

The response of the male freshwater goby to natural and synthetic male courtship sound playback following exposure to different female sexual stimuli

M. LUGLI ¹, G. PAVAN ² and P. TORRICELLI ³

¹ *Dipartimento di Biologia Evolutiva e Funzionale, Università di Parma, Viale delle Scienze, 43100 Parma, Italy*

² *Centro Interdisciplinare di Bioacustica e Ricerche Ambientali, Via Taramelli 24, 27100 Pavia, Italy*

³ *Dipartimento di Scienze Ambientali, Università di Venezia, Campo della Celestia, 30122 Venezia, Italy*

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Among teleosts, field and laboratory experiments have shown the playback of male courtship sounds often elicits competitive courtship and sound emission in the territorial male. However, the importance of female stimulation for the male response to the sound is poorly understood. In this study, playback experiments with the freshwater goby, *Padogobius martensii*, examined the response of resident males (i.e. males individually housed within laboratory tanks for at least 5 days) to natural and synthetic male courtship sounds after exposure to chemical or visual stimuli from a ripe female. All playback tests consisted of one experimental (sound playback) and two control treatments, all of equal duration. Three experiments were conducted on a first group of 12 males using natural courtship sounds. Experiment 1 consisted of playing back the sound to a male that had not exposed to female stimulation for at least 24 hr. The same male was exposed to the "female pheromone" by dropping a few cm³ of female holding water into the male's tank, just prior to playback (Experiment 2), or to the view of a live ripe female in a close-by tank, during playback (Experiment 3). The sound playback failed to elicit positive responses by males not exposed to female stimuli (Experiment 1), and did not increase courtship activity of the male in visual contact with the female (Experiment 3). However, it increased swimming activity and facilitated courtship and sound production in the male after an increase in sexual arousal by prior chemical stimulation (Experiment 2). In Experiment 4, the synthetic version of the courtship sound and one deprived of pulse-rate modulation and harmonic content (pure tone) were played back to 10 resident males following prior chemical stimulation. The synthetic courtship sound, but not the pure tone, was effective in eliciting positive responses by the sexually aroused male. Functional implications of the responses to the courtship sound for the territorial male are discussed. The results are relevant to the understanding of the role of sound communication and interception among gobies and to develop playback experiments in the field.

KEY WORDS: *Padogobius martensii*, sexual arousal, acoustic orientation, frequency modulation, competitive courtship, sound interception.

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INTRODUCTION

The emission of sounds during reproduction has been documented in many teleost species (FINE et al. 1977, MYRBERG 1981), but its significance to the recipient remains poorly understood. The inherent methodological and theoretical difficulties of the underwater research are well known (reviewed in HAWKINS & MYRBERG 1983). Another reason may be the contextual use of the sound shown by many territorial species. Breeding sounds may be emitted only at given locations or periods of the day (WINN 1972), are usually specific to particular courtship stages (female outside or inside the male's nest, MYRBERG 1981, LUGLI et al. 1995), and are often accompanied by chemical and visual stimuli (e.g., TAVOLGA 1956). Therefore, presenting the sound stimuli to a receiver in the wrong motivational state or stimulatory context may generate inappropriate responses, or lack thereof. For instance, it is well known that the presence of female sex pheromones is widespread among teleosts (reviewed by STACEY & SORENSSEN 2002). Available evidence (mainly anecdotal) indicates prior contacts with female-associated stimuli (e.g., the female holding water) may strongly affect male responsiveness to the courtship sound (TAVOLGA 1958, KENYON 1994, see below). However, the importance of recent experience for the fish's response to sound stimuli has been seldom subjected to experimental investigation. The functional interpretation of the playback response of subjects tested under laboratory conditions may be an additional problem, especially if the context of sound production under natural conditions is not known precisely (MYRBERG 1981). Most of these difficulties may be overcome by conducting experiments in the field, where sounds are usually played back to motivated subjects in the appropriate functional context. The extensive experimental work done by MYRBERG and his students on the acoustical communication system of the damselfish, *Stegastes* (= *Pomacentrus*) *partitus*, is a notable case in point (e.g., MYRBERG et al. 1978, 1986). The main drawback of this approach is that one has little control over many contextual variables (e.g. presence and number of competitors) and motivational or experiential factors of the tested subjects (e.g. time elapsed since the last encounter with a female), all potentially affecting the playback response of subjects. Therefore, if the primary interest is to understand the mechanisms, the sound playback should be most conveniently conducted under laboratory conditions after taking care to provide the receiver/s with appropriate acoustic stimuli presented in

an appropriate functional context. Laboratory experiments may also help the making of inferences about functional aspects of the response to the sound, provided that the stimulatory situation of the test is applicable to specific contexts of sound detection under (semi-)natural conditions (MYRBERG 1981).

