sight. Once the individuals were identified from the projected area of
the mantle, pictures were also taken of both sides of the individuals in order to identify them from all angles. The pictures were put in a small album and the individuals named. The album was used as a reference during video analysis to quickly identify individuals on tapes. By the time all cuttlefish were identified, 13 adults were available. Seven were identified by unique markings on the mantle, three were identified by injuries to their mantles, and three were marked by punching a small hole in their fin. One cuttlefish was marked on the superior end of the right fin, another on the posterior end of the right fin, and the third on the superior end of the left fin. The sex of each cuttlefish observed was verified after death via dissection and examination of their gonads.

Table 3. I imeline for observation period
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Observation	Identification and Initial Observations	Preliminary Observations	Sex Ratio Manipulation	Mating in F2 generation
Time Period	5/24/99–6/24/99	6/24/99–7/1/99	7/12/99-7/29/99*	1/29/00*

\* video obtained

### **Observations**

### Focal sampling

I randomly listed each individual on a data sheet and then performed focal samples on each in order of their appearance. The individual was located in the tank and behaviors that took place within a 1 min period were noted. This sampling was repeated over four days, with two observations of each individual being made in the morning, and two observations of each individual being made in the evening. These observations took place during the Preliminary Observation Period and resulted in an ethogram of reproductive behaviors and male-male interaction behaviors. Another type of focal sampling that was used was a 15-min scan sample. While all animals were together, each individual's behavior was sampled twice, once in the morning and once in the evening, over six days during the Preliminary Observation Period. During the Sex Ratio Manipulation Period where I manipulated the sex ratios, each individual was observed for 30 min.

### Scan sampling

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The scan sampling method that was used was a scan of the entire tank only noting which animals were interacting and with whom they were interacting. Scans of 25 min duration were used each day during the Preliminary Observation Period. During the Sex Ratio Manipulation Period, one hr scans were used each day, during 5 consecutive days. The interactions of concern for both scan and focal sampling included: male-male interactions, mating, guarding, and egg laying. Details of these accounts include duration, initiation, termination, and individuals involved (see Table 4 and Table 5 for descriptions of behaviors).

After the Preliminary Observation Period, the tank was divided into two sections using a seine net, plastic ties, and weights. The sex ratio was then manipulated (from 7/19/99 to 7/26/99) to observe cuttlefish behavior under different sex ratios. The individuals were placed in the intended sex ratio the night before the observation and allowed to acclimate prior to observation. However, due to senescence of the animals, replication of the ratios was not possible. For discussions, information taken from the Sex Ratio Manipulation Period was combined.

## Analysis of Behavior

Three hours of videotape were obtained during the Sex Ratio Manipulation Period and three hours were obtained during the Mating Period in the F2 Generation using a Sony Digital 3-CCD camera (DCR VX-1000) with an Amphibico VH1000 underwater housing. The primary behavior that was obtained during the Sex Ratio Manipulation Period was of male-male agonistic behavior. The primary behavior that was obtained during the Mating Period in the F2 generation was of focal pairs before and after mating bouts. The videotape was analyzed using The Observer VideoPro behavioral software from Noldus Technologies. The analysis of the video generated data that was examined further by comparing frequencies of specific behaviors between individual males, behavioral transition frequencies for approaching males and approached males, and frequency of mating behaviors for individual males. Data from the sampling methods was analyzed to determine if the frequency of approaches were related to dominance status. When a male swam directly towards another male, the act was deemed as an approach. The number of approaches and the individuals involved in the approach were calculated during the focal and scan sampling of the Sex Ratio Manipulation Period.

These calculations were then compared with a Chi-Square Goodnesss-of-Fit Test (Zar, 1984) for large males versus small males to determine if size affected an individual's tendency to approach others. A Chi-Square Goodnesss-of-Fit Test (Zar, 1984) was also used to compare the number of matings received by large males with matings received by small males to determine if matings were received randomly.

