AGGRESSIVE TERRITORIAL ETHOGRAM OF THE OF THE RED-MOUTHED GOBY, *GOBIUS CRUENTATUS* (GMELIN, 1789)

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Abstract: We present a detailed description of the territorial behaviour of *G. cruentatus*. We staged simulated intrusions in a captive setting to overcome the inherent difficulty of observing these interactions in the field. An 'intruder' was introduced in an experimental arena containing a 'resident' male. The response of the resident was video-taped and audio-recorded. The tested animals (two groups of 5 animals each) played in turn both the resident and the intruder roles. A total of 40 territorial interactions were recorded. Twenty-six behavioural units (BU) were identified and described according to their physical and functional interpretations; average duration and frequency of occurrence of each BU were reported. During the contests, resident fish mainly produced sounds. According to the 'bourgeois strategy', contests were settled in a maximum of 10 min, with the owner of the territory usually resulting the winner. Differences in body size seemed not to influence the contest outcome, whereas resource value did.

Key words: behaviour, agonistic contests, sound emission, Gobiidae.

Introduction

Territorial defence is a substantial behaviour of many fishes and in particular gobies (Nyman 1953; Yanagisawa 1982; Lugli 1987; Casaretto 1988; Torricelli *et al*. 1988a). Gobies are benthic freshwater and marine species, generally found in shallow water (Tortonese 1975). Most have a sedentary attitude related with a cryptic behaviour adopted to reduce predation risk and to increase efficiency in capturing prey. To further reduce predation and impact of unfavourable environmental conditions (Shulman 1984, Koppel 1988, Forsgren and Magnhagen 1993), gobies use holes or crevices proportional to body size (Lindquist 1985; Koppel 1988; Hixon and Beets 1989) as refuge off the breeding season and as nest during spawning. Gobies are highly territorial defending vigorously nests and shelters from conspecific intruders during the whole year, using both visual and acoustic signals.

In animal agonistic contests, three kinds of asymmetries may be important to define the winner (Maynard Smith and Parker 1976): (1) asymmetries in fighting ability or "resource holding potential" (RHP; Parker 1974) (e.g., due to size, strength, or site familiarity), (2) asymmetries in resource value (e.g., winning individual gains most; Smith and Parker, 1976) and (3) uncorrelated asymmetries (i.e., asymmetries uncorrelated with RHP or resource value that can control outcome, e.g., ownership).

An asymmetry in size affects outcomes of territorial contests in Datehaze goby (*Amblyeleotris japonica*), with bigger fish in the winning role. When opponents have the same size, the residents win the contests (Yanagisawa 1982). This is also the case of the freshwater goby *Padogobius martensi* (Parmigiani *et al*. 1988), although the effect

of role is not detectable outside the breeding season (Torricelli *et al.* 1988a). In the grass goby *Zosterisessor ophiocephalus* an asymmetry in size determines the outcome, since the bigger fish always wins, irrespective from its role (Casaretto 1988; Ota *et al.* 1999).

Gobies are soniferous species. They produce a complex sound repertoire as part of the species social behaviour (reviewed by Lugli *et al.* 1997; Bass and McKibben 2003), which has been recognized to be not fortuitous. Most of the described sounds are emitted during courtship and mating behaviour (Lugli *et al.* 1995; Lugli *et al.* 1997; Lugli and Torricelli 1999; Lindstrom and Lugli 2000; Malavasi *et al.* 2003), however, gobiids competing with a conspecific for territory also produce sounds (Mok 1981; Takemura 1984; Casaretto *et al.* 1988; Ladich and Kratochvil 1989; Lugli *et al.* 1995; Lugli 1997). Sounds were recorded during aggressive encounters between conspecifics, when an intruder enters in a resident's territory. Specifically, the presence of the intruder induced sound production by the resident. In many fish species (Myrberg 1972, 1981; Hawkins and Amorim 2000), sounds are part of a threat visual display involved in territory defence, inducing the retreat of the opponent.