The freshwater goby, *Padogobius martensii* (= *P. bonelli*), is a highly territorial species living in stony rivers of Northern Italy. Communication during the breeding behaviour of this species involves the use of all the three major sensory modalities, i.e., visual, chemical, and acoustical. The importance of these communication channels for the reproductive behaviour of *P. martensii* has been determined by numerous laboratory experiments. In brief, the simple sight of the female is an adequate stimulus for eliciting intense courtship activity by the isolated male in his aquarium tank (LUGLI pers. obs). The ripe female communicates its sexual maturity by releasing into the water a sexual pheromone that stimulates swimming activity and promotes courtship behaviour in the male (BISAZZA et al. 1989, LUGLI pers. obs). The female *P. martensii* is generally mute during courtship (TORRICELLI et al. 1986). The male attracts the female to the nest by means of visual and acoustic displays (TORRICELLI et al. 1986). The male approaches the female with short, jerky movements accompanied by body vibrations, the pectoral fins beating the substrate and the emission of sounds. These courtship movements are followed by quick leading movements of the male toward the nest. The male produces two types of courtship sounds (LUGLI et al. 1995). The tonal sound, typical of the early phase of courtship, is a short (< 0.5 sec), highly stereotyped vocalization made of rapidly repeated pulses whose spectral and temporal features do not vary among different individuals (TORRICELLI et al. 1990). The pulse-train sound, emitted only when the female enters the nest cavity (a hollow under a stone) and during actual spawning, is a short (< 0.5 sec) train of low-frequency pulses that can be produced alone or in combination with the tonal sound to form a complex sound. Both the pulse-train sound and the complex sound were labelled as "spawning sounds" by LUGLI et al. (1995).

Simple playback experiments using synthetic sounds showed that the tonal sound played back within experimental tanks is capable of eliciting oriented approaching and attachment to the sound source by resident ripe *P. martensii* females (LUGLI et al. 1996). This finding, along with observations on laboratory groups of breeding gobies (TORRICELLI et al. 1993, LUGLI pers. obs, see also the Discussion), suggests that a possible function of the tonal sound is mate attraction. The effect of this type of sound to recipient male *P. martensii* has not been investigated. Comparative evidence indicates that the courtship sound produced by other territorial species, when played back to courtship-ready males in the field or under controlled laboratory conditions, commonly stimulate competitive courtship behaviour in these males (WINN 1972, MYRBERG 1981, KENYON 1994). However, studies conducted in other species failed to see any clear effect of the courtship sound playback on the isolated male (STOUT 1975). The importance of prior sexual stimulation for the response to the courtship sound has been recognized by some authors (TAVOLGA 1958, KENYON 1994), but has never been examined with proper experimental investigation.

The main goal of this investigation was to examine the response to playback of courtship tonal sound by male *P. martensii* kept isolated, or exposed to the "female pheromone" (chemical sexual stimulus) and to the sight of a ripe female (visual sexual stimulus). The results will clarify the importance of sexual arousal for male responsiveness to the courtship sound and will help understanding the

role of acoustical interception (MYRBERG 1981) during the breeding behaviour of *P. martensii*. A further aim of the work was to determine the importance of some acoustical parameters, such as the pulse rate modulation, for signal recognition using synthetic sound playback.

GENERAL METHODS

Subjects and housing

The fish used for playback experiments were adult male *P. martensii* collected by means of hand-nets from the stream Stirone (Parma, Northern Italy), from onset to the middle of the reproductive season (March-June). In the laboratory, males were singly housed within polycarbonate tanks (44 × 24 × 26 cm) for a minimum period of 2 days (see below). Each tank was provided with 1-2 cm layer of sand, one external water filter and one automatic heater, placed in the left-rear corner of the tank, which maintained the water temperature at 20-21 °C. One artificial tunnel-shaped shelter (10 × 9 × 2.5 cm) was placed on the bottom of each tank, at 5 cm from the right-side wall, to serve as hiding place for the male. All tanks were laid on 2-cm thick small rectangles of vibration-absorbing material to reduce the negative effects of low-frequency background noise on the detection of playback stimuli by the subject and hydrophone (see below). Two 300 w halogen bulbs illuminated the room for an automatically regulated photoperiod that approximated the natural light-dark cycle. An automatically regulated air-cooler assisted the tank-mounted heater to maintain the water temperature at around 21 °C. The water temperature of tanks was measured at the end of each experiment by means of a digital thermometer (resolution: 0.1 °C). The temperature varied from 19.5-23.0 °C across the whole period in which experiments were conducted. These temperature values were within the range of values found in the river during the breeding season of the species. Animals were fed daily (except on Sunday) with live earthworms and frozen chironomid larvae. In order to ensure responsiveness of subjects to sexual stimuli during the playback tests, males were daily exposed to visual and chemical stimulation from the ripe female for the whole duration of the investigation. The male was presented with a ripe female placed in the male's tank for 10-15 min (visual stimulation). In addition, 50 cm³ of water were taken from a tank housing a ripe female and poured into the male's tank (chemical stimulation). Subjects were judged courtship-ready by the experimenter when the male promptly reacted to the presence of the female in the tank by performing the typical visual and acoustic displays described in early studies (TORRICELLI et al. 1986). In most subjects, the presentation of the female elicited an intense courtship activity after only 2 days of acclimation, whereas other subjects achieved the necessary courtship readiness (for unknown reasons) only after several weeks of housing. Consequently, the housing period before playback varied greatly among subjects (2-40 days).

Playback apparatus and tapes

The playback equipment (i.e., tape-recorder + amplifier + waterproof loudspeaker) and electro-acoustic receiving chain (i.e., hydrophone + pre-amplifier + tape-recorder) used in the present study were the same as those used in LUGLI et al. 1996 (see also TORRICELLI et al. 1990 for details). The loudspeaker and the hydrophone were placed in the tank against the side-wall opposite to the shelter, 10 min before the beginning of the experiment. Playback levels were adjusted to approximate the normal amplitude of sounds recorded from males of *P. martensii* during laboratory courtship interactions. Spectrographic analyses of transmitted signals (see TORRICELLI et al. 1990 for the sound processing method) showed their temporal features were not altered during playback.

