

MATING BEHAVIOR AND FERTILIZATION SUCCESS OF THREE ONTOGENETIC STAGES OF MALE ROCK SHRIMP *RHYNCHOCINETES TYPUS* (DECAPODA: CARIDEA)

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A B S T R A C T

The mating behavior and fertilization capacity of three different ontogenetic stages of male rock shrimp *Rhynchocinetes typus* were examined. The first ontogenetic stage is the typus morphotype, which is similar in morphology to the female. The last ontogenetic stage, the robustus morphotype, is characterized by extremely long third maxillipeds and powerful chelae. During ontogenetic development between these two morphs, males undergo several intermediate molts, which are termed “intermedius.” In mating experiments with pairs of single males and females, all ontogenetic male stages (typus, intermedius, robustus) behaved in a similar manner. First, they followed the female, then embraced it with the second pereopods and held it beneath their bodies, engaged by the pereopods, the 3rd maxillipeds, and the abdomen. Various stimulating and checking behaviors preceded the placement of the first spermatophore, which usually coincided with the start of the spawning process. Most spermatophores were placed during the first 30 minutes of spawning. Following spermatophore placement, males guarded the females during almost the entire spawning process, which could last longer than 120 minutes. The number of spermatophore transfer events during the mating process varied significantly between the two extreme ontogenetic male stages, being typus greater than robustus. No significant differences were found in the percentage of eggs lost by females that were fertilized by the three ontogenetic male stages. These results suggest that all male stages have the same potential to mate successfully with females in a competition-free environment. However, we propose that male mating success may change drastically when different ontogenetic male stages compete directly for access to reproductive females.

Many crustacean females are receptive only during a relatively short period, often immediately following molting. In many of these species, this leads to a highly male-biased operational sex ratio resulting in strong competition among males to gain access to receptive females. Once mated with a female, males may utilize various tactics to ensure that no other male can successfully inseminate the female. One tactic to protect the male sperm investment is prolonged post-copulatory mate-guarding which occurs in some brachyuran crabs (Jivoff, 1997a, b; Sainte-Marie *et al.*, 1997; Pinheiro and Fransozo, 1999), and is also reported for the caridean shrimp *Macrobrachium rosenbergii* (De Man, 1879) (see Ra’anan and Sagi, 1985). Males may also remove sperm from a previous male (see e.g., Elnor and Beninger, 1995). Another tactic is the application of sperm-plugs, known to occur among brachyuran crabs (Diesel, 1990) and a wide variety of penaeid shrimp (Bauer and Lin, 1993). Both pro-

longed mate-guarding and the application of sperm-plugs are uncommon among caridean shrimp. In caridean shrimp species, pairs only form for comparatively short periods during which sperm packets are transferred (< 30 s), after which the female is liberated again (e.g., Bauer, 1976; Boddeke *et al.*, 1991). Following this short mating act, a female could possibly mate with another male. Thus, there appears to be an imminent potential for sperm competition in caridean shrimp.

After reaching sexual maturity, males of many crustaceans continue to grow and gain competitive advantages over smaller males (e.g., Jivoff, 1997a). Large males may monopolize food resources as well as reproductive resources (i.e., females and reproductive habitats). In many species, growth may be accompanied by considerable morphological changes. For example, allometric growth of some body structures is reported in many brachyuran crabs (Hartnoll, 1982). In the freshwater shrimp *Macrobrachium rosen-*

bergii, these changes during male ontogeny are so drastic that different male morphotypes are distinguished (Kuris *et al.*, 1987). Two different male morphotypes have also been described for the common rock shrimp *Rhynchocinetes typus* H. Milne Edwards, 1837 (Torres, 1983). Although Torres (1983) discussed that these morphotypes may be genetically distinct, recent studies demonstrated that they represent different stages during male ontogeny (Correa and Stotz, unpublished data), similar to what is known for *M. rosenbergii* (Kuris *et al.*, 1987; Sagi and Ra'anani, 1988). Males first become mature during the female-like *typus* morphotype, after which they molt through various intermediate stages to the *robustus* morphotype, which is the last molt stage. Although it is currently not known exactly how long it takes for a male to grow from the *typus* morphotype to the *robustus* morphotype, the process requires at least three months (Correa and Stotz, unpublished data). Thus, *typus* males are sexually comparatively naive, whereas *robustus* males could be considered relatively experienced.

The occurrence of different morphotypes among males has evolved in different ways in many animal taxa (see for example Moczek and Emlen, 1999, and papers cited therein). In many cases, the occurrence of more than one male morphotype within a population has important implications for male behavior when males are seeking access to scarce resources primarily related to reproduction. Behavioral patterns may range from basic social hierarchies to more complicated patterns in which single individuals might use more than one tactic depending on the social environment. In other cases, single male morphotypes use only one tactic throughout their lives, but within the population different tactics coexist among males (Dominey, 1984).

The rock shrimp *Rhynchocinetes typus* is very common along shallow (from 0 to ap-

proximately 40 m depth) rocky areas of the coast of Chile (Vasquez and Castilla, 1982). During the day, they hide among rocks, and at night, they forage over the surface of the rocks. Ovigerous females and juveniles are found in commercial traps throughout the year (Vasquez and Castilla, 1982; Arana and Henríquez, 1983). However, there is a marked increase in the proportion of ovigerous females during the austral summer, while simultaneously the overall proportion of females caught by traps decreases (Vasquez and Castilla, 1982). These changes could reflect a reduced availability of unmated females, implying that male-male competition for females exists in *R. typus*. The observed morphological differences between the ontogenetic male stages suggest that they exhibit different behaviors when searching for and mating with females. In the present study, we describe the mating behavior of three ontogenetic male stages of *R. typus* in a competition-free environment in order to provide information that is essential for the evaluation of male mate competition in future studies.

