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Cleaning interactions between shrimps (Palaemonidae) and freshwater stingrays (Potamotrygonidae) in the Paraná River, Southeastern Brazil

Domingos Garrone-Neto*, Otto Bismarck Fazzano Gadig, Jansen Zuanon*** and Lucélia Nobre Carvalho******

We report the first record of cleaning symbiosis between a river stingray, *Potamotrygon falkneri*, and a palaemonid shrimp, *Macrobrachium jelskii*. Shrimps of different sizes were observed cleaning adult stingrays partially buried in sandy substrate and shallow water, during the day in the Paraná River, Southeastern Brazil, in four events. The presence of ectoparasites in the *P. falkneri* individuals was not detected during the cleaning interactions, and the shrimps were probably consuming dead tissue and/or mucus. Thus, the cleaning activity of the palaemonid shrimps was considered commensal and not casual. Despite *P. falkneri* including palaemonid shrimps in its diet, the low diurnal activity of this predator and a possible state of gastric repletion by the stingrays may have facilitated the cleaning interaction.

Aqui nós relatamos o primeiro registro de simbiose de limpeza entre uma raia fluvial, *Potamotrygon falkneri*, e um camarão palemonídeo, *Macrobrachium jelskii*. Camarões de diferentes tamanhos foram observados limpando raias adultas parcialmente enterradas em substrato arenoso de locais rasos durante o dia, no rio Paraná, região sudeste do Brasil, em quatro eventos. A presença de ectoparasitas nos indivíduos de *P. falkneri* não foi detectada durante as interações de limpeza e os camarões provavelmente estavam consumindo tecido morto e/ou muco. Assim, a atividade de limpeza dos camarões palemonídeos foi considerada comensal e não casual. Apesar de *P. falkneri* incluir camarões palemonídeos em sua dieta, a baixa atividade diurna deste predador e um possível estado de repleção gástrica pelas raias pode ter facilitado a interação de limpeza.

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Fig. 1. a, General view of study site. Note low turbidity in Paraná River during dry season (May to October); **b,** detail of sandy substrate where stingrays can easily camouflage; note algae patches where shrimps can hide and forage. (Photos: a, Gabriel Raposo; b, Domingos Garrone Neto).