The sound stimuli used for these experiments were either natural (Experiments 1-3) or synthetic (Experiment 4) courtship sounds. The natural sound sequence (Fig. 1) was obtained by repeating on a tape a sequence of four, regularly spaced sounds emitted by a laboratory *P. martensii* male courting a ripe female at temperature of 20 °C. The resulting repetition rate (about one sound every 3 sec) was within the range of values observed during courtship (TORRICELLI et al. 1986). The synthetic sound sequence (Fig. 2) was constructed by repeating on a tape a synthetic sound at intervals of 3.2 sec. The sound, obtained by digitally adding pure tones, had a duration of 418 msec, a maximum pulse repetition rate of 173 Hz, and a pulse rate modulation of -0.142 Hz/msec. These values of the synthetic sound parameters are those predicted for the "typical" *P. martensii* courtship sound emitted at a temperature of 20 °C (for the linear regression analysis of *P. martensii* sound features with water temperature see TORRICELLI et al. 1990).

Besides the sequence of natural and synthetic courtship sounds, a white noise sequence (Experiments 1-3) and a pure tone sequence (Experiment 4) were prepared to serve as control treatments. The white noise sequence was obtained by repeating every 3 sec a computer generated white noise segment (frequency spectrum continuum from 50 to 500 Hz, duration: 470 msec; see fig. 1C in LUGLI et al. 1996), and recording it on a cassette. This noise sequence, that included the frequencies of the sound of *P. martensii*, allowed a distinction to be made between selective responding to the sound and a general response to any acoustic stimulus. The pure tone sequence (Fig. 2) was obtained by repeating on a tape, every 3.2 sec, a computer generated pure tone whose characteristics (duration: 418 msec; frequency: 164 Hz) were those predicted for the duration and mean fundamental frequency of the "typical" *P. martensii* courtship sound emitted at a temperature of 20 °C (TORRICELLI et al. 1990). The use of pure tones for playback is recurrent among studies examining the behavioural effects of sounds among fishes (e.g., WINN 1972, MCKIBBEN & BASS 1998). Notice in the case of *P. martensii*, the pure tone sequence allowed a distinction to be made between selective responding to the "typical" sound and general responding to a sound deprived of pulse rate modulation and harmonics above the fundamental.

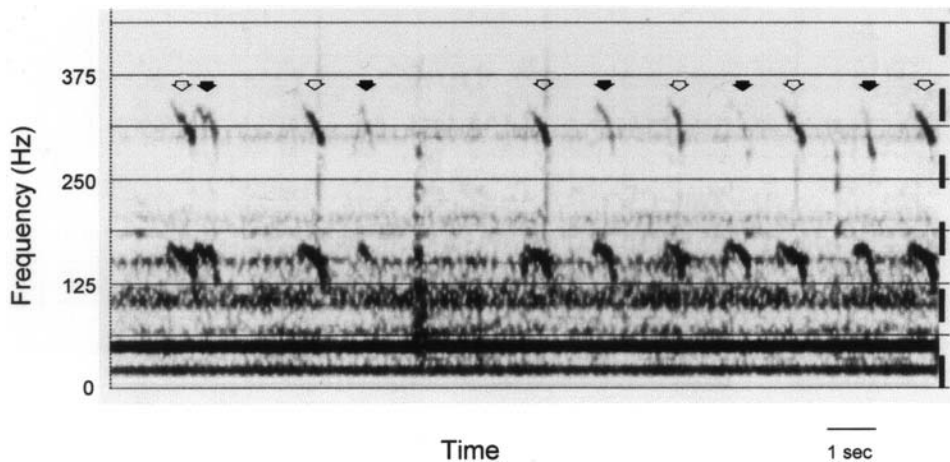


Fig. 1. — Sonogram (Y-axis divisions: 62.5 Hz) of a playback sequence with natural courtship sounds recorded during the course of a replicate of Experiment 2 (see Results). The length of the frame is 16 sec. Note playback sounds (empty arrows) are mixed with sounds produced by the male (filled arrows) as response to the playback stimuli. Horizontal bands on the spectrogram are harmonics of electrical noise from the recording apparatus. Analysis filter bandwidth: 256 Hz; Hamming window applied (Kay 5500 DSP sonograph).