MATERIALS AND METHODS

Collection and Selection of Rock Shrimp for Experiments

Shrimp were collected by autonomous diving from the shallow subtidal zone of Bahía La Herradura, Coquimbo, Chile (29°59'S, 71°22'W). Individual shrimp were caught with a hand-held net, placed in a collecting basket, and shortly thereafter transferred to flowing sea water in the laboratory. Males and females were kept in separate tanks with *ad libitum* food supply (ascidian colonies with their epibionts, crushed mollusks, and dead fish) before they were used in the experiments. During the period of this study, water temperatures varied between 17.0 and 19.2°C.

Three ontogenetic male stages were distinguished according to the presence of hairs on the first cheliped, the numbers of spines at the tip of the 3rd maxilliped, number of spines at the propodus of the first pereopod, and the relationship between the lengths of the last segment of the 3rd maxilliped and the carapace (Table 1, Fig. 1). Males were selected randomly within each male cate-

Table 1. Morphological characters used to select the three ontogenetic male stages of *Rhynchocinetes typus* for the experiments in this study.

Characters	Ontogenetic male stages		
	Typus	Intermedius	Robustus
Hairs on 1 st cheliped	no	yes	yes
Relationship, last segment 3 rd maxilliped/carapace length	<< 1	> 1 and < 2.1	> 2.1
Number of spines at tip of 3 rd maxilliped	7	7	1
Number of teeth at tip of propodus of 1 st cheliped	4	4	1

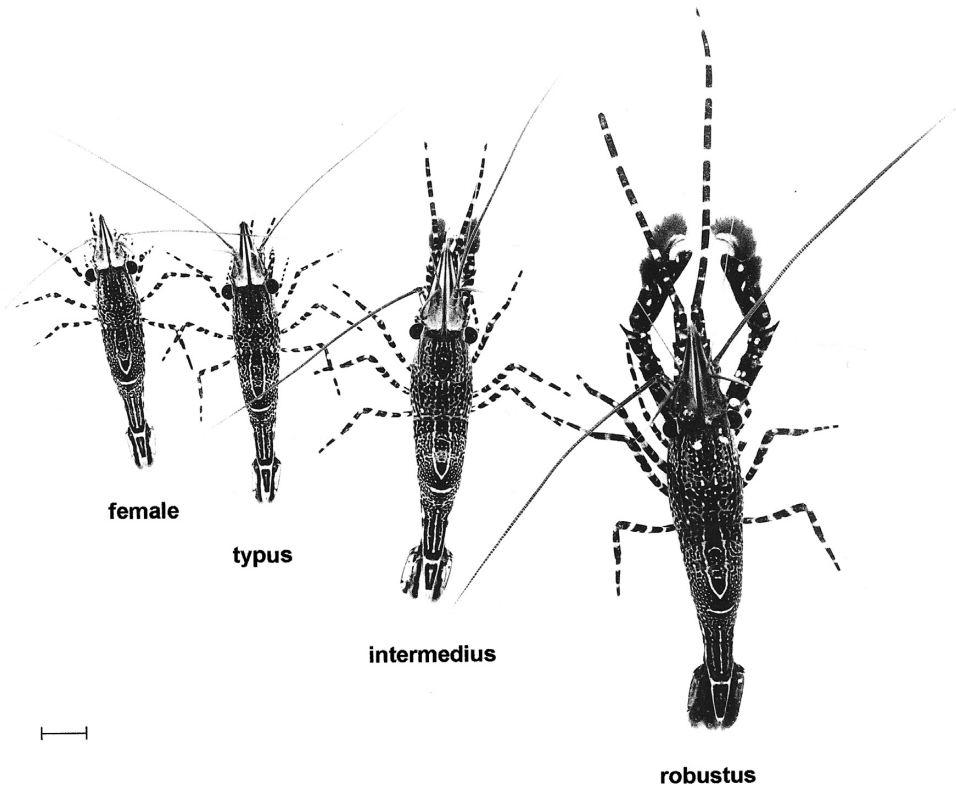


Fig. 1. Photographs of female and the three ontogenetic male stages of *Rhynchocinetes typus*; scale bar = 10 mm.

gory for use in the mating experiments. In order to ensure full sexual maturity in males of the first morphotype (typus), only males larger than 15 mm carapace length were considered for the experiments. This means that in the case of the typus males, only the largest males from this category were used in the experiments. Most males were maintained in the holding tanks without access to females for at least 5 d before being used in the mating experiments, although occasionally a male may have been used the day following its capture in the field.

Recently molted and receptive females were collected each afternoon from the sea water tanks. When exuvia were found in the tanks, the recently molted females with still-soft exoskeletons were identified and immediately separated into individual containers. These females were used for observations of the mating behavior to be conducted the following day. Thus, all mating observations were conducted using females 12–36 h after they had molted. Preliminary observations indicated that females are not receptive immediately after molting but some hours thereafter, which is why we chose to use females 12–36 h after molting for this particular experiment. For the experiments on the fertilization potential of males, each recently molted female was immediately placed in an individual container together with a male. Thus, all experiments on male fertilization potential were started with females 0–24 h after molting. In these experiments, because males and females remained together for at least 48 h, it was guaranteed that females reached receptivity. Females were randomly selected for the different treatments

(male stages and control). All experimental replicates in which either males or females showed adverse behavior (males molting during experiment, females never spawning) were discarded and repeated with new rock shrimp (see below).