Table 4.Description of male-male interaction behaviors and their abbreviations as used in the sequence<br/>diagrams (see Fig. 10 and Fig. 11 for illustrations of these behaviors). This is not an exhaustive<br/>list of adult S pharaonis male-male interactions. The behaviors can and have been observed<br/>during male-female interactions (Table 5) as well; also two or more behaviors can occur<br/>simultaneously.

Behavior	Abbreviation	Description
Approach head to body	A-hb	male approaches head on towards side of body
Approached by head to body	A-by-hb	male approached by another male head on towards side of body
Aproach side to side	A-ss	male approaches to a side parallel position
Approached by side to side	A-by-ss	male approached by another male in a side parallel position
Rotate	Rot	males rotate in a circle in either A-ss or A-hb position
Touch	Tou	an interaction escalates to a male touch another male on side of mantle, this rarely happens
Chase	СН	a male chases another male
Flee	Flee	a male flees the area after being approached or after approaching
Left by	Left	a male is left by the male that flees the area
Blackbar pattern	BBP	the Zebra Display with a black bar along the lateral margin of the mantle also a white continuous or dashed line below the black bar on the medial edge of the fin

Behavioral frequencies were generated from the video analysis by totalling the number of times a behavior was followed by another behavior, and dividing that by the total number of times the initial behavior was observed. The frequencies or transition rates were then displayed for easy interpretation in a state-space representation or a sequence diagram (Haccou & Meelis, 1994). Two sequence diagrams were generated for

Table 5.Description of male-female interaction behaviors and their abbreviations as used in the sequence<br/>diagram (see Fig 12 and Fig. 13 for illustrations of these behaviors). This is not an exhaustive<br/>list of adult S pharaonis male-female interactions. The behaviors can and have been observed<br/>during male-male interactions (Table 4) as well; also two or more behaviors can occur<br/>simultaneously

Behavior	Abbreviation	Description
Approach	Appr	male swims towards a female closely
Hover	Hov	male stays closely over female, sometimes slightly ahead, behind or left or right of femlae
Hover and cruise	H-cr	male hovers over and cruises along with female
Hover with first pair of arms up	H-1st	male hovers with his pair of arms extended upwards
Hover and cruise with first pair of arms up	H-cr-1st	male hovers and cruises with first pair of arms extended upwards
Touch head	ТН	male touches female's head gently with tips of his arms
Touch arms	ТА	male touches female's arms gently with tips of his arms
Touch mantle	ТМ	male touches female's mantle gently with tips of his arms
Arm spread	AS	male spreads arms out laterally
Initiate head to head position	нн	male moves into the head to head position to initiate mating
Mating	Mat	male and female appear to be copulating (i.e., male is transferring spermatophore)

male-male interactions, one from the perspective of the approacher, and the other from the approachee's perspective. Another sequence diagram was generated from the males perspective in a mating sequence.

### RESULTS

#### Identification

Four groups of cuttlefish were identified on the basis of sex, size, and display patterns: large males, average-sized males, small males, and females. Large (> 270 mm) and average-sized males (240-270 mm) were associated with distinctive chromatophore patterns, while small males (<240 mm) were associated with a more muted pattern. Although large and average-sized males differed in their mating success rate, their body patterning and behavior was similar and hence they are grouped together for the purposes of behavioral discussions.

Large and average-sized males (Fig. 2) were easily identified by their wide use of *Intense Zebra Display* (94.7%; Table 6), flamboyant postures (100%), and aggressive behavior (100%) towards other males and females. In contrast, small males (Fig. 3) rarely displayed elaborate patterning (5.3%), were never observed displaying flamboyant postures, and were not aggressive towards other males or females. Because small males had a muted zebra pattern that consisted of faint stripes on their mantle and fourth arms, they were initially identified as females until they were observed mating. Females (Fig. 4) were usually mottled with very faint stripes visible on the mantle, but no stripes visible on the fourth arms. Females never approached males or other females aggressively. The sexes were correctly identified as verified by posthumous dissection.