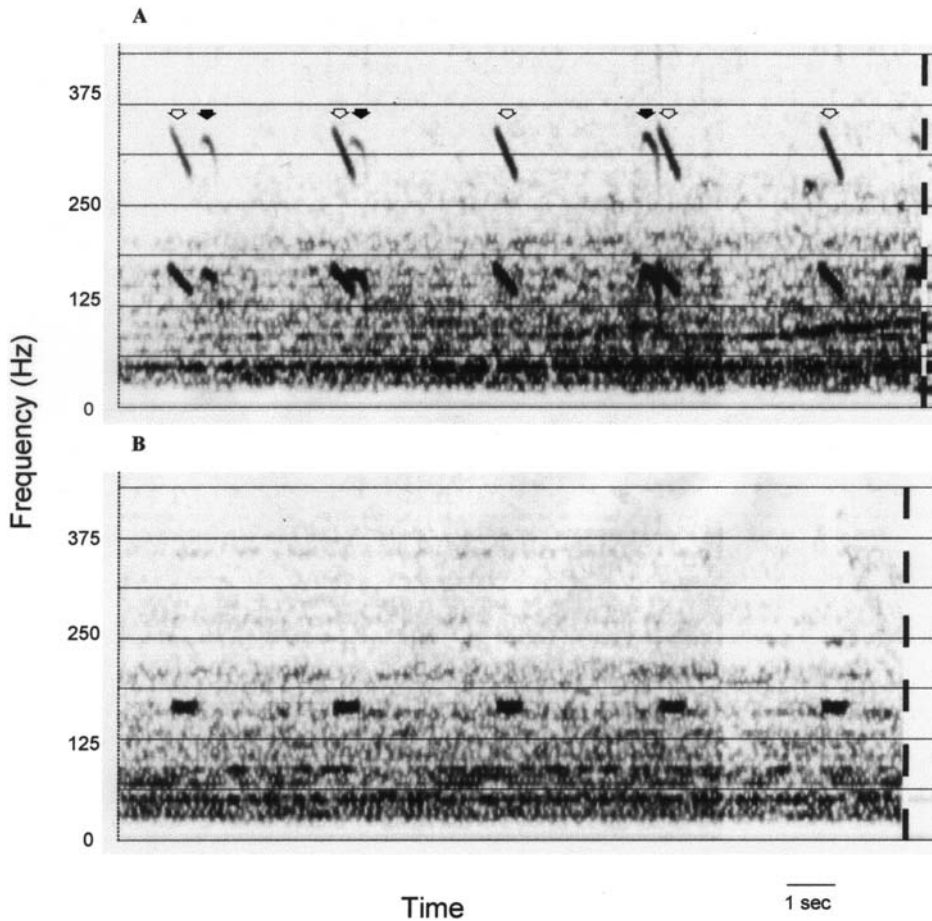


Fig. 2. — Sonogram of a playback sequence with synthetic courtship sounds (A) and one with pure tones (B) recorded during the course of a replicate of Experiment 4 (see Results). The length of the frame is 16 sec. Notice in the top sonogram (A) playback sounds (empty arrows) are mixed with sounds produced by the male (filled arrows) as response to the playback stimuli. For details on scale units and analysis parameters see the legend of Fig. 1.

Procedure and data collection

Each playback test consisted of one experimental treatment (sound playback) and two control periods (described in the Methods of each section), all of equal duration and separated by a pause of 1-min. The order of presentation of controls and experimental periods was established following a randomized blocks design which allowed complete interspersion of treatments (HURLBERT 1984). The response to sound playback was evaluated by measuring the behavioural activity of the male in each treatment period and comparing the measurements among the three treatments using both parametric and non-parametric tests (see Methods of each section for details).

EXPERIMENTS 1-3

The main aim of these experiments was to determine whether the courtship sound could affect the behaviour of male *P. martensii* in three different stimulatory contexts: the detection of sounds by males without any recent exposure to sexual stimuli (Experiment 1), following prior chemical sexual stimulation (Experiment 2), and during ongoing courtship of a ripe female in visual contact with the male (Experiment 3).

Methods

Twelve males (60-70 mm, TL) were submitted to 3 different playback experiments, two subsequent experiments being separated by a 48 hr interval. In all experiments, the treatments consisted of a 5-min sequence of natural sounds (sound playback, P), a 5-min sequence of noise periods (noise playback, N) and a 5-min period of silence (S). Experiment 1 involved playback stimulation alone. In Experiment 2 (chemical stimulation test), 50 cm³ of water was taken from a tank housing a ripe female and dropped in to the male's tank 10 min before the start of the experiment. Experiment 3 (visual stimulation test) consisted of exposing the subject to the sight of a ripe female. A small transparent polycarbonate tank (13 × 9 × 10 cm), with a ripe female inside, was placed externally against the left-side wall of the male's tank. The playback experiment started 1 min after the beginning of courtship by the male. The female's tank was removed at the end of the experiment. In Experiment 1 and 2 the effect of sound playback on males was assessed by determining, for each treatment, (a) the amount of swimming activity by the male (i.e., number of seconds spent in motion by the subject), (b) the amount of time spent in the loudspeaker area, defined as the portion of the bottom area extending from the left-side wall to the imaginary parallel line 15 cm from it (i.e., two body lengths of the fish), (c) the presence of courtship movements and sound production, (d) the presence of approach responses to the speaker. In Experiment 3, the visual contact with a ripe female elicited courtship activity and sound production in all subjects. The effect of the sound playback was assessed counting the number of leading movements (i.e. leads) and emitted sounds in each treatment. In addition, the presence of possible approach responses toward the speaker was noted. Differences among treatments in a given response variable were tested with the randomized blocks ANOVA (SOKAL & ROHLF 1981), when data met the assumption of the parametric ANOVA (swimming activity), or with the Friedman test for randomized blocks, in the other cases (time spent in the loudspeaker area, number of displays). When results of a given parametric or non-parametric test were significant at the confidence level of 0.05, differences among all possible pairs of means were tested respectively by correcting the type I error for the number of planned comparisons ($k = 3$) (SOKAL & ROHLF 1981), or by using the Friedman multiple comparisons analysis (SIEGEL & CASTELLAN 1992). When the results of the Friedman test for randomized blocks approached significance ($0.1 > P > 0.05$), the tendency of the response variable to differ in the experimental treatment in comparison to control treatments was examined with the Friedman method for multiple comparisons against a control group (i.e. P vs N, P vs S; $\alpha = 0.05$) (SIEGEL & CASTELLAN 1992).