The red mouthed goby (*Gobius cruentatus*, Gmelin 1789) is a Mediterranean and West-Atlantic saltwater benthic gobiid species, distributed between 2 and 50 meters depth. Maximum size is 180 mm (total length), but it is common between 100 and 150 mm (total length). It lives on both sandy and rocky bottoms, adopting longer than larger holes and crevices in the rocks as shelters, which are used during the whole year (Wilkins and Myers 1991, 1993). As many other gobiids, *G. cruentatus* spends most of the time inside the shelter hiding to enhance predation success and to avoid predators (Wilkins and Myers 1992). It is a territorial species with threat visual displays, often accompanied by sounds (Costantini *et al.* 1998; Sebastianutto *et al.* 2005)

The aim of this study is to describe qualitative and quantitative features of behaviour displayed by *G. cruentatus* during aggressive territorial encounters with conspecifics.

Materials and methods

A total of 10 individuals (7 females and 3 males) of the red-mouthed gobies were collected outside the breeding season at depths ranging from 3 to 8 meters. Total length was measured (13.16 ± 1.44 cm) and sex was determined observing the shape of the genital papilla (Tortonese 1975). Specimens were kept individually in small tanks (36x20x22 cm) provided with artificial shelters. To avoid visual contact between fish, an opaque board was positioned externally between the walls of adjoining tanks. Automatically regulated lighting, which followed the natural light-dark cycle, was provided. Temperatures values measured in the tanks across the whole observation period ranged between 18-20 °C.

Experimental procedure

During each 10 min experiment, a fish was introduced into the main arena (a tank of 60x30x40 cm) where another fish had been previously maintained singly for a week, becoming therefore the territorial fish (hereafter referred as 'the intruder' and 'the resident', respectively).

Fish were allowed to interact for 10 min; after the interaction the intruder was returned to its tank. A total of 40 experimental sessions were run between November 1999 and March 2002; agonistic encounters were not run during the *G. cruentatus* breeding season. Due to inherent difficulty in holding *G. cruentatus* in captivity for long time, two different groups of 5 animals were used for the experimental sessions in order to get 20 territorial interactions from each group. Since the fish behavior in the two groups was tested for homogeneity and did not differ significantly (all ps >0.5, Mann-Whitney U Test), data were merged. Each fish in each group was tested both as resident (R; n = 4

experiments) and as intruder (I; n = 4 experiments).

Behaviour was video-recorded by a hand held video camera SONY VIDEO 8 TR 805 (10X) connected to a VHS videotape recorder. Sounds produced by fish were also simultaneously recorded on the same video-track. Sounds were collected with a pre-amplified hydrophone Reson TC 4032 (sensitivity of – 159 dB re 1 V/µPa) suspended just above the artificial shelter in the main arena, stored on a DAT recorder Pioneer DC-88 (sampling rate 44.1 KHz) and monitored on headphones.

Data analysis

To give a qualitative description of *G. cruentatus* behaviour, video-recordings were examined and behavioural units were classified according to previous behavioural studies on gobies (Torricelli *et al.* 1986; Casaretto 1988; Marchesan 1994; Sunobe 1998; Ota *et al.* 1999).

All audio-video recorded experiments were analysed frame-by-frame and every interaction was classified and logged on previously prepared *ad hoc* check-lists using the Etholog 2.25(@Ottoni) software. Behaviours of the resident (40 encounters) and the intruder (40 encounters) were quantified and compared using the non-parametric Wilcoxon matched pairs test (Fowler and Cohen 1990). At the end of each experiment the ownership of the artificial shelter was ascribed to the intruder or the resident, according to the outcome of the territorial interactions between the two fishes.

The sequence of events expressed by each fish was explored. For both the resident and the intruder sequence data were entered in contingency tables, with preceding behaviours in rows and immediately following behaviours in columns. Chi-square analysis at 0.05 level of probability was run with these contingency tables. Cells with residuals of chi-square exceeding the level of significance were identified (Fowler and Cohen 1990). Intra-role sequence analysis of behavioural events was represented

graphically. Each behavioural unit was outlined as a circle proportional in size to its frequency of occurrence, and sequences between preceding and immediately following behavioural units were showed by connecting circles with arrows proportional in width to their relative significant chi-square residuals (only significant chi-square residuals higher than a set threshold of 11 were expressed graphically). Since in natural conditions the intruder is not forced to interact with the resident nor is unable to flee; there can be a bias in the intruders' behavioural outcome due to the experimental design.

