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AN INTERFERENCE VISUAL CENSUS TECHNIQUE APPLIED TO CRYPTOBENTHIC FISH ASSEMBLAGES

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CRYPTOBENTHIC FISH ASSEMBLAGES
VISUAL CENSUS METHODS

ABSTRACT. – We compare the accuracy of an interference visual census technique (IVC), in which dismantling of the habitat is performed, to traditional underwater visual census (VC) and anaesthetic census. We compare the performance of these techniques applied to a temperate cryptobenthic fish assemblage using two strategies: sampling over the whole depth extent of the rocky bottom, and stratified sampling over the main microhabitats present at the study site. The number of species encountered was lower using the traditional VC. Fish density estimates were significantly higher using the interference technique compared to the traditional VC, in the transect strategy. These differences were larger for clingfishes and some gobies which occurred preferably under cobble and small rocks. No differences were found when comparing the IVC and anaesthetic census in the habitat strategy, for each microhabitat considered. We conclude that dismantling the habitat increases the performance of the visual census technique and is therefore a valuable approach when applied to temperate cryptobenthic fish assemblages.

INTRODUCTION

Sampling marine habitats with minimal lasting interference effects is fundamental for studies in ecology. Underwater visual census (VC), firstly used by Brock (1954) in a pioneering study on Hawaiian fishes, are nowadays applied to different types of fish ecology studies (Edgar *et al*. 2004), including those on assemblage structure (Prochazka 1998), ecological processes (Nanami & Nishihira 2003) and biogeographic patterns (Gasparini & Floeter 2001). Biases in sampling introduced by visual census are however recognized by most authors and there have been a number of suggestions on how to reduce them (Luckhurst & Luckhurst 1978, Sale & Sharp 1983, Bellwood & Alcala 1988, Lincoln Smith 1988, Kulbicki 1990, Samoilys & Carlos 2000). In particular, when dealing with cryptobenthic species, the use of small areas and minimum fish sizes (e.g. recording only fishes larger than 5 cm) have been appointed as possible solutions to reduce bias (Harmelin-Vivien *et al.* 1985). However, traditional VC biases remain to be fully tested (Edgar *et al*. 2004).

Traditional visual census methods have been frequently used to count benthic and nektobenthic fishes but it is generally accepted that they cannot correctly sample cryptobenthic fish species (Sale & Douglas 1981, Brock 1982, Willis 2001). Miller (1979) defined cryptobenthic fish as "small bodied fish that exploit restricted habitats where food and shelter are obtained in, or in relation to, conditions of substrate complexity and/or restricted living space, with a physical barrier likely to be interposed between the small fish and sympatric predators". As suggested by different authors, habitat complexity can greatly influence the observed distribution patterns of cryptobenthic fish assemblages (Harmelin-Vivien *et al.* 1985, Connell & Jones 1991). However, many of the studies that tried to assess biases in counting fish have mainly dealt with tropical species (Sale & Douglas1981, Brock 1982, Fowler 1987, Lincoln Smith 1988, Bortone *et al.* 1989, Kulbicki 1990, Samoilys & Carlos 2000) and visual *in situ* evaluation methods of fish populations were essentially developed in tropical environments. Coral reefs in particular are amongst the most diverse marine habitats where numerous species can typically be found in a relatively small area (Ackerman & Bellwood 2000). It is thus conceivable that the use of the same techniques in temperate regions may offer different results. Given that some microhabitats are composed of small movable items that create interstitial spaces where many of the cryptobenthic fish hide (Gonçalves *et al*. 2002) it may be worthwhile to include a more thorough sampling of particular microhabitat types in the visual census techniques.

In this paper we have two main goals. First compare the performance of a traditional censusing technique and a modified visual technique to anaesthetic sampling by randomly sampling the rocky bottom. Second, compare the performance of the modified technique and the quantitative (anaesthetic) sampling across microhabitat types.

