

BEHAVIOR OF REMIPEDIA IN THE LABORATORY, WITH SUPPORTING FIELD OBSERVATIONS

Stefan Koenemann, Frederick R. Schram, Thomas M. Iliffe, Lara M. Hinderstein, and Armin Bloechl

(SK, correspondence; AB) Institute for Animal Ecology and Cell Biology, University of Veterinary Medicine Hannover Foundation, Bünteweg 17d, 30559 Hannover, Germany;

(FRS) Burke Museum, University of Washington, Seattle, Washington 98195, U.S.A.;

(TMI, LMH) Department of Marine Biology, Texas A&M University at Galveston, Galveston, Texas 77553-1675, U.S.A.

(SK: stefan.koenemann@tiho-hannover.de)

A B S T R A C T

We observed in the laboratory the behavior of six individuals of an as yet undescribed species of *Speleonectes* (Remipedia) over a period of 76 days. The live specimens were collected from an anchialine cave on the Yucatan Peninsula and maintained in separate aquaria at the Zoological Museum Amsterdam. In addition, field observations were conducted in the same cave to compare the laboratory results with naturally occurring behaviors. We found a variety of complex behavioral traits that include several new and unexpected findings. For example, our observations suggest that remipedes are not obligatory, but rather facultative carnivores, and that filtering particles might be the predominant mode of feeding. A digital video with examples of various behavioral traits can be downloaded at <http://www.tiho-hannover.de/einricht/botanik/research.htm>.

INTRODUCTION

Among the most intriguing arthropods that have emerged in recent years are crustaceans of the class Remipedia (Yager, 1981). To date, we recognize 18 species (Koenemann et al., 2003, 2004; Wollermann et al., 2007; Koenemann et al., 2007a), while new species are constantly emerging from the caves in the Caribbean islands, the Yucatan Peninsula, Mexico, and other tropical locations. Remarkably, some 25 years after their discovery, essential aspects of the biology of Remipedia are either still not known, or poorly understood. Unresolved issues include, for example, their phylogenetic relationships and evolutionary history (Koenemann et al., 2007b), reproductive and developmental modes (Koenemann et al., 2007c), and reconstructions of their life cycle.

There are two relatively detailed investigations that focus on the behavior and ecology of Remipedia. Kohlhage and Yager (1994) provided an analysis of swimming and the metachronal movement of trunk limbs. Another study of living remipedes in the laboratory by Carpenter (1999) confirmed several instances of predatory behavior, with detailed comments on the function of individual cephalic appendages. However, aside from these studies and a few brief published notes, we still know little else about the behavior of Remipedia. Therefore, we wish to take this opportunity to record some new observations based on living remipedes both in the laboratory and in their natural habitat.

During a recent diving expedition investigating anchialine caves on the Yucatan Peninsula, we were able to collect live specimens of Remipedia. Six individuals of an as yet undescribed species of the genus *Speleonectes* Yager, 1981 were brought back to the Zoological Museum Amsterdam (ZMA) and kept in aquaria. These were observed over a period of more than two months. We also conducted field observations in the same cave to compare the laboratory results with naturally occurring behaviors. Our observations

reveal that the behavior of Remipedia comprises a variety of complex traits, including some new and unexpected findings.

For example, it is widely accepted that remipedes are carnivores based on the three pairs of raptorial, prehensile cephalic limbs, a distinct apomorphic feature shared by all species. This assumption is partially corroborated by our observations, made both in the laboratory and in the field by two of us (TMI and LMH). However, our laboratory observations suggest that remipedes might not be obligatory carnivores but that filtering particles out of the water column is another important mode of feeding.

MATERIAL AND METHODS

In the laboratory, we studied six living individuals of a species of *Speleonectes* that is morphologically closely related to *S. tulumensis* Yager, 1987. The animals were collected from Cenote Crustacea, a cave on the Yucatan Peninsula located several kilometers south of Puerto Morelos, Quintana Roo, Mexico, where this species occurs in comparatively high abundance. Five individuals were adults with body sizes ranging from 33.9 to 40.8 mm (40 to 42 trunk segments), and one smaller individual, probably a sub-adult, measured 20.6 mm (35 trunk segments).

The animals were maintained in two separate tanks in a completely dark climate room at ZMA. The larger aquarium (150 liters) was connected to an Eheim bio-filter pump system; fine sediment and small carbonate rocks taken from the original collection site (anchialine cave) were used as bottom material. The commercial bacterial culture 'Bactomarine' was added to the bio-filter of the large tank. A smaller tank, not connected to a pump or filtering system, contained small-sized aquarium gravel as bottom material. After three weeks, small quantities of 'Bactomarine' were also added to the water of the small tank. A coral sea salt mix was used to maintain salinity between 32 to 35 ppt (49 to 53 mS/cm), which fell within the salinity range measured in several caves. Water temperature was kept constant around 24 degrees C, and the pH ranged from 6.8 to 7.7.

