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Can barber goby *Elacatinus figaro* control *Neobedenia melleni* infections on dusky grouper *Epinephelus marginatus*?

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Abstract

The interaction between cultured barber goby *Elacatinus figaro* (cleaner fish) and the dusky grouper *Epinephelus marginatus*, and the efficiency of the cleaner fish in removing ectoparasites were evaluated. When the interaction between these two species was observed, cleaner fish showed a preference for the largest groupers. In a second trial, treatments: TWC (Control) – two groupers without a cleaner fish, T1C – two groupers with one cleaner fish, T3C – two groupers with three cleaner fish and T6C – two groupers with six cleaner fish were tested in four replicates. After 8 days, monogeneans were removed and identified as *Neobenedenia melleni*. The highest mean abundance of parasites was found on the groupers in the TWC group (37 parasites per host) and the lowest on those in the T6C group (4.1 parasites per host). By increasing the number of cleaner fish, a higher cleaning efficiency was obtained, as observed in T6C, where almost 90% of the parasites were removed. Possibly, this removal would have been complete if the number of cleaners had not been reduced in the treatments due to the mortalities observed. This study demonstrates the possibility of using gobies to remove monogeneans and in improving grouper health.

Keywords: monogenean capsalid, cleaner fish, parasite control, marine fish farming

Introduction

The use of intensive farming systems for marine fish culture, which employs high stocking densities and high feeding levels, contributes to the reduction in water quality and can facilitate the establishment and increase in existing parasite infections (McGhie, Crawford, Mitchell & O'Brien 2000). These pathogens can result in serious economical impacts, not only causing mortality losses but also increasing the costs of production through the treatment of disease or reducing the quality of production (Nowak 2007). Thus, the main concern becomes the prevention and disease control (Leong 1997; Rückert, Palm & Klimpel 2008).

Diseases have been reported in marine fish species of commercial importance, particularly ectoparasites belonging to the group of monogenean capsalids *Benedenia* sp. and *Neobenedenia* sp. (Paperna, Diamant & Overstreet 1984; Al-Marzouq & Al-Rifae 1994; Deveney, Chisholm & Whittington 2001; Ernst, Whittington, Corneillie & Talbot 2002; Jithendran, Vijayan, Alavandi & Kailasam 2005). Chambers and Ernst (2005) reported that infections with *Benedenia* sp. are the major barrier against the expansion of sea-cage aquaculture of kingfish *Seriola lalandi*. In cultured Asian seabass *Lates calcarifer* in Australia, *Neobenedenia melleni* caused mass mortalities in few weeks (Deveney *et al.* 2001). According to Ernst *et al.* (2002), about 20% of the production costs of species of

cultured *Seriola* (amberjacks) in Japan are spent in controlling *Benedenia seriolae*.

Cleaner fish can be an alternative to the use of conventional treatments to control ectoparasites (Cowell, Watanabe, Head, Grover & Shenker 1993; Deady, Varian & Fives 1995; Tully, Daly, Lysaght, Deady & Varian 1996; Grutter, Deveney, Whittington & Lester 2002). According to Cowell *et al.* (1993), the use of cleaner gobies was highly effective in removing monogeneans from cultured red tilapia in seawater. Zimmermann, Rotman, Alarcon, Stevens, Matzie and Benetti (2001) described the successful use of the goby *Elacatinus oceanops* as a cleaner fish in the cultivation of red snapper *Lutjanus analis* and greater amberjack *Seriola dumerili*. Benetti, Orhun, O'Hanlon, Zink, Cavalin, Sandenberg, Palmer, Delinger and Bacoat (2007) suggest that the use of the same goby is beneficial for maintaining cobia *Rachycentron canadum* brood fish. In addition to the benefits of parasite removal, cleaner fish possibly provide a positive effect on the welfare of their clients generated by the tactile stimulation of their pectoral and pelvic fins on client fish; a process compared to massage in humans (Bshary, Oliveira, Oliveira & Canario 2007). These authors have shown that fish with access to cleaner fish had lower levels of cortisol, indicating less stress, compared with those not exposed to cleaner fish.