Results

The results of Experiment 1 (playback alone) are reported in Fig. 3. The swimming activity by the subject did not differ among the three treatments (ANOVA: $F_{(2,22)} = 1.42$, NS). However, the amount of time spent in the loudspeaker area tended to differ among treatments (Friedman test: $\chi^2 = 5.66$, $P = 0.056$, $df = 2$). Pair-wise comparisons showed subjects spent significantly less time in the loudspeaker area during P than during S (P vs S, $P < 0.05$; P vs N, NS). Furthermore, no subject performed any courtship display or approached the loudspeaker during the experiment. In Experiment 2 (chemical stimulation test), as expected there was a sudden increase of the swimming activity (not quantified) by most subjects when the "female water" was added to the male's tank prior to the test. The increased

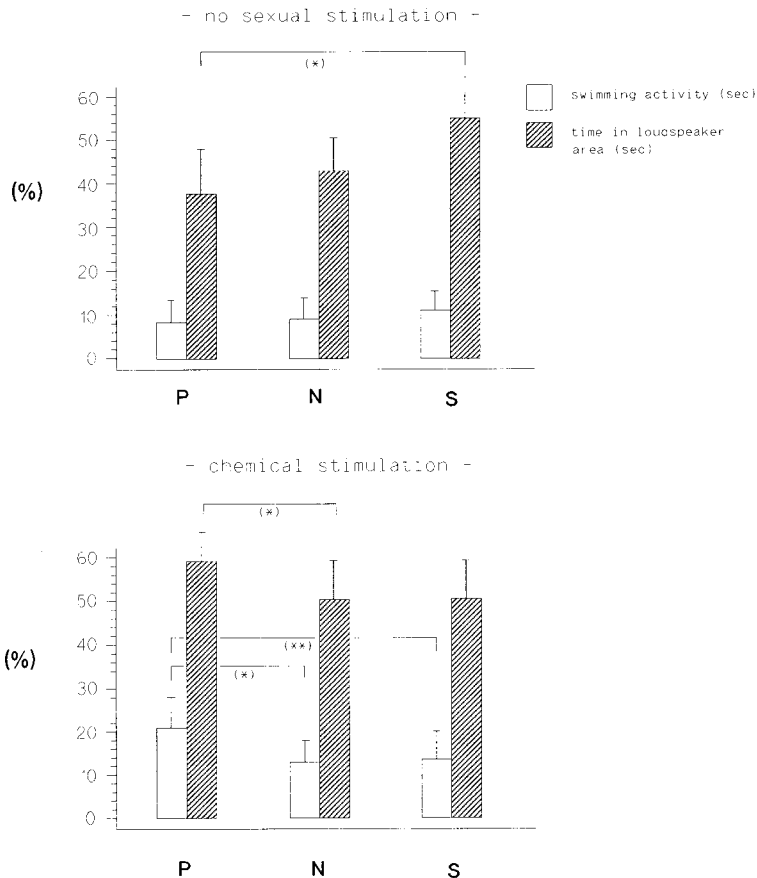


Fig. 3. — Mean value (+ 1 SE) of the swimming activity and total time spent within the loudspeaker area expressed as a percentage of the treatment duration (300 sec) by males in the three treatments (P = natural sound playback, N = noise playback, S = silence) of Experiment 1 (no sexual stimulation) and Experiment 2 (chemical stimulation). Significant differences among pairs of treatments are indicated with asterisks (* $P < 0.05$, ** $P < 0.01$).

swimming activity was accompanied in a few cases by not-orientated courtship movements of short duration. Typically, the male wandered in random fashion around the tank by swimming slowly above the bottom or up and down against the tank's walls, as if he was searching for the female. The swimming activity tended to wane as more time elapsed from the initial stimulation. During the playback test, the effects of chemical stimulation on the swimming activity of subjects were still noticeable (Fig. 3), as shown by the significantly higher total duration of swimming during S of Experiment 2 in comparison to the same treatment of Experiment 1 ($t = 1.92$, $P = 0.039$, $df = 11$; one-tailed Student t-test for paired comparisons). The total duration of swimming differed significantly among treatments of Experiment 2 (ANOVA, $F_{(2,22)} = 8.2$, $P < 0.01$; Fig. 3). Pair-wise comparisons showed swimming activity was significantly increased during P as compared with control periods (P vs N: $F_{(1,11)} = 26.5$, $P < 0.001$; P vs S: $F_{(1,11)} = 9.6$, $P < 0.025$), whereas no differences were found between the two control periods (N vs S: $F_{(1,11)} = 0.02$, NS). As in Experiment 1, the amount of time spent in the loudspeaker area tended to differ among treatments (Friedman test: $\chi^2 = 5.14$, $P = 0.076$). Pair-wise comparisons showed subjects spent significantly more time in the loudspeaker area during P than during N (P vs N, $P < 0.05$; P vs S, NS). Furthermore, five males reacted to the sound playback by emitting a few sounds during the experimental treatment. Four of these males emitted a few sounds also during the control period following the experimental treatment, indicating the presence of after-effects on courtship activity of the subject produced by the sound playback. Straightforward movements toward the loudspeaker were never observed, however. Finally, the results of Experiment 3 (Fig. 4) showed that neither the number of sounds, nor the number of leading movements, nor the total number of displays (i.e., no. of sounds + no. of leading movements), differed significantly among treatments (Friedman test: $\chi^2 = 0.5$, NS, no. of sounds; $\chi^2 = 0.2$, NS, no. of leading movements; $\chi^2 = 0.5$, NS, total no. of displays). Furthermore, if the treatments from each experiment are arranged according to their position (1st, 2nd or 3rd) in the playback sequence (i.e. regardless of the type of treatment), comparison of the total number of displays among the three treatment positions showed the presence of a significant decrease in the intensity of courtship of subjects during the course of the experiment (Friedman test: $\chi^2 = 7.8$, $P < 0.05$).