Results
1. Inventory of displayed behaviours

A total of 26 behavioural units have been recognized and described according to their functional context.

Locomotion behaviour

G. cruentatus propulsion is diodontiform by undulating tail, body associated with repeated synchronous adduction of the large pectoral fins. Swimming towards an evident target is here defined as *directed swimming* (**dir. sw.**). *Scraping swimming* (**sc. sw**) is defined when a fish advanced moving its pectoral fins and scraping simultaneously the substrate, whereas *oscillating swimming* (**o. sw**.) is referred to a fish swimming by oscillating the body and simultaneously raising itself from the substrate with folded dorsal and anal fins. Sometimes fish were observed stopping for few seconds in a *vertical position* (**ver**.), with the body parallel to the tank walls. The fish also performed potential comfort movements, such as turning to one side (*changing position*, **ch. pos.**) or moving slowly in the arena without a specific direction (*wandering*, **w**.)

Rest and alert positions

Alone in the arena, fish used to stay close to the shelter, above the substrate in a horizontal position with the pectoral fins spread (*stationary position*, **st**.). Fish often propped up the anterior part of the body

resting on the lower edge of the pectoral fins and on the ventral fins, assuming a *reared stationary position with spread fins* (**r. st. sf.**); in the upward posture all the pectoral and caudal fins were spread. Rearing up was usually observed during both aggressive and non-aggressive interactions (like in blennids, Wirtz 1978), resulting in an ambivalent drive to staying or leaving.

When an intruder was introduced into the experimental tank, the resident usually remained stationary spreading its fins, as described in Wirtz (1978). The intruder was approached by the resident, resulting in the two fish staying one in front of each other (*orthogonal display*, **od.**) or parallel or anti-parallel one to each other (*lateral display*, **ld**.).

Confrontation behaviour

When fish reared up their body, the orthogonal and lateral displays (see above) became a *reared lateral display* (**r. ld.**) or a *reared orthogonal display* (**r. od**.), respectively. The fins of both the opponents were spread. The lateral display is considered a "comparison pattern"; territorial fish often perform it to threaten an opponent, sometimes before an aggressive action (Baerends and Baerends-Van Roon 1950; Myrberg 1972; Bradbury and Vehrencamp 1998).

In presence of an intruder, the resident generally opened the mouth while rearing up, this movement assumed a threatening meaning. Opening the mouth has never been observed in a fish left alone. We can distinguish a *reared stationary position with spread fins gaping* (**r. st. sf. g.**) or *a changing position gaping* (**ch. pos. g.**). When two fish were close to each other, a *reared orthogonal display gaping* (**r. od. g.**) or a *reared lateral display gaping* (**r. ld. g**.) were noticeable.

Threatening and chasing behaviour

During aggressive interactions, a fish stationing in front or near the opponent sometimes moved a little forward to the adversary as it would thrust him, but it did not complete the thrust (*false thrust,* **f. t.**). In some cases, the animal contacted or even bit the opponent (*thrust*, **t**). One fish may also hit the other with its head or tail (**beat**); in this case, the opponent could remain stationary, bending its flank and curving its tail laterally, with a C-like body shape (*arched flank* **a. f.**). When no one of the two fish retreated from the contest, the two fishes swam very fast side by side, in a circle, with an anti-parallel orientation, hitting or biting each other (*circling*, **cir.**).

One of the fish, the winner in this case, could chase the opponent for a varying distance (*pursuit*, **pu.**).

Several alternatives existed in the fight when the fish usually assumed a reared stationary position with spread fins.

Inferiority and retreat

If attacked, a fish could retreat swimming backwards by pectoral fin movements (*backward swimming,* **b. s.**). Sometimes the fish did not bend itself but it tilted its body laterally, leaning on the substrate with half of the body. This posture was defined the *flank position* (**f. p.**). In this position, the ventral posterior part of the body was turned towards the opponent.

