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An assessment of biomass and diel activity of fish at an artificial reef (Adriatic sea) using a stationary hydroacoustic technique

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The biomass of fish assemblage inhabiting the Senigallia artificial reef (northern Adriatic Sea, Italy) was evaluated in the period July–November 1996. Density and biomass were assessed through a stationary hydroacoustic technique using an appropriately adapted SIMRAD EY500 system. Part of the system was placed inside the reef and it was linked by radio-modem to the Institute. Four ES120 split-beam acoustic transducers were used. The data gave useful information about the daily behaviour of the fish assemblage living at the reef: during the whole period the lowest densities were generally recorded in the early afternoon, whilst the highest abundances were commonly observed late in the night and early in the morning. It was confirmed that in late summer–early autumn, most of the reef fishes migrated from the coastal shallow waters to offshore. Throughout the study period fish abundance was higher inside the reef and decreased significantly at a distance of about 80 m. Moreover, the fish assemblage did not appear to be homogeneously distributed inside the reef. The highest values were recorded in the central part of the area where several structures of different shape were placed.

Keywords: Adriatic Sea, artificial reef, fish biomass, split-beam hydroacoustic.

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Introduction

A knowledge of the fish assemblage occurring on an artificial reef is essential to the evaluation of its effects on the community of the original habitat induced by the deployment of the artificial structures such as the potential increase in biomass and the understanding of the relationships between natural and artificial habitats, as well as management of the available resources. In recent years a particular need has been for quantitative data both on the biomass and the spatial and temporal variations of the fish assemblages inhabiting relatively long-standing artificial reefs that are mature from an ecological point of view (Steimle and Meier, 1997). This is not a trivial task because artificial reefs are open systems, directly connected with the surrounding marine environment and many species frequently move both horizontally between the reef and the open sea as well as vertically in the water column.

Visual census carried out by scuba divers or through a remotely operated vehicle (ROV) is the most common method utilized for the assessment of the fish assemblage on natural rocky bottoms and at artificial reefs (Bortone and Kimmel, 1991; Okamoto, 1991; Charbonnel *et al.*, 1997). Nevertheless, this technique is limited by both the environmental conditions and the behaviour of the different species. For example, Demartini *et al.* (1989) note that sampling may be drastically endangered when the water transparency is less than 3 m. In addition, the spatial heterogeneity of the artificial modules and the behaviour of cryptic species may reduce the probability of a specimen being found and so leading to an underestimation of the real abundance (Wickham and Russel, 1974; Buckley and Hueckel, 1985; Harmelin-Vivien *et al.*, 1985).

Non-visual techniques comprise fishing surveys with relatively non-destructive gears (hook-and-lines, set nets, traps) which have the additional advantage of

