

Facultative cleaning behaviour of juvenile *Diplodus sargus* (Sparidae) and its ecological role in marine temperate waters

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ABSTRACT: The diversity and abundance of cleaner species have been frequently associated with ectoparasite load and ecological wealth of tropical fish communities. Cleaning behaviour in temperate regions has received less attention, with few labrid species being described as cleaners. The context and frequency of cleaning behaviour by juvenile white seabream *Diplodus sargus* are described. Surface observations from pontoons in yachting marinas were carried out based on a method used in a recent first report of cleaning behaviour by this northeastern Atlantic and Mediterranean sparid. A total of 51 h of observations revealed that these juveniles (<10 cm total length [TL]) display similar or higher cleaning rates (13.1 cleaning events per hour) compared to other temperate cleaners. The high cleaning rates, high abundance of young *D. sargus* on rocky shores along their distribution area and preferential targeting of adults by coastal fisheries highlight the ecological importance of *D. sargus*. The most common client species include grey mullets (Mugilidae), which represent 93.5% of total cleaning events registered. Regarding TL, clients were 4.6 to 6.6 times larger than cleaners. Environmental factors such as water temperature (14.0–24.0°C), wave exposure (6.0–17.0 s) and wind speed (2.0–8.0 m s⁻¹) influence white seabream cleaning rates. Thus, a combination of factors may affect the health of temperate client fish communities. On a different perspective, these results also highlight the potential of juvenile *D. sargus* in integrated multitrophic aquaculture. In conclusion, white seabream cleaning behaviour plays an important role in temperate fish communities and its relevance in different habitats should be further assessed.

KEY WORDS: Cleaner fish · Temperate communities · White seabream · Ectoparasites · Symbiosis

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1. INTRODUCTION

Cleaning symbiosis is an association in which an organism defined as 'cleaner' removes parasites, dead tissue or unwanted food particles from the epidermis of a cooperating 'client' (Galeote & Otero

1998). This behaviour has been described in a variety of terrestrial vertebrates, but it is especially common within marine ecosystems (Limbaugh 1961, Grutter 1999), with over 200 fish species already described as cleaners (Van Tassell et al. 1994, Arnal et al. 2006, Vaughan et al. 2017). Furthermore, Rosa

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et al. (2014) highlighted the ecological relevance of this behaviour for coastal communities and the possible threats it may face under a climate change context.

According to their behaviour, fish are classified as obligatory or facultative cleaners. The first group depends mostly on the food obtained from these interactions throughout their entire lifespan, while fishes from the second group exhibit this behaviour during a specific phase of their life-cycle and rely on other food sources (Arnal & Côté 2000). Several studies indicate that cleaners choose their clients based upon their size and parasite loads (Grutter 1995, 1999, Arnal et al. 2000). Frequently cleaners defend small territories and tend to be sedentary, while many clients have a roaming lifestyle, risking being preyed upon during visits to these cleaning stations (Cheney & Côté 2001, Oates et al. 2012).

A substantial number of factors intrinsic to each community influence the frequency of cleaning interactions, which may vary greatly amongst species (Floeter et al. 2007). Besides the relative importance of cleaning interactions and general difference in cleaning rates, obligatory cleaners seem to share some morphological characteristics. Physical traits such as small body size and contrasting striped patterns are common among obligatory cleaners, helping clients to recognize cleaners through common visual cues (Stummer et al. 2004).

Despite the number of species currently described as cleaners, the ecological relevance of cleaning interactions has been frequently debated (Cheney & Côté 2005). While cleaner benefits remain obvious, difficulties assessing client gain during these interactions have been pointed out (Cheney & Côté 2005). Episodes of cheating during cleaning activity have been described, with 'cleaners' biting healthy tissue from their clients, resulting in mucus loss and tissue injuries (Bshary & Schäffer 2002, Grutter & Bshary 2003). However, clients seem to actively choose the cleaners they interact with in order to avoid these occurrences and react adversely when cheating occurs (Bshary & Schäffer 2002, Bshary & Grutter 2005, Pinto et al. 2011).

In any case, cleaning interactions have an impact on the ecological relationships between clients and cleaners. Field experiments showed that completely removing cleaners from specific reefs affects the community with several fish opting to roam into other areas (Limbaugh 1961, Bshary 2003), highlighting the ecological importance of cleaners as key organisms within their respective communities (but see Grutter 1996a).

Cleaning interactions have been frequently studied in tropical fishes (Quimbayo et al. 2017, Sampaio et al. 2017, Vasco-Rodrigues et al. 2017) including the Indo-Pacific bluestreak cleaner wrasse *Labroides dimidiatus* (Grutter 1997) and the Caribbean and western Atlantic genus *Elacatinus*, namely the Caribbean sharknose cleaning goby *E. evelynae* (Johnson & Ruben 1988, Sazima et al. 2000, Whiteman & Côté 2002, Bertoncini et al. 2009, Narvaez et al. 2015).

However, few studies have focused on species in temperate regions (Limbaugh 1961, Van Tassell et al. 1994, Galeote & Otero 1998). Most cleaner species belong to the families Labridae and Gobiidae with a worldwide distribution (Arnal et al. 2006, Baliga & Law 2016), but there is no trend supporting the assumption that this behaviour is more frequent in tropical than in temperate waters (Hobson 1968).

An increasing number of studies in temperate regions have led to the recognition of additional cleaner species (Zander & Sötje 2002, Weitzmann & Mercader 2012), such as the northeastern Atlantic *Symphodus melops* (Potts 1973) and *Centrolabrus exoletus* (Henriques & Almada 1997) and the Mediterranean *Centrolabrus (Symphodus) melanocercus* (Baliga & Law 2016) (see Almada et al. 2002, Hanel et al. 2002 for taxonomic clarifications). To a lesser extent, *Coris julis*, *Thalassoma pavo* (Labridae) (Van Tassell et al. 1994) and *Lepadogaster candolii* (Gobiesocidae) (Weitzmann & Mercader 2012) have also been reported as cleaner species in the temperate northeastern Atlantic and Mediterranean. For this reason, many species are currently being used as cleaners in fish farms, which has been particularly evident in the salmon aquaculture industry (Robalo & Mirimin 2018).