Laboratory observations were conducted in three daily sessions, each lasting between 30 and 60 min. In addition, we made videos between direct observation intervals and overnight. Total observation time (direct observation and video-taping) constituted approximately 147 hours over a period of 76 days. We conducted several light reaction tests including abrupt and punctual illuminations of the tanks and/or individuals in the tanks. In all instances, the observed individuals did not exhibit any noticeable reaction to light. This is corroborated by field observations when

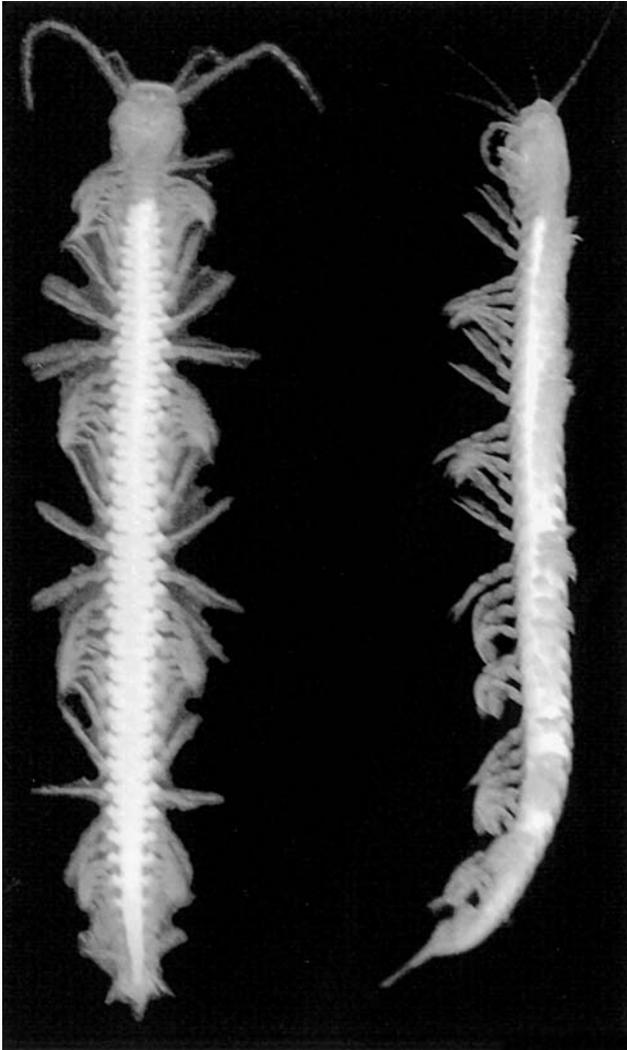


Fig. 1. Individual of the remipede *Speleonectes* sp. from Cenote Crustacea showing 'vertical swimming' behavior, with metachronal, synchronous strokes of trunk appendages. Left: ventral view; right: lateral view.

individuals were illuminated with dive lights and showed no response. Nevertheless, during direct observation periods and most video recordings, we used dim, indirect light sources, and occasionally an additional flashlight for short inspections. Some video tapes were recorded using the infrared mode, i.e., without additional light sources. A digital video that summarizes various behavioral traits can be downloaded at <http://www.tiho-hannover.de/einricht/botanik/research.htm>.

During the initial phase of the observations, four individuals were kept in the large tank (including the sub-adult specimen) and two individuals in the smaller tank. Whenever an individual died, individuals in both tanks were relocated, so that each tank contained at least two individuals. The first individual (20.6 mm sub-adult) died after two days; the remaining five individuals died successively after 20, 31, 57, 62 and 76 days (see below Grooming and Death of Individuals).

Field observations of the same species of *Speleonectes* were conducted on 29 and 31 July 2006 in Cenote Crustacea, the original collection site. More than 100 individuals were surveyed over a period of about one hour. All cave diving complied with standards and procedures required by NSS-CDS (National Speleological Society—Cave Diving Section) and AAUS (American Academy of Underwater Sciences). The behavior of the remipedes was noted by divers swimming slowly along submerged cave passages. Observations consisted of noting the location of the remipede with respect to the bottom sediment as well as other individuals. Swimming orientation was recorded, either vertical or horizontal, and, if horizontal,

whether the individual was swimming on its dorsal or ventral surface. When feeding behavior was observed, the remipede was followed, but not interfered with. In addition, food in the form of freshly killed cave shrimp (*Typhlatya* sp.) was offered to remipedes by hand and by placing shrimp on the cave sediment.

RESULTS AND DISCUSSION

Behavioral Observations in the Laboratory

Swimming and Locomotion.—Apart from several different swimming modes described below, we made the following general observations:

1. The beating movement of the biramous trunk limbs is ceaseless, even during so-called resting periods, as described by Parzefall (1986). On each side of the trunk, five to seven limb pairs perform metachronal, synchronous strokes that appear as continuous, upward (anteriad) rolling waves (Fig. 1; for a detailed analysis of leg movement see Kohlhage and Yager, 1994).
2. The second antennae are also constantly moving; performing very fast, flap-like strokes from anterioposteriad (Fig. 2). Carpenter (1999) proposed that the antennae in *Speleonectes epilimnius* are used to comb detritus and plankton from the peduncular aesthetascs of the first antennae (antennules) and move them towards the atrium oris. The fact that the strokes of the second antennae are performed ceaselessly suggests that filtering particles out of the water column is a critical mode of feeding in Remipedia.
3. When feeding on large prey, the prehensile cephalic appendages are used to continuously manipulate the prey object (as described by Carpenter, 1999).
4. At least two pairs of the three prehensile cephalic limbs, the maxillae and maxillipeds, seem to beat irregularly laterad to mediad when individuals were not feeding on prey. These movements are not performed constantly, but most of the time and asynchronously, with left and right limbs moving differently (Fig. 3). Movement of the maxillules was not distinguishable with the naked eye.
5. The first antennae (antennules) perform irregular, asynchronous sweeping strokes.