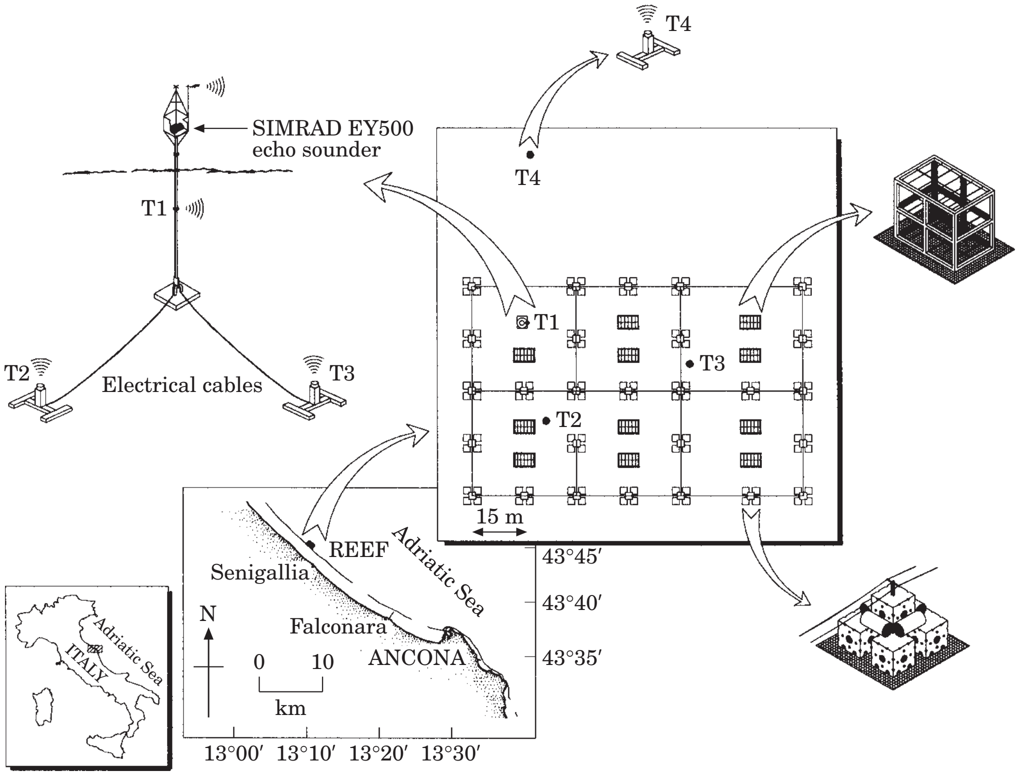

Figure 1. Map of the Senigallia artificial reef. Schematic view of the stationary hydroacoustic transducer deployment and location of the EY500 system on the fixed pole.

providing individual specimens for further analysis. Nevertheless with these too, the results can be affected by several factors, such as the behaviour of the different species, the difficulty of setting the gears close to structures and the selectivity of the gear being used (Miller and Hunte, 1987; Somerton *et al*., 1988; Bombace *et al*., 1997). In fact comparison between visual observations and fishing surveys with a trammel net at an Adriatic artificial reef showed that the former method was particularly suitable to assess obligate or partially-obligate reef-dwelling species, such as cryptic fish living inside the holes of the artificial substrates and nekto-benthic and pelagic fish swimming around the structures. On the other side, the fishing sampling gave a broader picture of the fish community living in and around the reef: the trammel net caught both species of the original sand-muddy habitat and nekto-benthic and pelagic reef species, but it did not appear to be a good sampler for cryptic animals and was likely to underestimate pelagic fish because of its limited height (Bombace *et al*., 1997).

Starting from these experiences, the aim of the present study was to test a fixed hydroacoustic technique as an alternative or integrative method to assess the fish assemblage inhabiting an artificial reef.

Area description

The study was carried out at the Senigallia artificial reef which was built-up in 12 m of water off the northern Adriatic coast, about 30 km NW of Ancona and 2 km offshore, on a sand-muddy seabed far from natural hard seabed (Figure 1).The reef was constructed in 1987 and consists of 29 pyramids, each made of five 2-m cubic concrete blocks, and twelve concrete cages for shellfish culture placed in a rectangular arrangement (Fabi and Fiorentini, 1994). It has a warming light placed on a fixed pile rising about 5 m above the sea surface.

The water temperature ranges from 7°C in winter to 26–27°C in summer, without a significant difference between the surface and the bottom. Because of the low coastal-water temperature most fish species migrate towards the deeper waters offshore in fall and gradually return in spring as the water warms (Bombace, 1992). Poor underwater visibility is very common because of riverine input and the suspension of sand and mud due to storms, therefore making it difficult to carry out regular visual observations over a long time. Because of this the evolution of the fish population around the reef was surveyed using a bottom trammel net. The survey