Experiment 1: Observations of Male Mating Behavior

Pairs consisting of one male and one female were each introduced to a glass aquarium (20 cm × 30 cm surface area, 12 l volume). During an acclimatization period of 15 min, the male and the female were separated by a plastic sheet placed in the middle of the aquarium. The experiment was initiated by removal of the plastic sheet after which males and females could approach one another. Individual pairs were observed for periods of at least 120 min. When mating was initiated during those 120 min, we continued to observe the mating pair throughout the entire mating process, which may have occasionally lasted for longer than 3 h. We recorded the exact moment when a male approached and grasped a female and the moment when the male released the female after successfully mating with it (= total mating time). During the mating process, we alternated periods of 15-min duration during which we intensively observed each mating pair (high-intensity observations) with 15-min periods during which we only monitored whether the pair remained together (low-intensity observations). During the low-intensity observations, we also recorded the total number of events in which spermatophores were

placed by the male under the female's abdomen. Because we were primarily interested in the details of the mating process, we intensely monitored each mating pair every other 15 min for intervals of 15-min duration, which we conducted repeatedly throughout the whole mating process. These high-intensity observations commenced immediately after the male had taken hold of the female. During these high-intensity observations, we carefully monitored the different behaviors exhibited by the shrimp. The characteristics of these behavioral events are described herein and their occurrence was recorded quantitatively during the high-intensity observations. At the end of the mating process, when male and female shrimp separated, one additional 15-min interval of high-intensity observations was conducted. The observations were concluded when no more direct interactions between the male and female shrimp were observed. During all high-intensity observations, we additionally recorded the spawning behavior of the females. Individual mating experiments were considered unsuccessful when males did not pay attention to females after 120 min. For each treatment (typus, intermedius, robustus), 10 replicates were conducted.

A size analysis between the males of the different stages used in this experiment revealed that intermedius males were significantly larger than the other two male stages (one-way ANOVA; $P < 0.05$; Tukey test; $P < 0.05$) (typus: 18.2 ± 1.5 mm; intermedius: 20.5 ± 1.1 mm; robustus 18.8 ± 1.8 mm; mean carapace length \pm SD). Normally, one would expect typus $<$ intermedius $<$ robustus, and the size differences found herein may have been a result of the small sample size we used. Because in this experiment males from 15.5 to 21.5 mm carapace length mated successfully with females, we believe that the size differences revealed in the above analysis did not affect the outcome of our experiments.

Experiment 2: Examination of Male Fertilization Potential

To examine the fertilization potential of the three ontogenetic male stages, the numbers of fertilized eggs were compared for females mated with each of the male stages (typus, intermedius, robustus). Each recently molted female was placed in an individual container (18 cm \times 18 cm surface area, 3 l volume) together with a male immediately after being located. Thus, these females may have molted a maximum of 24 h prior to being placed together with a male. To inhibit shrimp from feeding on lost eggs, shrimp were separated from the container bottom by a rigid plastic mesh and fed with small pieces of fish. The female and the container were examined carefully each day. During examination, the female on its mesh was briefly lifted from the container, examined against a light source for the presence of the egg mass, and then placed in a clean container with sea water. The container bottom was searched carefully for all lost eggs, which were counted and removed. All containers had flowing sea water and were cleaned with a sponge and abundant sea water every day. Twenty-four hours after a female was found with the egg mass attached to its abdomen, the male was removed from the container. To provide control females, we left one group of receptive females without a male. For each treatment (typus, intermedius, robustus, control), 10 replicates were conducted. In 3 of 40 cases, females never spawned during the 5 d of the experiment. Furthermore, in 3 of 30 cases, the male molted during the experiment. All of these replicates were discarded and repeated with new individuals.

A size analysis between the males of the different stages used in this experiment revealed significant differences (one-way ANOVA, $P < 0.05$). The sizes of both intermedius and robustus stages differed significantly from typus males (Tukey test; $P < 0.05$) (typus: 16.1 ± 1.0 mm; intermedius: 21.5 ± 1.8 mm; robustus: 19.6 ± 2.2 mm; mean carapace length \pm SD). In spite of the random selection of the females for this experiment, their sizes were significantly different (one-way ANOVA, $P < 0.05$). Females paired with typus males (12.6 ± 1.1 mm) were significantly smaller than those paired with intermedius (14.0 ± 0.8 mm; carapace length \pm SD; Tukey test; $P < 0.05$).

All experiments were conducted in March, 1999, and all shrimp were returned to their natural environment after being used in the experiments.

RESULTS

Male Mating Behavior (Experiment 1)

During this experiment, 17 successful mating sequences were observed and recorded. Males of all three ontogenetic stages exhibited similar behavioral events. Some of these behavioral events occurred only once during each mating sequence, whereas others were performed repeatedly during a single mating (Fig. 2).

(1) *Contact*.—The male established initial bodily contact with the female by touching it with its antennae. Some males immediately directed their full attention to the female after first contacting it with their antennae, leading to the next event. Other males repeatedly examined the female with their antennae before proceeding to the next event.

(2) *Seizure*.—The male approached the female from behind and, after mounting the female, immediately proceeded to the subsequent state.

(3) *Cage*.—In this position the male confined the female below his body by forming a cage with the pereopods, the 3rd maxilliped, and the abdomen (Fig. 3). The male guarded the female in this "enclosed" position throughout the mating process. Due to the lingering duration of this cage position (23 min to 3h 42 min), it represents a state (*sensu* Martin and Bateson, 1986) rather than an event. The following events (3A–D) were performed repeatedly by the male during this cage state (Fig. 2). During the end phase of the cage state, the males did not anymore poke (see below) the female's abdomen or place spermatophores but continued to guard the female.