Three generations of *S. pharaonis* were cultured at UTMB. Mantle lengths and weights were recorded for most individuals at the time of death (Table 7; Fig. 5). Size differences exist in males of the same age, but there is insufficient data to construct a size distribution.

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Fig. 2. Large male *Sepia pharaonis* 



Fig. 3. Small male Sepia pharaonis



Fig. 4. Female Sepia pharaonis

## Male-Male Approaches

Data from the sampling methods was analyzed to determine if the frequency of approaches were related to dominance status as well as to determine if size affected an individual's tendency to approach others. Small males were rarely observed approaching large or small males (5.8% of observed approaches). Large and average-sized males seldom approached small males (13.5%) but frequently approached other large and average-sized males (80.8%). This approach pattern was not random (n = 208 approaches;  $\chi^2 = 213.80$ , P < 0.001, d.f. = 3).

and females. Data collected from the Sex Manipulation Period.						
Behavior category	Relative Frequency Observed In					
	Large males	Small males	Females			
Body Pattern Displays (Intense Zebra Display)	94.7% (72/76)	5.3% (4/76)	0%			
Flamboyant postures (front two arms up, arm spread)	100% (61/61)	0%	0%			
Approaches (male to male or female to male)	100% (57/57)	0%	0%			
Aggressive Behaviors (approach with arms spread, chasing, touching other males)	100% (46/46)	0%	0%			
Mating Behavior (males approaching females, males touching females, hovering, head to head)	88.2% (15/17)	11.8% (2/17)	involved in all mating behavior			

Table 6.The comparison of relative frequency of specific behaviors between large males, small males,<br/>and females. Data collected from the Sex Manipulation Period.

Generation	Generation Males Fem		nales	<b>Unknown Sex</b>		
Parental	ML (mm)	WT (g)	ML (mm)	WT (g)	ML (mm)	WT (g)
5 months	202 145	696.1 362.9				
6 months					177 177	585.5 532.1
7 months	260	1355				
8 months					240 225	1216.8 711.9
9 months					240	1174.2
10 months	262	1256.7			250	1045
F1						
5 months					140	400
6 months	170	443.1	214 205	1187.5 1140.5	141 186 195	235.7 660.7 983.2
7 months	206	879.8	220 215 204	975 1173 901.7		
8 months	249 260 208	1541.6 1662.2 1028.6	210 223	978.8 1215.6	206 198	800.6 956.1
9 months	304 252	2481.4 1419			225	1148
11 months	250	?				
F2						
7 months					190 194 215 195	950 951.5 1372.6 ?
8 months	214	1942.3	206 195 208	1052.4 862.8 893.9	165	426.6
9 months	246	1263.8			276	1942.3

 Table 7.
 Mantle lengths and weights for three successive generations of S. pharaonis (shaded areas were obtained from J. Minton, unpublished data). Measurements were taken at the time of death.



Fig. 5. Comparison of individual male mantle lengths measured at the time of death. Differences in individual sizes appear within the same age category and are denoted by different hatching patterns.

While observing male-male approach behavior, a body pattern, dubbed the *Blackbar Pattern*, was repeatedly observed. The *Blackbar Pattern* (Fig. 6) is the *Zebra Display* accompanied with a black bar along the lateral margin and a white continuous or dashed line below the black bar on the edge of the fin. This pattern is often accompanied with an extension of the fourth arm. Occasionally a blue iridescent line appeared below the black bar instead of the white line. *Blackbar Pattern* was observed in 65.6% of males that exhibited flight behavior when approached (Table 8). Males that approached displayed *Blackbar Pattern* only 7.4% of the time.

Table 8.The frequency of a Blackbar Pattern in conjunction with approach or fleeing behavior; data<br/>from the Sex Manipulation Period.