Gobies of the genus *Elacatinus* are regarded as the most specialized cleaner fish in the tropical Western Atlantic (Losey 1971, 1974; Colin 1975; Johnson & Ruben 1988; Wicksten 1995). The barber goby *Elacatinus figaro*, an endemic species of the Brazilian coast (Carvalho-Filho 1999), performs an important ecological role in coral reefs, working in the cleaning of a variety of clients (more than 30 species according to Sazima, Sazima, Francini-Filho & Moura 2000), ranging from small herbivores to large carnivores, such as groupers and snappers (Monteiro-Neto, Cunha, Nottingham, Araújo, Rosa & Leite 2003; Floeter, Vázquez & Grutter 2007). For many years, it was among the most exploited ornamental species along the Brazilian coast, and because of the over-exploitation, *E. figaro* was included in the list of threatened fish species by the Brazilian Environmental and Renewable Natural Resources Institute (IBAMA) (Normative Instruction No. 5 of 21, May 2004). Recently, successful production of barber goby has been achieved in captivity (Meirelles, Tsuzuki, Ribeiro, Medeiros & Silva 2009; Tsuzuki 2011).

To investigate sustainable alternatives to the problems caused by infections with monogenean in marine fish culture, this study sought to determine if the barber goby produced in captivity presents a cleaning behaviour in dusky grouper *Epinephelus marginatus*, used as a model: to verify the efficiency of this cleaner fish in removing ectoparasites and to identify the optimal ratio between groupers and cleaners for an efficient cleaning.

In Brazil, the dusky grouper has been produced experimentally with promising results by the Fisheries Institute, Southeast Brazil. Nevertheless, this species is highly susceptible to infections of *N. melleni*. Sanches and Vianna (2007) pointed out serious problems of infection of this parasite in cultured dusky grouper, causing the destruction of the eyes, bacterial infections and high mortalities.

Materials and methods

Animals and general maintenance conditions

This study was conducted at the Marine Fish Culture Laboratory II (LAPMAR II), Federal University of Santa Catarina, Florianópolis, SC, Brazil.

The cleaner fish used in this study was the barber goby *E. figaro,* obtained from the reproduction of the 3rd generation (F3) of fish produced in the laboratory. Animals were reared according to methods described by Côrtes (2009) and Meirelles *et al.* (2009). After the transition from live to inert food, juveniles were fed with dry diet NRD (5–6) (INVE, Dendermonde, Belgium – 570 g kg^{-1} crude protein and 145 g kg^{-1} lipid), minced fish and shrimp. Fish were kept in the laboratory until they reached 5 months old and an average size of 3.5 cm.

Forty wild dusky groupers *E. marginatus* were maintained in a floating cage (2 × 2 × 2 m), 6 m depth, located in Itaguá Bay, Ubatuba-SP. The water current was around 0.1 ms^{-1}, and had a mean salinity and temperature of 34 g L^{-1} and 28°C respectively. Groupers were fed to apparent satiation with chopped sardines once every 2 days until the start of the trials. During the experiment I and II, they were fed to apparent satiation once a day in the afternoon.

Experiments of the cleaning interaction and parasite cleaning efficiency were carried out at the Fisheries Institute, Ubatuba, São Paulo, Southeast Brazil, during the summer season in January 2010, period when the highest occurrence of

ectoparasites on groupers was observed (Sanches & Vianna 2007). In these experiments, water temperature ranged between 27 and 29°C, salinity was kept at 34 g L^{-1} and dissolved oxygen ranged from 5.1 to 6.4 mg L^{-1}.Water nitrite and nitrate were not detected while the total ammonia remained stable (0.25 mg L^{-1}) with a slight increase from the fifth day onwards (between 0.25 and 0.5 mg L^{-1}). A natural photoperiod of 13 light:11 darkness was used throughout the experiment.

Experiment I - Cleaning interaction between cultivated barber gobies and dusky groupers

Three groupers weighing 350, 420 and 1215 g and measuring 30, 32 and 44 cm (total length), respectively, were stocked in a 2000-L circular tank connected to a mechanical and biological filter in a recirculation system. A mesh was placed over the outflow pipe, to prevent gobies from escaping. After acclimation in tanks for 3 days, three barber gobies were introduced. Two ceramic pots with holes in the sides were placed at the bottom of the tank to provide a hiding substrate for the gobies and to simulate a cleaning station.