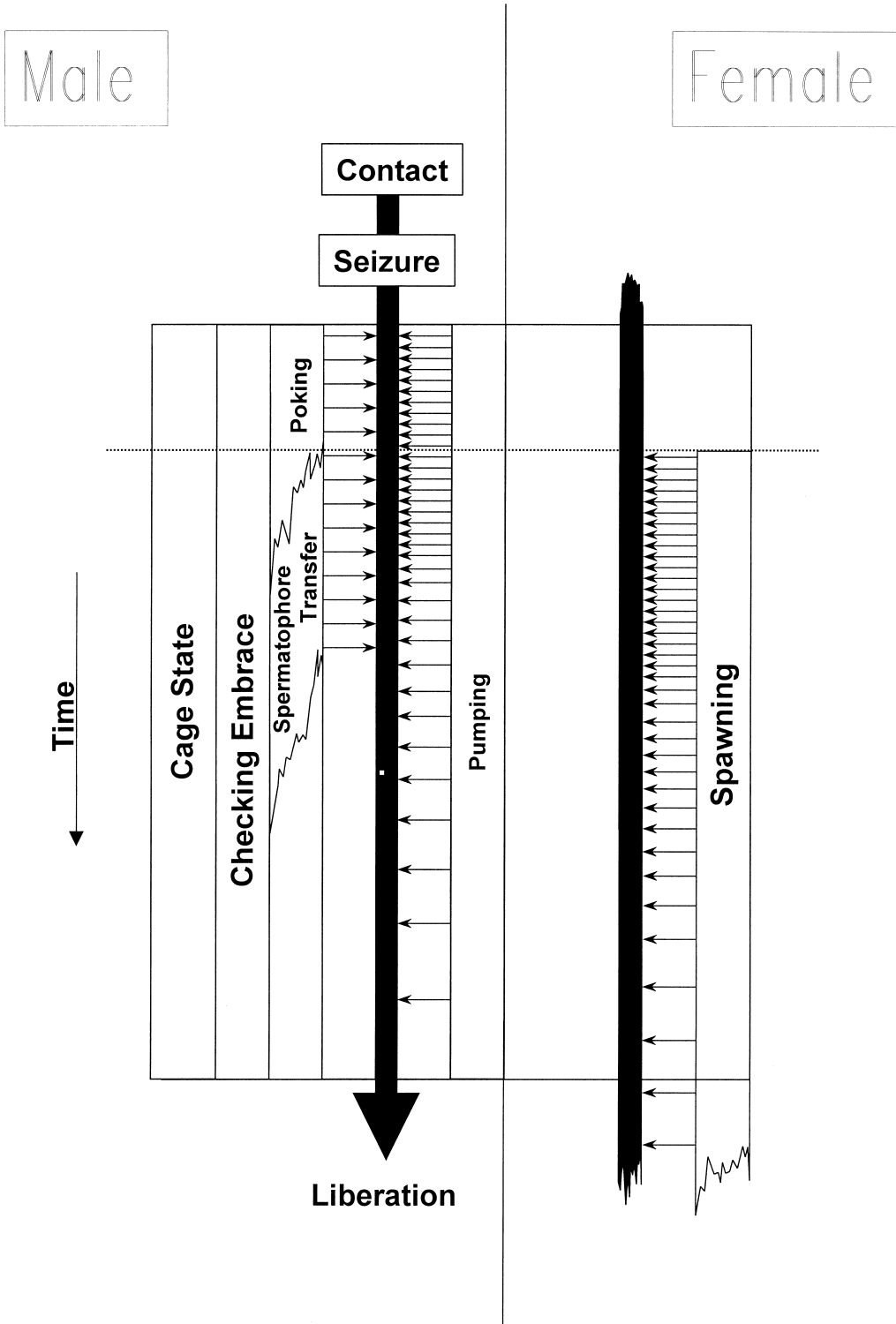


Fig. 2. Ethogram of behavioral states and events exhibited by male and female *Rhynchocinetes typus* during the mating process; all three ontogenetic male stages showed the same behavior; horizontal arrows indicate specific events.