Several distinctive phases were noted while the animals were in swimming mode.

1. Horizontal 'backstroke' swimming. This type of swimming, with the ventral side up, was the predominant mode of movement (approximately 65% of total observation time in the laboratory). This mode of swimming was performed in the water column, at the surface, and along the bottom (Fig. 4).
2. Horizontal swimming with the ventral side down occurred less frequently and was always performed along the bottom or on rock surfaces.
3. Vertical swimming occurred when individuals 'hung' vertically in the water column, always with their heads directed towards the surface and apparently remaining at the same spot (Figs. 1 and 3). Fast forward mode of videos (5 × speed) revealed swaying motions during vertical swimming, individuals swinging from left to right. These movements were performed relatively slowly and could only be distinguished at higher video

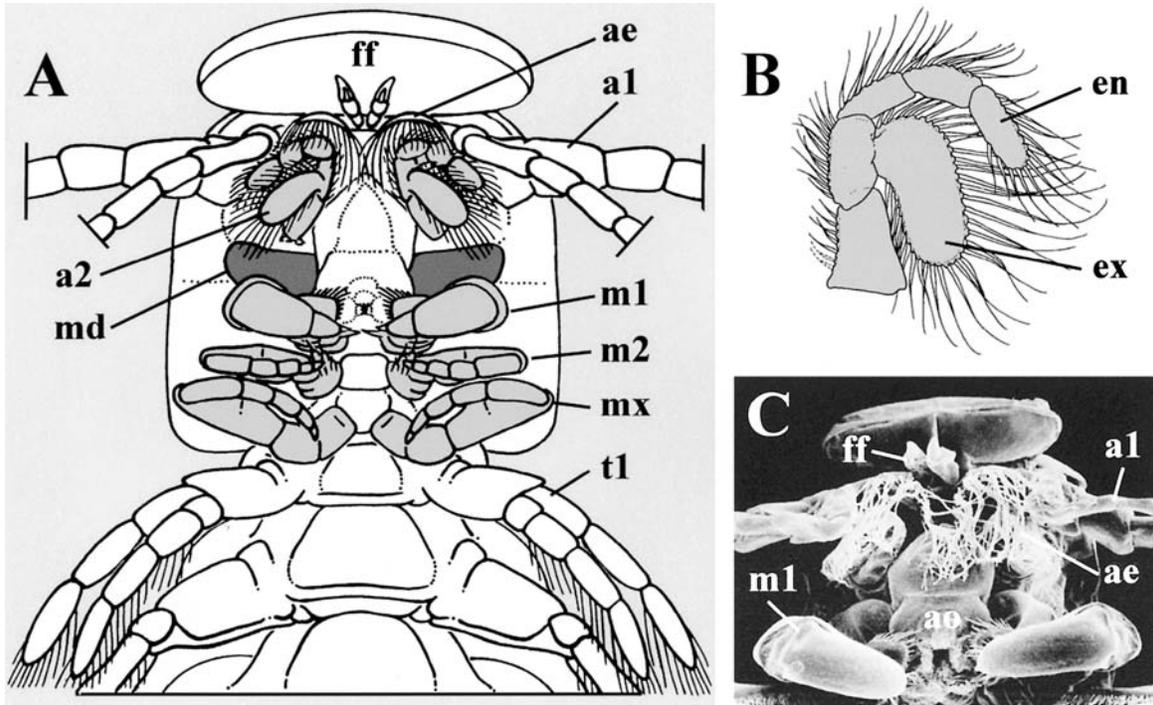


Fig. 2. Cephalic appendages in Remipedia. A, Head of a generalized nectiopodan remipede (ventral view); second antennae and prehensile cephalic limbs shaded by light gray, mandibles shaded by dark gray (modified from Emerson and Schram, 1991). B, Enlarged second antenna *Speleonectes parabenjamini* (modified from Koenemann et al., 2003). C, SEM photograph of *Lasioneectes entrichoma*, ventral view of anterior head (modified from Schram and Emerson, 1986). Abbreviations: a1 = first antenna (antennule); a2 = second antenna; ae = peduncular aesthetascs of first antenna; ao = atrium oris; ff = frontal filaments; en = endopod; ex = exopod; m1 = first maxilla (maxillule); m2 = second maxilla; md = mandible; mx = maxilliped; t1 = first trunk limb.

speed. During the first 3 weeks, vertical swimming was performed during 80% of total observation time and could last up to 40 min. We suspect at this time that vertical swimming might be related to a feeding mode, i.e., particle or filter feeding.