MATERIAL AND METHODS

This study was performed during January and February 2004 in the Arrábida Marine Park (Portugal) at two stations, Risco (38º27'03''N, 9º01'24''W) and Cozinhadouro (38º26'54''N, 9º02'12''W), which were characterized by the highest diversity

of coastal fish species (Gonçalves *et al*. 2003). The highly heterogeneous underwater habitats result from the disintegration of calcareous cliffs that border the shoreline. Different microhabitats: sand, gravel, cobble, small rocks (< 30 cm) and large rocks (> 30 cm), were patchily distributed on this area. Fish sampling was performed in the morning with good sea-weather conditions. The local cryptobenthic fish species were easily identified according to distinct morphological and colouration characteristics, except for the gobiesocids *Lepadogaster lepadogaster* and *L. purpurea*. Since it is very difficult to distinguish between these species in the field (Henriques *et al*. 2002), they were generally indicated as *Lepadogaster* sp. Data on the cryptobenthic fish assemblage was collected using three techniques:

Visual Census (VC): This technique has been used by several authors (Harmelin-Vivien *et al*. 1985, Willis 2001, La Mesa *et al*. 2004, La Mesa & Vacchi 2005). In a 0.25 m^2 quadrat the observer recorded all fish, taking note of the microhabitat where they were firstly seen. The use of a flashlight allowed the observer to look for fish inside clefts and small holes but no habitat manipulation was performed.

Interference Visual Census (IVC): This technique was applied to the same quadrats as the VC. After counting all visible fish over the substrate (VC) we systematically looked for fish hidden under rocks and cobbles, buried in gravel or sand. This technique was therefore not strictly a "visual" technique since it involved lifting all microhabitat items (smaller than 30 cm in maximum length). In each quadrat all fish were identified and their position recorded. After displacement, the microhabitat items were put back in their place. This procedure could have attracted fish from nearby areas, but given the small quadrat area used we are convinced that these cases (less than 3 % of the occasions) were spotted and excluded from the census.

Anaesthetic Census: Quinaldine (2-methyloquinolina) diluted in alcohol at 15:1 (Patzner 1999) was used to count all fish present in each 0.25 m^2 quadrat, by squirting it into cavities, clefts and under all microhabitat items present. Approximately 125 ml of the anaesthetic was slowly applied per quadrat from the boundaries to the centre. The search for fish started immediately after this procedure. Although we used open stations, the relatively small quadrat size allowed us to record all fishes before they escaped. We also controlled the potential influence of the anaesthetics on fish outside the quadrat by searching from the boundaries to the centre of the sampling point and therefore detecting any anaesthetised fish that entered the quadrat. The searching effort and method was similar to the one applied in the IVC.

Table I. – Microhabitat area sampled in the transect and habitat strategies using underwater visual census (VC), interference visual census (IVC) and anaesthetic census (see text for details).

	Transect strategy (m^2)		Habitat strategy (m^2)	
	VC/IVC	Anaesthetic	VC/IVC	Anaesthetic
Sand	1.10	0.52	1	1
Gravel	0.35	0.45	1	1
Cobble	0.73	1.08	1	1
Small rocks	0.71	0.30	1	1
Large rocks	9.36	9.90	1	1

We applied these techniques in two sampling strategies: sampling over the whole depth extent of the rocky bottom, and stratified sampling over the main microhabitats present at both stations. While the first strategy aimed at sampling each microhabitat proportionally to its occurrence (random sampling), the second strategy aimed at balancing the sampling effort among the main microhabitats present (Table I). This later strategy allowed us to evaluate bias in sampling the different microhabitat types since by sampling all microhabitats equally we could ascertain that our results would be consistent in all microhabitats.

Strategy 1: Sampling over the rocky bottom: Eight parallel transects were established five meters apart over the subtidal rocky bottom, from the deeper sandy area (depth range 8.9 m to 11.2 m, average = 10.3, S.E. = 0.3) to the infralittoral (depth range 1.3 m to 2.3 m, average = 2.0, S.E. = 0.2). Four transects were sampled with the visual techniques (VC and IVC) whilst the other four were sampled with anaesthetic census. Transect length varied according to the extent of rocky bottom (range = 55 m to 70 m, average = 61.25, S.E. = 3.15). On each transect, a 0.25 m^2 quadrat was sampled every 5 m. The quadrat area chosen was smaller than in previous studies (e.g. Willis 2001). The choice for such an area was a compromise between the time necessary to sample each quadrat before the anaesthetic dispersed, especially in the more complex microhabitats, and the size of the microhabitat patches sampled in Strategy 2 (see below). A total of 98 quadrats were sampled, half using the visual techniques and the other half using anaesthetic census. The sampling procedure began by examining the first quadrat on the visual transect after which the diver swam to the parallel transect and sampled the first quadrat on the anaesthetic transect. This procedure was repeated until the infralittoral area was reached. Cover percentage of each microhabitat present in each quadrat was visually estimated.