At the last stages of the fight, the loser could remain motionless, paling and fluttering both its body and the dorsal fin (*fluttering*, **flu**) or could move rapidly away and taking distance from the other one (**flee**).

2. Outcome of the experimental sessions

Out of a total of 40 experimental sessions, the resident won significantly 77.5% of the contests (Chi square test, $p < 0.001$). Five sessions (12.5%) had no winner because the fish did not interact at all. Table 1 summarizes the wins of each fish according to its role. Biggest fish (fish 4) lost all its matches as intruder, while the smallest one (fish 7) won all the encounters as resident and three out of four encounters as intruder. Although further studies are necessary, this may suggest that animal size has a no evident role in the contest.

Table 1. Number of wins of each tested fish according to its role.

Fish	Length (mm)	Wins as R	Wins as I	Retreats as R	Retreats as I	Equality as R	Equality as I
1 - male	136	4	0	0	3	0	1
2 - female	134	4	0	0	4	0	0
3 - female	138	2	0	0	4	2	0
4 - female	160	4	0	0	4	0	0
5 - female	137	4	0	0	3	0	1
6 – female	123	2	0	2	3	0	1
7 – male	118	4	3	0	1	0	0
8 – male	156	3	1	1	2	0	1
9 – female	125	2	0	1	4	1	0
10 – female	139	2	1	1	3	1	0

3. Quantitative analysis

Frequency of occurrence of each behavioural unit (n. acts *per* 10 min) both in the resident and intruder fish is reported in Figure 1. The frequency of occurrence of behavioural units differ significantly between the residents and intruders (Wilcoxon test, p=0.005)

The average duration of each behavioural unit expressed by both resident and intruder is expressed in Figure 2. The average duration of each behavioural unit does not differ significantly between the residents and intruders (Wilcoxon test, p=0.86)

The intra-role sequence of the events observed during the encounters is represented in Figure 3 (resident fish) and in Figure 4 (intruder fish). In this analysis, the sound production (examples of sonograms in Figure 5) has been considered as a behavioural unit *per se* (*sound emission*, **S**) and included in the sequences. Considering the number of significant transitions, the event frequency and the Chi-square residuals, we observe that aggressive and very aggressive behaviours are prevalent in the resident's ethogram whereas neutral and inferiority behaviours are predominant in the intruder's ethogram. Figure 6 portrays the species aspect and sketches out some relevant behavioural units