For 4 days, fish behavioural observations (interactions related to different body size and approaching behaviour) were recorded five times a day at 07:00, 10:00, 13:00, 16:00 and 19:00 hours. Each observation lasted for 10 min and the interactions between all fish were photographed with flash turned off and video-documented.

Experiment II – Estimation of the level of ectoparasite infection on groupers and the cleaning efficiency of the barber goby

Before the start of the cleaning experiment, the mean abundance of parasites was calculated according to Bush, Lafferty, Lotz and Shostak (1997) in 10 fish from the floating cage (as previously described). This number represents 32% of total groupers used for the experiment. This initial number was necessary to check the parasites presence and the influence of the groupers size on ectoparasitic fauna.

Groupers were individually treated in a freshwater bath for 6 min to remove ectoparasites. After each treatment, the freshwater was filtered through a 45-μm mesh sieve for parasite collection. Parasites were fixed in formalin solution 5% for counting, to generate the average of initial mean abundance of parasites. A total of 15 parasites were stained with Gomori trichrome and mounted in Canada balsam or Hoyer's for identification according to Whittington and Horton (1996).

Groupers (n = 10) used to obtain this initial average were divided into two groups: the largest groupers (n = 5) with 685 ± 152 g (mean ± SD) and 37 ± 3 cm in weight and length, respectively; and the smallest groupers (n = 5) with 390 ± 127 g and 26 ± 3 cm in weight and length respectively.

The day after initial mean abundance determination, the cleaning efficiency of *E. figaro* was evaluated in four treatments for 8 days. New groupers from the same floating cage were distributed in four replicates as follows: (1) Treatment TWC (control) – maintenance of two groupers without the presence of cleaner fish, (2) Treatment T1C – two groupers with one cleaner fish, (3) Treatment T3C – two groupers with three cleaner fish, (4) Treatment T6C – two groupers with six cleaner fish.

The experiment was conducted in 16 octagonal-shaped tanks, each with a capacity of 3500 L, and supplied with seawater in a recirculation system with a rate of daily tank renewal of water at around 250–300%. A mesh was placed over the outflow pipe, preventing the gobies from escaping. The tanks of each treatment were selected randomly. In each experimental unit, two groupers, one of larger size (647.0 ± 230.0 g and 35.8 ± 3.7 cm) and one of smaller size (372.0 ± 144.0 g and 30.1 ± 4.0 cm) were placed together. Mean weight and length of the groupers did not show any marked variation among the treatments ($P > 0.05$).

Ceramic pots were placed upside down (with a hole on the top of the pot) at the bottom of the tanks where the cleaners were present. In these tanks, the cleaners were introduced 2 days before the groupers to enable a possible acclimation of the gobies to the tanks. Four times a day, at 08:00, 11:00, 14:00 and 17:00 hours, the groupers were observed for feeding and swimming behaviour, while the cleaners were monitored in relation to cleaning behaviour and interaction with the groupers. These observations followed the same criteria determined in the first experiment.

At the beginning and at the end of the trial, groupers were anaesthetized with a benzocaine solution (50 mg L^{-1}), measured (±1.0 mm) and weighed (±0.01 g). To evaluate the mean abundance of parasite 8 days after experiment,

fish were killed, bathed in freshwater and examined for parasite counting.

Statistical analysis

To verify the similarity between the weight and length of the groupers in the different treatments, analysis of variance (one-way ANOVA) followed by Tukey test ($\alpha = 0.05$) was applied.

Differences in the initial mean abundance of parasites compared with the average of the control treatment (TWC), as well as the mean abundance of parasites in the treatments T1C, T3C and T6C compared with the mean abundance in control fish (TWC) were detected using analysis of variance (one-way ANOVA) followed by Dunnett's test ($\alpha = 0.05$).