Strategy 2: Stratified sampling over the main microhabitats: Five microhabitats were sampled using 0.25 m^2 quadrats: sand, gravel, cobble, small rocks (< 30 cm maximum length) and large rocks (> 30 cm maximum length). At each microhabitat patch, eight quadrats were randomly deployed, half of which were sampled with the visual techniques (VC and IVC) while the other half were examined using anaesthetic census. Sam-

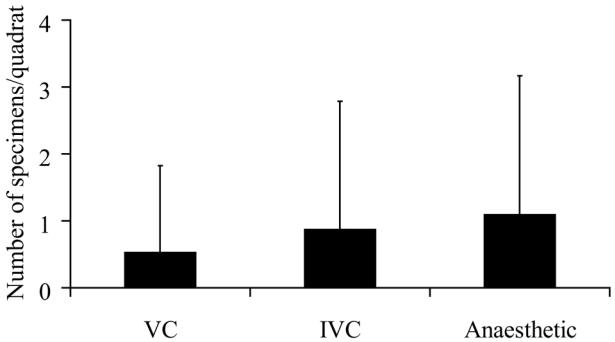

Fig. 1. – Mean density of cryptobenthic fishes (number of specimens per quadrat ± SD) recorded in the transect strategy (random) by visual census (VC), interference visual census (IVC) and anaesthetic census (ANA).

pling was performed in narrow depth intervals (1 m depth range) to avoid possible confounding depth effects in the data.

Data analysis: To evaluate the efficiency of the visual techniques we used two one-way ANOVA and tested for differences between VC and anaesthetic and between IVC and anaesthetic in the transect (random) strategy. To compare both visual techniques, a visibility index was calculated based on the percentage of specimens recorded by the IVC but missed by the VC in both strategies. To compare the efficiency of the IVC to quantitative census (quinaldine) in the different microhabitats (Strategy 2), we used a two-way ANOVA and post-hoc Student-Newman-Keuls tests to find out where differences lay. All data were transformed following a square root + 1 transformation to meet homoscedasticity assumptions.

RESULTS

A total of 15 species belonging to 8 families were observed in our study site (Table II). The overall densities obtained returned an average value of 2.37 individuals/m^2 (S.E. = 0.38, range 0-12) for the VC, 4.46 individuals/m^2 (S.E. = 0.54, range 0-16) for the IVC and 6.20 individuals/m^2 (S.E. = 0.65, range 0-28) for anaesthetic census. The total number of species encountered using each technique was: VC = 7, IVC = 11, anaesthetic census = 12 (Table II).

For Strategy 1 (random sampling) the VC recorded significantly less fish than the anaesthetic census (ANOVA: $F = 11.2$, $p < 0.001$), whereas no significant differences were found between the IVC and anaesthetic census (ANOVA: $F = 1.61$, $p = 0.207$) (Fig. 1).

Using data from both strategies we calculated the percentage of fish counted with the IVC that was missed by the VC, and ascribed a visibility index to each species (Fig. 2). Three distinct groups can be identified. One composed by the gobiesocids *Lepadogaster* sp. which were completely missed by the VC; a second group composed by the gobies *Gobius paganellus* and *Gobius xanthocephalus* which were partially missed without interfer-

Table II. – Number of specimens of each species recorded by visual census (VC), interference visual census (IVC) and anaesthetic census. * *Lepadogaster* sp. was used to refer to two co-occurring species, *L. lepadogaster* and *L. purpurea* which are very difficult to distinguish in the field (Henriques *et al.* 2002).