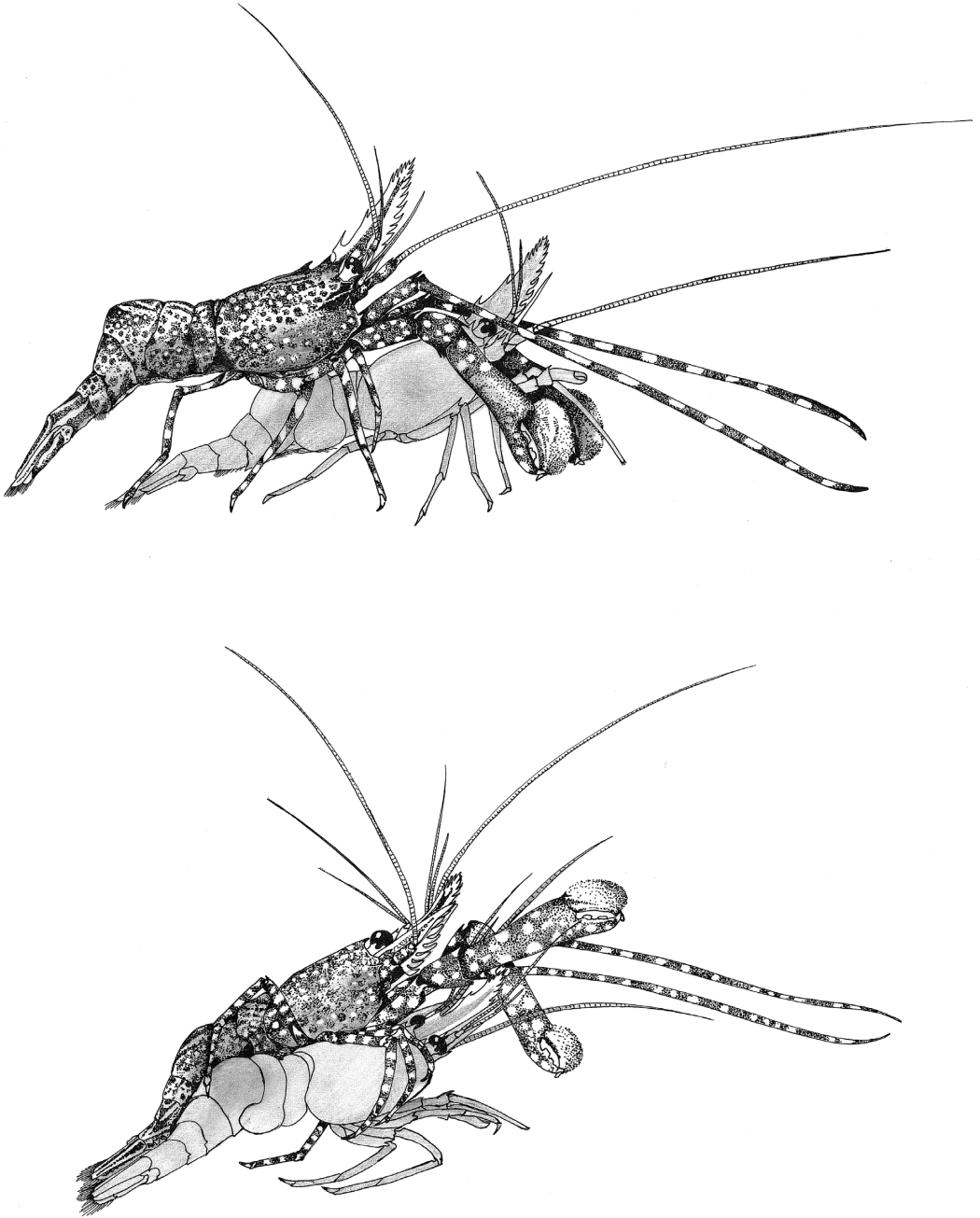


Fig. 3. Schematic drawing of the posture of male and female *Rhynchocinetes typus* (top) during the "cage" position and (bottom) during a spermatophore transfer event.

(3A) *Checking Embrace*.—During the entire cage state, the male embraced the female with its 2nd pereopods. The male continually touched the female's ventral region near the gonopores with these pereopods, which it then

directed to its own mouth region. Occasionally, the male also directed the 2nd pereopods to the ventral abdominal region of the female. These behaviors occurred continually while the cage state was well established.

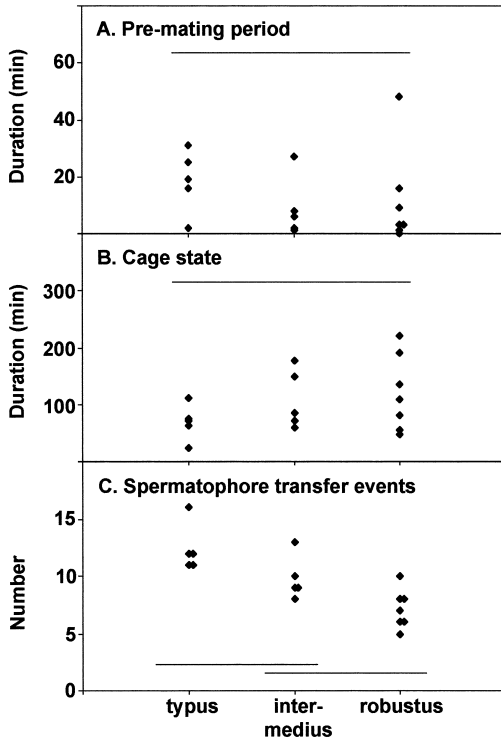


Fig. 4. Duration of (A) the pre-mating period, (B) the mating process (= cage state), and (C) the number of spermatophore transfer events for the three ontogenetic male stages of *Rhynchocinetes typus*; lines in each graph connect male stages for which no significant differences were found (ANOVA, followed by Tukey test; $\alpha = 0.05$).

(3B) *Poking*.—At various times during the first phase of the cage state, the male also touched the female's ventral abdominal region with one of its 4th pereopods. The male usually used only one of its 4th pereopods at a time, poking the female's abdomen from either the right or the left side.

(3C) *Pumping*.—One common behavior exhibited by males during the cage state was the execution of short gusts (1 to 3 sec) of repeated and quick up-and-down movements of the entire body with the pereopods firmly attached to the substrate. The frequency of these characteristic movements was high (approximately 10 pumping events in 10 min) during the beginning of the mating process but decreased towards the end (approximately 4 pumping events in 10 min).

(3D) *Spermatophore Transfer*.—This event consisted of a short sequence of behaviors

that culminated in the transfer of spermatophores. First, the male lifted its abdomen slightly and rapidly shook its pleopods. The male then took the female's carapace in a clinch with its first pereopods and grasped the lateral edge of one of the last tergites of the female abdomen with one of its 5th pereopods. It slightly raised the female's abdomen, thus twisting its ventral side towards its own body, immediately balanced its own body sideways, and with a rapid pleopod beat transferred spermatophores onto the ventral region of the female's abdomen (Fig. 3). Males exhibited between 5 and 16 spermatophore transfer events during a single mating process. It was not possible to determine whether males transferred only one or more spermatophores during one transfer event.

(4) *Liberation*.—To liberate the female, the male opened its checking embrace of the female with its 2nd pereopods, then lifted the 3rd pair of maxillipeds. The male then let the female walk out of the "cage" toward the anterior part of its body.