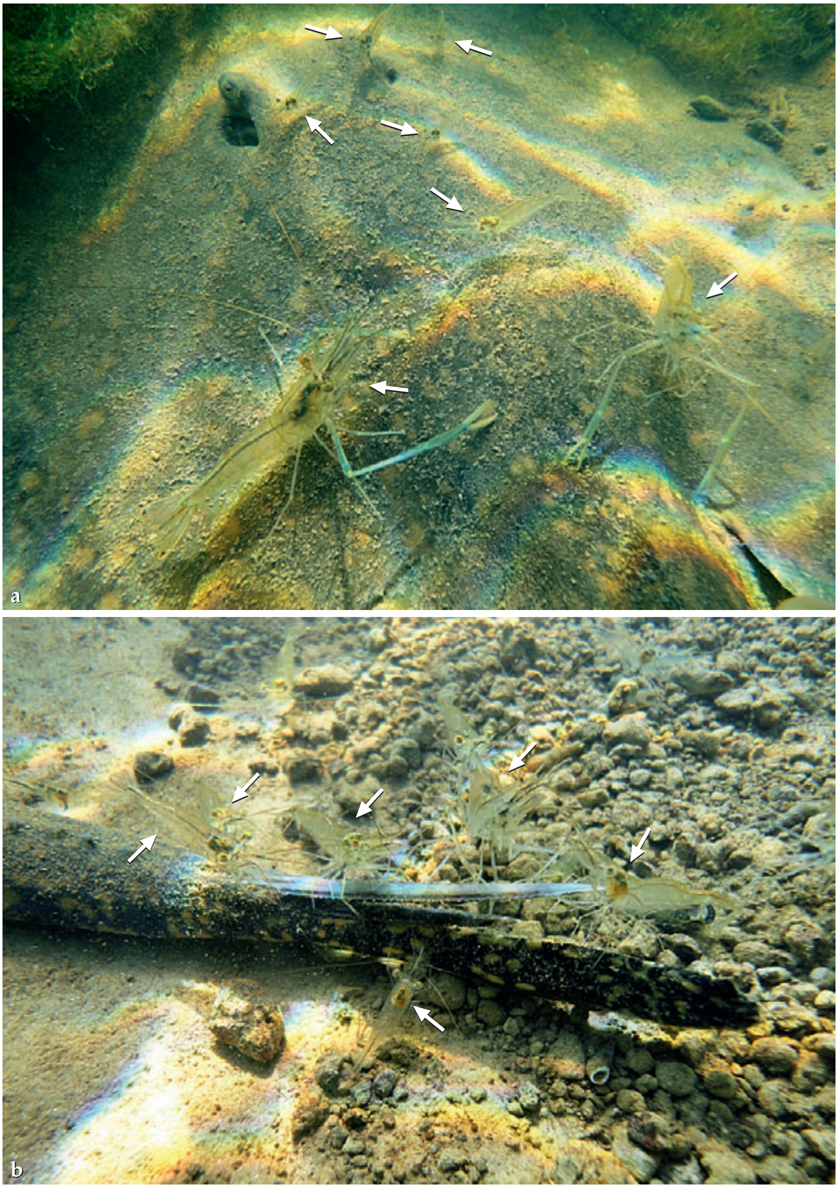

Fig. 2. Some palaemonid shrimps *Macrobrachium jelskii* acting as cleaners of freshwater stingray *Potamotrygon falkneri* in Paraná River, Southeastern Brazil. **a,** shrimps cleaning dorsal portion of stingray (n = 7, indicated by arrows); and **b,** gathered around and cleaning caudal stingers (n = 6). (Photos: Domingos Garrone Neto).

Introduction

Cleaning symbiosis is one of the most remarkable interspecific relationships in aquatic organisms, where the 'cleaner' have the habit of removing ectoparasites (e. g. fungi, bacteria and small crustaceans), diseased or injured tissue and mucus from the body of fishes and invertebrates – 'customers' or 'hosts' (Pott, 1968; Losey, 1972). This type of interaction has been considered as mutualism (positive) when the incidence of ectoparasites on the customers is high and as commensalism, or even parasitism (negative), when its incidence is low (Grutter & Poulin, 1998; Poulin, 1993).

Cleaning symbiosis has been repeatedly recorded in marine environments, including interactions between teleost fishes and shrimps in coral reefs (Bunkley-Williams & Williams, 1998; Becker & Grutter, 2004; Guimarães et al., 2007). In contrast, this behavioural interaction is rarely reported for freshwater environments. Interactions between teleost fishes and freshwater shrimps can be negative, such as predator-prey relationships (Gibson et al., 1995) and mutilation (Sabino, 1995), or positive as numerical or social mimicry (Carvalho et al., 2006). The few records involving freshwater stingrays and shrimps refer to negative interactions, where potamotrygonids are known as important predators of several small invertebrates, including palaemonid shrimps and teleost fishes in Brazilian rivers (Silva & Uieda, 2007; Garrone Neto & Sazima, 2009). Thereby, here we present the first report of cleaning symbiosis between the potamotrygonid stingray *Potamotrygon falkneri* and the palaemonid shrimp *Macrobrachium jelskii* in a freshwater environment, discussing the possible evolutionary steps for this behaviour.

Material and methods

The study was conducted in the upper course of the Paraná River, Southwestern Brazil (about 20°47' S 51°37' W). The interactions were recorded underwater while snorkeling (Sabino, 1999) at day (10.30–15.00 h), during the dry season in June 2012 and in October 2013. A total of 2 h 10 min of underwater observations was made at depths of 0.5 to 1.5 m, using the 'ad libitum' and 'behaviour' sampling methods (Martin & Bateson, 1986). The observational sessions were recorded using digital photography and notes were made on PVC boards, based on the methods presented by Sazima (1986) and Sabino (1999).

Stingrays identification was done in situ during the underwater observations, based on the coloration of the animal's dorsum (following Silva & Carvalho, 2011), without the necessity of catching the animals; a few shrimps specimens were collected and analyzed in laboratory (cf. Melo, 2003). The size of the observed individuals was estimated using a hand net of known dimensions as scale, placed close to the animals in a way to assess the disc width for the stingrays (DW, the greatest distance between both pectoral fin margins) and the total length of the shrimps (TL, the distance between the rostrum and the uropod). The sex recognition and sexual maturity of the individuals in the field was possible only for the stingrays, based on the presence or absence of claspers in the animals (easily observed dorsally) and on information about the size of sexual maturity for *P. falkneri* in the same area (Garrone Neto, 2010). In order to enable future verification of the stingray's identification, two additional specimens of *P. falkneri* not involved in the present observations were collected, fixed in formalin and preserved in ethanol, and stored at the Universidade Estadual Paulista Elasmobranchs Collection as voucher specimens (UNESP-CLP 0010.01, 0010.02).