Sparids in general, and *Diplodus sargus* in particular, represent a striking example that illustrates the strong deficiency regarding field observations of this symbiotic behaviour in temperate regions. Several *Diplodus* species contribute greatly to fish assemblages in Atlanto-Mediterranean rocky habitats (Rosecchi 1987, Sala & Ballesteros 1997, Dias et al. 2016). Sympatric species such as *D. sargus*, *D. vulgaris* and *D. puntazzo* have a diverse omnivorous diet while coexisting in the same ecosystem. Several studies have aimed to investigate the feeding habits of these species in order to understand, among other factors, potential food partitioning, prey preference and habitat use (Rosecchi 1987, Sala & Ballesteros 1997, Figueiredo et al. 2005, Leitão et al. 2007). In these studies, the composition of the diet of *D. sargus* proved to be highly opportunistic. Notably, Rosecchi (1987) identified small ectoparasite copepods (*Cali-*

gus pageti) commonly found on the epidermis of grey mullets (Mugilidae). Nevertheless, this fact was interpreted as occasional cleaning of conspecifics. Other authors confirmed the presence of ectoparasites in the stomach contents of *D. sargus* (Mariani 2001) and another highly similar species, common in the Mediterranean Sea, *D. puntazzo* (Moosleitner 1980, Van Tassell et al. 1994).

Behavioural reports of cleaning activity by congeneric sparid fish were only described later for the south American silver porgy *D. argenteus* (Krajewski 2007), and recently for *D. sargus* (Abecasis & Abecasis 2015). Both studies represent first reports and neither of them aimed to quantify the cleaning behaviour in sparids in order to evaluate their ecological role in each community. Based on field observations, this work aims to describe the context and frequency of the cleaning behaviour of juvenile *D. sargus*. The ecological relevance of this facultative cleaner species is compared to the information currently available for other temperate cleaner species.

2. MATERIALS AND METHODS

2.1. Field surveys

Field observations were performed in 3 marinas along the west coast of Portugal in Oeiras (38° 40' 34" N, 9° 19' 05" W), Tróia (38° 29' 36" N, 8° 54' 10" W), and Póvoa de Varzim (41° 22' 08" N, 8° 45' 49" W) from June 2014 to October 2015, and on the Spanish Mediterranean coast in Barcelona (41° 24' 50" N, 2° 13' 39" E) in June 2019. The number of observations for each location is shown in Table 1. In addition, non-quantitative cleaning behaviour reports were registered in other locations along the distribution area of this species, namely in the Azores, North Spain and Italy. All sampling sites are shown in Fig. 1.

Direct observations were made while standing on pontoons carefully avoiding disturbing the fish. Video recordings of some observations were also made. SCUBA divers and underwater cameras were ineffective, as both cleaners and clients flee in the presence of the observer, even in semi-confined areas such as marinas

and coastal lagoons. This was probably the reason why this conspicuous behaviour was only recently described by Abecasis & Abecasis (2015), based on visual observations from floating piers in yachting marinas. The same methodology was adopted in this work.

Overall concordance between observers was achieved with video recordings and visual estimations of the total lengths (TLs) of cleaners and clients. Preliminary data collected during this calibration phase was discarded from the analysis. To minimize limitations to visual observations, data were collected in sheltered areas near rocks along the margins of the marinas.

2.2. Cleaners and clients

Cleaners were classified according to their size in 5 classes, with intervals of 2.5 cm up to 10 cm, with the last class including fish with more than 10 cm TL.

Table 1. Distribution of observation hours, cleaning events, nips and duration of cleaning events for *Diplodus sargus* at each location

Location	Observations (h)	Cleaning events	Nips	Total duration of cleaning events (s)
Marina de Oeiras	31	427	1105	3147
Marina de Póvoa de Varzim	9	101	304	838
Marina de Tróia	5	78	289	880
Port Fòrum Barcelona	5	44	129	382
Total	50	650	1827	5247

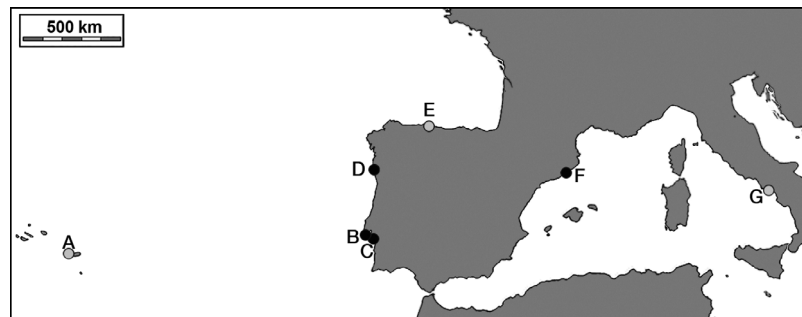


Fig. 1. Locations where cleaning behaviour by juvenile *Diplodus sargus* was observed. (●) Locations where the quantitative data used in this work was gathered; (○) locations where cleaning behaviour by juvenile *D. sargus* was observed but no quantitative data was collected. (A) Termas da Ferraria; São Miguel (Azores); (B) Marina de Oeiras (continental Portugal); (C) Marina de Tróia (continental Portugal); (D) Marina de Póvoa de Varzim (continental Portugal); (E) Puerto Deportivo de Gijón (Spain); (F) Port Fòrum Barcelona (Spain); (G) Marina di Puolo (Italy)

Clients, being conspicuously larger than cleaners, were also classified in 5 size classes, with intervals of 10 cm up to 40 cm, with the last class including all fish with more than 40 cm TL.

To evaluate if cleaner fish target any particular section of their client's body, cleaning events were described according to the targeted area: (1) head, starting from the tip of the snout to the base of the pectoral fin; (2) flank, starting from the base of the pectoral fin to the base of the anal fin; and (3) tail, from the anal fin to the tip of the tail.