EXPERIMENT 4

The use of synthetic sounds mimicking the natural sounds, or simplified versions of them (e.g. pure tones), for playback has long been a common practice for investigating the sound parameters relevant for signal recognition among fishes (e.g. TAVOLGA 1958, WINN 1972, MCKIBBEN & BASS 1998). In general, results from these studies have shown that synthetic signals, even with deeply altered spectral content (e.g. pure tones), are as effective as natural sounds, as long as the specific temporal patterning of the sound units is preserved. For instance, the playback of pure tones of similar duration and repetition rate as the natural sounds has generally proven stimulatory in all tested species (the frill-fin goby, *Bathygobius soporator*; TAVOLGA 1958; the oyster toadfish, *Opsanus tau*, WINN 1972; the plainfin midshipman, *Porichthys notatus*, IBARA et al. 1983; but see HA 1973).

The aim of this experiment was to determine whether the synthetic courtship sound, or the pure tone signal, could elicit the responses observed in the aroused male *P. martensii* during playback of natural sounds.

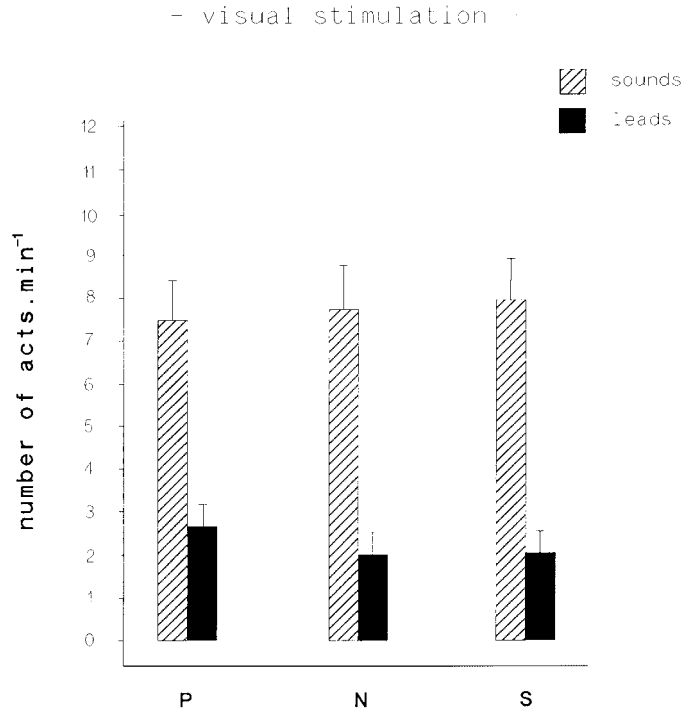


Fig. 4. — Mean value (+ 1 SE) of the frequency of courtship displays by males in the three treatments (P = sound playback, N = noise playback, S = silence) of Experiment 3.

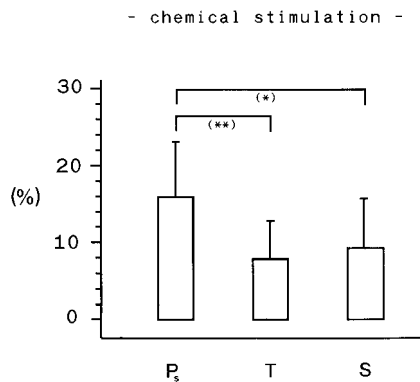


Fig. 5. — Mean value (+ 1 SE) of the swimming activity expressed as a percentage of the treatment duration (300 sec) by males in the three treatments of Experiment 4 (Ps = synthetic sound playback, T = tone playback, S = silence). The presence of significant differences among pairs of treatments is indicated with asterisks (* $P < 0.05$, ** $P < 0.01$).

Methods

In this experiment, 10 males (56-70 mm, TL) were submitted to a 3-min sequence of synthetic courtship sounds (sound playback, P_s), a 3-min sequence of tone periods (tone playback, T) and a 3-min period of silence (S). As for Experiment 2, test subjects were sexually aroused by dropping 50 cm³ of water from a tank housing a ripe female into the male's tank 10 min before the start of the experiment. The effect of playback acoustic stimuli was assessed by determining the amount of swimming activity of the male, and the presence of courtship displays, or approach responses to the speaker. Differences among treatments in the swimming activity were tested using ANOVA for randomized blocks (SOKAL & ROHLF 1981). Differences among all possible pairs of treatments were tested as reported in the Methods of Experiments 1-3.

Results

The swimming activity differed significantly among treatments (ANOVA: $F_{(2,18)} = 7.5$, $P < 0.01$; Fig. 5). Pair-wise comparisons showed swimming activity increased significantly during P_s as compared with both T and S (P_s vs T: $F_{(1,9)} = 15.6$, $P < 0.005$; P_s vs S: $F_{(1,9)} = 9.17$, $P < 0.025$); however, no differences were found between the T and S ($F_{(1,9)} = 0.6$, NS). Furthermore, the presence of courtship displays (i.e. courtship movements accompanied by a few sounds) was noted in two males during P_s, and never during T, or S. Approach responses directed toward the loud-speaker were absent.

