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Importance of ectoparasites and mucus in cleaning interactions in the Mediterranean cleaner wrasse *Symphodus melanocercus*

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**Abstract** In fish cleaning associations, the net benefits gained by clients and cleaners from cleaning have still not been clearly evaluated. In particular, the role of ectoparasitism and the importance of client mucus characteristics remain unclear for most cleaner fish species. This paper investigates the cleaning behaviour of the Mediterranean cleaner wrasse *Symphodus melanocercus*, based on observations, cleaner gut contents, client ectoparasites and mucus characteristics. We showed that this fish is a specialised cleaner fish, similar to some other tropical cleaner species. Gnathiid isopod larvae and caligid copepods represented a large proportion of the items preyed on by *S. melanocercus*. Although their feeding activity was related to their client ectoparasite load, it was also significantly linked to client mucus load, which would indicate that the cleaning behaviour of *S. melanocercus* is not purely altruistic. Finally, as client visit to cleaning stations is related to their ectoparasitism, we propose that ectoparasite removal is likely to be a benefit for the client fishes of *S. melanocercus*.

**Introduction**

The behaviour of cleaner fishes, which remove ectoparasites, mucus and scales from the body of client fishes, has been widely investigated (Gorlick et al. 1978; Losey 1987; Poulin and Grutter 1996; Sayer et al. 1996; Côté in press). Many species have been described as cleaner fishes (van Tassel et al. 1994). They may be obligate cleaner fishes, cleaning throughout their life-span, or facultative cleaner fishes, cleaning only as juveniles or as females. Among obligate cleaner fishes, variations in ectoparasite removal and feeding on benthic animals have been observed (Grutter 1997a). Within a species, cleaners may be very specialised, relying almost exclusively on ectoparasites, or nonspecialised, feeding on benthic animals and ectoparasites and mucus from their client’s body surface (Grutter 1997a, 1999a).

The net benefits gained by clients and cleaners from cleaning are yet to be evaluated properly. Although most of the cleaner fish removal experiments failed to find any change in client health or behaviour (Youngbluth 1968; Gorlick et al. 1987; Grutter 1997b; but see Losey 1972 and Limbaugh 1961), two studies revealed that cleaners have an impact on their client’s ectoparasite size and abundance (Gorlick et al. 1987; Grutter 1999b). Reliable data on fish ectoparasite load (in particular for Gnathiidae and Caligidae) are, however, scarcely available, and the role of ectoparasitism has still not been clearly demonstrated from field observations. Cleaner fishes ingest mucus from the body surface of their client fishes (Gorlick 1980). However, mucus is an amorphous material which cannot be clearly recognised in stomach content analysis. Thus, the importance of mucus in cleaning interactions should be assessed using another methodology. Gorlick (1980) found a qualitative agreement between the preference of the cleaning wrasse *Labroides phthirophagus* for specific clients according to their mucus load and energetic value. The importance of client mucus characteristics in cleaning remains unclear. This is regrettable considering that mucus may be a more reliable food source for cleaner fishes than ectoparasites (Gorlick 1980), and may present an important glycoprotein source (Nakagawa 1988; Shephard 1994).

The majority of past studies have concentrated on tropical cleaner fishes. To our knowledge, from 1968 only 13% of the publications on cleaning have involved...
temperate cleaner fishes (Arnal, personal observation). This can be explained by the fact that temperate cleaner fishes are generally considered to be less specialised than tropical ones (Limbaugh 1961; van Tassel et al. 1994; Zander and Nieder 1997; Galeote and Otero 1998), and that temperate regions offer less favourable conditions for field observations (Potts 1968; Breen 1996). Furthermore, the costs and benefits of cleaning interactions between temperate cleaner and their client fishes remain to be evaluated (Losey 1987; Poulin and Grutter 1996).