To evaluate the cleaning frequencies of juvenile *D. sargus*, 2 different methods were implemented: (1) group scans, in which fish could freely abandon or enter the observation area that consisted of a 2 × 2 m square with presence of juvenile *D. sargus* chosen randomly within the shallow area of the yachting marina. A total of 50 observation periods, 1 h each, were performed to evaluate the frequency of this behaviour per area; (2) focal observations of individual fish were performed following 50 randomly chosen juvenile *D. sargus* that were followed for as long as they could be sighted during a total of 105 min (2.10 ± 1.76 min). These observations were performed to evaluate individual frequency of this behaviour.

Cleaning events were registered starting with the first physical contact between cleaner and client and ending with the separation of the pair (sensu Johnson & Ruben 1988). These events could involve 1 or several nips. For each cleaning event, total duration, number of nips and client reaction were recorded. Client reaction was considered 'positive' whenever the client remained motionless or slowly swimming, and 'negative' whenever the client reaction changed in response to contact, resulting in a jolt. A visual inspection was reported whenever a juvenile *D. sargus* swam directly into close range (less than half the client's body length) of a potential client and no contact was observed between them. Visual inspections that were not followed by a cleaning event were further described in order to evaluate which fish was responsible for the separation: the client swimming away from the cleaner or the cleaner swimming in another direction, losing interest in its potential 'client'. These interactions are shown in Fig. 2.

Client behaviour was also recorded. Cleaning requests commonly include

head-up or head-down displays at an angle of 45 to 90° accompanied by an interruption of the normal swimming pattern (sensu Galeote & Otero 1998, Stummer et al. 2004). Ambiguous situations were discarded.

In order to evaluate whether there is a preference for a particular set of target species, local abundances of potential clients must be accounted for. A preference for specific clients could be explained by (1) a bias towards species with a particular set of characteristics; or (2) randomly targeting potential clients according to their abundances in the study area. To evaluate a possible bias of juvenile *D. sargus*, 10 visual censuses were conducted to estimate relative abundances of local ichthyofauna (Table 2).

Statistical analysis was performed with R version 3.6.0 (R Development Core Team 2019). The number of cleaning events observed in group scans along the Portuguese coast was analysed via Generalized Linear Models (GLM) with a Poisson distributional family. Environmental variables (listed in Table 3) were used as explanatory variables. Daily mean wave period, wave height and wind speed data were obtained using the Global Forecast System model available at www.windguru.cz. All other environmental variables were collected during field observations. The most parsimonious model was selected based on the Akaike Information Criterion and incident rate ratios (IRR) were obtained by exponentiating the Poisson regression coefficients. Model residuals were checked and no significant deviation from the assumed distribution was found.

Frequency of nips by cleaners with different TLs targeting distinct client body areas and positive and

Table 2. Distribution of the cleaning interactions between juvenile *Diplodus sargus* and each client species and relative client species abundances in the study area. Information regarding species with low relative abundance that were never observed to interact with *D. sargus* is not shown

Host	Cleaning events	% of total cleaning events	Cleaning solicitations	Relative abundance
Mugilidae	608	93.54	5	0.34
<i>Sarpa salpa</i>	14	2.15	2	0.01
<i>Boops boops</i>	11	1.69	3	0.24
<i>Diplodus sargus</i>	10	1.54	2	0.16
<i>Seriola</i> sp.	3	0.46	0	0.00
<i>Dicentrarchus labrax</i>	2	0.31	0	0.00
<i>Symphodus melops</i>	1	0.15	0	0.00
<i>Oblada melanura</i>	1	0.15	0	0.01
<i>Atherina presbyter</i>	0	0.00	0	0.21
<i>Diplodus vulgaris</i>	0	0.00	0	0.02
Total	650		12	