	Yes	No
Approach	7.4%	92.6%
Flee	65.6%	34.4%

**Blackbar Pattern** 

The frequency of approaches, and flight behavior were compared in an attempt to identify a dominance hierarchy among the males (Fig. 7). The largest male was observed



Fig. 6. Blackbar Pattern shown by a subordinate male



Fig. 7. The relative frequency of males, ranked by size (mantle lengths (ML) in mm) from largest to smallest, approaching, being approached and fleeing.

to approach most frequently and the smallest male was not observed approaching. While all males were approached by other large and average-sized males, the smallest male was approached the least. Differences in flight behavior also appeared: the largest male fled the least of all large males. Similarly, the smallest male fled the least of all males.

The frequency of guarding (*Hover* and *Hover Cruise*) may also be a good indication of the dominance status of a male. The frequency of pre-copulatory guarding (i.e., courtship) of females was observed for each male (Fig. 8). The largest male was observed to guard most frequently (69.2%) while the smallest male was never observed guarding.

Due to observations taking place at the end of the life cycle of the F1 generation, few courtships and matings were observed (11). Matings only occurred during the Preliminary Observation Period (4 large males, 2 small males, and 2 females) and the Sex Ratio Manipulation Period (where 3 large males were paired with one female). Guarding seemed to be dependent upon the presence of multiple large males. When this occurred, large males often seemed relentless in hovering above females (Table 9). Females responded by either fleeing rapidly from the males, or allowing males to guard them for long periods of time. There was no attempt made to mate or guard by either male when a large male, a small male, and a female were placed together. Similarly, a large male did not guard or attempt to mate with a female in the absence of other large males. As soon as another large male was placed in this situation, the first large male immediately began guarding the female and shortly thereafter initiated mating (pers. obs).

Table 9. The percentage of time observed that individual females were being approached, or hovered by a male; data from the Sex Manipulation Period and the Mating in F2 generation Period.

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		Total Time Hovered	Percentage of Time
	Total Time	or Approached by a	Hovered or Approached
Females	Observed (in sec)	Male (in sec)	by a Male
1	971.5	831.0	85.5%
2	483.2	478.3	100%
3	3100.2	3075.4	99.2%
4	2275.0	1821.4	80.1%
5	4141.3	4141.3	100%



Fig. 8. The relative frequency of pre-copulatory guarding of individual males ranked in size (mantle lengths (ML) from largest to smallest.

### **Agonistic Behavior Sequences**

Agonistic behavior was observed between males both in the presence and absence of females. Documentation of this behavior was obtained via video camera recordings (Fig. 9). The typical sequence of events obtained from viewing the video for a male-male encounter followed a general pattern: 1) a male approached another male head on or towards the other male's side; 2) the approached male usually displayed a *Blackbar* Pattern in response to this approach (see also Fig.6); 3) the males then rotated around each other; 4) the approached male extended his 4<sup>th</sup> arm towards the approaching male; sometimes the approaching male responded by also extending his 4<sup>th</sup> arm; 5) the approached male normally fled the area. Behavioral transition frequencies were calculated from the video analysis and are shown in Figs. 10 and 11. Seven of the most common behaviors were chosen to calculate the transition frequencies to simplify the diagrams. The thickness of the arrows in these diagrams corresponds with relative frequency of the action. Thus, the thicker the arrow, the greater the relative frequency. The diagrams are useful to demonstrate differences among the sequences for particular actors. The sequence was first calculated from the approacher's perspective (shown in Fig. 10). This sequence always started with an approach, either Approach Side to Side (23.6%) or Approach Head to Body (76.4%) behaviors are described in Table 4). Touch behavior (7.3%) was rarely seen, but when a sequence escalated to this behavior, it always resulted with the approaching male being Left By the approached male. In 81.8% of the interactions, *Rotate* behavior was followed by *Left By* behavior. *Chase* behavior was followed by Left By behavior in 75% of the interactions. This sequence also ended



Fig. 9. The sequence of events in a typical male-male approach. 1) a male approaches another male in the *Head to Body* position; 2) the approached male displays the *Blackbar Pattern*; 3) both males rotate and extend their fourth arms; 4) the approached male flees the area