The sequence of these events was similar for the three ontogenetic states of males (Fig. 2). Once held in the cage position, the female assumed a more or less passive role. Spawning of the first eggs generally coincided with the placement of the first spermatophore by the male. During spawning, the female contracted her body slightly and directed her anterior pleopods towards her gonopores located in the coxae of the 3rd pereopods. During each of these repeated contractions, a certain quantity of eggs are probably extruded, and we consider these contractions to reflect the spawning process (i.e., extrusion of eggs). The frequency of spawning contractions was highest during the first phase of the spawning process. Once initiated, spawning continued throughout the remaining mating process. Many females spawned for more than 120 min. In most cases, spawning continued after the liberation of the female, but the large majority of the eggs were spawned during the cage state.

Quantitative Comparison of Mating Efforts Between Ontogenetic Male Stages

During this first experiment, five of ten *typus*, five of ten *intermedius*, and seven of ten *robustus* males mated successfully with fe-

Table 2. Average number (mean \pm SD) of specific events (male poking of female abdomen, placement of spermatophore, spawning contraction by females) during the mating process of *Rhynchocinetes typus*; mating pairs were intensely monitored every other 15 min.

Time (h:min)	Event	Typus	Intermedius	Robustus
		$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$
0:00–0:15	Poking	2.40 + 2.88	6.40 + 5.73	1.71 + 2.56
	Spermatophore	10.00 + 1.58	3.40 + 4.67	4.00 + 4.12
	Spawning	20.60 + 12.28 (n = 5)	7.20 + 10.26 (n = 5)	12.86 + 14.10 (n = 7)
0:30–0:45	Poking	0	4.40 + 6.07	0.50 + 1.22
	Spermatophore	0	0	1.00 + 2.24
	Spawning	19.50 + 5.2 (n = 4)	10.20 + 10.76 (n = 5)	9.86 + 6.31 (n = 7)
1:00–1:15	Poking	0	1.75 + 3.50	0.20 + 0.45
	Spermatophore	0.60 + 1.15	0.25 + 0.50	0
	Spawning	10.33 + 7.77 (n = 3)	10.50 + 9.15 (n = 4)	7.20 + 4.71 (n = 5)
1:30–1:45	Poking		0.50 + 0.71	0.33 + 0.58
	Spermatophore		3.50 + 4.95	1.00 + 1.00
	Spawning		20.00 + 14.14 (n = 2)	5.67 + 5.51 (n = 3)
2:00–2:15	Poking		0	0
	Spermatophore		1.50 + 0.71	0.50 + 0.71
	Spawning		9.50 + 4.95 (n = 2)	8.50 + 3.54 (n = 2)
2:30–2:45	Poking		0	0
	Spermatophore		0	0
	Spawning		15 (n = 1)	2.50 + 2.12 (n = 2)
3:00–3:15	Poking		0	0
	Spermatophore		0	0
	Spawning		9 (n = 1)	3.50 + 4.95 (n = 2)
3:30–3:45	Poking			0
	Spermatophore			0
	Spawning			0 (n = 1)

males (Table 2). All males showed the same mating behaviors and performed the same events in the same sequence. Among the males that successfully mated during the experiment, the pre-cage phase lasted between 0 and 48 min (13 ± 14 min; mean \pm SD), and the total mating time lasted between 23 and 221 min (102 ± 56 min). No significant differences were found between ontogenetic male stages, neither in duration of the pre-cage phase nor in the duration of total mating time (ANOVA, $P > 0.05$; Fig. 4A, B). The total number of spermatophore transfer events toward female's abdomen during the total mating process was significantly higher for typus males (12.4 ± 2.1 events; mean \pm SD) than for robustus males (7.3 ± 1.8 events; ANOVA, followed by Tukey test; $P < 0.05$; Fig. 4C). All male stages performed the highest numbers of spermatophore transfer events

during the first 15 min of the mating process (Table 2). Since the mating process lasted comparatively long in robustus and intermedius males, these males continued to engage in spermatophore transfer for up to 2 h, albeit at much lower numbers. Similarly, the occurrence of poking the female's abdomen rapidly decreased after the first 15-min interval.

Females that were mated with typus males spawned at a much higher frequency during the first and third 15-min intervals than did those females mated with robustus and intermedius males (Table 2). All females continued to spawn throughout the mating process and also after being liberated by the males. During the first 15-min interval after being liberated, females that had been mated with robustus males spawned at a lower frequency than did females mated with intermedius male stages (one-way ANOVA; $P < 0.05$; Fig. 5).

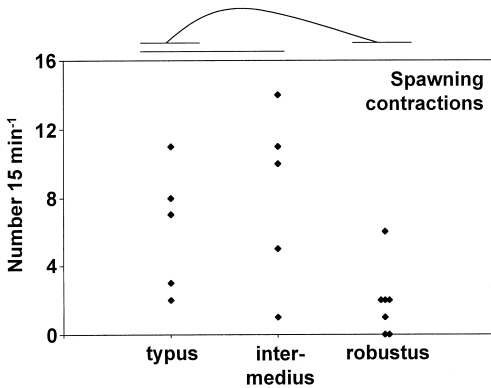


Fig. 5. Number of spawning contractions by female *Rhynchocinetes typus* after being liberated from males; lines on top of graph connect male stages for which no significant differences were found (ANOVA, followed by Tukey test; $\alpha = 0.05$).