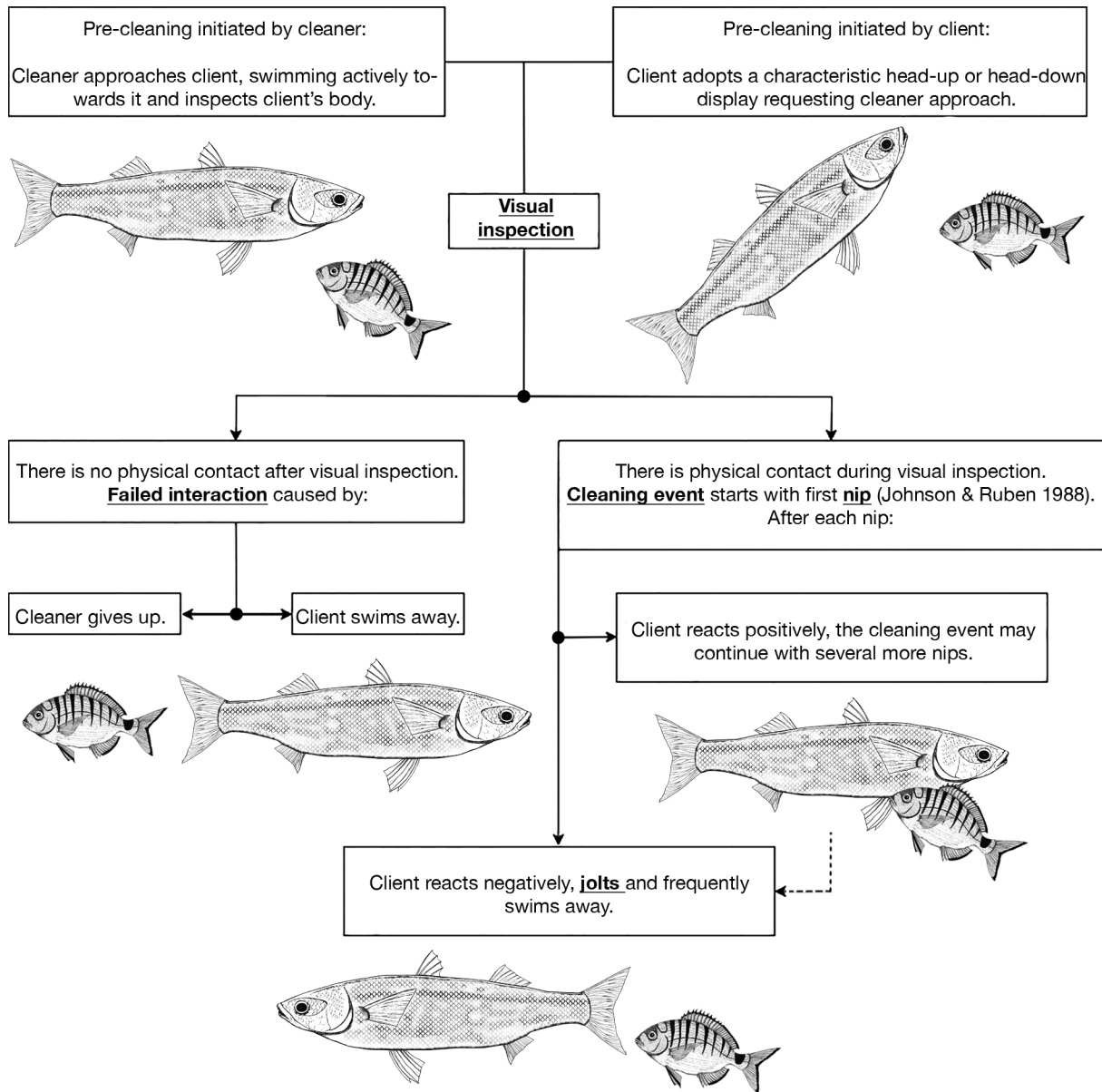


Fig. 2. Schematic representation of the interactions between *Diplodus sargus* (the cleaner) and its clients, represented here by a mugilid. Note: terms underlined and **bold** are not used uniformly in the literature

negative reaction to nips by clients of different sizes were analysed with ACTUS2 (Estabrook et al. 2002), using 1000 simulations under a 0.05 significance level.

2.3. Ectoparasites in stomach contents

Rosecchi (1987) and Mariani (2001) previously reported the presence of ectoparasites in the stomach contents of juvenile *D. sargus*. Four juvenile *D. sargus* were captured in 2015 (TL 7.4 ± 1.4 cm)

within the study area to confirm a direct relationship between cleaning behaviour observations and the presence of ectoparasites in stomach contents. Fish were sacrificed with a lethal dose of anaesthetics (300 mg l^{-1} MS222 tricaine methane sulphonate; Pharmaq) in strict accordance with the recommendations of the Animal Care and Use Committee of ISPA Instituto Universitário (ORBEA-ISPA). This study did not involve endangered or protected species and was not performed in a marine protected area, therefore no permission for capturing fish was needed.

Table 3. Range of observations (minimum, mean and maximum) for quantitative environmental variables and number of observations for qualitative environmental variables

Environmental variable	Minimum	Mean	Maximum	
Water temperature (°C)	14.00	18.92	24.00	
Visibility (m)	0.30	1.28	3.00	
Daily mean wave height (m)	0.80	1.83	4.90	
Daily mean wave period (s)	6.00	10.96	17.00	
Daily mean wind speed (m s ⁻¹)	2.00	4.73	8.00	
Number of observations				
Tidal period	High: 14	Ebb: 14	Low: 8	Flood: 8
Moon phase	Full: 6	Waning: 9	New: 16	Crescent: 14
Time of day	Morning (08:00–12:00 h): 15	Noon (12:01–15:00 h): 15	Afternoon (15:01–19:00 h): 15	

3. RESULTS

3.1. Context and duration of cleaning events

Considering both successful cleaning events and unsuccessful interactions, 1761 visual inspections were performed by *Diplodus sargus* during the 50 h of observation.

The 650 successful cleaning events (rate $13.0 \pm 7.7 \text{ h}^{-1}$; mean \pm SD) involved 1827 nips (rate $36.5 \pm 33.5 \text{ h}^{-1}$) (see Video S1 in Supplement 1 at www.int-res.com/articles/suppl/m629p165_supp1). Considering each individual cleaning event, the mean number of nips was 3.0 ± 4.3 , and the average duration of interactions was $8.7 \pm 13.7 \text{ s}$.

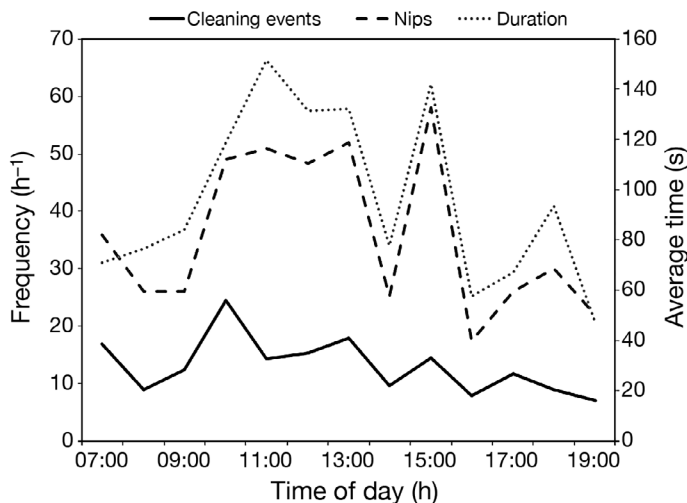


Fig. 3. Average number of cleaning events, average number of nips and average duration of cleaning events throughout the day for *Diplodus sargus*

Of the 1111 inspections not followed by cleaning events (number of failed inspections $22.2 \pm 25.0 \text{ h}^{-1}$), 830 (74.7%) were due to avoidance by the potential client and 281 (25.3%) were due to loss of interest by the cleaner *D. sargus*.

Cleaning events were observed in all months, except March 2015 when no juvenile *D. sargus* were observed at the sampling sites, from first daylight to sunset. The variation in the average number of cleaning events, average number of nips and average duration of cleaning events per hour throughout the day is depicted in Fig. 3.

The selected GLM model showed that environmental variables such as water temperature ($p < 0.001$), wave period ($p < 0.001$) and wind speed ($p < 0.01$) had a positive effect on the frequency of cleaning events observed in group scans (see Table S1 in Supplement 2 at www.int-res.com/articles/suppl/m629p165_supp2.pdf). A significant decrease in cleaning events was observed towards the end of the day (morning–noon $p < 0.01$ and morning–afternoon $p < 0.001$) and differences between moon phases ($p < 0.05$) were also found (Table S1).