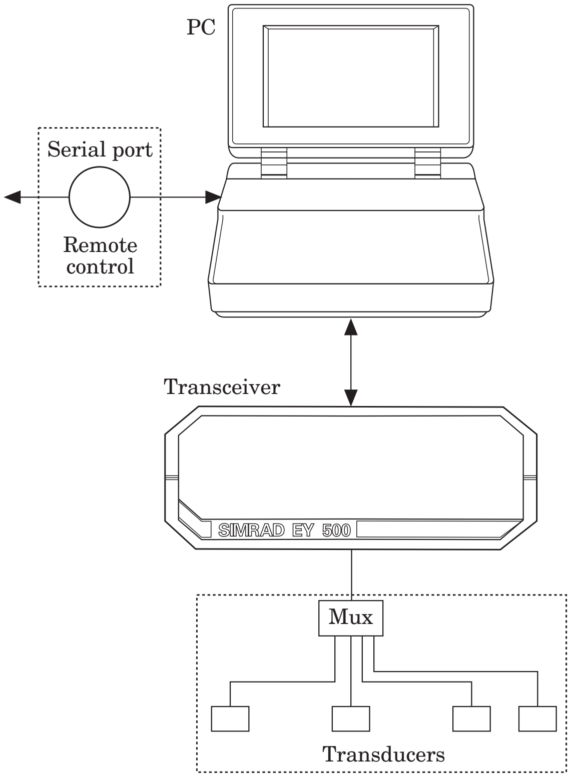

Figure 2. EY500 system equipment

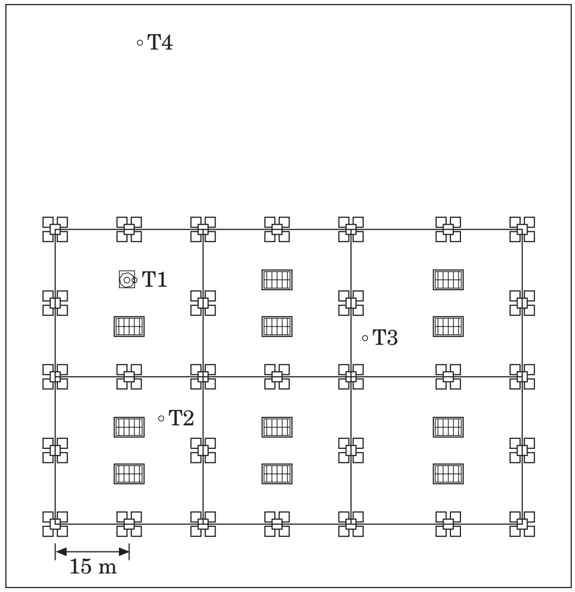

Figure 3. Location of the stationary hydroacoustic transducers to measure the relative density of fishes associated with the artificial reef.

started one year before the deployment of the structures and continues to date (2001). Sampling has been carried out by day (from dawn to dusk) and by night (from dusk to dawn). Because nocturnal sampling captured more species and larger number of individuals (Bombace *et al.*, 1997), it was adopted as the standard survey method in the recent years (Fabi *et al.*, 1999a; Bombace *et al.*, 2000).

Experimental design and data acquisition

The hydroacoustic equipment consisted of a SIMRAD EY500 echosounder (Figure 2) and comprised: a transceiver; a personal computer; a power supply (four electric batteries: 12V–100Ah, charged with solar panels); a transducer multiplexing (SIMRAD MP500); a timer to periodically power the system; and four transducers (ES120 split-beam transducers: operating frequency 120 kHz, beam angle 7.1°).

All the equipment, with the exception of the ES120 split-beam transducers, was packed inside a waterproof case on the fixed buoy placed within the reef area (Figure 1). It was linked by a radio-modem (V_{TX} 4800 baud) to a personal computer installed in the Institute, which automatically controlled data acquisition and provided the correct functioning of the EY500 system in real time. From the first, particular attention was given to the electrical input of the EY500 equipment placed on the pile. The batteries were continually charged via four solar panels and, in order to increase their lifespan, a timer cyclically turned the EY500 system on and off. In accordance with previous studies (Stanley and Wilson, 1998) the system was started by the timer for 16 min every 2 h, encompassing four periods (dawn, noon, dusk and midnight) over a 24-h interval.

During each 16 min period the transducers sampled sequentially for a 4 min interval and the acoustic data, received from the echosounder, were stored on the computer's hard-disk in a telegram-based structure. At the same time, every 60 sec, the system transferred through the radio-modem the data integration to the Institute to allow the control of data acquisition. The four transducers were set to measure *in situ* fish target-strength distribution and density at the Senigallia artificial reef (Figure 3) in the following way: transducer 1 (T_1), placed 4 m deep on the pile of the fixed light signal, was horizontally oriented towards the centre of the reef; transducers 2 and 3 (T_2, T_3) were located on steel frames placed on the seabed inside the artificial reef and upward-oriented; transducer 4 (T_4) was oriented towards the surface, as T_2 and T_3, but located on the seabed outside the artificial reef, about 80 m far.

Transducer calibration was undertaken prior to the beginning of the study at Ancona inlet on 15 July 1996. Calibration procedures followed the guidelines given by Foote and MacLennan (1984) and SIMRAD operator's manual (1995), whereby a target sphere of known

Table 1. Sampling intervals.

Sampling interval	S1	S2	S3	S4	S5	S6	S7	S8
Start	21 Jul 96	2 Aug 96	11 Aug 96	20 Aug 96	2 Sep 96	25 Sep 96	2 Oct 96	1 Nov 96
End	30 Jul 96	8 Aug 96	17 Aug 96	29 Aug 96	9 Sep 96	30 Sep 96	12 Oct 96	14 Nov 96

backscattering cross-section was suspended below each transducer in turn. The sphere of 23-mm diameter and TS of −40.4 dB was situated at a 4-m distance from the transducer surface and a data set containing sphere echo samples was obtained while moving the sphere randomly in the beam.