Fertilization Potential of the Three Ontogenetic Male Stages (Experiment 2)

Although all three ontogenetic male stages mated successfully, one of the intermedius males and three of the typus males failed to fertilize their females during this experiment (Fig. 6A). The four females that were not successfully mated spawned within 72 h after molting (one female 24–48 h and three females 48–72 h after molting). These unmated females each lost its entire egg mass during the subsequent days. Similarly, almost all control females, which were deprived of males, spawned 48–72 h after molting (one female 24–48 h, eight females 48–72 h, and one female 72–96 h), and each lost its entire egg mass during the following days. Almost all females that had successfully mated with males (26 in total) had spawned 24–48 h after molting (23 females 24–48 h, and three females 48–72 h after molting), i.e., sooner than those without males. All but two of the successfully mated females lost a few eggs during the days they were held following mating. Most females only lost single eggs during this time (< 10 eggs per female), but a few females lost relatively large numbers of eggs. Those females mated with typus males lost about 5% of the total number of eggs they spawned, whereas those successfully mated with intermedius and robustus lost less than 1%, but treatments were not significantly different (Kruskal-Wallis, $P > 0.05$; control females not included in analysis because no eggs were fertilized; Fig. 6B).

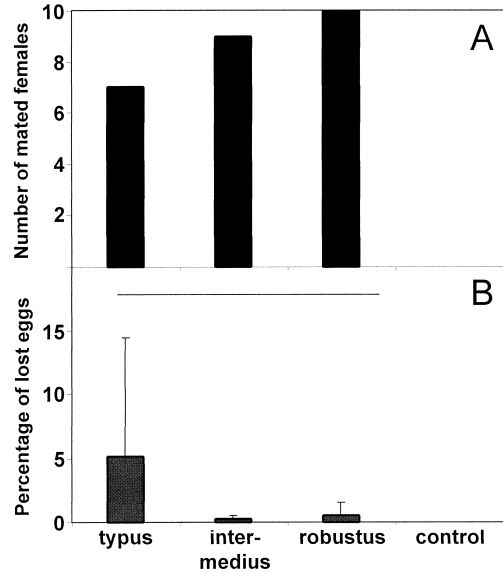


Fig. 6. (A) Number of successful matings and (B) average percentage (mean \pm SD) of eggs lost by females fertilized by three ontogenetic male stages of *Rhynchocinetes typus*; 10 females were mated with each male stage, females that lost more than 95% of their brood during the first five days of the experiment were assumed not to have been fertilized by males; line in (B) connects treatments for which no significant differences were found (Kruskal-Wallis; $\alpha = 0.05$).

DISCUSSION

Mating behavior in marine and freshwater shrimp is highly diverse (Bauer, 1996). In many species, mating is of very short duration. The actual placement of a spermatophore by the male takes place during a short interaction (< 30 sec) with the female (Hoffman, 1973; Berg and Sandifer, 1984; Saint-Brisson, 1985; Yano *et al.*, 1988; Bauer, 1992). Usually, no mate-guarding occurs, and other males may (pseudo-)copulate with the female (see e.g., Bauer, 1996). Because in many caridean shrimp the molting process is apparently not synchronized, few receptive females will be available at any one time, and competition for females may be particularly high among males. It is known that intrasexual competition may have an important role in shaping mating system in a wide variety of animal taxa (Emlen and Oring, 1977). The existence of different male morphotypes, as reported for *Macrobrachium rosenbergii* and *Rhynchocinetes typus*, and, furthermore, the adoption of alternative reproductive behaviors by individuals (as re-

ported for the *M. rosenbergii*; Ra'anan and Sagi, 1985) is likely to be related to this intense competition (Dominey, 1984). In the present study, we demonstrated that in a competition-free environment all three ontogenetic male stages of *R. typus* exhibited similar mating behaviors albeit with different intensities. These differences in the type of mating investment may be a result of male sexual experience and may have important implications for male mating behavior in a competitive environment.

Mating Behavior in *Rhynchocinetes typus*

Whereas most penaeid shrimp have internal fertilization with subsequent application of sperm plugs (Bauer and Lin, 1993), fertilization in the majority of carideans is external. Males place one or more spermatophores under the female's abdomen (Bauer, 1976; but see Boddeke *et al.*, 1991), and the only way to ensure that no other males fertilize the eggs would be by prolonged mate-guarding. However, prolonged mate-guarding, as it occurs in *R. typus*, is not commonly reported for caridean shrimp. The only other shrimp species for which some form of mate-guarding has been reported is the freshwater shrimp *Macrobrachium rosenbergii* (see Ra'anan and Sagi, 1985). In this species, the dominant male guards females for a period before and after copulation, although it was not reported for how long and in what manner the females are guarded (see Ra'anan and Sagi, 1985). In the present study, it was shown that in a competition-free environment all three ontogenetic male stages of *R. typus* are capable of guarding females during the mating process.

While guarding the females, all males engaged repeatedly in poking and checking behavior. These behavioral events were most common before females commenced to spawn. During the checking embrace, the males continually touched the female genital region with their 2nd pereopods, which they directed immediately thereafter to their mouthparts. For penaeid shrimp, Bauer (1992) reported that male *Sicyonia dorsalis* Kingsley, 1878, prodded the female genitalia with their eyes and antennules during the "following" behavior, and he suggested that this behavior served to investigate or stimulate the female. For *Litopenaeus setiferus* (Linnaeus, 1769) and *L. vannamei* (Boone, 1931), Misamore and Browdy (1996) also re-