4. A resting phase occurred when individuals either lay on their backs on the bottom, oftentimes slowly gliding along the substrate, or leaning vertically against rocks; metachronal strokes of trunk limbs slowed; but second antenna, maxillae, and maxillipeds were still moving as during active swimming.
5. Finally, we recorded fast (startled) swimming movements that occurred during an escape response, for example, when handled or when attacking prey. Fast swimming is a combination of an increased frequency of trunk limb strokes and swift, snake-like propulsion involving the whole trunk.

Feeding, Foraging, and Hunting.—During the entire period of observation, a variety of possible prey and food was offered at irregular intervals, including both non-living food resources, and living organisms.

Larger non-living food particles included ‘Tetra’ pellets and flakes, frozen shrimp (*Crangon* sp.) and krill, body parts of freshwater mysids (*Mysis* sp.), and dried cladocerans. Particles either drifted at the surface or sank to the bottom. However, no individual could be observed feeding on any larger, i.e., detectable, particles. Since vertical swimming was the most frequently and persistently performed behavior, we

assume that this behavior served to filter small particles out of the water. During vertical swimming periods, the mouthparts and second antennae were noticeably moving (Fig. 3). We also interpret periods during which individuals swam with the

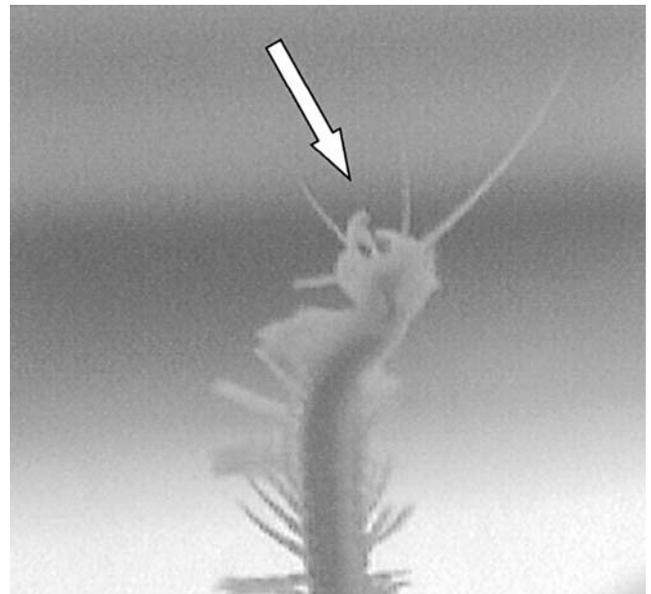


Fig. 3. Individual of *Speleonectes* sp. swimming vertically in the water column. Arrow points at irregular and asynchronous movements of first maxillae and maxillipeds. Taken from video film.

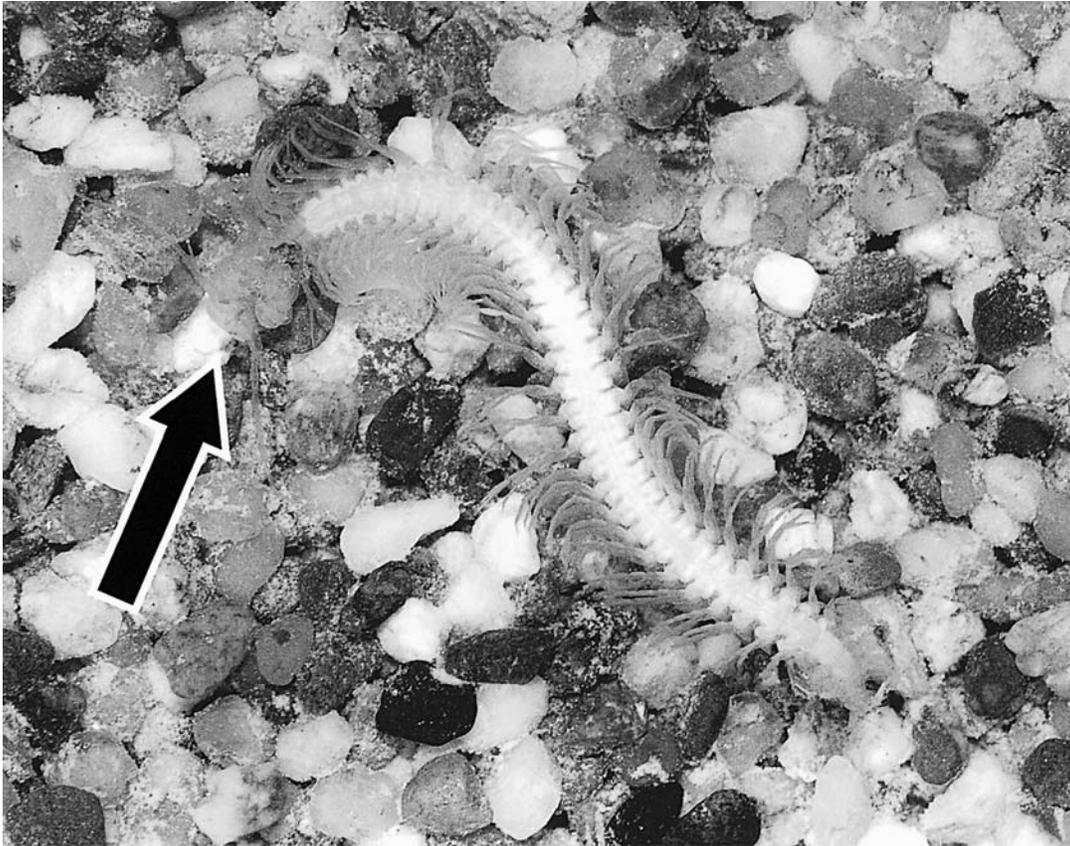


Fig. 4. Horizontal 'backstroke' swimming. Individual of *Speleonectes* sp. swimming with ventral side up along the sediment. Arrow points at head.