Results

Experiment I – Cleaning interactions between cultivated barber gobies and dusky groupers

The interaction between both fish was observed on the first day of the experiment, 18 min after the introduction of a goby into the experimental tank (Fig. 1). The first approach of the goby was through a 'dance' performed by a different swimming behaviour where the goby positioned its body parallel to the grouper and swam forwards and backwards, possibly for the groupers to recognize or visualize the cleaner colour pattern. Generally, the interaction occurred between one cleaner fish and one grouper with some observations of more than one goby cleaning the same grouper. At one point, in the second day of the experiment, three gobies were observed simultaneously inspecting the largest grouper.

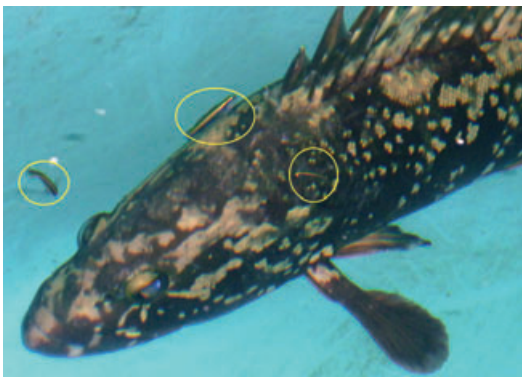

Figure 1 Cleaning interaction between barber gobies and a dusky grouper.

It was observed that the barber goby preferred to inspect the largest grouper (70% of the interactions). The highest frequencies of interaction were observed at midday, between 10:00 and 13:00 hours, with some eventual interaction occurring in the afternoon (16:00 hours). In the first and last observation times of the day, no interaction was recorded.

Experiment II – Estimation of the level of ectoparasite infection on groupers and the cleaning efficiency of the barber goby

During the experiment, there were six mortalities, one grouper in two tanks of treatments TWC, T1C and T3C. Both groupers of treatment TWC died on the sixth day of the experiment, and those from treatments T1C and T3C died on the seventh day. In these treatments, the final number of groupers was six ($n = 6$). The causes of death were not identified. In treatment T6C, no mortality was observed with the resulting final number of eight groupers ($n = 8$).

The parasites were identified as the capsalid monogenean *N. melleni* (MacCallum, 1927) Yamaguti, 1963 (Monogenean: Capsalidae) with the following measurements: total body length with haptor 2.82 ± 0.31 (2.10–3.24) mm; body width 1.31 ± 0.32 (0.80–1.80) mm; haptor length 0.76 ± 0.14 (0.46–0.92) mm; haptor width 0.78 ± 0.13 (0.60–1.00) mm. The largest groupers ($n = 5$) showed a mean abundance of 24.0 ± 4.0, and the smallest ones ($n = 5$) showed a mean abundance of 24.6 ± 3.0 parasites. These values did not differ significantly ($P > 0.05$). Thus, the initial mean abundance was obtained through the values of all fish ($n = 10$), which was 24.3 ± 5.1 (17–33) parasites per grouper (Fig. 2).

Groupers ($n = 10$) used to obtain the average of initial mean abundance presented 537.0 ± 203.0 g and 34.0 ± 5.0 cm in length and weight respectively. These values did not differ significantly ($P > 0.05$) when compared with the average weight and length of groupers used in all treatments. As groupers were subjected to the same environmental and husbandry conditions, it was assumed that they presented a similar average infection of the groupers used at the beginning of the experiment.

At the end of the experiment, no significant difference ($P > 0.05$) was observed between the mean intensity of infection on the largest groupers

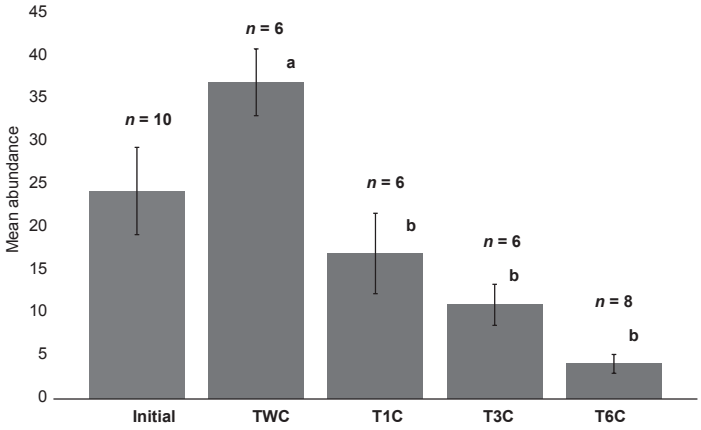

Figure 2 Mean abundance (±SD) of *Neobenedenia melleni* from dusky groupers in treatments T1C (two groupers with one cleaner fish), T3C (two groupers with three cleaner fish) and T6C (two groupers with six cleaner fish), compared with the mean abundance of *N. melleni* on control fish (TWC: two groupers without the presence of cleaner fish).