ported probing of the female thelycum with the antennules, a behavior that they termed "probe." Because the male-female position in the caridean shrimp *R. typus* is substantially different from that of other shrimp during the mating process, the males cannot probe the female genitalia with their antennules. Instead, they utilize their 2nd pereopods, which they then direct toward their mouth. Because mouthparts are important sites for chemoreception, we consider it most likely that the checking embrace serves to examine the reproductive stage of the female. Additionally, males may use this behavior to determine whether females have been fertilized by other males. Presently, we do not know how a male would react upon finding spermatophores from a previous male attached to the female's abdomen. For crayfish and snow crabs it has been reported that males would remove spermatophores deposited by a previous male with their mandibles or first gonopods (Reynolds *et al.*, 1992; Elner and Beninger, 1995); however, no such observations are known from caridean or penaeid shrimp. Furthermore, this checking embrace may help the males of *R. typus* to determine the exact moment at which spawning starts, but it may also have some stimulatory function. During the first phase of the cage state, the males also continuously engaged in poking the female abdomen with their 4th pereopod. Whether this poking behavior has stimulatory or checking function cannot be clearly distinguished. Because females that were paired with males in the 2nd (= fertilization) experiment started to spawn sooner than those without males, it is likely that males somehow (e.g., by checking embrace and/or poking) stimulate the females to start spawning. In combination, the checking embrace and poking behavior by male *R. typus* probably serve to synchronize placement of the first spermatophore and commencement of spawning. In *R. typus*, spermatozoa contained in a spermatophore become activated immediately upon contact with seawater (Dupré and Schaaf, 1996). Placing spermatophores too early or too late may result in waste of spermatozoa and non-fertilization of eggs, which is why synchronization between placement of the first spermatophore and commencement of spawning is important in these shrimp.

Not all pairs of females and males of *Rhynchocinetes typus* established in the present

study mated successfully. Mating appeared to be more successful for pairs in the 2nd (= fertilization) experiment than for pairs in the 1st (= observation) experiment. There are at least three possible explanations for the differences in mating success between the two experiments. In the observation experiments, the conditions (standing water, disturbance by observer), could have imposed a negative influence on the shrimp, thereby affecting their mating behavior. However, all shrimp appeared to behave normally, and those that mated successfully did not react to movements of observers. Another possible explanation for the lower mating success in the observation experiment (50–70% successful matings) is the fact that pairs were observed only 120 minutes, and mating was considered as unsuccessful if they failed to initiate the mating process during that period. Furthermore, females were used 12–36 hours after molting and, possibly, during the observation some of them were not at their optimal receptivity. This time interval was chosen because preliminary tests revealed that females are not receptive immediately (< 12 h) after molting. Zhang *et al.* (1998) found that in the banded coral shrimp all matings were successful when females were used within 24 hours after molting, but the rate of successful matings dropped to 25% for females 36 hours after molting. Yet, our preliminary tests did not indicate such a sharp decline in receptivity for *R. typus* females. Rather, females appeared to remain receptive until they finally extruded their egg masses, which usually occurred 48–72 h after molting.

In both experiments (observations and fertilization), the robustus morphotype mated most successfully with the females. In the fertilization experiment, three of ten typus morphotypes apparently failed to mate with females. Several possible explanations can be given for the low mating success of the typus morphotype compared to the robustus morphotype: i) because this is the first mature male morphotype in *Rhynchocinetes typus*, not all typus males may have reached full sexual maturity (morphological, behavioral, and physiological maturity—*sensu* Elnor and Beninger, 1995); ii) because the typus morphotype appears to guard females for comparatively short periods, it is possible that females show some resistance to mating with these males in expectation of “better” males;

iii) the lack of other males in these experiments may have affected the responsiveness of the test males toward the females. Generally, these explanations are applicable for shrimps in a competition-free environment such as in our experiments. We consider it likely that in the field the social environment (that was not provided in the present experiments) may play a pivotal role in male mating behavior. The behavior of typus males may depend more on the social environment than does that of robustus males.

Mating Investment in *Rhynchocinetes typus*

Female guarding may have two main functions: to protect female rock shrimp *R. typus* from subsequent inseminators, and to protect females from losing eggs while they attempt to evade harassment by other males.

Interestingly, robustus morphotypes placed significantly fewer spermatophores under the female's abdomen than did the typus morphotypes. Thus, robustus morphotypes apparently invested more time but less sperm than did the typus morphotype, while the intermedius stage exhibited intermediate time and sperm investment. Although sperm production is often considered energetically inexpensive, several studies indicate that this assumption may not be true for decapod crustaceans. Jivoff (1997a) found that male blue crabs *Callinectes sapidus* Rathbun, 1896, required about 15 days for the ejaculate of a second mating to reach the same weight as that invested during a first mating. Related experiments conducted by Kendall and Wolcott (1999) confirmed that more than 9 days are necessary for male *C. sapidus* to replete sperm reservoirs. For penaeid shrimp, it has been reported that males require about 8–12 days to regenerate a spermatophore (Leung-Trujillo and Lawrence, 1987, cited in Ogle, 1992). These observations indicate that sperm are not an unlimited resource in male decapods and that it may be advantageous for males to use their sperm supply in carefully balanced portions. In *R. typus*, the robustus morphotypes may be much more experienced than the sexually naive typus morphotypes. The latter may have utilized their sperm reservoir less sparingly than the former. Possibly, the sexually naive typus males also fail to place spermatophores at the optimal location on the female's abdomen (see e.g., Ogle, 1992), necessary to exploit the full fertiliza-

tion potential of a single spermatophore. Females fertilized by typus males lost almost 5% of their eggs during the first five days of incubation, which could be a consequence of a limited male fertilization potential or the lack of male assistance and protection during the last period of the spawning process. These observations indicate that typus males are capable of fertilizing females but are apparently not yet as skilled as robustus males.

Outlook

Our results show that the ontogenetic changes in male morphology in *Rhynchocinetes typus* are accompanied by changes in the type of mating investment, while the mating behavior did not differ among male stages. In the present experiments, all three ontogenetic male stages were provided competition-free access to receptive females. On the other hand, females were denied any choice of males. We hypothesize that in a competitive environment males gain access to females in a hierarchical order, as is known for other species (e.g., fish—Taborsky, 1994). Because in *R. typus* the robustus morphotype has the most powerfully developed 1st pereopods and 3rd maxillipeds, males of this morphotype are most likely to win access to receptive females. Whether subordinate males change their behavior in such a competitive environment remains to be tested in future experiments.

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