4. Sound production

As detailed elsewhere (Sebastianutto *et al*., submitted) *G. cruentatus* emitted short, stereotyped vocalizations below 1.5 kHz of four distinct types. Here they can be cursorily summarized as: a train of broadband pulses of short duration (TP), a dense harmonic-like sound (DHS) consisting of few harmonic parallel bands below 800 Hz, a noisy harmonic-like sound (NHS) where harmonic components are overcome by additional noise, and a complex sound (C) made of a first dense part followed by a train of pulsed elements. Illustrations of TP and DHS are given in Figure 5.
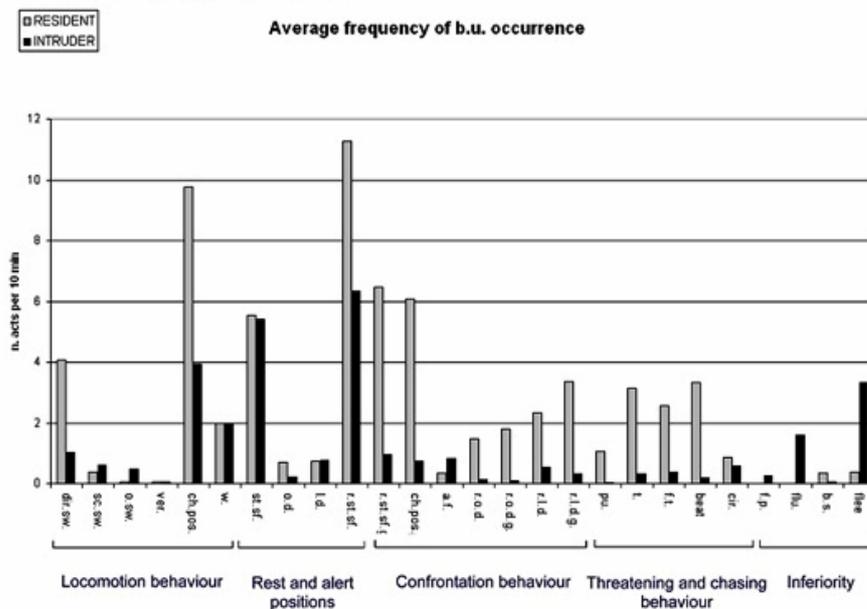
Figure 1. Frequency of occurrence (mean number of observed acts per 10 min) of each of the 26 *G. cruentatus* **behavioural units in the resident (grey) and the intruder (black). List of abbreviations:** *arched flank* **(a. f.),** *backward swimming* **(b. s.), beat,** *changing position* **(ch. pos.),** *changing position gaping* **(ch. pos. g.),** *circling* **(cir.),** *directed swimming* **(dir. sw.),** *false thrust* **(f. t.),** *flank position* **(f. p.), flee,** *fluttering* **(flu.),** *lateral display* **(l. d.),** *orthogonal display* **(o. d.),** *oscillating swimming* **(o. sw.),** *pursuit* **(pu.),** *reared lateral display* **(r. l. d.),** *reared lateral display gaping* **(r. l. d. g.),** *reared orthogonal display* **(r. o. d.),** *reared orthogonal display gaping* **(r. o. d. g.),** *reared stationary position with spread fins gaping* **(r. st. of. g.),** *scraping swimming* **(sc. sw.),** *stationary position* **(st.),** *reared stationary position with spread fins***(r. st. of.),** *thrust* **(t),** *vertical position* **(ver.),** *wandering* **(w.).**
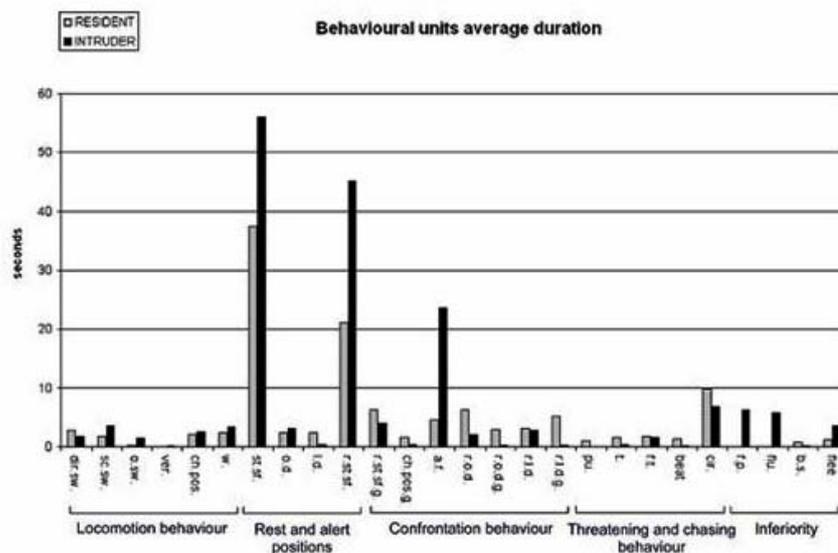