compared with the smallest ones within each treatment. The mean final abundance for each treatment was obtained from the values of all fish in each treatment.

At the end of the trial, the mean abundance, standard deviation and the range in each treatment were as follows: control (TWC): 37.0 ± 3.9 (32–42); T1C: 17.0 ± 4.7 (10–23); T3C: 11.0 ± 2.4 (8–15) and T6C: 4.1 ± 1.1 (3–6) parasites per host. Means of treatments T1C, T3C and T6C were significantly lower than the mean found in TWC ($P < 0.01$) (Fig. 2). The treatment with six cleaner fish (T6C) showed the highest cleaning efficiency with a reduction of almost 90% in the parasites when compared with the control treatment. On the other hand, treatments T1C and T3C enabled a reduction of 54% and 70% in the mean abundance respectively. Control treatment (TWC) showed a significant increase in parasite load, when compared with the initial mean ($P < 0.01$) raising the average in 52% of mean abundance. These results were in agreement with the daily observations that showed more interactions between cleaners and groupers in two tanks of the treatment T6C, preferably in the afternoon.

The observations also showed that groupers in all treatments (fish from control and those exposed to cleaner fish) were frequently close to the bottom of the tanks, mainly leaning against the water pipes or the ceramic pots. This behaviour did not allow the gobies to reach their shelters, so they were frequently seen in the lateral sides of the tanks or close to the surface.

In most tanks, a dominance of the largest grouper over the smallest one was observed, especially at feeding time when the smallest grouper was virtually unable to eat.

The interactions were observed to occur near the shelters or substrates where the groupers remained stationary with their bodies bent at an angle of about 45°. Most of the time, cleaning was performed mostly in the posterior region of the grouper's body, with some records of gobies cleaning inside the gills and on the head. In some tanks, the groupers were seen to expand their operculum, allowing the gobies to access the gills. This event was observed especially in tanks of treatment T6C. The objective of this observation was qualitative (interaction behaviour between groupers and cleaners).There were times during the cleaning events when groupers were bothered with the presence of the gobies on their bodies. They raised their dorsal fin and made sudden movements, which immediately drove the gobies away, disrupting the cleaning interaction.

One goby was found dead on the first day of the experiment and it was immediately replaced. At the end of the experiment, 24 gobies were missing probably due to predation by the groupers, as none of them were seen dead in the bottom of the tanks during daily observations. The number of gobies observed in the tanks in the last days of the experiment decreased, especially on the seventh day. Treatment T1C (1 cleaner fish per tank) showed a final number of two from initially four gobies (50% survival), in T3C, there were four (33% survival), with one cleaner in each experimental unit. The treatment T6C had a final number of 10 cleaner fish (42% survival), two tanks with two and other two tanks with three cleaners.

After the trial, some groupers from the control treatment (TWC) showed exophthalmia and skin haemorrhages.

Discussion

The cleaning interaction observed in this study between the cleaner fish *Elacatinus figaro* and the dusky grouper *E. marginatus* promptly occurred when both fish were placed together. It should be emphasized that the cleaner fish used in this study were produced in captivity, and after the metamorphosis phase or weaning period, they were only being fed with dry or inert diets. Moreover, these cleaners never had come in contact with other fish species and had never fed on ectoparasites. These facts demonstrate the innate cleaning behaviour of the barber goby. Such behaviour was also observed by Zimmermann *et al.* (2001) in the goby *E. oceanops* produced in captivity, acting in the cleaning of mutton snapper *L. analis* and amberjack *S. dumerili* brood fish. These authors observed the beginning of the interaction 30 min after the introduction of gobies into the broodfish tanks.