The three transducers located inside the reef started to collect data from 21 July 1996, the other began on 2 August 1996. The system collected data for 116 days until 14 November 1996. The whole period was subdivided into eight intervals, each having a duration of about eight days (Table 1), linked to battery life. During each interval the system operated continuously 24 h/day until the hard disk was full; the resetting of the system was influenced by the weather conditions.

Data analysis

The echo integrator technique gives reliable estimates of fish abundance. If v(t) is the voltage produced by the echosounder at time t after the transmit pulse, the energy (E_i) is the integral of the squared amplitude of v(t) with respect to time. Thus the echo integrator output due to one transmission is:

$$E_i = \int_{t_1}^{t_2} |v(t)|^2 dt \quad (1)$$

If there are N transmissions, the echo integrator accumulates the echo integrals:

$$E = \sum_{i=1}^{N} E_i | N \quad (2)$$

The fish density ρ is proportional to E and is calculated from the echo integrator equation (MacLennan, 1990):

$$\rho = [C\bar{g}/(\psi\sigma)]E \quad (3)$$

where σ is the expected value of the acoustic cross-section (m^2), C is the calibration factor which depends on the sensitivity of the transducer, ψ is the equivalent beam angle, and $\bar{g}$ is the TVG correction factor. The measurements C, ψ and $\bar{g}$ come from the equipment calibration.

As the integrated backscattering area (S_a) is defined as:

$$S_a = [C\bar{g}/\psi]E \quad (4)$$

thus using equations (3) and (4), the formula for converting integrated backscattering area (S_a) into fish density per unit of area (ρ) takes the form:

$$\rho = S_a/\sigma \quad (5)$$

where σ is equivalent to a given Target Strength (dB) and is calculated through the following formula:

$$\sigma = 4\pi \cdot 10^{(TS/10)} \quad (6)$$

Abundance may be expressed either as number of fish or as total weight in the stock. When considering the acoustic estimation of fish abundance it is convenient to work with the weight and consistent units must be used throughout the analysis. Therefore if the abundance (A) is required as a weight while the acoustic cross-section (σ) function is given for individual fish, the latter must be converted to acoustic cross-section per unit weight of fish (σ_w):

$$A = S_a/\sigma_w \quad (7)$$

This was done by reference to the method of MacLennan and Simmonds (1992) which considers that the Target Strength of a single fish is given as:

$$TS_n = a_n + b_n \cdot \log L \quad (8)$$

the corresponding function TS_w, Target Strength of unit weight of fish, has the same form with different constants:

$$TS_w = a_w + b_w \cdot \log L \quad (9)$$

where L is the standard length of fish animal. Thus if the relationship of fish weight w (g) is given by a generalized function of standard length L (cm):

$$w = a_f \cdot L^{b_f} \quad (10)$$

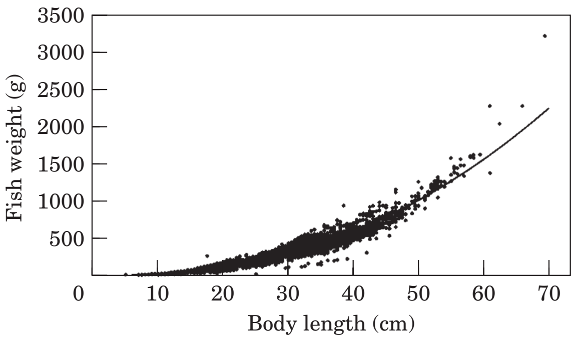

Figure 4. Length–weight relationship from the data of all the pelagic and nekto-benthonic fish species sampled at the Senigallia artificial reef with trammel net as from 1987 to 1996.