During cleaning events, juvenile *D. sargus* did not target their client's body randomly. Although total observed nips directed to the client's head, flank or tail were similar (601 nips directed to the head, 579 nips directed to the flank and 647 nips directed to the tail), there were differences between cleaners of different sizes. Analysis of contingency tables using ACTUS2 (Estabrook et al. 2002) indicated that smaller *D. sargus* (<2.5 cm TL) show no preference, whilst slightly larger ones (2.5–5.0 cm TL) significantly avoided the head section of their clients. In contrast, larger juveniles (5.0–10.0 cm TL) preferentially targeted their client's head section ($\chi^2 = 55.5$, $df = 6$, $p < 0.001$) (Table S2).

3.2. Cleaners and clients

D. sargus juveniles were observed cleaning fish from 8 different taxa and the number of cleaning events per client species is shown in Table 2. A large proportion of clients (93.5%) were grey mullets, with the most common species being thicklip grey mullets *Chelon labrosus* and golden grey mullets *Liza aurata*. In general, mugilids are one of the largest and most

abundant groups of fish within the study area. Although *C. labrosus* represent the majority of the individuals observed in the sampled sites, their morphological similarities with other mugilid species (Reay & Cornell 1988) led us to identify all subjects as 'Mugilidae' and no further attempt was made to identify them to the species level.

Cleaning solicitations, with client immobilization and conspicuous body tilting (cleaning solicitation) followed by a successful cleaning event, were observed 12 times during the observation period for 4 different taxa: mugilids ($n = 5$), *Boops boops* ($n = 3$), *Sarpa salpa* ($n = 2$) and adult *D. sargus* ($n = 2$).

Clients reacted positively to physical contact by hovering motionless or soliciting cleaning on 1312 occasions (71.8%) as a response to continuous nips, sometimes by multiple cleaners (Video S2). Clients reacted negatively, swimming away and/or jolting, 515 times (28.2%) as a reaction to a cleaning attempt or nip (Video S3).

Considering only negative reactions, clients terminated 421 interactions (81.7%) after contact and cleaners lost interest and moved away in the remaining 94 events.

Analysis of the client reactions showed significantly high numbers of negative reactions by individuals with 10.0–20.0 cm TL ($\chi^2 = 15.0$, $df = 3$, $p < 0.01$) (Table S3), meaning that only smaller clients tended to react negatively to an interaction with a cleaner.

It is worth noting that there was a reduction in nip frequency (cleaning success) with increasing *D. sargus* size for all client size classes (Fig. 4). This is further emphasized by the fact that smaller *D. sargus* (<5 cm) cleaned clients of all size classes while larger *D. sargus* (>10.0 cm) cleaned only larger clients (>30.0 cm).

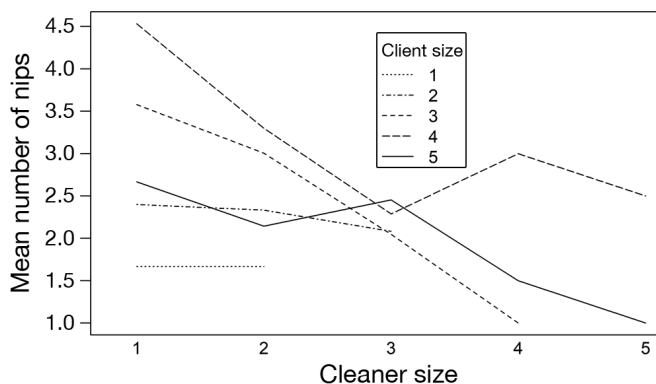


Fig. 4. Mean number of nips with increasing *Diplodus sargus* size for each client size class. Cleaner size 1: <2.5 cm; 2: 2.5–5 cm; 3: 5–7.5 cm; 4: 7.5–10 cm; 5: >10 cm. Client size 1: <10 cm; 2: 10–20 cm; 3: 20–30 cm; 4: 30–40 cm; 5: >40 cm

The size relationship between client and cleaner seems to play an important role in these interactions. Considering successful interactions, clients were on average 4.6 ± 2.3 to 6.6 ± 2.9 times larger than their cleaners.

Mugilids represent the most abundant group within the study area with *B. boops* and *Atherina presbyter* (see Almada et al. 2017) being also very abundant. However, only a few cleaning events were directed towards these 2 species. In contrast, potential clients such as *S. salpa* showed the opposite pattern, with low abundances in all field surveys, although they were the species second most frequently cleaned by juvenile *D. sargus*. Overall, the abundance of client species is important but it does not explain preferential cleaning behaviour by this opportunistic species.

Interestingly, *D. sargus* juveniles were also observed cleaning 2 potential predators: *Dicentrarchus labrax* and *Seriola* sp., which are commonly observed preying on small fish within the study area.

Juvenile *D. sargus* were not the only sparids displaying cleaning behaviour in the study area. Although they have rarely been seen in the Atlantic, juvenile *D. puntazzo* have occasionally been observed cleaning other fish in the Mediterranean. However, other congeneric species, namely *D. vulgaris*, frequently observed in the study area, have never been observed cleaning other fish.

3.3. Individual focal observations

Fifty randomly chosen individuals were followed for 105 min (2.10 ± 1.76 min). Of these, 21 (42%) were observed visually inspecting potential clients resulting in 44 failed interactions and 23 cleaning events, with a frequency of 13.1 successful cleaning events per hour. The average duration of each cleaning event was 8.2 ± 16.6 s, suggesting that *D. sargus* are involved in cleaning activities approximately 3% of their time.

3.4. Ectoparasites in stomach contents

Only 1 of 4 juvenile *D. sargus* captured had ectoparasites in its stomach contents. Three *Lepeophtheirus* sp. (Caligidae) (Fig. S1) were found in this fish. These results show that cleaning behaviour and the presence of parasites in the cleaner's stomach is simultaneously observed in the same location and during the same period of time, but do not exclude the possibility that these fish are also feeding on client fish mucus or tissue.