In this study, the first approach of the gobies was through a 'dance', which according to Grutter (2004) is a form of interspecific communication in cleaning interactions. Sazima and Sazima (2004) reported that some cleaners presented a specific swimming pattern, indicating the willingness of the clients to be cleaned. Some authors suggest that this behaviour may reduce the risk of aggression by the client (Losey 1971; Sazima, Moura & Gasparini 1998). Furthermore, the ability of the client to recognize the cleaner fish may be directly related to the colouration pattern and the contrasting colours of the body. Arnal, Verneau and Desdevise (2005) reported that the colouration of the genus *Elacatinus*, ranging from yellow to blue would be related to the contrast with the corals, which these species inhabit. According to these authors, this contrast would be essential for the recognition of this fish species by the clients in the natural environment.

In the first experiment of this study (cleaning interaction), the cleaners showed a preference to inspect the largest grouper. Bansemer, Grutter and Poulin (2002) observed that the cleaner wrasse *Labroides dimidiatus* inspected larger clients for longer periods in the natural environment, such as in this study with cultured goby. Furthermore, several authors suggest that fish with larger size have a higher parasite load (Grutter & Poulin 1998a). Therefore, cleaners identify the larger clients as a source of higher food availability (Poulin 1993; Grutter 1995; Grutter and Poulin 1998b). According to Soares, Cardoso and Côté (2007), gobies of the genus *Elacatinus* sp. examined from natural environment give preference to clients with high parasite load.

However, considering that most ectoparasites of client fish are very small (0.14–2.7 mm) (Grutter 1994) and have the ability to camouflage (e.g. some monogeneans) (Whittington & Horton 1996), cleaner fish might not be able to identify the parasites before approaching the client. In turn, the size of client fish can be identified by the cleaner from a distance. The mucus secreted by the client fish is another food source sought by cleaners (Gorlick 1980; Grutter & Bshary 2003) and its production is related to body size (body surface). Thus, the selection of clients based on body size may influence the initial choice of a client by the cleaner (Grutter, Glover & Bshary 2005).

In the experiment of cleaning efficiency, no difference between the parasite load of the largest and smallest groupers was found, both of them having a similar number of parasites at the beginning and at the end of the cleaning treatment. Therefore, it can be assumed that initially, the attraction of cleaners to larger groupers might have occurred due to the size of the client as the gobies may not have been able to visualize the parasites.

Elacatinus figaro interacts with a large number of clients of different species to obtain different sources of food (ectoparasites and other material found on the outer surface of fish) (Sazima *et al.* 2000). However, the patterns of interaction that guide this symbiotic relationship are still largely unknown. Which species receive the cleaning benefit, and how frequently it occurs are currently not well understood (Grutter *et al.* 2005). This is partly due to the fact that both the client and the cleaner can initiate the interaction (Losey 1971).

Neobenedenia melleni is recognized as a potential lethal pathogen causing irreversible damage in several marine species, apart from encouraging the emergence of secondary infections such as *Vibrio* sp. (Sanches & Vianna 2007). Sanches (2008) reported the importance of acting in the early stages of infection with this parasite. Noga (2010) commented that *N. melleni* causes serious skin damage and has a predilection for the eye. In this study, groupers kept without cleaner fish presented exophthalmia and skin haemorrhages unlike the

groupers maintained with cleaners, showing the importance of the cleaner fish in maintaining grouper health. Grutter (1999) demonstrated convincingly that cleaners significantly reduce the level of ectoparasite infection on host fish, and her work strongly suggested that if infected client fish are maintained in captivity and are unable to elicit the services of cleaners, then infection levels are likely to increase.

At the end of the experiment (eighth day), in the treatment with the presence of one cleaner fish (T1C), a reduction of 54% in parasite load was observed. At the same period, the very low number of parasites observed in treatment T6C (six cleaners) indicates that *E. figaro* was able to near eliminate (88.9%) the ectoparasites of the client fish in a short period of time compared with control. Possibly, this removal would have been complete if the number of cleaners had not been reduced in the treatments due to the mortalities observed.