ventral side up at or just below the surface served to (filter-) feed on small particles. In contrast to vertical swimming, however, swimming at the tank surface lasted only a few minutes and was comparatively rapid, with frequent, abrupt turns of the trunk to change directions. Like vertical swimming, this behavior was also repeated numerous times during observation periods.

On a few occasions, larger food items, e.g., body parts of *Artemia*, had disappeared during non-observation times and might have been consumed by remipedes.

Living prey organisms included *Artemia* sp., *Caridina japonica*, freshwater mysids (*Mysis* sp.), *Gammarus duebeni*, *Tubifex*, and mosquito larvae. Since *Gammarus duebeni* is an aggressive, omnivorous predator, individuals were partly immobilized before putting them into the tanks.

The remipedes ignored potential prey organisms in 99% of total observation time; they neither showed any noticeable interest, nor did they appear to particularly avoid prey organisms. On numerous occasions, swimming remipedes appeared to accidentally 'bump' into individual prey organisms; they responded in all cases with a swift startled motion, swimming away from the prey at increased speed. In some instances, however, individuals did not swim immediately away after the startled reaction but stayed close to the object or even swam in narrow circles around the prey.

Predatory capture was observed four times. On two occasions, a remipede attacked and grabbed either a mysid,

or an individual of *Artemia* (recorded on video). Two other instances also involved a mysid and an *Artemia*, although in these cases the actual attack was not observed, but the strikes must have occurred only a few moments before observation since prey organisms had been freshly added and the remipedes swam around with individual prey in their fangs.

The actual grabbing of prey occurred spontaneously, without any noticeable initiation or preparatory behavior. The grab began with an explosive, 'jump-like' motion towards the prey, which was seized with the prehensile cephalic limbs and vigorously shaken, and presumably also stabbed with the maxillules to inject venom (van der Ham and Felgenhauer, 2007). The initial, aggressive and vigorous part of the attack lasted 1 to 2 min. During this phase, the entire trunk of the remipede was curled up and occasionally even coiled around the prey in a snake-like manner. After the initial attack, the remipede swam around or lay on the bottom (always with the ventral side up) with the prey in its fangs. This phase lasted up to three hours, with alternating swimming modes involving rapid swimming with sudden turns, and slow gliding or resting on the bottom. During this whole phase, the prehensile limbs constantly moved and pulled the prey apart until it was completely ingested.

Predation on other living prey organisms (except mysids and *Artemia*) was not observed.

Heretofore, one other mode of feeding in remipedes has been completely overlooked. The limb motions of the

antennae and posterior mouthparts described above indicate adaptations to collecting and manipulating particles. That this does occur should have been obvious long ago. While remipedes have rather transparent bodies, their guts appear constantly packed with dark (sometimes reddish brown) material similar in color to the bottom sediments. Furthermore, while our remipedes in the laboratory inevitably fasted, or at least seldom took live prey, their guts never cleared out. Finally, to sustain constant, unceasing activity, in some cases over weeks and months, these animals must have a food source to maintain themselves. Thus, it seems from their observed limb movements during swimming and grooming, and the material in the gut that remipedes are also particle or detritus feeders.

Interaction and Contacts between Individuals.—While swimming in the water column, direct contact between two remipedes appeared to occur accidentally and as frequently as ‘bumping’ into potential prey. Such contacts were followed by a startled, fast escape response. However, single individuals occasionally swam back and forth in circles over a small area on the bottom with their ventral side in contact with the sediment. This behavior lasted up to 40 seconds and occurred on average about once per hour. In approximately 20% of all observed cases, a second individual visited the same spot shortly after the first individual had left, also swimming back and forth 2 or 3 times with its ventral side down. Similarly, the grooming spot of an individual was frequently visited by a third individual after the first visitor had left the spot.

Circular gliding around a particular spot on the bottom was also performed by two individuals that swam very close to each other. Whenever the two individuals came into contact, for example, by passing each other in parallel or perpendicular directions, a startled response reaction was either much weaker than upon contacts in the water column, or did not occur at all. Direct contacts could occur several times during circular bottom swimming.

Grooming.—Grooming always occurred on the bottom in a curled-up position, usually in narrow double or triple coils, and lasted between 10 seconds and 3 min. The prehensile mouth limbs were used to groom any part of the trunk within reach.

A distinct change of grooming behavior was noticeable in the course of the total observation period. During the first weeks of observation, the periods of grooming behavior were relatively short (10 to 30 seconds) and occurred less frequently (approximately once in 3 h). Duration and frequency increased gradually (several minutes, up to 8 times per hour) towards the end of the observation periods. Grooming was performed most excessively shortly before an individual died.