Fig. 10. Sequence diagram of 55 male-male interactions from the approacher perspective Abbreviations found in Table 4.

most frequently with the approaching male being *Left By* the approached male. It also ended when the approaching male would *Flee* from the approached male. The sequence of male-male interactions was also calculated from the approachee's perspective (shown in Fig. 11). This sequence either began with *Approached by Side to Side* (25%) or *Approached by Head to Body* (75%). *Approached by Head to Body* was followed by *Blackbar Pattern* in 64% of the interactions. *Blackbar Pattern* followed *Approached by Side to Side* in 46.1% of the interactions. When *Blackbar Pattern* was expressed by the approached male, 55.2% of the interactions resulted in the approached male *Flee*ing the area. In the event that the males exhibited *Rotate* behavior, 40% of the interactions resulted in the approached male being *Left By* the approaching male, and 40% of the interactions resulted in the approached male *Flee*ing the approaching male.

### Mating by Large Males vs. Small Males

Eleven matings were observed (Table 10) during both the scan and focal observations in the Sex Ratio Manipulation Period. All matings listed in Table 10 were observed when all remaining individuals were housed together and allowed to move about freely, with the exception of one, which was observed in a 3:1 male:female ratio. While there was a trend for both the largest and the smallest males to obtain the greatest frequency of matings, this trend was not significant ( $\chi^2 = 5.63$ ; 0.05 < P < 0.10; d.f. = 2; Table 10). Mean mating duration for large males was twice as long as the mean mating duration for small males; however, it is not clear if this represents a significant difference



Fig. 11. Sequence diagram of 52 male-male interactions from the approachee perspective. Abbreviations found in Table 4.

because of the low frequency of mating overall and the small sample size for measures of mating duration. Small males engaged in fewer components of overall mating behavior (approaching, hovering, touching, head-to-head positioning) than large males, primarily displaying only head-to-head behaviors (=mating) (Table 6).

Size	Males	Size (ML)	Mating Frequency	Obs. Freq.	Exp. Freq.	Mean Duration
	Hi-C	280 mm*	5			208 sec
Large	Redd	290 mm	1	6	3.67	218 sec
	Frank	280 mm	0			-
Average	Wayne	250 mm	0	0	3.67	-
	Martini	240 mm	0			-
Small	L-3	210 mm	4	5	3.67	94 sec
	Tiny T	210 mm	1			103 sec

Table 10. Mating frequency of males ranked by size with consideration to duration.

\* male died three weeks before others but was largest male at time of death

## Mating Sequence

Four observed matings were obtained on video from the F2 Generation Mating Period. The general sequence of events was as follows: 1) a male approached a female; 2) if the female did not flee immediately, then the male hovered above her or to the side of her closely; 3) the male stroked the female on her head, her arms, and the dorsal side of her mantle (sometimes this continued for over an hour) (Table 9) ; 4) eventually the male initiated mating by turning around and getting in a head-to-head position (details of copulation are not known but probably very similar to *S. officinalis*); 5) the female usually terminated the mating and fled from the male; 6) the male followed the female and hovered over her (i.e., post-copulatory guarding) as she cruised around. Behavioral transition rates were calculated from the video analysis (shown in Fig. 12). Each interaction began when a male *Approach*ed a female. The male was observed equally to either *Hover* or *Hover and Cruise*. When *Initiate Head to Head Position* behavior occurred, it was always followed with *Mating*. Each observed *Mating* was followed with *Hover* or *Hover and Cruise*.



Fig 12. Sequence diagram of 22 male-female interactions from the male perspective. Abbreviations found in Table 5.

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### DISCUSSION

## **Identification**

One goal of this study was to be able to differentiate male from female *Sepia pharaonis* via external characteristics. After many hours of observation and the use of a photographic atlas, this goal was achieved. Large, average, and small males always showed striping on their fourth arms, albeit sometimes faint, especially noticeable under the eye. Striping on the fourth arms was completely absent in females. This method proved to be highly reliable for adults (no animals were misidentified); unfortunately, it was not useful for identifying the sex of hatchlings or juveniles because they lack striping on their fourth arms. This method of distinguishing males from females is similar to that used for *S. officinalis* (J.W. Forsythe, pers. comm., 1999).