Our results demonstrate that barber gobies can reduce the mean abundance of *N. melleni* on dusky groupers. Cowell *et al.* (1993) suggested that gobies are able to remove ectoparasitic monogeneans of seawater-cultured tilapia in a short period of time. Zimmermann *et al.* (2001) reported the efficiency of the cultured goby *E. oceanops* in removing ectoparasites on red snapper *L. analis* and amberjack *S. dumerili* brood fish. However, in this study, the capacity of removing ectoparasites per cleaner fish (T1C treatment), at the present infection level, was moderate. These findings suggest that more than one cleaner fish is required for treatment efficiency.

Cleaners of the genus *Elacatinus* usually interact with potentially dangerous clients such as piscivorous species (Côté, Arnal & Reybolds 1998; Wicksten 1998). Soares *et al.* (2007) commented that in the natural environment, cleaners of the *Elacatinus* genus prefer predatory clients. This characteristic may lead to breakdowns in the cleaning symbiosis relationship, eventually ending up in predation (Lobel 1976; Francini-Filho, Moura & Sazima 2000). The latter authors reported two cases of predation by the grouper *Cephalopholis fulva* on the cleaner wrasse *Thalassoma noronhanum* that was acting outside the cleaning stations. Lobel (1976) observed the cirrhitid hawkfish *Cirrhites pinnulatus* preying on the Pacific cleaner *Labroides phthirophagus* when they were also acting outside the cleaning stations. In addition, Machado, Daros, Bertoncini, Hostim-Silva and Barreiros (2008) reported that gobies were part of the dusky grouper's diet in southern Brazil.

Thus, it is most probable that the cleaners that had disappeared during the experiment were predated upon by the groupers, as no dead cleaner fish were found in the tank. Moreover, the permanence of the gobies inside the shelters placed in the tanks for the simulation of cleaning stations was inhibited due to the fact that the groupers dominated these territories. Moreover, Grutter (2004) stated that the probability of aggression of the client with the cleaner increases when levels of parasite infection decreases, which reduces the need for cleaning services. This author also reported that as the client becomes hungry, the likelihood of preying on the cleaner increases. Two possibilities may explain the disappearance of gobies from the tanks. One of them could be explained by the low parasitic load, responsible for reduced cleaning services, as supported by Grutter (2004), increasing the aggressive behaviour by the grouper. An alternative explanation is related to territory domination by the client fish favouring the predation of cleaner fish.

In this study, the dominance of the largest grouper during feeding may have caused a condition of starvation in the smallest one, a plausible fact that can account for the missing cleaner fish. Thus, the hungry grouper may have identified the cleaner fish as a food source. By analysing the interaction between piscivorous client fish, the coral trout *Plectropomus leopardus* and cleaner fish *Labroides dimidiatus*, Grutter (2004) found that hungry fish ate cleaners in less than 8 min after being placed in the same tank. This author also reported the ingestion of dead cleaners, when offered to clients.

Studies evaluating the ability of parasite removal have been proposed for a variety of cleaner species. Positive results from these studies would allow for the utilization of this species in the biological control of ectoparasites in fish culture. However, the use of cleaner fish depends on their production and market availability. Thus, this study is of great importance because it used a cleaner fish that shows viability of production in captivity (Côrtes 2009; Meirelles *et al.* 2009; Tsuzuki 2011).

Due to the feasibility of production and capacity of removing ectoparasites observed by the barber goby on grouper, the use of six gobies is recommended to control *N. melleni*, especially in brood

fish, as they need optimum conditions for reproduction and spawning.

Conclusions

The cleaning behaviour can be observed in cultured individuals (cleaner fish) that had never been in contact with other species of fish and ectoparasites. These cleaner fish were previously fed on dry and inert diets.

The use of cleaner fish was efficient to control *N. melleni* on cultured groupers. It was evident that as the number of cleaner fish increases in the treatments, better cleaning efficiency was observed. Nevertheless, the cleaning efficiency would have been greater if there were no predations in this study. In future studies involving this cleaner fish, the authors suggest the use of floating shelters for the gobies to hide in.

Further work will deal with the use of this cleaner fish in controlling ectoparasites and possibly reducing stress of commercially important marine brood fish, such as cobia *R. canadum* and snook *Centropomus* sp.

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