Carpenter (1999) made similar observations of grooming behavior combined with secretion of mucus during his study. He suggested that changes in the water chemistry, in particular, an increase in pH was a probable cause for this behavior; under laboratory conditions in relatively small containers, a higher pH may be due to a loss of carbon or an increased excretion of ammonia. However, we think that it is also possible that grooming is a stress-related response

triggered by an increased number of external, irritating micro-organisms, for example, bacteria and/or protozoa (see also Microscopic observations).

Death of Individuals.—Our remipedes exhibited extended periods of grooming behavior shortly before dying. Hours before expiring, individuals appeared bloated and of a pale whitish color. The metachronal beating of trunk limbs grew gradually weaker until it was reduced to a slight twitching. At this stage, individuals lay at a standstill on their back on the sediment, and their trunk limbs and somites were covered with fine particles of sediment and detritus.

On one occasion, a remipede was observed swimming head-on into an individual *Artemia*. However, the startled response was much more extreme than usual: the remipede went into strong spasms and sank onto the bottom. After 30 seconds, it was completely covered with sediment. The specimen did not recover from this incident and died the next day, with all symptoms as described above. We have no explanation for this response, but similar reactions to food have been noted by Carpenter (1999). Shortly after consuming portions of crushed *Artemia*, aquatic oligochaetes, or fish flesh, three individuals of *Speleonectes epilimnius* Yager and Carpenter, 1999 became encrusted with mucus and accumulated debris, and died within two days.

Our observations confirm the results reported by Carpenter (1999). Intensive grooming behavior appears to be a response to an increased (or modified) secretion of mucus, and occurs in almost all cases shortly before death. This response is most probably stress-related. In addition to a changing water chemistry (see Grooming), other possible causes for stress may include either deficiencies (of nutrients, trace elements, or symbiotic protists), or over-abundances (of pathological or lethal bacteria, and viruses). For example, Yager (1991) observed gregarines throughout the mid guts of three species of remipedes. However, the ultimate physiological and/or ecological causes for similar stressful behavior in remipedes kept under laboratory conditions may be a very complex combination of factors.

Although our observations were almost identical with those described by Carpenter (1999), there is one interesting difference. While his animals lived up to 26 days, our remipedes survived a considerably longer period of time. We think that this discrepancy may reflect a crucial difference of the experimental designs. While Carpenter kept his remipedes in small culture containers, we used much larger volumes of water (50 and 150 L) that are more likely to maintain a longer stability of the *in vitro* environments, and thus increase the survival time of individuals. In yet another experiment with living remipedes, survival time could be increased up to 100 days in relatively small containers (about 5 L) by regularly replacing the tank water with fresh cave water (Yager, personal communication).

Reproduction.—Based on the anatomy and morphology of their reproductive systems, remipedes are considered simultaneous hermaphrodites (Itô and Schram, 1988; Yager, 1989a, 1991, 1994). Our examinations of individual remipedes and observations of their behavior confirm this

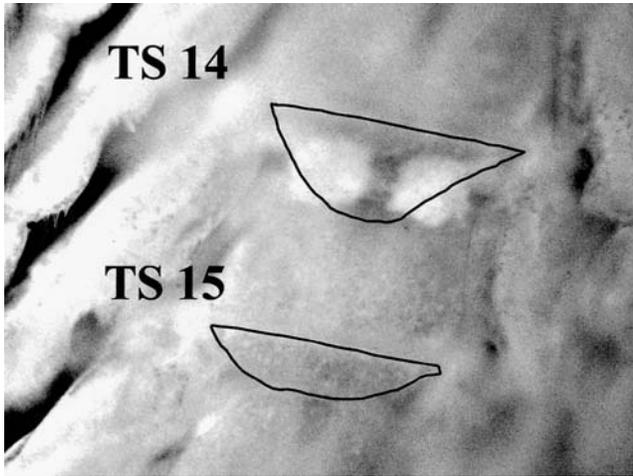


Fig. 5. Alcohol-preserved specimen of *Speleonectes benjamini*. Detail of ventral trunk showing two white spermatophores under the sternal bar of trunk segment 14. Sternal bars of trunk segments (TS) 14 and 15 traced by black lines.

assumption. Since the female pore is located on the seventh trunk segment and the male pore is on the fourteenth, it has been informally suspected that remipedes might possibly engage in mutual, simultaneous copulation. However, remipedes have never been seen in copulo, nor have any remipedes been seen to carry or brood eggs.

In watching our living remipedes for weeks on end, ceaselessly swimming, it occurred to us that mutual, simultaneous copulation was probably impossible, unless remipedes were to stop their constant metachronal beating of the limbs—something as noted above does not even occur when animals are apparently at rest.

However, we noted that the remipedes spent a certain amount of time on the bottom or on the surface of rocks in our tanks. One peculiar behavior we witnessed involved a remipede curling up on its side on the bottom in a secluded corner of the tank, with another remipede “on hold” nearby. When the first remipede straightened out and swam away, the second waiting remipede would slowly swim to the spot vacated by first and “examine” the surface with its first antennae. We saw this behavior more than once, but we did not see anything that looked like egg laying and/or subsequent deposit of any spermatophore. It suggests, nevertheless, that remipedes perhaps do not copulate but rather fertilize eggs laid in appropriate places. The lack of direct field observations suggests eggs are laid in crevicular habitat rather than in macro spaces accessible to human divers.