Figure 2. Mean duration of each of the 26 *G. cruentatus* **behavioural units in the resident (grey) and the intruder (black). See list of abbreviations of the behavioural units in Figure 1.**
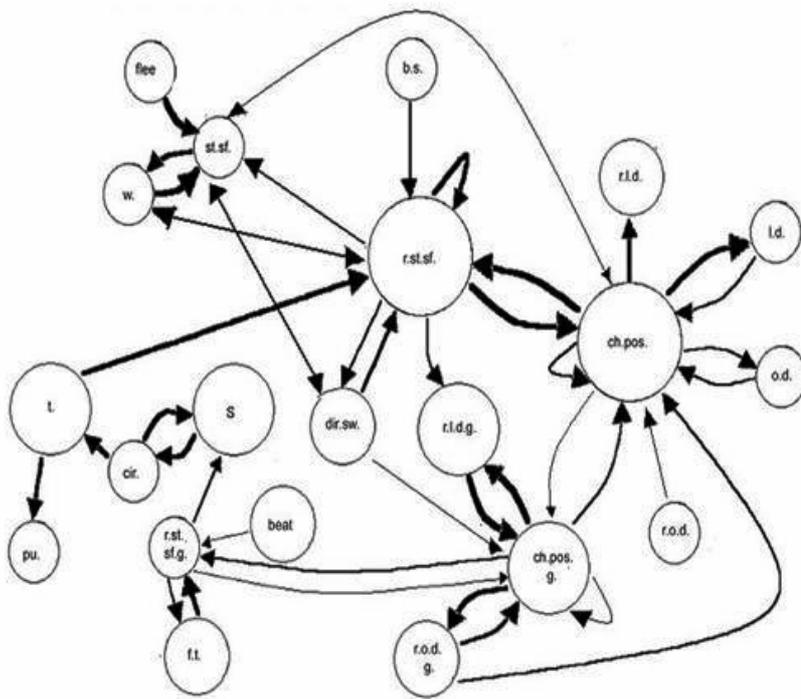
Figure 3. Sequence of behavioural events of the resident fish. Each behavioural unit is outlined as a circle proportional in size to the event frequency. Sequences between preceding and immediately following behavioural units are shown connecting circles by 4 types of arrows whose thickness is proportional to the relative χ^2 residuals ($11 < \chi^2 < 16$; $17 < \chi^2 < 23$; $24 < \chi^2 < 30$; $\chi^2 > 31$). See list of abbreviations of the behavioural units in Figure 1.
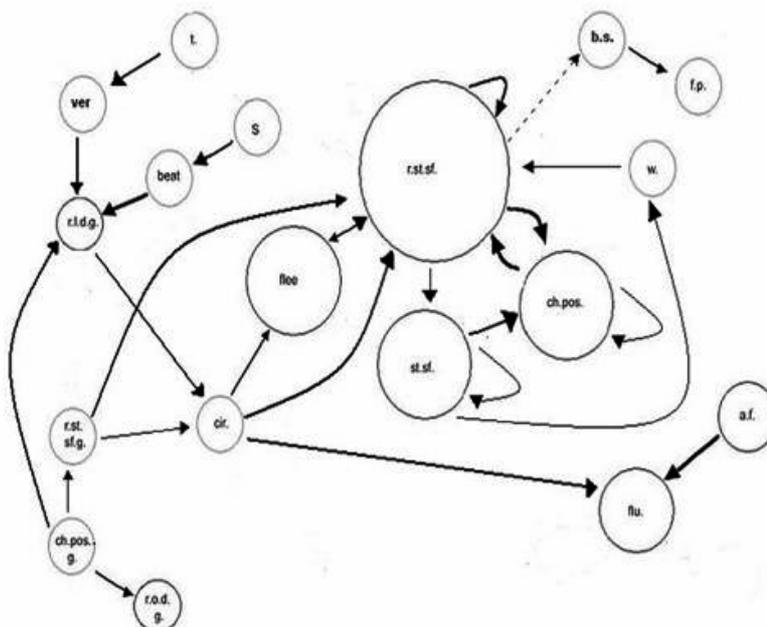