Furthermore, I found that there were two behavioral classes of males which correspond to size differences, in my study. Large and average-sized males displayed the *Intense Zebra Pattern*, had flamboyant postures, aggressively approached males, and frequently approached and guarded females. In contrast, small males rarely displayed the *Intense Zebra Pattern*, did not approach males or females aggressively, and rarely guarded females. Despite these behavioral differences in the two classes of males, they both obtain matings. In fact, the largest male(s) and the smallest male(s) obtained matings, while those males between these two extremes did not obtain matings during the observation period even though they display "large" male behaviors. Thus, it appears that at least two reproductive strategies exist in this species: a large male strategy of intense

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displaying and approach towards other similarly sized males, and a small male strategy of muted patterns and female-like behaviors. Although it is known that size varies greatly in adult cuttlefish of similar age, the distinction of two classes of males has not been made. In the more commonly studied cuttlefish, *S. pharaonis*, two male mating strategies have not been reported (Tinbergen, 1939; Adamo & Hanlon, 1996).

The existence of two male mating strategies may be unique in Sepia pharaonis, but it has also been observed in teuthoids, as well as some vertebrates. In teuthoids, sneaker males are found in Loligo squid (Hanlon et al., unpubl. data as cited in Hanlon & Messenger, 1996). Most male squid temporarily pair with females, accompanying them into the egg laying area after mating in either the *Head to Head* or the *Parallel Position*. Sneaker males differ in that they are smaller and female-like, and come in quickly towards a temporary pair to mate with the female in an angled position. Another example of alternate mating strategies is found in some frog species (Wells, 1977). Smaller males are non-vocal and apparently lack territories. They position themselves near a larger, calling male and try to intercept the females that the larger male is attracting (Wells, 1977). In this example, however, size is associated with age, unlike the situation seen in S. *pharaonis*. Wells (1977) explains that the smaller males are younger. An increase in age coincides with an increase in size, possibly enabling the older and larger frogs to defend better territories. In S. pharaonis, size varies among males of the same age, so they appear to maintain the same mating strategy throughout their lives, dependent on their size.

Overall, the animals used in this study were larger than those sampled in the wild. The males in this study ranged in size from 145 mm ML to 304 mm ML. Females ranged in size from 195 mm ML to 223 mm ML. In contrast, wild caught *S. pharaonis* females had a larger size range than males (50–240 mm ML and 40–200mm ML; Gabr et al., 1998). The difference in size could be due to food availability in the wild vs. laboratory settings or to geographical variation. The cuttlefish used here were raised from eggs obtained from Thailand, while those measured by Gabr et al. (1998) were obtained from the Suez Canal. Silas et al. (1985) reported that ranges in size differed along the two coasts of India. They also reported that the maximum size of males was larger than that of females, and stated that females had a faster growth rate up to 24 months when males then surpassed them.

# Male-Male Approaches

Large and average-sized males approached other males more frequently than small males. Furthermore, the largest male of the group (Redd) approached other cuttlefish more frequently than all other males. This may suggest that Redd was the most dominant male of the group; however, dominant status may change over time. During initial observations prior to data collection, it appeared that Frank was the most dominant male because he was observed frequently approaching other males, causing them to flee, and guarded and mated with females. For reasons unknown, the hierarchy appeared to change within a week to result in HI-C as the most dominant male. After Hi-C's death Redd became the most dominant male. Further investigation is needed to determine the factors involved in the shift of the dominance hierarchy.