One of the specimens of *Speleonectes benjamini* Yager, 1987 collected during a recent diving expedition to Abaco Island (March 2006), had two perfectly globular masses of tissue (about 1/2 mm in diameter) on the ventral side of the 14th trunk segment (Fig. 5). Each of them lay close to the openings of the noticeably swollen male gonopores, both under (sic!) the flap-like sternal bar. Unfortunately, we were not able to analyze these masses because they dissolved during the attempt to preserve them for further analysis, but it is possible that these were spermatophores. To date,

Table 1. Observed remipede behavior in Cenote Crustacea.

Behavior	Number of Occurrences
Horizontal swimming, dorsal side down	59
Horizontal swimming, dorsal side down, touching sediment	17
Vertical swimming	25
Feeding	1

spermatophores in remipedes have only been described as relatively small, internal assemblages of sperm cells (Yager, 1989a, 1991).

This observation implies that the modified sternal bars on the 14th trunk segment in some species of Remipedia might serve as an attachment plates and/or protective covers for spermatophores. The fact that many species of remipedes do not have enlarged or modified sternal bars on the 14th trunk segment is puzzling. It might be an indication that the modes of reproduction are variable among species and more complex than thus far assumed.

Clearly, attention must be paid by field collectors to observing and collecting individuals on or near the bottom, in addition to sediment samples from various regions within the caves.

Behavioral Observations in the Field

Distribution and Abundance.—Many hundreds individuals of *Speleonectes* can be observed below the halocline in the deeper, fully marine water in Cenote Crustacea. This abundance is quite unusual compared to other anchialine cave systems inhabited by remipedes, where the number of observed individuals is usually less than 10.

Upon entering from the open sinkhole pool of Cenote Crustacea, remipedes were first noted about 150 m into the cave where the passage drops below the halocline at station number 1 on the cave guideline. From this point on, the passage consists of a 6-10 m wide by 4 m high, joint-controlled tunnel with deep vertical cracks in both ceiling and floor down the center line of the cave passage. The halocline is present near the ceiling of this passage, with hundreds to thousands of cave shrimp (*Typhlatya* sp.), occurring in brackish water above the halocline and in much smaller numbers in fully marine waters below it. From stations number 1-9, the distribution of remipedes was relatively sparse. However, from stations number 9-15, population densities increased with a patchy distribution of loosely assembled clusters. Clusters seemed more prevalent over large concave depressions in the sediment. Most remipedes were found concentrated near the center of the passage.

Swimming and Locomotion.—Except for escape responses, swimming consisted of a continuous metachronal beating of the trunk appendages. No remipedes were seen resting or completely still. When individual remipedes were first sighted by a diver, their swimming behavior was assigned as follows (Table 1):

1. Horizontal ‘backstroke’ swimming (dorsal side down). Similar to laboratory results, this was the predominant swimming mode, occurring at all depths.

2. Horizontal 'backstroke' swimming while touching sediment. Individuals touched the sediment when they swam close to the sediment surface, with quick dips down to the bottom so that the dorsal side contacted with the sediment. There was no lingering contact with the sediment and recovery to the backstroke swimming position was achieved by swimming up vertically or rolling over (from dorsal side up to dorsal side down). This behavior was observed in both single and multiple (clustered) individuals.
3. Vertical swimming: Second most common swimming mode observed; individuals either remained in place or used this orientation to change their depth in the water column.

Feeding, Foraging, and Hunting.—During this study, only one remipede was observed to actively feed on *Typhlatya*. The remipede constantly swam, holding the shrimp with its maxillae, while the other mouthparts manipulated the prey as it was ingested.

When this remipede was first sighted by divers, it already had a *Typhlatya* grasped in its mouthparts. The remipede maintained continuous horizontal swimming, with back-and-forth movements in the water and erratic turns for at least 10 min.

Three years earlier, in July 2003, divers observed and photographed another remipede in the act of feeding on a *Typhlatya* in Cenote Crustacea (Fig. 6). Both our current and prior observations were made in the water column, suggesting that the shrimp either were alive and swimming when they were captured, or had recently died and not yet settled to the substrate. Felgenhauer et al. (1992) observed *S. tulumensis* swimming dorsal side up away from baiting stations with fish in the cephalic appendages. Schram and Lewis (1989) recorded *Lasionectes entrichoma* Yager and Schram, 1986 feeding on a caridean shrimp while swimming in the more commonly observed dorsal side down position.

Attempts to feed dead *Typhlatya* by hand to remipedes were unsuccessful. No reaction was noticed from the remipedes except for a startled response upon being touched by an object (prey). This lack of reaction may be due to the abundance of prey species (*Typhlatya*) and resulting well-fed state of the remipedes in Cenote Crustacea.

Interaction and Contacts between Individuals.—No direct interactions or contact between remipedes were observed, even though numerous individuals were found in close proximity.

