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research paper



## New insights into sound production in *Carapus mourlani* (Carapidae)

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**ABSTRACT.**—*Carapus mourlani* (Petit, 1934) is a commensal of sea stars and has the ability to produce sounds. Interestingly, we show here that this fish has a larger repertoire than previously recorded. *Carapus mourlani* can produce trains of 2–6 pulses, suites of double-pulses, staccatos of 2–17 weak pulses, and hums. These sounds are most probably emitted for species identification and for conspecific attraction, but we do not know their exact functions because they were all produced once the fish were inside the host. The ability to produce various specific messages indicates this behavior is important to the biology of this symbiotic, nocturnal species.

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Some carapid species (from the *Carapus*, *Encheliophis* and *Onuxodon* genera) are well-known for their ability to live, as commensals or parasites, inside invertebrate hosts such as Holothuroidea, Asteroidea, and bivalves. *Carapus* commensal species use their host as a shelter and leave it to hunt small prey, such as annelids, crustaceans, and small fishes. Parasite species feed on the internal tissues of their host, mainly the gonads (Arnold 1956, Trott 1970, Parmentier and Das 2004, Parmentier and Michel 2013). Another interesting aspect of this group of fishes is their ability to produce sounds (Parmentier et al. 2003a,b, 2008, Kéver et al. 2014). In captivity, series of pulsed sounds have been recorded for *Carapus boraborensis* (Kaup, 1856), *Carapus homei* (Richardson, 1846), and *Encheliophis gracilis* (Bleeker, 1856), only after an individual had entered a sea cucumber that was already occupied by a conspecific or a heterospecific organism (Parmentier et al. 2003b, Lagardère et al. 2005). In the Mediterranean *Carapus acus* (Brünnich 1768), pulses were recorded when the fish was swimming in the observation tank, whereas *Carapus mourlani* (Petit, 1934) produced calls when competing for access to the oral aperture of a sea star (Parmentier et al. 2006a).

In parts of the Pacific Ocean where they live in the same geographic area (Markle and Olney 1990), *C. boraborensis*, *C. homei*, and *E. gracilis* can inhabit the same host species, but heterospecifics are rarely found in the same host specimen (Parmentier and Vandewalle 2005). Sounds would be used, at least, for species-specific identification and, in some cases, for sex identification (Lagardère et al. 2005). Because sound attenuation by the sea cucumber tegument is negligible at the frequencies emitted by carapids, they have the potential to call for mates from the safety of their hosts

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(Lagardère et al. 2005). In the species *Onuxodon fowleri* (Smith, 1955), known to live inside pearl oysters, the sounds can be amplified by the shell cavity enhancing the communication distance (Kéver et al. 2014). Therefore, the ability to communicate acoustically would be an advantage during host colonization, territory defense, or reproduction. However, spawning behavior is not known in this family and additional studies are required to clearly understand the function of their sounds. It is difficult to obtain direct information because sounds are made inside the host, where it is not possible to observe the associated behavior.

The acoustic signals produced by carapids seem to be species-specific. In *C. boraborensis*, the sounds are composed of trains of 11–30 pulses of a duration of 25–30 s (Parmentier et al. 2003b, Lagardère et al. 2005). *Carapus homei* mainly produces brief sequences (about 10 pulses), lasting for 3–5 s, of a slower regular beat although in some cases trains can be made of double pulses. *Encheliophis gracilis* produce “drum rolls” consisting of trains of 5–6 pulses at a period of 40 ms (Parmentier et al. 2008). They can also produce single beats (Parmentier et al. 2003b). Preliminary studies on *C. mourlani* off Madagascar and *C. acus* in the Mediterranean Sea failed to record such trains of calls. *Carapus acus* produced only single and isolated pulses, whereas *C. mourlani* produced isolated single- or double-pulsed calls. These results are quite surprising because both *C. acus* and *C. mourlani* possess the same sound-producing mechanism as the other *Carapus* and *Encheliophis* spp. (Courtenay and McKittrick 1970, Parmentier et al. 2008, 2016).

Thus, it would be surprising if species such as *C. acus* or *C. mourlani* are able to produce only isolated sounds. In the present study, we aimed to investigate further the calls produced by *C. mourlani*.