Among cleaner fish species, the Labridae family is well represented, with 61 and 10 species in tropical and temperate waters, respectively (Côté in press). In the Mediterranean Sea, the most conspicuous cleaner wrasse is Symphodus (Crenilabrus) melanocercus (Risso, 1810). Few studies have described the cleaning behaviour of this cleaner wrasse, and many of them are mostly descriptive (Wahlert and Wahlert 1961; Potts 1968; Lejeune and Voss 1979, 1980; Zander and Nieder 1997). Symphodus melanocercus and its client fishes show behaviour during cleaning interactions similar to that of tropical cleaner wrasses (Potts 1968). They live in fixed territories, or “cleaning stations”, which they defend aggressively against conspecifics (Potts 1968; Lejeune and Voss 1980; Zander and Nieder 1997). The relationship begins with a client’s visit to the cleaning station. This may be initiated either by the cleaner fish, soliciting fishes by swimming towards them, or by client fishes which come to the cleaning station without being solicited (Wahlert and Wahlert 1961; Potts 1968; Lejeune and Voss 1979). The frequency of client visits is generally related to their density (Grutter and Poulin 1998; Arnal et al. 2000). Visiting clients may adopt a stereotyped horizontal, head-stand or tail-stand posture, considered “pose behaviour” (Potts 1968; Lejeune and Voss 1979). Then, S. melanocercus may inspect its client fishes, picking at their body surface (Potts 1968). At any moment, the interaction may be terminated by the client fleeing away or, less frequently, by the client becoming aggressive toward the cleaner, probably when cleaner bites are aversive (Potts 1968; Lejeune and Voss 1979; Zander and Nieder 1997). Stomach content analyses of S. melanocercus have rarely been performed (Potts 1968; Lejeune and Voss 1979) and exploitable numerical data are not available. It appears, therefore, difficult to assess, for S. melanocercus, its level of specialisation in cleaning and to compare its feeding behaviour to that of tropical cleaner fish species.

In this paper we investigate the cleaning behaviour of S. melanocercus, based on behavioural observations and on direct measurement of cleaner gut contents, client ectoparasites and mucus characteristics. We assessed three questions: (1) What is the cleaning activity of S. melanocercus? (2) What is the composition of the diet of S. melanocercus? (3) Are the behavioural patterns of cleaner fish and their client species, in the Mediterranean Sea, related to the client’s mucus characteristics and/or ectoparasite load?

Materials and methods

Data collection

Study site and behavioural observations

The study was carried out at Banyuls-sur-Mer (Mediterranean Coast, France), in July 1999. All observations were made at Be Grosse, near the Laboratoire Arago. The study site was located at a depth ranging from 10.4 to 12.4 m, over a bottom covered by large boulders and pebbles. We identified six cleaning stations at the beginning of the study (i.e. cleaner wrasse territories) and mapped them. We carried out observations by SCUBA-diving, between 0830 and 1800 hours. A single diver recorded all data directly on underwater paper from a distance of 3–5 m from the cleaner fish (according to the visibility). A period of 3–5 min prior to data recording allowed the fish to become used to the presence of the diver. We made a total of 1380 min of observations of cleaning stations.

Each cleaning station was observed 8–11 times for a total of 200–265 min. We recorded, for each cleaning station, the number and species of clients solicited or visiting without being solicited by the cleaner. We considered that cleaners solicited their clients, when they left their cleaning station, swimming toward them. For each cleaning event, we noted whether the client posed, whether the interaction resulted in an inspection, the inspection duration, and the number of bites delivered to the client. Finally, we noted whether or not the interaction was terminated by the client fleeing away (Table 1).

Assessment of fish density

Fish density was obtained from visual counts. This was made by a single diver, swimming slowly along a 50 m transect line, and recording on a plastic slate all the client fish species present within 2 m on either side of the line (see Leum and Chaot 1980; Galzin 1987, for similar methods). The sampling time was separated into three periods, four replicate counts were performed at 0930, 1400 and 1700 hours; we observed a minimum delay of 15 min between each count. Transect lines were positioned in the area where behavioural observations occurred, to assess more precisely the densities of client fish. We thus obtained 12 transects of 100 m² (50 x 2 m) each (Table 1).

Gut content analysis

Symphodus melanocercus (n = 20) were collected haphazardly by divers, following Grutter (1994). We used a 8 x 1.5 m barrier net with a 8 mm mesh; fish where captured with a hand net and placed in small plastic bags with an overdose of anaesthetic. Fish were preserved whole immediately after the dive (a maximum of 30 min after capture) in 10% formalin. Five fishes were collected in each of the four collection periods: 0800–1000, 1000–1200, 1400–1600 and 1600–1800 hours, to control for daily variations in cleaner food consumption (Grutter 1996, 1997a; Arnal and Côté 2000). Each fish was weighed (mg) and measured (SL). Gut contents were categorised following Grutter (1997a) into: gastrihiod isopod larvae, caligid copepod adults and pre-adults, other parasitic copepods (Penicillus fistula and copepod larvae), fish scales, and benthic organisms (molluscs, ctenidarian polyps), and free-living copepods. Gastroidi heads, with or without an attached body, were used to estimate the number of gastroidiids, following Grutter (1996). Although mucus is sometimes considered in gut content analysis of cleaner fishes (e.g. Grutter 1997a, 1999a), we could not readily distinguish it from other digested material in this study. We used two methods to assess cleaner wrasse gut contents (Hyslop 1980): the percentage cover of each food category was estimated visually in each of the 28 squares (1.5 x 1.5 cm) of a marked 9 cm diameter dish, then, discrete items were counted and removed from the dish to prevent double-counting.