Figure 4. Sequence of behavioural events of the intruder fish (see the legend of Figure 3). See list of abbreviations of the behavioural units in Figure 1. One unconnected group of behavioural sequence is recognizable. Although not significant, the most frequently observed transitions (n=5) between these groups is indicated by the dotted line in order to complete the behavioural pattern outline.
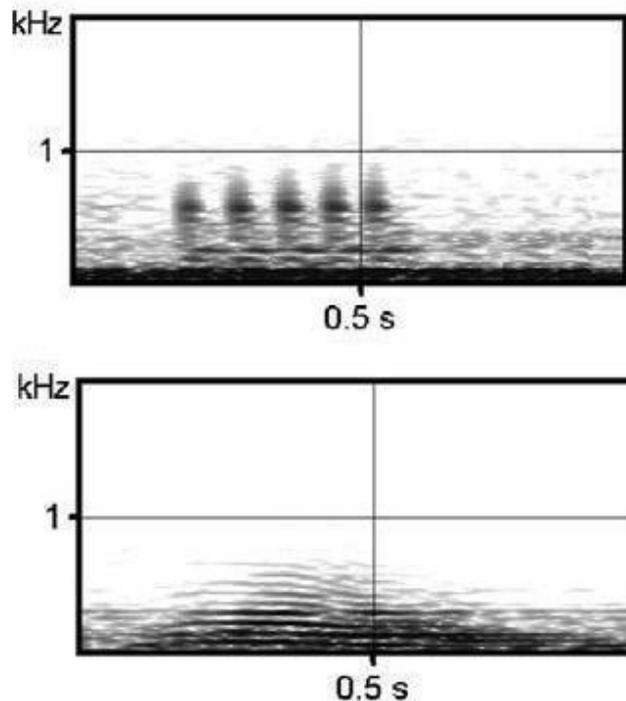
Figure 5. Spectrogram (Hamming window, FFT size 512, bandwidth 20 Hz) of two sounds emitted by a tank-confined male (total length 118 mm; water temperature 18.3). From the top: train of pulses (TP) and dense harmonic-like sound (DHS) .

During the experimental sessions, a total of 279 sounds were recorded. 262 sounds (93.9 %) were produced by the residents, whereas the intruders emitted only 17 sounds. Interestingly, a strong link was observed between the behavioural unit 'circling' and the sound emission (see the resident sequence; Figure 3).

Table 2 summarizes the number of sounds emitted by each animal according to the role. Clearly, male fish produced most of the sounds. However, further studies are necessary to assess if sex plays a decisive role in sound production.

Discussion and conclusion

Gobius. cruentatus aggressive patterns concur with the behavioural description in other gobiid species (Tavolga 1956, Kinzer 1960, Yanagisawa 1982, Cole 1984, Torricelli *et al*. 1988, Casaretto 1988, Ota *et al*. 1999), except for the case of the behavioural units defined as 'scraping swimming', 'flank position' and 'fluttering'. Fluttering and flank position

have been previously observed only in mature females of *Zosterisessor ophiocephalus* during courtship (Ota *et al.* 1999). It is possible that males mime female behaviour, in order to reduce the aggressiveness of the opponent. However, reproductive behaviour of *G. cruentatus* has not been investigated. Nevertheless, female mimicry is a peculiar feature of the behaviour of kleptogamic parasitic males in many fish species and gobies (Taborsky 1994).