## MATERIALS AND METHODS

Thirty-two *C. mourlani* (total length 6–10 cm) were collected on scuba dives in the lagoon (length 62 km, width 15 km) of Makemo atoll (16°36'S, 143°42'W) in October–November 2013. All experiments were carried out close to the beach with imported materials for aquaria. *Carapus mourlani* were collected by hand from the general body cavity of the sea star, *Culcita novaeguinea* Müller and Troschel, 1842 ( $n = 40$  sea stars) within the lagoon. Host and fish were stored in a community tank with sea water. Recordings were made at 26 °C in a circular plastic tank (radius 90 cm and water height 50 cm). A specimen of the host was placed in the center of the tank, and several individuals were introduced successively into the aquarium. Sounds were recorded with a hydrophone (HTI Min-96, –164.4 dB re 1V for a sound pressure of 1 $\mu$ Pa; High-Tech Industries, Long Beach, MS, USA) connected to a Tascam DR-05 recorder (44.1 kHz sampling rate; TEAC, Wiesbaden, Germany). In each case, the hydrophone was placed just above the sea star, between 5 and 10 cm from the sound source.

The effects of reverberation, resonance, and tank size can induce potential artifacts on the characteristics of sounds recorded inside small glass tanks (Akamatsu et al. 2002). The computed resonant frequency of the recording tank was 1.64 kHz; therefore, a high-pass filter of 1.5 kHz was applied to all recordings. However, according to comparative experiments conducted on the red drum, *Sciaenops ocellatus* (Linnaeus, 1766), sounds could be somewhat distorted despite the filter (Parmentier et al. 2014). This, however, does not affect the pulse period, the number of pulses, or

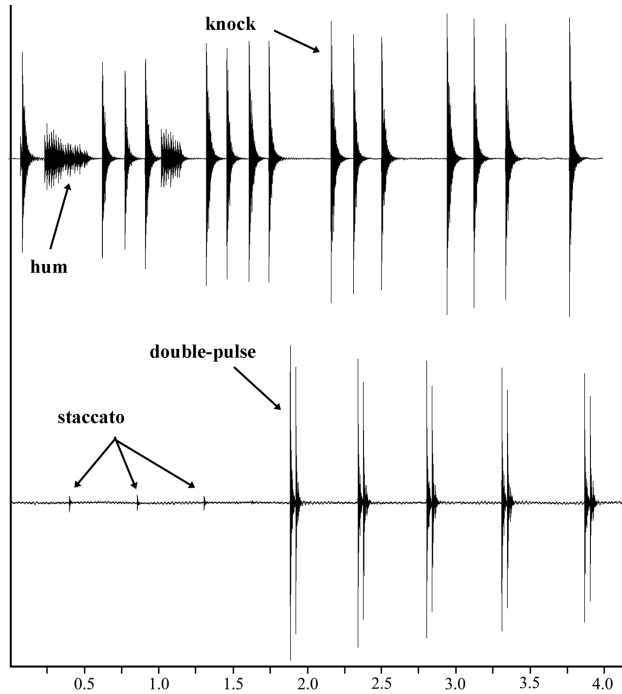


Figure 1. Oscillograms of the four kinds of sounds recorded from *Carapus mourlani*.

the period between consecutive calls. Sounds were thus digitized at 44.1 kHz (16-bit resolution), low-pass filtered at 1.5 kHz, and analyzed using AvisSoft-SAS Lab Pro 4.33 software. Due to the remoteness of the location, our fieldwork did not allow perfect recordings, thus only sounds with a good signal-to-noise ratio were used in the analysis. Temporal features were measured from oscillograms and frequency parameters were obtained from power spectra. The shape of the oscillogram indicates that the sounds were somewhat distorted because of plastic tank resonance and it is not desirable to measure pulse lengths under these conditions (Parmentier et al. 2014). The following sound parameters were then measured: number of pulses in a sound; pulse period (measured as the average peak-to-peak interval between consecutive pulses, in ms); and dominant frequency (Hz).

## RESULTS

All sounds were produced once the fish had entered into a host, in which at least one individual of the same species was already present. The amount of sounds produced peaked just after the entry to the host and subsequently diminished. To obtain these sounds, up to five different specimens were allowed to enter the same sea star. Four different sounds were recorded (Fig. 1). The first three sounds (knocks, double pulses, and staccatos) are clearly made of isolated pulses, but the last can be compared to a buzz.

Knocks are separate pulses, produced in trains of 2–6 pulses (mean = 3, SD 1,  $n = 34$ ). In this case, the period between each pulse is on average 136 (SD 25) ms ( $n = 86$ ) (Fig. 2).