Family	Species	VC	IVC	Anaesthetic
Blenniidae	*Parablennius gattorugine* (Brünnich, 1768)			2
Blenniidae	*Parablennius pilicornis* (Cuvier, 1829)	8	8	11
Callionymidae	*Callionymus reticulatus* Valenciennes, 1837			1
Gobiesocidae	*Apletodon dentatus* (Facciola, 1887)			1
Gobiesocidae	*Diplecogaster bimaculata* (Bonnaterre, 1788)		1	
Gobiesocidae	*Lepadogaster candolii* Risso, 1810		1	9
Gobiesocidae	*Lepadogaster* sp.* (Bonnaterre, 1788)		17	35
Gobiidae	*Gobius cruentatus* Gmelin, 1789	5	5	1
Gobiidae	*Gobius paganellus* Linnaeus, 1758	1	5	5
Gobiidae	*Gobius xanthocephalus* Heymer and Zander, 1992	9	19	17
Gobiidae	*Pomatoschistus pictus* (Malm, 1865)	8	9	10
Muraenidae	*Muraena helena* Linnaeus, 1758		1	
Scorpaenidae	*Scorpaena porcus* Linnaeus, 1758	1	1	
Syngnathidae	*Nerophis lumbriciformis* (Jenyns, 1835)			1
Trypterigiidae	*Tripterygion delaisi* Cadenat and Blache, 1971	9	10	15
	Total	42	77	108

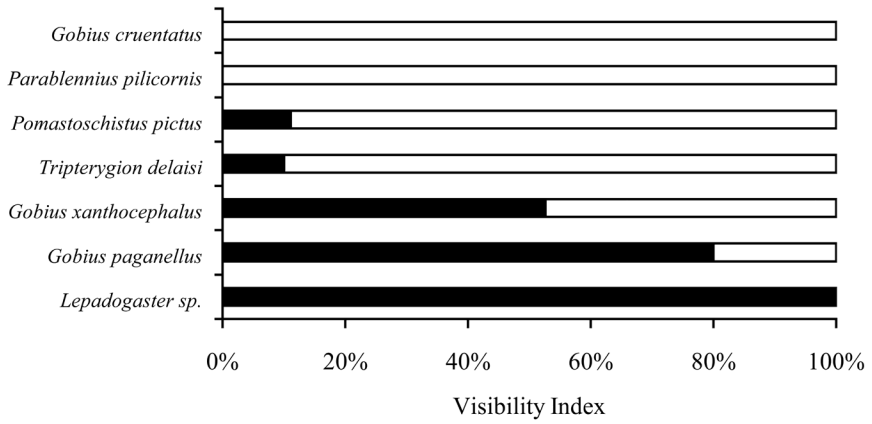

Fig. 2. – Percent of the number of specimens counted using both visual census techniques, the interference visual census (IVC) (black) and the underwater visual census (VC) (white), for species with over five individuals.

Table III. – Factorial ANOVA results for the comparison between the interference visual census (IVC) and anaesthetic census (ANA) in the different microhabitats recorded in the habitat strategy.

	df	MS	F	p
IVC - ANA	1	0.78	9.12	0.005
Microhabitat	4	0.68	7.95	0.000
IVC – ANA vs. Microhabitat	4	0.02	0.19	0.943
Error	30	0.09		

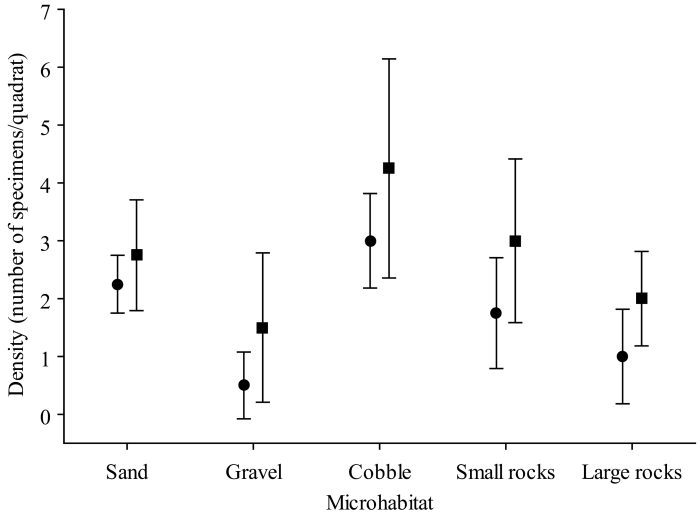

Fig. 3. – Mean density of cryptobenthic fish (number of specimens per quadrat ± SD) recorded in the habitat strategy by interference visual census (IVC) (circle) and anaesthetic census (square) in each microhabitat.