the constant coefficients are related by the formulae:

$$a_w = a_n - 10 \cdot \log a_f \quad (11)$$

$$b_w = b_n - 10 \cdot b_f \quad (12)$$

The coefficients a_f and b_f were computed throughout the maximum likelihood fitting method applied to the length and weight data of all the pelagic and nekto-benthic fish species collected by trammel net at the reef in the same period. The original data and the corresponding length/weight curve are reported in Figure 4. The results obtained were $a_f = 0.089$ and $b_f = 2.381$. Considering a mixed pool of fish species, for the definition of Target Strength as a function of standard length of a single fish, the relationship as proposed by McCarteney and Stubbs (1971) was used:

$$TS_n = -66.84 + 24.5 \cdot \log L \quad (13)$$

Therefore, given the coefficients $a_n = -66.84$ and $b_n = 24.5$, the following values of $a_w = -56.38$ and $b_w = 0.69$ were calculated. The generalized formula (9) for the Target Strength of unit weight of fish can be re-written as:

$$TS_w = -56.38 + 0.69 \cdot \log L \quad (14)$$

As a mean fish length of 27 cm was estimated from all the sampled data, consequently $TS_w = -55.39$ and the equivalent acoustic cross-section per unit weight of fish is:

$$\sigma_w = 4\pi \cdot 10^{TS_w/10} = 363 \cdot 10^{-7} \quad (15)$$

S_a values and the σ_w were used to calculate the mean fish biomass (7) at each sampling interval and at each hour of the day. For each transducer, over the 4-min interval of acquisition, the resultant integrated values of Area density (fish/hectare), S_a total (dB) and TS distribution were calculated using EP500 software (Ver. 5.0) and stored on a folder in ASCII-files. Gain and threshold levels for the EP500 analyses were set to detect a minimum target of −50 dB or approximately equal to that from an individual 10-cm fish according to Thorne *et al.* (1989). Subsequently, in order to held all the acoustic data in a Microsoft Access database, a Borland Pascal program was written specifically to provide the data from the ASCII files. Fish density per hectare and fish biomass obtained were standardized to a water volume [number $\cdot$ m^{-3} and g $\cdot$ m^{-3}].

The data recorded by the vertically oriented transducers were related to a water column whose upper and lower limits were established on the basis of several considerations. The lower allowable limit of the transducer is twice the Rayleigh distance (RD), which is the square of the largest transducer dimension (l) divided by the acoustic wavelength (λ) defined as the ratio of the operating frequency (f) and the sound speed into the water (c):

$$RD = \frac{l^2}{\lambda} = l^2 \cdot \frac{f}{c} \quad (16)$$

As the transducers had a 0.10 m-diameter circular base and an operating frequency of 120 kHz, the RD resulted:

$$RD = 0.10^2 \cdot \frac{120\,000}{1490} \cong 0.8\text{m} \quad (17)$$

Considering the frame on which the transducer was located (60 cm height) and the calculated Rayleigh distance, the lower limit was established at 2.2 m from the seabed. To ensure that near-surface schools of fish were included and bubbles arising from surface turbulence excluded, the upper boundary was set at 30 cm under the sea surface, though in rough sea conditions this last limit was further lowered to 30 cm from the trough of the deepest wave.

The horizontally-aligned transducer enabled near-field density estimates to a maximum distance of 25 m from the buoy. Also in this case fish density per hectare and fish biomass were standardized to a water volume.

Because the acoustic estimates of biomass from the original (non-transformed) data might be oversensitive to extreme values and confidence intervals are large, McConnaughey and Conquest (1992) suggested that density and biomass of aggregated stocks should be indexed with an estimator such as the geometric mean. This estimator was computed by exponentiating the mean of the ln(x+1)-transformed data and subtracting one. Using it may reduce the errors associated with conventional stock-assessment practices and provide more effective management of over-dispersed stocks.

Table 2. Acoustic estimates of mean fish biomass (g/m^3) recorded in the period 21 July–4 November 1996 at the Senigallia artificial reef by the four transducers (T1–T4). (GM = Geometric Mean; LCI = Lower Confid. Interval. UCI = Upper Confid. Interval.)

Sampling interval	T1 LCI	T1 GM	T1 UCI	T2 LCI	T2 GM	T2 UCI	T3 LCI	T3 GM	T3 UCI	T4 LCI	T4 GM	T4 UCI
S1	4.36	5.90	7.53	17.81	24.44	32.03	5.61	9.06	12.87	—	—	—
S2	4.46	5.59	6.77	33.79	45.23	58.79	6.82	10.80	15.23	0.80	1.77	2.77
S3	1.88	2.41	2.95	13.04	18.49	24.67	1.06	2.68	4.40	0.44	1.04	1.65
S4	2.68	3.58	4.51	29.28	39.94	52.60	6.13	10.46	15.34	0.71	2.50	4.41
S5	2.15	3.11	4.10	4.51	7.24	10.21	2.17	4.22	6.42	0.53	1.17	1.83
S6	0.40	0.69	0.97	4.06	7.75	11.87	1.92	3.66	5.50	0.83	1.84	2.89
S7	0.38	0.63	0.89	1.74	3.56	5.49	5.12	8.27	11.72	0.54	1.01	1.49
S8	0.12	0.24	0.37	1.46	2.27	3.11	1.19	2.24	3.33	0.30	1.33	2.39
Whole period	2.40	2.73	3.05	14.11	16.12	18.22	5.36	6.38	7.43	1.11	1.47	1.84