DISCUSSION

The effect of prior exposure to sexual stimuli on the response of the male to courtship sound playback

The results of this study have shown that visual and chemical stimuli from the ripe female *P. martensii* strongly affect the behaviour of the courtship-ready male in his tank. The sight of the female in a close-by tank elicits vigorous courtship and sound emission by the male (Experiment 3), whereas the chemical detection of the female that occurs after a small amount of female holding water is introduced into the male's tank increases his swimming activity and facilitates male courtship behaviour (Experiment 2). Chemical cues are likely to play an important role in the social behaviour of gobies (reviewed in STACEY & SORENSEN 2002). TAVOLGA (1956) pioneered the field by showing in the marine goby, *B. soporator*, that introduction of ovarian fluids from the gravid female into the male's tank can stimulate male courtship behaviour and the accompanying sound production. TAVOLGA (1958) also examined the behaviour of the male *B. soporator* in response to playback of the male courtship sound under a variety of conditions. The importance of prior female stimulation for the playback response of the male *B. soporator* was unclear, however. In the present study, the results of Experiments 1 and 2 showed chemical stimulation from the female prior to the test was necessary in order to observe positive responses by the male *P. martensii* to the playback of

courtship sounds. The sound playback failed to elicit positive responses in isolated males without any recent exposure to female stimuli (Experiment 1). However, sound playback increased swimming activity and, in a few cases, courtship movements and production of a few sounds, once the male's sexual motivation was aroused by adding a small amount of "female water" to the experimental tank just prior to the test (Experiment 2). The importance for the territorial male of maintaining chemical contact with the female in order to show positive responses to the playback sound has been documented also in the bicolour damselfish, *S. partitus* (KENYON 1994). Responses of male bicolour fish to the courtship chirp playback could only be obtained in the laboratory by continuously adding "female water" to the males' experimental tank. Another result of Experiment 2 was the lack of directional responses toward the sound source by the sexually aroused males. Similarly, bicolour males did not approach the speaker during chirp playback, provided that the loudspeaker was in the male goal or in the empty goal (KENYON 1994). The lack of orientated responses by subjects in Experiment 2 was unlikely to be an artifact due to the altered acoustic environment of small tanks (PARVULESCU 1967, AKAMATSU et al. 2002). In fact, tank-confined females *P. martensii* showed clear directional responses toward the loudspeaker during playback of the courtship sound (LUGLI et al. 1996). The result of Experiment 1, that males not pre-exposed to sexual stimuli tended to avoid the loudspeaker area during sound playback, further supports the above conclusion. The latter finding also suggests that the courtship sound has a modulating effect on the behaviour of the male *P. martensii*, i.e., it enhances in the aroused male the behavioural responses elicited by prior chemical stimulation, and causes in the unmotivated male avoidance of the area around the sound source. There is here an interesting parallelism with the results of analogous experiments examining the effects of aggressive sound playback on the isolated male *P. martensii* exposed or not to prior aggressive stimulation with their own mirror image (LUGLI 1997). Hearing the aggressive sound by aggressively aroused males evoked behavioral responses (i.e. approaching, aggressive displaying and emission of sounds toward the loudspeaker) similar to those exhibited by the male toward the mirror prior to the playback test. Furthermore, the same playback stimuli determined avoidance of the site of sound stimulation in the unmotivated male. Thus, the courtship and aggressive sound of *P. martensii*, when combined with signals from other sensory channels in the same functional context, produced seemingly equivalent modulating effects on the behaviour of the intercepting male (see below). The main difference between the response of the aroused male during the aggressive and courtship playback was the importance of the loudspeaker as orientation cue for the aggressive behaviour but not for courtship. This might be related to the nature of the stimulus (chemical vs visual) presented to the male prior to testing in the two situations. Chemical stimuli spread through the environment in complicated ways and they do not provide accurate information about the location of the sender (BRADBURY & VEHCAMP 1998). On the contrary, the sight of a conspecific can provide the motivated male with the necessary spatial information for directing his activity toward the site of prior visual stimulation during the sound playback. Therefore, one might ask whether the sexually aroused male *P. martensii* would have shown directional responses in the presence of the sound stimuli, had the male being exposed to the live female prior to the playback test.

The presentation of courtship sounds to an isolated *P. martensii* male in his tank is the analog of a process of sound interception by a male in his territory, under natural conditions. The overhearing of courtship sounds originating from

adjacent territories, when associated to the concurrent detection of the sexual chemical stimulus, would inform the intercepting male *P. martensii* about the presence of a mature female in the area. Hence, the increased swimming activity and occurrence of courtship and sound emission, as shown by the aroused male in the laboratory, would facilitate the encounter of the territorial male with the unseen ripe female and the attraction of the female to the sound source. The female *P. martensii* is very attentive to the direction of the sound (LUGLI et al. 1996, LUGLI per. obs): a few sounds emitted by a out-of-sight male may quickly orient her toward the new sound source, even if courted vigorously by nearby male. The tendency to avoid the sound source shown by the unmotivated male *P. martensii* (Experiment 1) is harder to explain in functional terms. LUGLI (1997) interpreted the avoidance of the site of sound stimulation shown by the unmotivated male *P. martensii* during playback of the aggressive sound as a strategy to avoid unnecessary aggressive interactions with neighbours by the territory owner. Given the spectral resemblance of the aggressive and the courtship acoustic signals of *P. martensii* (TORRICELLI et al. 1990), it is possible that without additional information from the female, the male would interpret the sounds emitted during courtship as being aggressive in nature and, hence, avoid the sound site to lower the risk of a potentially harmful interaction. This could be an adaptive strategy among species emitting sounds in the aggressive and sexual context with similar characteristics and lacking individual differences in main spectral and temporal features.