ence; and a third group with *Tripterygion delaisi*, *Poma - toschistus pictus, Parablennius pilicornis* and *Gobius cruentatus* which were mostly recorded prior to interference. Therefore, without habitat dismantling during the visual census, the first two groups of species would have been partially or completely missed.

The factorial ANOVA comparing the IVC and anaesthetic census data collected in the habitat strategy revealed significant differences between techniques and habitats but there was no interaction between these factors (Fig. 3, Table III). Post-hoc tests revealed that there were no differences between techniques in each of the microhabitats sampled. The only observed differences occurred between different habitats: gravel and all the other microhabitats (sand: $p < 0.05$; cobble: $p < 0.001$; small rocks: $p < 0.05$); large rocks with cobble ($p < 0.05$).

DISCUSSION

Cryptobenthic fish diversity observed in this study was lower than that reported in other studies on temperate fish assemblages: e.g., 39 species from 9 families in South Africa (Prochaska 1998), 33 species from 17 families in New Zealand (Willis 2001), and 20 species from 5 families in Italy (La Mesa *et al.* 2004). This relatively low diversity is probably due to the smaller sampling size used in our test of the IVC. However, overall average densities obtained in our study with both the anaesthetic census and the visual census are comparable to those described by Prochazka (1998) and Willis (2001) using rotenone sampling: 3.41 specimens/m^2 and 3.61 specimens/m^2, respectively. Using a VC technique applied to northern Adriatic blennioids, Ilich and Kotrshall (1990) reported an average density of 4 specimens/m^2. In the Ciclopi Islands, Central Mediterranean Sea, La Mesa *et al.* (2004) using the same technique found an average density between 0.60 and 0.67 specimens/m^2.

The observed differences between the traditional VC and the IVC were revealed by the visibility index. At the species level, major differences were related to the clingfishes *Lepado - gaster* sp. which occur almost exclusively under stones (Henriques *et al.* 2002) and were completely missed by the VC. The gobies *G. xanthocephalus* and *G. paganellus*, use the space under small microhabitat items and were also underestimated by the traditional visual technique. Other benthic species such as *G. cruentatus, P. pilicornis, T. delaisi* and *P. pictus* present a less cryptic behaviour and were equally detected by both visual techniques. Therefore, traditional VC techniques underestimate different species at different degrees. In particular, species with cryptic habits are the most affected. By dismantling the substrate, a significant increase in the number of specimens detected is achieved.

In order to explore this result, a comparison of both visual techniques with a quantitative survey (anaesthetic counts) was performed. While differences were large between VC and anaesthetic counts, when interference was applied and specimens under microhabitat items were recorded (IVC) there were no significant differences with the quantitative survey. Moreover, when microhabitats were sampled proportionally (habitat strategy), no differences between the IVC and anaesthetic counts were found for each microhabitat type.

Most studies that quantitatively sampled these fish assemblages used visual census techniques that did not involve disturbing the bottom by lifting items where fish could be hiding (Sale & Douglas 1981, Bortone *et al.* 1989, Willis 2001). Sampling other groups of marine animals, such as some invertebrates (e.g. Chapman 2002) is frequently done with interference techniques. In low complexity microhabitats, such as sand, interference has

been used to improve censusing of cryptic fishes (Forrester 1995) but this has not been tested in other microhabitats. In this study we conclude that lifting small microhabitat items where fish could be hiding significantly increases the performance of the underwater visual census technique.

The interference visual census technique may render better abundance estimates, closer to those obtained with anaesthetics, depending on the specific behaviour of some species and the ability to sample some microhabitats. In the future, this IVC technique should be tested in different temperate cryptobenthic fish assemblages and its efficiency should be evaluated in other microhabitats.

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