4. DISCUSSION

4.1. Cleaning behaviour by juvenile *Diplodus sargus*

A high cleaning frequency (13.0 cleaning events per hour) by juvenile *Diplodus sargus* under 10 cm TL, and the absence of cleaning activity by larger individuals, shows that cleaning behaviour is restricted to earlier life stages. According to the age/growth pattern reported by Gordoa & Molí (1997) (further explored by Abecasis et al. 2008), cleaning activity is probably restricted to individuals up to 1 yr old, meaning that seasonal interruptions of cleaning activity are probably short or absent, as a new cohort of post-larvae replaces the previous one with the onset of a new settlement phase. Field observations confirmed the occurrence of cleaning activity throughout the year, although a maximum frequency is expected during spring and summer accompanying the highest abundances of juvenile *D. sargus*. Considering daily activity patterns, the frequency of cleaning behaviour declined during the afternoon. These results are identical to the general feeding activity pattern described by Figueiredo et al. (2005) for this species. This pattern is also similar to other cleaner species such as the tropical *Labroides dimidiatus* (Grutter 1996b) and *Bodianus rufus* (Johnson & Ruben 1988). Considering other variables, higher temperatures may result in higher activity rates, which may be less conspicuous in tropical species subjected to narrower temperature ranges. As described by Nunes et al. (2013), increasing wave exposure could limit access to additional food items, thus reducing reef fish activity outside the sheltered areas sampled in this study. An omnivorous opportunistic species such as *D. sargus* could increase cleaning frequency in sheltered areas as a response to compensate their feeding habits during these periods. The positive relationship between cleaning rates and wind speed described in this study could have the same effect. Additional studies are needed to better understand the increasing cleaning rates observed during new moon compared to waning moon quarters.

The presence of ectoparasites in the stomach contents of individuals inhabiting coastal lagoons was previously suggested by Rosacchi (1987) and Mariani (2001). However, the relative importance of *D. sargus* as a cleaner species was largely overlooked, probably due to limitations related to stomach content analysis (e.g. limited number of specimens under 10 cm TL, preservation and consequent identification of consumed ectoparasites). Recently, Abecasis & Abecasis

(2015) reported cleaning behaviour by juvenile *D. sargus* stressing that its ecological relevance was yet to be evaluated.

Grey mullets (Mugilidae) were preferential client species and the most abundant taxa in the study area. Preferential cleaning behaviour and high cleaning rates could be mainly dependent on the abundance of specific clients (Galeote & Otero 1998, Arnal et al. 2000). However, the inclusion of additional species revealed no direct relationship between species abundances and frequency of interactions, suggesting that more complex mechanisms are involved. Grutter (1995) and Arnal et al. (2000) reported that cleaning frequency was also related to factors such as client size. Other authors have highlighted the importance of client behaviour to determine both cleaning frequency and interaction outcome (Arnal & Côté 2000, Zander & Sötje 2002, Soares et al. 2008). Regarding client size, larger clients are usually more attractive to cleaners than smaller clients due to their higher parasite loads (Grutter 1995) and richer mucus (Arnal & Morand 2001b). While size and local abundance of species are usually negatively correlated, the combined effect of high abundance and larger body size could help to explain the high frequency of interactions with a particular client (Floeter et al. 2007). Considering that grey mullets were the most abundant and among the largest fish species within the study area it was expected that they would also be preferential clients. On the other hand, *Boops boops* (Sparidae) and *Atherina presbyter* (Atherinidae) were highly abundant but commonly excluded as potential clients. This was probably due to the fact that their abundances were mainly due to the presence of schools of small juveniles with sizes that do not reach the threshold reported here. Sazima et al. (2000) reported previously that clients were on average 1.5 times larger than their cleaners, but the differences observed here are even higher, with *D. sargus* clients being usually 4.6 to 6.6 times larger, thus excluding smaller fish as potential clients.

Regarding client behaviour, clients may actively request cleaning interactions. Solicitation of cleaning was rare but this behaviour was observed in 4 different client species. In the large majority of cases, swimming slowly or resting near the surface triggered the cleaning behaviour of juvenile *D. sargus*. Interestingly, a single juvenile *D. sargus* interacted with an adult *Sarpa salpa* in a cleaning event that totalled 150 s, involving 65 nips, to none of which the client reacted negatively. *S. salpa* is common in the northeastern Atlantic and Mediterranean and is known to interact with other cleaner species, often

soliciting cleaning interactions (Henriques & Almada 1997, Arnal & Morand 2001b, Sabatino et al. 2007). This may suggest that *S. salpa* possesses the mechanisms required to recognize cleaners and interact with them. In addition, it is also interesting to note that *D. sargus* juveniles approached and cleaned potential piscivorous predators. Cleaners generally approach potential predators with caution and predators are very rarely cheated upon (Bshary 2002, Barbu et al. 2011). This suggests that the cleaning behaviour of juvenile *D. sargus* often requires client/cleaner cooperation, highlighting their potential ecological role in northeastern Atlantic and Mediterranean fish communities. These 2 examples do not exclude the fact that a combination of cooperative and cheating interactions may coexist. In fact, the number of positive and negative reactions was different among grey mullets of different sizes. Smaller clients reacted negatively more often to inspections and to nips, while larger individuals often remained indifferent during cleaning interactions. This could be related to (1) larger clients suffering less damage, having higher tolerance and being less intimidated by cleaner approach and contact even if they cheat on some occasions (Pinto et al. 2011); and/or (2) client learning processes (Sabatino et al. 2007) starting with small unwilling clients, resulting in forced 'hit and run' interactions (Johnson & Ruben 1988), with larger and less naïve clients being more likely to contact cleaners. In fact, a mutual learning process may underlie the interactions between *D. sargus* and their clients, with smaller naïve *D. sargus* choosing randomly or avoiding risks around the anterior part of the client's body, and larger and more experienced ones showing a preference for the head, gills and base of the pectoral fins. Considering that Alaş & Öktener (2017) reported a higher persistence of parasites in the head region of their hosts, experienced cleaners may show preference for the anterior region of their client's body due to higher parasite loads in this area.