Reactions to Divers.—When physically disturbed by divers, remipedes exhibited a quick escape movement involving a rapid, ametachronal recoil in the opposite direction. The remipedes did not react to divers' lights. One diver was apparently bitten while attempting to herd a remipede into a collection vial, but no adverse reaction occurred.

Microscope Observations of Laboratory Specimens

During the examination with a microscope of dead individuals, a single specimen of the ciliate *Euplotes* was found in one sample. Since a contamination via tap water (in



Fig. 6. Individual of *Speleonectes* sp. eating a caridean cave shrimp (*Typhlatya*). This photograph was taken in Cenote Crustacea, Puerto Morelos, Quintana Roo, Mexico in July 2003 by Colin Kliewer.

Amsterdam) can be excluded, the ciliates may either have been introduced via the addition of living prey organisms or it arrived in the containers with original cave water we used for transporting the remipedes. The latter option appears more plausible since the ciliate matched the description of the marine stygobiont *Euplotes iliffei* Hill, Small, and Iliffe, 1986. However, it remains unclear whether this ciliate is a parasite or a commensal organism that the remipedes might be dependent on. In a previous study, Felgenhauer et al. (1992) also found epibiontic organisms, such as protists, ciliates, and suctorians, primarily on the ventral

surface of *Speleonectes tulumensis*, and they hypothesized that epibionts may be exuviotrophic or parasitic, and feeding from tissue fluids of remipedes.

Microscopic examination of dead individuals also revealed hundreds of small droplets of transparent lipids or fluid mucoids that had been secreted from tiny lateral pores along the trunk somites. These droplets were attached to the external body surface, and may be the cause for the adherence of sediment prior to death. Yager (1991) reported very large lipid-like intracellular droplets in the mid gut of *Godzillioognomus frondosus* Yager, 1989 and *Speleonectes benjamini*. In hypogean isopods, lipid stores are important energy resources during depressed rates of metabolism (Hervant and Renault, 2002). Isopods store lipids in digestive glands and in adipocytes that are distributed all over the body beneath the integument and vary in lipid content (Wägele, 1992).

Our observations suggest that lipids play an important role in the metabolism of Remipedia. However, why lipids or mucoids are secreted during a metabolic collapse requires further investigation. For example, the secretion of lipids would probably require an extension of gut-associated cells from the gut to the body wall as found in mystacocarids (see Fernández et al., 2002, and references therein). Felgenhauer et al. (1992) indicate that mucus might facilitate digestion in the aquatic environment by providing a protective coating that holds together food particles, digestive secretions or digestates. They also observed glands on the cuticle of remipedes of unknown function. Boxshall (1992) described various hypodermal and integumental glands found in calanoid and harpacticoid copepods; these glands apparently secrete mucoids for different purposes. For example, mucous gland cells are often found associated with lipid producing cells in tube building copepods. However, Boxshall suggested that mucus secretion may also be used in feeding by the calanoids.

In addition to secretion of mucus, all dead individuals had whitish strands protruding from their anal openings (up to 3 times longer than the anal somite). During field observations, recently collected specimens could be observed pumping water anally as has been reported for *Godzillioognomus frondosus* (Yager, 1991).

Anal intake of water has been observed for a number of small crustaceans and is considered to be a respiratory or osmoregulatory adaptation, particularly for organisms inhabiting water deficient in dissolved oxygen (Yager, 1991).

CONCLUSIONS

Our observations do not support an exclusively raptorial or "arachnoid" mode of feeding for Remipedia as suggested by Schram and Lewis (1989). Instead, filtering small particles out of the water column appears to be an important feeding mode, whereas feeding on large prey organisms occurs only sporadically and selectively. Although these results were obtained under laboratory conditions, we observed that the abundance of large prey in anchialine cave systems is typically very low. Consequently, alternative food resources such as detritus or micro-organisms are likely to play an important role for remipedes in their natural

environments; and hence these might provide the energy necessary to sustain the ceaseless beating of the trunk appendages. However, the question whether particle/filter-feeding represents the ancestral condition in Remipedia, as proposed by Brooks (1955) in his reconstruction of the enantiopod remipede fossil *Tesnusocaris* (although challenged by Schram and Emerson, 1991), remains still unanswered.

There is still much we do not know about the reproduction, life history, and behavior of remipedes. We are convinced, however, that more attention needs to be paid to the cave sediments and habitat not only to collect animals, but also to observe their behavior in their natural environment. Careful collection of bottom sediment might possibly yield fertilized egg masses.

Normally, remipedes occur in low abundance, but for unknown reasons, Cenote Crustacea has exceptionally high numbers and some field and lab behaviors may be atypical when compared to other species and environments. For example, the occurrence of mucus in laboratory held specimens may promote grooming behavior, which has not been observed yet in the natural environment. Some of our future research will focus on examining the potential roles of POC, DOC, microbiology, and energetics on behavioral responses in Remipedia. In particular, interaction of remipedes with bottom sediments has not been investigated yet.

Further observations of living remipedes are needed, and we believe that such observations should combine laboratory and field studies carried out at sites close to the native habitat utilizing laboratories set up specifically for this purpose. Year-round field studies and collections from the caves, as well as observations in captivity, will undoubtedly further contribute to understand the basic biology of these elusive and mysterious animals.

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