Based on 40 experimental sessions, our results indicated that in *G. cruentatus* the bourgeois principle "fight if owner, retreat if intruder" (Maynard Smith 1976, 1982), was used as an arbitrary rule to settle conflict and the chance for a fish to win a fight was much higher on its "own" *vs*. an "alien" territory. This seems to be true also in case of a size disadvantage of the resident. As resulted, the same fish playing the resident's role typically expressed threatening displays, showing inferiority as intruder.
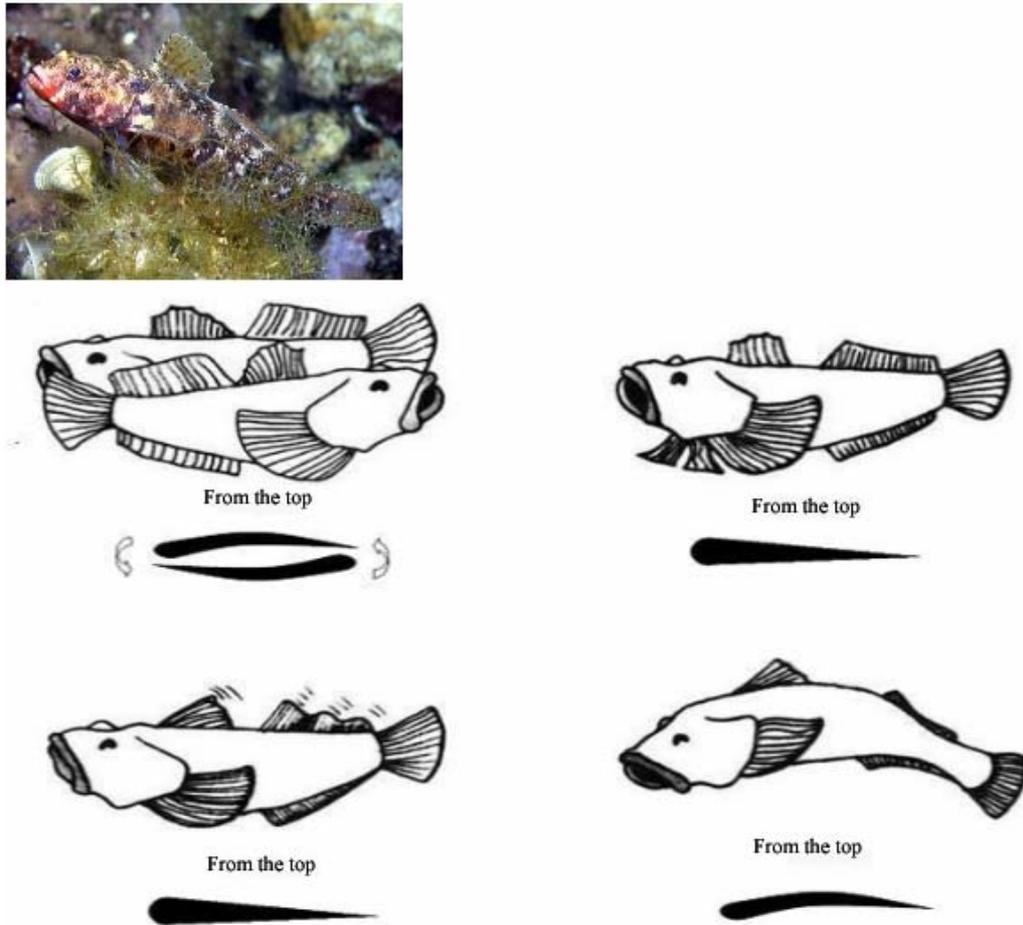
Figure 6. Top left: a specimen of adult *G. cruentatus*. Below, from top left, clockwise: graphical representation of the behavioral units called 'circling', 'reared stationary position with spread fins gaping', 'flank position' and 'fluttering'.

Table 2. Number of the sound emissions of each tested fish according to its role

Fish	Length (mm)	N. sounds as R	N. sounds as I
1 - male	136	66	0
2 - female	134	10	0
3 - female	138	0	1
4 - female	160	18	2
5 - female	137	11	1
6 - female	123	14	2
7 - male	118	15	10
8 - male	156	87	1
9 - female	125	30	0
10 - female	139	11	0

This tendency is also clear considering the amount of sound emissions per role. In *G. cruentatus*, as in the case of *P. martensii* (Torricelli *et al.* 1988b), the resident fish emits almost all the vocalizations. Acoustic signalling is known to play an important role in agonistic contexts in fish (Ladich 1997) and may influence the outcome of contests (Valinski and Rigley 1981; Ladich *et al.* 1992a). In *P. martensii* most of the vocalizations are concentrated before the first bite (Torricelli *et al.* 1988a), whereas in *G. cruentatus* sounds are mainly produced during the phases of uncertainty and confrontation (Sebastianutto *et al.*, submitted). In particular, most of the vocalizations were connected to the behavioural unit 'circling', that was the most interactive and energetic part of the agonistic contest, during which the opponents used both acoustic and visual threat displays to define the outcome of the contest (as in Ota *et al.* 1999). Sounds could be used by *G. cruentatus* for mutual assessment in order to solve the conflict between competitors (Sebastianutto *et al.*, submitted), as demonstrated for many other fish species (Ladich 1997, Lugli 1997, Hawkins and Amorim 2000).

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