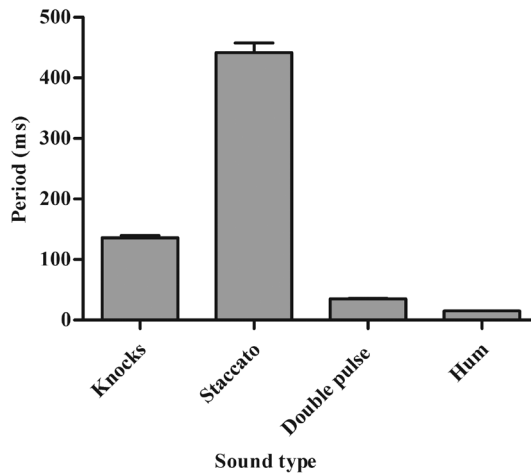


Figure 2. Comparison of the period length in the four kinds of sounds recorded from *Carapus mourlani*. Error bars represent standard errors of the mean.

Calls can also be made of suites of double pulses that are produced in series of 2–6 units, usually separated by a period of 473 (SD 185) ms. These double-pulses can be emitted in alternation with single pulses. Within double pulses, the period is 35 (SD 4) ms ( $n = 36$ ) and the second pulse is always weaker than the first (Figs. 1, 2). As was described in *C. mourlani* from Madagascar (Parmentier et al. 2006a), double pulses appear to be a repetition of the single pulse that form the knocks.

*Carapus mourlani* is also able to produce staccatos that are trains of 2–17 pulses of long duration (up to 10 min). These calls are separated by various periods of time, between 0.7 and 5 s (Fig. 2). In this case, pulses are emitted at a period of 442 (SD 113) ms ( $n = 52$ ). These sounds are 8–11 times weaker than both knocks and double-pulses (Figs. 1, 3).

The last type of sound recorded can be likened to a hum and was rarely emitted. They were usually preceded by the production of a least one knock (Fig. 1). These sounds are trains of pulses with decaying amplitudes. Hums last from 65 to 345 ms ( $n = 14$ ) depending on the number of pulses (from 5 to 23). The pulse period is 15 (SD 1) ms ( $n = 52$ ).

The first three kinds of sounds have their main frequency range between 80 and 550 Hz. In hums, the fundamental frequency of the whole sequence is around 64 Hz, but the dominant frequency corresponds to the 2<sup>nd</sup> or 3<sup>rd</sup> harmonics. Up to seven harmonics were found in the whole hum. However, these harmonics are a feature of the repetition of the pulses and do not correspond to the frequency structure of the individual pulses. Dominant frequency of a single pulse is between 45 and 130 and there is no harmonic in this case.

## DISCUSSION

Carapidae and other Ophidiiformes are fascinating due to their highly specialized sound-producing apparatus (Rose 1961, Courtenay 1971, Parmentier et al. 2002, Kéver et al. 2012). This mechanism is based on the same principle in *Encheliophis*

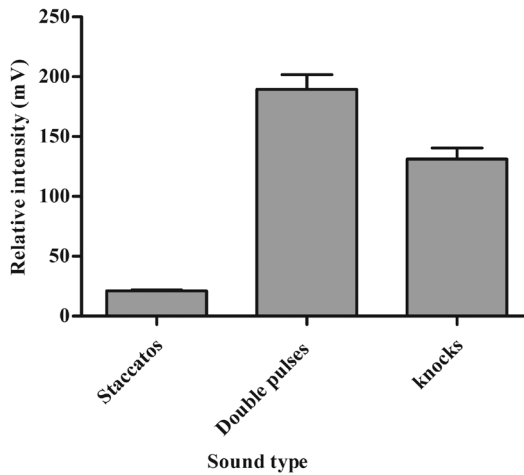


Figure 3. Comparison of the sound amplitude in staccatos, double pulses, knocks, and hums from *Carapus mourlani*. Values concerning the hums are not placed in this figure because hum signal corresponds to decaying pulses with various amplitude. Error bars represent standard errors of the mean.