Observations of approach behavior revealed that specific chromatophore patterns were used by males involved in encounters. Common displays of *Zebra Pattern* and *Acute Disruptive Pattern* are seen in several species of cuttlefish and some squid (see Table 1) during interactions. Other display patterns seem to be species-specific. For example, *S. officinalis* males display a *Lighter Face* during their agonistic encounters, presumably to stop the escalation of a fight, although many bouts still escalate to physical contact and sometimes biting. In *S. pharaonis*, a newly described pattern–the *Blackbar Pattern*–was associated with flight behavior. When one male approached another, the male that fled would frequently display *Blackbar Pattern*. Occasionally, Blackbar Pattern was displayed by the approaching male, but this was fairly infrequent (<10% of the observations). Thus, far, the Blackbar Pattern was the only new pattern observed; however, other patterns may be related to specific behaviors with further investigation.

## **Guarding Behavior**

Males were observed guarding females both before and after copulation. Quite frequently males would guard females for long periods of time (sometimes over an hour) (Table 9) with no attempt to initiate mating (pers. obs.). Males also approached a temporarily paired male and female. The paired male would either display back to the approaching male by raising its first pair of arms and exhibiting *Intense Zebra* or he would leave the female with little to no agonistic displays. Both responses were observed in large and seemingly dominant males, as well as small males, but the rate of displacement was not investigated. Boal (1996) used the rate of displacement as an indicator of dominance within a group of five adult male *S. officinalis*. However, Boal's dominance hierarchy experiment focused on the displacement of a male from an area and did not include males being displaced from guarding a female. A similar experiment with *S. pharaonis* would be valuable and may better illuminate the nature of the dominance hierarchy, as well as the role of guarding behavior in this species.

Guarding behavior in *S. officinalis* and *S. pharaonis* appears to be strikingly different. In *S. officinalis* pre-copulatory guarding (*Hover*) and courtship are minimal, with immediate attempts at mating being made by the male (Boal, 1996; Hanlon et al., 1999). However, the differences seen in guarding behavior between the two species may be due to experimental conditions which differed as well. In both studies of *S. officinalis* mating behavior, cuttlefish were communally-reared until sexually distinguishing characteristics became apparent, and then males were separated from females until the time of the observation. In my experiment, all males and females were continually housed together. During the Sex Ratio Manipulation Period, the tank was divided by a seine net, and at certain times during that period, males and females were separated. Yet when the males were later allowed access to the females, they made no attempt to rush the females or initiate mating immediately, unlike *S. officinalis*. Thus, I would anticipate that if males and females were held in separate tanks, and not introduced until the beginning of the

observation period, that males would not rush females immediately attempting to mate, again unlike *S. officinalis*.

## Agonistic Behavior Sequence

Agonistic encounters between males were continuous throughout my behavioral observations. The amount and degree of escalation did not change after the death of all females. It has been suggested in *S. officinalis* that female choice has nothing to do with the outcome of an agonistic bout, the size, or the dominance status of the male (Boal, 1997). My results indicate that such factors may have an effect on the outcome of female choice in *S. pharaonis*. However, since female choice was not examined directly, this is merely speculation. What I examined, however, was the sequence of behaviors involved in agonistic encounters and how those sequences varied for the male initiating the approach and the male being approached. Thus far, transition sequences have not been created for any other species of cuttlefish.

From an examination of these sequences, differences in agonistic behavior again are apparent between *S. officinalis* and *S. pharaonis*. In *S. officinalis*, agonistic bouts often lead to physical contact, grappling, and sometimes biting (M. Karson, pers. comm., 1999); such escalation also occurs in *S. latimanus* (Corner & Moore, 1980), however it is not known to what extent, because this species has never been observed in a laboratory setting. Escalation to physical contact was extremely rare during agonistic encounters in *S. pharaonis* and was limited to a slight touching of the mantle. Most agonistic sequences consisted of an approach, rotation of the pair then flight of the subordinate male (usually the approached male). The only exception to this occurred when I attempted to remove a male from the tank and he jetted backwards while inking, bumping into another male. This second male then grabbed onto the fleeing male, and marred his mantle. At this point, the fleeing male swam away from the male who had grabbed him.