Visual observations do not show if cleaning interactions involve the removal of clients' ectoparasites or body mucus. As a first step to overcome this limitation, the frequency of cleaning behaviour observed in juveniles was compared with the ectoparasite load (Caligidae) in the stomach contents of *D. sargus* reported in the literature. Individual focal observations revealed that 24% of individuals ($n = 12$) were engaged in cleaning events. Assuming that jolts may represent cheating occurrences with no removal of parasites from their hosts, only 18% of the individuals ($n = 9$) might have been involved in honest clean-

ing activities. These results from behavioural observations are in agreement with the occurrence of ectoparasites in the stomach contents of 10 and 17.9% of the *D. sargus* examined by Rosecchi (1987) and Mariani (2001), respectively.

4.2. Relative importance of *D. sargus* as a temperate facultative cleaner

Cleaner fish contribute to the health of fish communities by controlling the ectoparasite load of numerous client species (Grutter & Lester 2002). Studies focused on tropical ichthyofauna highlight the importance of this behaviour in the diet of obligatory cleaner species such as the Indo-Pacific bluestreak cleaner wrasse *L. dimidiatus* (Grutter 1996b) and the Caribbean sharknose cleaning goby *Elacatinus evelynae* (Johnson & Ruben 1988, Whiteman & Côté 2002, Côté & Soares 2011).

Studies on temperate cleaner species are much less abundant, which is probably related to harsh sea conditions and the fact that temperate species are often facultative and not obligatory cleaners (Limbaugh 1961, Ayling & Grace 1971, Potts 1973, Narvaez et al. 2015). In the temperate northeastern Atlantic and Mediterranean, cleaners are almost exclusively wrasses (Labridae) (Almada et al. 2002), with the main ones being the rock cook *Centrolabrus exoletus* in the Atlantic (Henriques & Almada 1997) and *C. melanocercus* in the Mediterranean (Galeote & Otero 1998, Arnal & Morand 2001a, Sabatino et al. 2007) (see Treasurer 2018 for additional studies focusing on aquaculture applications).

Compared to tropical obligate cleaners, the cleaning rates of *D. sargus* were consistently low. However, these rates were unexpectedly high when compared with those from known sympatric temperate cleaners (Fig. 5). Although individual focal observations revealed a high number of interactions, they also showed that *D. sargus* spend less time cleaning (3% for *D. sargus* compared to 13% for *C. melanocercus*; 118.6 ± 8.4 s per 15 min period; Arnal & Morand, 2001b), which points to the lack of specialization and lower dependency on cleaning activities of this opportunistic cleaner species.

Considering the overall diversity of client species, *D. sargus* targeted only 8 species while *C. exoletus* targeted 12 client species (Henriques & Almada 1997) and *C. melanocercus* targeted 19 client species (Sabatino et al. 2007) (Table 4). Cleaning was higher for *D. sargus* (*D. sargus* 13.0 cleaning events per hour, mean number of cleaning events per client spe-

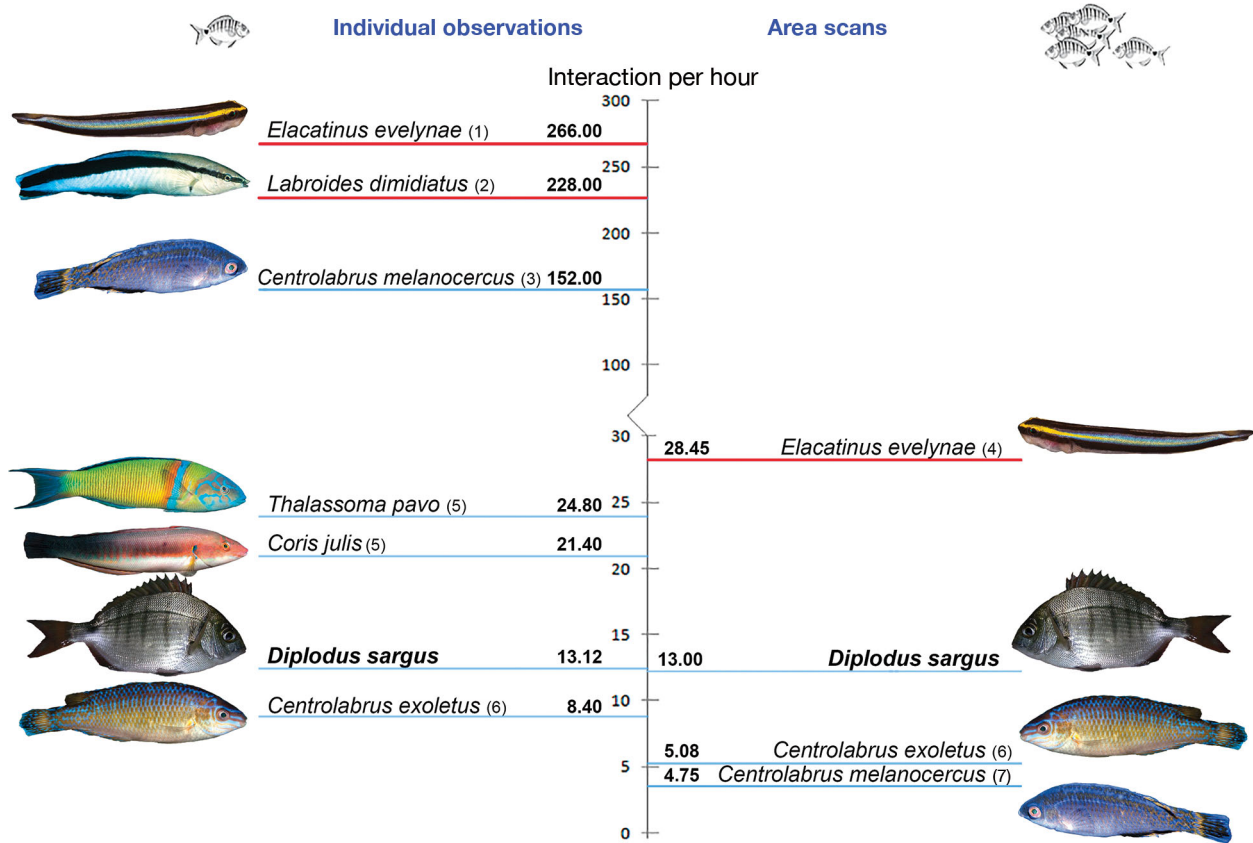


Fig. 5. Comparison of the cleaning frequencies of 5 different temperate cleaners (underlined in blue) and 2 tropical cleaners (underlined in red), as obtained through focal individual observations and local scans. Data for *Diplodus sargus* were obtained in this study; other data were obtained from (1) Whiteman & Côté (2002), (2) Grutter (1996b), (3) Arnal & Morand (2001a), (4) Johnson & Ruben (1988), (5) Narvaez et al. (2015), (6) Henriques & Almada (1997) and (7) Galeote & Otero (1998)