The mean fish biomass and densities obtained with the three upward-oriented transducers (T2, T3, T4) and in the different sampling intervals were compared using a three-way variance analysis (Snedecor and Cochran, 1967). The factors were transducer, interval and hour-of-the-day, the latter being used in order to randomize unsuspected sources of variation in the effects of the other two predicted variables. Where there were significant interactions between factors a one-way analysis of variance was repeated on subgroups of data. Tukey's HSD test was used to make comparisons across all pairs of group means when corresponding ANOVA tests were significant ($P<0.05$). Prior to performing ANOVA tests, the normality (Kolmogorov-Smirnov test) and homogeneity of variances (Levene's test) of the data were verified. In many cases these assumptions were not verified and a common $\ln(x+1)$ transformation was applied. Because tests on biomass and density gave the same results only those for fish biomass were discussed in this paper.

The data recorded by the horizontally-aligned transducer (T1) were examined separately, because it explored just a distinct layer (4 m deep) of the water column. In this case the biomass values obtained in the different sampling intervals were compared by means of a one-way ANOVA.

Similarities among the mean fish abundance (biomass and density) at each hour of the day were also evaluated by means of Hierarchical Cluster Analysis (Anderberg, 1973). Starting from the Mean Normalized Abundance values (%), calculated by dividing the mean fish abundance at each hour by the maximum mean value, clustering was performed using the squared Euclidean distance and single linkage. All the statistical procedures were performed using the *SPSS Rel. 9.01* software package (1999).

Results

Upward-oriented transducers (T2, T3, T4)

The results are presented first as comparisons between different sites of the artificial reef over the period July–November 1996 (Table 2; Figure 5) and secondly, as description of the biomass fluctuations (Table 3). The latter was illustrated graphically on Figure 6, representing the Mean Normalized Abundance values (%) over each day.

Overall the mean fish biomass recorded inside the reef (Table 2) ranged between $6.38\ g \cdot m^{-3}$ (T3) and $16.12\ g \cdot m^{-3}$ (T2). Comparison between T2 and T3 showed a fish assemblage not homogeneously distributed inside the artificial reef, with the highest values of abundance recorded in the central part of the area (T2) and the statistical differences were highly significant ($P<0.001$, Table 4). Although the transducer effect was not assumed to be consistent across all the levels of the sampling interval factor ($P<0.001$), Table 4 shows that the mean fish biomass of T2 was significantly greater than T3 during all August (from S2 to S4). Fish biomass

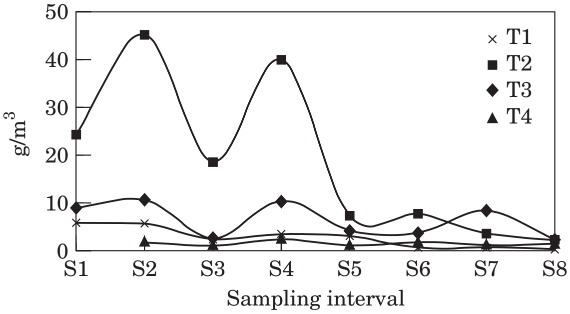

Figure 5. Geometric means of fish biomass recorded by the four transducers during the whole sampling period.

Table 3. Acoustic estimates of mean fish biomass (g/m^3) recorded in the period 21 July–14 November 1996 at the Senigallia artificial reef by the four transducers (T1–T4) at different hours of the day. (GM=Geometric Mean; LCI=Lower Confid. Interval; UCI=Upper Confid. Interval.)