Results

Four casual encounters resulted in the observation of individuals of *Macrobrachium jelskii* at different sizes (4–7 cm TL) interacting with two adult females of *Potamotrygon falkneri* (~30 cm DW), apparently involved in cleaning interactions. In all cases the interspecific interactions were noticed only under close proximity, since the palaemonid shrimps are usually hardly visualized underwater due to their translucent body and cryptic and disruptive markings (Melo, 2003; Carvalho et al., 2006). The stingrays were stationary, partially buried in soft sand substrate with algae patches, at shallow (about one meter of depth) and still water close to margins covered by native vegetation (Fig. 1). In each observed cleaning interaction, six to ten shrimps were distributed over the stingray's dorsum and tail. The shrimps moved along the stingray body, pinching the skin with its chela (pincers) in the dorsal disk borders and also in the tail spines (stings), apparently starting the cleaning activity from the tail and then moving to the dorsum and head region, around the eyes and spiracles (Fig. 2).

No signalling by the stingrays, such as an invitation posture, was perceived before the cleaning activity by the shrimps. Nevertheless, the ventral position of the stingray's mouth and the fact of being buried (hidden) in the substrate during resting possibly represent a low threat condition to the shrimps, so functioning as a clue that facilitates the start of the cleaning interaction. The shrimps were observed removing mucus and dead tissue, especially in the area of the tail spines, sometimes engaging in disputes for some areas to forage. After about 30–40 minutes of interaction, the stingrays started to slowly moving toward deeper places (> 2 m deep), simply abandoning the shrimps without any obvious signaling or other agonistic behaviour. The shrimps did not follow the stingrays, remaining close to the marginal vegetation and algae patches in shallow water (50–70 cm deep).

Discussion

Despite the fact that *P. falkneri* and other potamotrygonids include palaemonid shrimps in their diet (Lonardoni et al., 2006; Silva & Uieda, 2007; Garrone Neto & Sazima, 2009), the low diurnal activity of this predator (Garrone Neto & Uieda, 2012) may have facilitated the occurrence of the cleaning interactions. As *P. falkneri* usually hunts *M. jelskii* in shallow waters during the night (Garrone Neto & Sazima, 2009), it is possible that the absence of hunting behaviour by the stingrays during the diurnal cleaning interactions may be related to a condition of satiation after the nocturnal foraging. We cannot exclude other possibilities for the absence of aggressiveness by *P. falkneri* against *M. jelskii*, since other benefits such as tactile stimulation (Grutter, 1996; Grutter & Poulin, 1998) may mediate the process and to refrain a predatory response by the stingray. Although accepted as one hypothesis for the evolution of cleaning symbiosis between cleaning gobies and some sharks and marine rays (Sazima & Moura, 2000), it is premature to assume that this is occurring between potamotrygonids and palaemonid shrimps.

Differently from what is reported for some marine elasmobranchs, which search for cleaning stations in reefs environments (Sazima & Moura, 2000; O'Shea et al., 2010), the cleaning interactions between *P. falkneri* and *M. jelskii* seems to be occasional, when stingrays in a condition of feeding satiation rest partially buried near shrimps shelters in shallow water. Similarly, in some marine environments cleaning symbiosis is known to be facilitated when cleaner species, such as shrimps of the genus *Lysmata* (Hippolytidae) inhabit refuge holes in reefs that are also used by potential client fishes like moray eels (Quimbayo et al., 2012). Similar situations were observed for *Holocentrus adscensionis* and *Sargocentron hastatum*, which are predominantly nocturnal fishes but stay in or close to refuges inhabited by shrimps during the day. Nevertheless, the events of cleaning symbiosis observed between *M. jelskii* and *P. falkneri* have occurred in open places, without obvious refuges for the shrimps in the case of a possible aggressive response by the stingrays. In this case, the shrimp's translucent body, the simultaneous engagement of several shrimps in the cleaning, and the quick evasive response (jumping backwards) by the shrimps may have contributed to lessen the individual risk of predation, allowing the occurrence of such behavioural interaction.

Stingrays are notorious for producing copious amounts of mucus all over the body, and the occasional feeding by the shrimps during cleaning interactions possibly does not have negative effects on the health or fitness costs to the client. An analysis of the gut contents of shrimps involved in cleaning interactions with stingrays could help to elucidate the relative consumption of mucus, dead tissue and (possibly) parasites by the shrimps. As far as we know, this is the first report of cleaning behaviour by a freshwater shrimp. Based on the characteristics of the observed interactions, there is no reason to believe that it constitute a specialized behaviour, nor a species-specific relationship involving *P. falkneri* and *M. jelskii*. In this sense, observational studies of fishes and shrimps under natural conditions may reveal more cases of cleaning behaviour involving shrimps as cleaners. Further field observations and experiments in captivity are necessary and can reveal more details about this type of interaction, that still remains poorly understood in freshwater ecosystems.

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Sven O. Kullander, Michael Norén, Mikael Karlsson and Magnus Karlsson
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