Response of the courting male to the courtship sound playback

The results of the Experiment 3 clearly showed the intensity of the courtship activity of the male *P. martensii* during the sound playback is not increased beyond levels shown during control periods. Results from similar studies in other species support the presence of stimulatory effects of the courtship sound playback on courtship, or calling behaviour, of the territorial male (WINN 1972, STOUT 1975, MYRBERG et al. 1978, KENYON 1994). For instance, in the damselfish *S. partitus* the playback of the courtship chirp to territorial males in the field increases their courtship activity, as measured by the number of “dips” performed. Male responses to male courtship sound playback have frequently been used to study behaviour in fishes. MYRBERG et al. (1978) utilized the dip response of males to male chirp sounds to study species recognition, and MYRBERG & RIGGIO (1985) used the same technique to investigate the “dear enemy effect”. These studies were often conducted in the field by playing back sounds to territorial males courting “spontaneously” in the presence of free-ranging females. To our knowledge, the effects of the sound playback on the behavior of the male during a courtship interaction with a close-by female presented as a stimulus have been investigated systematically only in one species (STOUT 1975). In the cyprinid, *Cyprinella analostana* (= *Notropis analostanus*), the male increases the frequency of “solo-spawning” (a quivering motion associated to spawning behaviour) when “purrs” are played back in the presence of a freshly killed female. Interestingly, the sound playback had no effect on males courting continuously or showing no courtship activity (STOUT 1975). In the present study, the courtship activity of *P. martensii* males in visual contact with the female was continuous during all treatments of Experiment 3, with a tendency for the total number of displays to decrease during the course of the experiment (i.e. regardless of the playback sequence). We conclude, therefore, that sounds heard by

a territorial male *P. martensii* during a courtship interaction with a female have only minor effects, if any, on his behaviour. This conclusion supports an early anecdotal observation by TAVOLGA (1958) on the effect of the sound playback on the male *B. soporator*, during actual courtship "When the sound playback was begun while courtship was in progress, neither animal showed any response to the sound". Of course, in the case of *P. martensii* other interpretations are possible, for instance, the presence of inadequate stimulatory context for the male, due to the lack of chemical stimulation from the female.

Functionally, the presentation of courtship sounds to subjects in Experiment 3 may be considered an imperfect analog (due to the lack of the chemical stimulus) of a situation where sounds emitted by neighbouring males are detected by the territorial male courting a conspecific female. The phenomenon has been repeatedly observed during courtship interactions among territorial males *P. martensii* under semi-natural conditions (TORRICELLI et al. 1993, LUGLI pers. obs). Our results suggest that listening to the courtship sounds from other males does not affect the typical levels of courtship responsiveness of the male *P. martensii* toward the female. The lack of clear responses by the courting male to the courtship sound playback reported in other territorial species from different teleost families (i.e., Cyprinids, Pomacentrids, and Gobiids), may indicate the generality of this process among fish species where courtship occurs in close proximity to the female.

The sound spectral features important for signal recognition

Because fish sounds are structurally simpler than the calls of higher vertebrates, the playback of synthetic signals has proven a useful tool for investigating the importance of sound parameters for signal recognition among fishes (reviewed in MCKIBBEN & BASS 1998). Likewise, the use of synthetic signals for playback allowed some conclusions to be drawn concizing the behavioural relevance of the sound spectral features in *P. martensii*. The results of Experiment 2 showed that the playback of synthetic noise periods having the same duration and repetition rate as the courtship sound, but lacking the typical amplitude variation of a tone signal, did not increase swimming, or promoted courtship, by the aroused male *P. martensii*. The results of Experiment 4 showed the synthetic courtship sound evoked responses similar to those of the aroused male exposed to natural sounds. This study, therefore, demonstrates the effectiveness of the synthetic sound playback and the importance of waveform periodicity of the playback signal for the eliciting of positive responses by individuals of *P. martensii*, thereby confirming conclusions from previous playback studies on males and females *P. martensii* (LUGLI et al. 1996, LUGLI 1997). The results of Experiment 4 further showed waveform periodicity alone is not sufficient for evoking responses in *P. martensii*: aroused males did not increase swimming or show courtship activity during the playback of pure tones of constant frequency (164 Hz). The pure tone sound differed from the synthetic courtship sound in its lack of frequency modulation and the harmonic above the fundamental. The second harmonic of the synthetic courtship sound was at around 300 Hz and about 24 dB weaker than the fundamental. Recently, LUGLI et al. (2003) showed that *P. martensii* is a hearing-generalist with maximum sensitivity at 100 Hz and very low sensitivity at frequencies above 250 Hz, i.e., the steeply rising side of the species audiogram. In another companion study, LUGLI & FINE (2003) showed that the sound of *P. martensii* does not propagate further than 40-50 cm under the most favourable conditions in

the stream. Therefore, it is highly unlikely that members of this species can detect the weak harmonics above the fundamental except perhaps when in close proximity to the sound source. Furthermore, while the presence of a marked frequency modulation is always a salient feature of both the laboratory and field sounds of *P. marten-sii*, the lack of harmonics above the fundamental is a recurrent feature of courtship sounds recorded in both situations (see Fig. 1 for some examples). Altogether, these findings indicate that the harmonic structure of the courtship sound could be of little importance for signal recognition, and strongly suggest the lack of frequency modulation is the reason for the unresponsiveness of aroused males to the tone playback (Experiment 4). Interestingly, MCKIBBEN & BASS (1998) found female midshipman (*Porichthys notatus*) played back with two synthetic alternatives of the "hum" (the conspecific mating sound), showed no preference for the pure tone over the ± 5 Hz modulated version. However, females preferred the pure tone to a FM signal when the modulation was ± 10 Hz. Since the midshipman "hum" is a long, multi-harmonic tone of constant frequency (BRANTLEY & BASS 1994), it was concluded that increasing degrees of modulation are likely to make signals less attractive (MCKIBBEN & BASS 1998). Furthermore, the addition of the second harmonic to the pure tone had no effect on stimulus preference. Thus, the results of the playback study in *Porichthys* agree with those of the present study on the importance of the pattern of pulse rate modulation for signal recognition among fishes emitting sounds made up of rapidly repeated pulses.

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