## Mating by Large Males vs. Small Males

The ability for only the largest and smallest males to obtain matings was at first puzzling. It appeared that the largest males were interfering with other large and averagesized males by continuously engaging them in agonistic encounters. This left the small males free to pursue interactions with females. Large and average-sized males may ignore small males due to their female-like behavior and appearance, or may not consider the small males to be competitors. If the former were the case, however, one would expect to see large males attempting to mate with small males, yet this was never observed. If the latter, the lack of pre- or postcopulatory guarding of females by small males may have provided no stimulus for agonistic behavior from large or average-sized males. This may also explain why males would guard females in the presence of other large or averagesized males with no attempt to guard females in the presence of small males.

# Mating Sequence

Although only four matings were documented on video, 22 male-female interactions were obtained. From these interactions, it is clear that approaches and guarding (hovering) occur frequently, with no subsequent attempts at mating. Several circuits of behavior are noticeable from the transitional behavior frequency diagrams of mating obtained for *S. pharaonis* (Fig. 12). This rather complicated sequence is simplified by removing behaviors not directly involved in mating (Fig. 13). The typical mating sequence consisted of the following: *Approach* > *Hover and Cruise* (guarding) > *Touch Mantle* > *Head to Head* > *Mating*. Less commonly, was the more direct sequence of *Approach* > *Hover and Cruise* > *Head to Head* > *Mating*. In contrast, *S. officinalis* exhibits little to no pre-copulatory mate guarding (Boal, 1996; Hanlon et al., 1999). Therefore if a similar sequence were rendered for their behavior, it would consist of the following: *Approach* > *Head to Head* > *Mating*.

The video used for this analysis was also obtained from the subsequent generation of cuttlefish where individuals were not identified or measured. Otherwise further assumptions may have been made from this data.

## **Conclusions**

Unfortunately I was only able to look at one generation making it difficult to further examine the two male mating strategies observed and relating the strategies to male size. It is also not clear if this phenomenon occurs in nature. The lack of useful data on size distribution in the wild does not make it possible, at this time, to determine if there is a bimodal size distribution of mature males.

I also examined several kinds of behaviors to determine whether a dominance hierarchy existed in communally-held males. While a dominance hierarchy seemed to exist based on size, frequency of agonistic approaches, and frequency of guarding behavior, it



Fig. 13. Modified sequence diagram of 22 male-female interactions from the male perspective. Abbreviations found in Table 5

was not a determining factor for which males received matings. It is not thought to be a determining factor because small, seemingly subordinate males were also observed mating. It appears that there is non-random mating success among males because a few males accounted for the majority of the matings.

It is fairly certain that sperm competition occurs in cephalopods (Hanlon & Messenger, 1996). Cryptic female choice may also play a role in the paternity of offspring by females discharging sperm, selective use of stored sperm, and premature interruption of copulation (Eberhard, 1996). All three tactics have been observed in female *S. officinalis* (Hanlon et al., 1999) and in squid, multiple males are known to have fertilized the eggs within one finger. How differently obtained spermatophores are partitioned by *S. pharaonis* females is unknown. Perhaps females can hold those spermatophores obtained from small males in a different location from those obtained by large males, as is seen in squid. Clearly, further observations and paternity analyses of egg capsules would provide an answer for such questions.

The mating system observed in the laboratory is one of promiscuity for both males and females. Although a dominance hierarchy is suggested for males, it is not clear how this hierarchy aids males in obtaining matings. It is also not clear how or why the dominance hierarchy changes over time. Furthermore, it appears that *S. pharaonis* is far more social than the well-studied *S. officinalis* and this increased sociality may be responsible for the promiscuous mating system observed. However, natural sex ratios are not known for this species, making it difficult to keep laboratory conditions reflective of those in nature. Determining the natural sex ratio, as well as the natural distribution of

male sizes in a mating population, and using it in laboratory settings may result in a different mating system emerging.

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