Hour		T1			T2			T3			T4	
of the day	LCI	GM	UCI	LCI	GM	UCI	LCI	GM	UCI	LCI	GM	UCI
0	1.55	2.13	2.72	6.61	12.55	19.53	5.04	9.61	14.82	—	2.42	5.23
2	1.73	2.51	3.31	6.87	11.76	17.35	4.12	7.95	12.24	0.61	1.83	3.11
4	2.61	3.69	4.81	10.91	17.87	26.09	4.41	8.37	12.83	0.59	1.55	2.53
6	2.83	4.14	5.50	21.79	32.49	45.50	7.13	13.33	20.64	0.37	2.50	4.79
8	3.24	4.71	6.26	31.39	46.91	66.52	1.45	3.70	6.13	0.61	1.23	1.87
10	1.48	3.71	6.11	11.26	18.70	27.56	0.96	3.32	5.88	0.18	0.69	1.20
12	1.15	2.18	3.24	5.87	10.07	14.80	1.23	2.88	4.63	0.39	0.87	1.35
14	0.89	1.46	2.04	2.37	3.84	5.39	0.95	2.88	4.94	0.25	0.48	0.71
16	0.69	1.21	1.74	1.64	4.10	6.77	0.85	2.81	4.91	0.80	2.28	3.84
18	1.46	2.43	3.43	5.93	10.03	14.64	1.51	3.88	6.44	0.35	0.83	1.31
20	1.83	3.07	4.36	18.27	27.74	39.17	7.53	13.14	19.65	0.61	1.84	3.12
22	1.38	2.06	2.74	10.26	17.55	26.24	3.55	6.89	10.58	0.05	1.23	2.46

off the reef (T4) remained low throughout the study period, with scattered targets (1.47 g . m^{-3}, Table 2) and was significantly lower than those obtained inside the reef (P<0.001, Table 4). In particular, with the exception of the last sampling interval, this pattern was consistently observed across the sampling intervals among the values of T2 and T4 (Table 4). Also the mean fish biomass estimated by T3 was higher than T4 (Table 2), but in this case the difference was statistically significant (Table 4) only in early August (S2) and in November (S8).

The one-way analysis of variance conducted on the fish biomass (factor: Sampling Interval) confirmed for each transducer, with the exception of T4, a significant or highly significant difference among the sampling intervals (Table 4). Inside the artificial reef the fish biomass showed a rapid decline (Figure 5) at the beginning of September (S5). In further analysis, the Pearson correlation test revealed for T2 and T3 a negative relationship between the fish biomass and day factor, confirming that, throughout the study period, the fish biomass decreased as time passed. On the contrary the fish biomass at T4 site was fairly uniform throughout the period (P=0.730, Table 4).

Changes in fish biomass during the day were also observed via each transducer (Table 3). Significant variations in fish biomass were observed with time of day within period (P<0.001, Table 4). As the hour effect was assumed consistent across levels of the transducer factor (P=0.289, Table 4), a regular diel pattern was observed in each transducer (Figure 6). In general the fish biomass appeared negatively correlated with light intensity (i.e. fish biomass was highest at night hours and early morning).

The Hierarchical Cluster Analysis detected two distinct groups of data in terms of similar mean fish abundance (Figure 7): the upper group in the plot, including the hours of the day from 08:00 to 18:00; and the lower group, including the hours from 20:00 to 06:00.

Horizontally-aligned transducer (T1)

Even though throughout the study period the horizontal fish biomass did not vary consistently (2.40–3.05 g · m^{-3}; Table 2), highly significant differences were found among the eight sampling intervals (P<0.001). In general the data recorded by this transducer showed a gradual decrease from July to the end of September (Figure 5) and a consistent pattern with T2 and T3 data.

As for T2 and T3 the characteristic schooled distribution disappeared and abundance clearly declined rapidly within minutes of dawn. Abundance of fish inside the reef remained low throughout the day with few fish mostly near the surface (T1, Figure 6) until dusk, when a second peak of abundance occurred.

Discussion

The present study demonstrates the ability of the fixed hydroacoustic technique to get practical information on the fish assemblage living inside and around artificial reefs. In fact, as well as providing the evaluation of fish biomass, it gives data on the distribution of the fish population inside the reef and its daily behaviour. It also shows the area of influence of the artificial substrates on the surrounding habitat and thus provides useful data for the management and planning of the existing Adriatic artificial reefs.