spp. and *Carapus* spp. Primary sound-producing muscles are rostrally attached to the orbital roof and caudally to the antero-dorsal part of the swim bladder. This air sac has an anterior thinner zone of its wall called the swim bladder fenestra. This is situated just under the swim bladder plate, which is an osseous enlargement of the third epineural (Parmentier et al. 2003a, 2006b). During muscle contraction, the thinner zone allows the rostral displacement of the most anterior part of the swim bladder only and the stretching of the swim bladder fenestra (Parmentier et al. 2003a, 2006b). Sound is generated as the fenestra snaps back to its resting position. Having this complex apparatus means that these fish are able to produce various specific signals, suggesting this behavior is highly important to the fish's biology. The pulses are in a single unit or in long trains in *C. boraborensis*. They can be single, in trains, or in groups of two to three pulses in *C. homei*. They are single or in groups of five to six pulses in *E. gracilis* (Parmentier et al. 2003b, 2008, Lagardère et al. 2005). Moreover, carapid sounds can also show some variation. For example, temporal features can have a functional importance in sex identification in *C. boraborensis* (Lagardère et al. 2005). It has been shown that *C. mourlani* from Madagascar are able to produce sounds. Off Toliara, only isolated knocks and double-pulsed sounds were recorded (Parmentier et al. 2006a). These kinds of sounds were also found in the Tahitian population, highlighting that specimens from Madagascar and French Polynesia are able to produce the same kinds of sounds. The vocal repertoire of this species appears to be richer than expected because two additional kinds of sounds were recorded during the present study: long duration staccatos and hums.

Calling activities always take place inside the host, so it is difficult to associate sound and behavior. Moreover, all the fish and their hosts studied here were recorded in tanks, which may mean that the behavior is affected by the environment and the stress of being previously extracted from their hosts. However, the production of sounds once a second fish entered an already occupied host means that sounds are likely used for identification. This is confirmed by the finding that some carapid

species can alter their sounds according to the facing species inside the host; sounds presented a lower number of pulses and a modification in the pulse duration according to homo- or hetero-specific encounters between *C. homei* and *C. boraborensis* were observed (Lagardère et al. 2005).

Staccatos are interesting because the same kind of long-duration sounds were also recorded in *O. fowleri* (Kéver et al. 2014). In this species, sounds could be detected at 40 m from the emitter, which implies that these regular beats are made to attract conspecifics. This approach is more interesting for *O. fowleri* and *C. mourlani* than in *C. boraborensis* and *C. homei*, because the former species share their hosts with conspecifics and sounds could be used to attract them. Conversely, *C. boraborensis*, *C. homei*, and *E. gracilis* can compete for the same host species (Parmentier and Vandewalle 2005), so sounds may be used to repel other species. Some *E. gracilis* can also be (rarely) found in the sea stars *C. schmideliana* and *C. novaeguineae* (Arnold 1956, Branch 1969, Trott 1970), but their parasitic way of life indicates that they do not represent a danger for *C. mourlani* (Trott 1970, 1981, Parmentier and Das 2004). Different specimens of *C. mourlani* have been found in the same host (Meyer-Rochow 1977), but there are no records of *C. mourlani* and *E. gracilis* in the same host.

Finally, it is difficult to understand the function of the different humming sounds that the fish are able to emit and more investigations are required to fully understand them.

Two kinds of morphological organization are found at the level of the sound-producing apparatus. Some species (*C. boraborensis*, *C. acus*, and *C. mourlani*) possess a hook-shaped tendon that fits over a tubercle on the dorso-anterior wall of the swimbladder. The sound is produced when the tendon unhooks, snapping the swimbladder back to its resting position. In species such as *C. homei* and *E. gracilis*, the hooked tendon is not found and it seems the snapping back is simply due to muscle relaxation (Parmentier et al. 2008). It was first hypothesized that the absence of the tendon-hook system can explain why *C. homei* and *E. gracilis* are able to produce double- and triple-pulsed sounds with short periods and are able to make different kinds of sounds. The ability of *C. mourlani* to produce four different kinds of sounds, including hums and double pulses with a period of 15 and 35 ms, respectively, are not consistent with this morphology-based hypothesis. Differences between sounds would mainly rely on the neural system. Vocal pacemaker neurons can establish the patterned activity of sonic muscles and thus determine the contraction rate of vocal muscles that control the temporal properties of calls and other physical attributes of vocalizations (Bass and Baker 1990, Bass and McKibben 2003), and variations in these may account for the different sounds produced in the fish recorded here.

In addition to the primary sonic muscles, small secondary muscles can also be found in Carapini (Parmentier et al. 2003b). They attach to the skull (at the level of the epiotic bone) and on the first two epineural ribs. The distal tips of these bones have short ligaments that attach to the lateral anterior part of swim bladder. The contraction of these muscles necessarily involves the forward and outward displacement of the posterior tips of the epineural ribs. In this situation, the anterior part is transversally stretched, which could affect the sounds produced by the contraction of the primary sound-producing muscles. The increased stiffness of the swim bladder could help to sustain the sound (Parmentier et al. 2006b) and/or modulate the call amplitude. This ability may explain the variations in sound amplitude between the different kinds of call.

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