cies 1.6 ± 4.3 ; *C. exoletus* 5.1 cleaning events per hour, mean number of cleaning events per client species 0.4 ± 0.8 ; *C. melanocercus* 4.8 cleaning events per hour, mean number of cleaning events per client species 0.3 ± 0.3) but grey mullets accounted for 93.5% of all interactions, while the clients of *C. exoletus* and *C. melanocercus* were more evenly distributed (preferential target for *C. exoletus* was *Symphodus melops*, which accounted for 49.6%, and that for *C. melanocercus* was *S. tinca*, which accounted for 23.0% of total interactions).

The differences reported above point out that although *D. sargus* shows high cleaning rates compared to other temperate cleaner species, it is a rudimentary opportunistic cleaner spending less time cleaning and interacting with a limited number of client species.

Regardless of this lack of specialization, there is a major difference in the abundances of these cleaner species in temperate coastal areas. Juveniles of the sparid *D. sargus* aggregate in large numbers in near-

shore shallow and sheltered areas (García-Rubies & Macpherson 1995, Vigliola et al. 1998, Dias et al. 2016), while the labrids *C. exoletus* and *C. melanocercus* occur in comparatively low numbers in subtidal cleaning stations (Arnal & Morand 2001b, Zander & Sötje 2002, Sabatino et al. 2007). The high densities of juvenile *D. sargus*, specially from spring to autumn (Dias et al. 2016), compared with the much lower numbers of solitary and territorial *C. exoletus* and *C. melanocercus*, highlight the potential importance of *D. sargus* cleaning behaviour in temperate communities. In fact, *D. sargus* cleaning behaviour adds to the relevance of this pivotal species used as a model for population interconnectivity studies (Abecasis et al. 2013), monitoring the effectiveness of marine protected areas (Lloret & Planes 2003, Guidetti et al. 2008, Abecasis et al. 2015), or evaluating the impact of climatic changes including the potential effect of invasive species (Magliozzi et al. 2017). Fluctuations in the abundance of young *D. sargus* due to environmental changes or overfishing may have conse-

Table 4. Comparison of the cleaning frequencies per target species for each temperate cleaner species: *Diplodus sargus* (this work), *Centrolabrus exoletus* (Henriques & Almada 1997), and *Centrolabrus melanocercus* (Sabatino et al. 2007). The values for the 3 most important client species for each temperate cleaner species are displayed in **bold**

Host family	Host species	Frequency of interactions (h ⁻¹) and rank					
		<i>D. sargus</i>		<i>C. exoletus</i>		<i>C. melanocercus</i>	
		Freq.	Rank	Freq.	Rank	Freq.	Rank
Mugilidae	Unidentified	12.16	1	0.04	8		
Apogonidae	<i>Apogon imberbis</i>					0.03	18
Sparidae	<i>Boops boops</i>	0.22	3				
	<i>Diplodus annularis</i>					0.16	7.5
	<i>Diplodus puntazzo</i>					0.09	12
	<i>Diplodus sargus</i>	0.20	4	0.04	8	0.16	7.5
	<i>Diplodus vulgaris</i>			0.18	5	0.06	15
	<i>Oblada melanura</i>	0.02	7			0.03	18
	<i>Sarpa salpa</i>	0.28	2	0.06	6	0.66	3
	<i>Spondylisoma cantharus</i>			0.02	11		
Pomacentridae	<i>Chromis chromis</i>					0.06	15
Labridae	<i>Coris julis</i>			0.30	3	0.47	4
	<i>Ctenolabrus rupestris</i>			0.02	11		
	<i>Labrus bergylla</i>			1.64	2		
	<i>Labrus viridis</i>					0.13	9.5
	<i>Symphodus cinereus</i>					0.09	12
	<i>Symphodus mediterraneus</i>					0.13	9.5
	<i>Symphodus melops</i>	0.02	7	2.52	1		
	<i>Symphodus ocellatus</i>					0.06	15
	<i>Symphodus roissali</i>			0.20	4	0.19	6
	<i>Symphodus rostratus</i>			0.04	8	0.09	12
	<i>Symphodus tinca</i>					1.09	1
Moronidae	<i>Dicentrarchus labrax</i>	0.04	6				
Mullidae	<i>Mullus surmuletus</i>					0.03	18
Carangidae	<i>Seriola</i> sp.	0.06	5				
Serranidae	<i>Serranus cabrilla</i>					0.31	5
	<i>Serranus scriba</i>					0.91	2
Molidae	<i>Mola mola</i>			0.02	11		
	TOTAL	13.00		5.08		4.75	

quences for the health of local fish communities and should be further investigated.

From a different perspective, efforts to develop a prospective aquaculture exploration of white seabream (Papandroulakis et al. 2004, Ozorio et al. 2006), limited by their aggressive behaviour towards conspecifics (Gonçalves et al. 2015), could now follow an alternative path. Instead of evaluating solely the potential of this species as a primary target for the aquaculture industry, the high ectoparasite cleaning rates reported here also suggest that juveniles from this species could improve the conditions of other species reared in captivity, namely mugilid species (see Tancioni et al. 2016).

This is the first study quantifying cleaning behaviour by a non-labrid species in the northeastern Atlantic and Mediterranean. However, additional studies are needed to fully understand the contribution of juvenile white seabream to the health of temperate coastal fish communities.

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