The values of biomass and the distribution of fish obtained corroborated the earlier findings of Bombace *et al.* (1997), who described the Senigallia reef fish

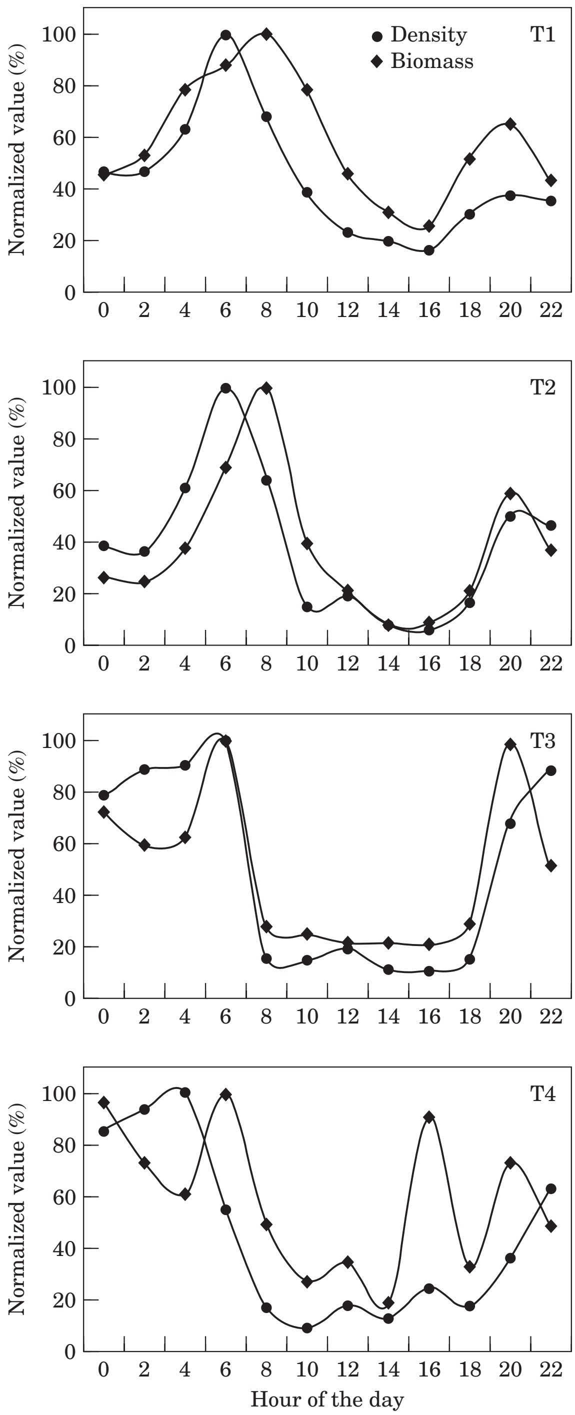

Figure 6. Mean fish density and biomass recorded by the four transducers in the different hours of the day. For each transducer the mean fish abundance (density and biomass) at each hour were normalized dividing by the maximum mean value.

assemblage using visual census and trammel net sampling, and confirmed the usefulness of these structures in the Adriatic sea in aggregating and managing the coastal fish resources. The values recorded by the off-reef transducer were generally lower than those collected by the inner ones, showing that the reef effect on the fish assemblage was already reduced at about 80 m from the structures. This agrees with the results of other studies indicating that the local area of influence of an artificial reef may range from 5 to 50 m, depending on the local environmental conditions and the reef size (Continental Shelf Associates, 1982; Gerlotto *et al.*, 1989; Fabi *et al.*, 1999b). Moreover the fish abundance did not appear to be homogeneously distributed inside the reef: the highest densities being recorded in the central part of the area, where there is a higher concentration of structures. As with previous studies (Fabi and Fiorentini, 1994), the acoustic records also confirmed that in late summer – early autumn most of the reef-fish species migrate from the coastal shallow waters to offshore, where during the winter months the water temperature is about 10–12°C.

The project gave useful information about the daily behaviour of the fish assemblage living inside the reef. According to Thorne *et al.* (1990), a density minimum was generally recorded during the early afternoon, with the highest abundance commonly observed late in the evening, night and early morning. Such diel variability of fish biomass might be connected either with fish horizontal migrations or with variability of fish acoustic response, expressed by TS-variability. However, as demonstrated by MacLennan *et al.* (1989, 1990), the "Target Strength" of fish species may change with time or between individuals, due to behavioural or physiological reasons which are not well understood. Because of this it is necessary to think of "Target Strength" as a stochastic parameter which is described by a probability distribution. Therefore the result of any "Target Strength" measurement is unpredictable. As far as the practice of acoustic surveying is concerned the inherent variability of "Target Strength" due to metabolism processes is not as important in the calculation of fish density as the expected value, that is the mean of the probability distribution (MacLennan and Simmonds, 1992). In the present study this value was determined on the basis of observable characteristics such as the species composition of target population and the size of the individuals. Behaviour of fish, which can be expressed by modulation of tilt angle, could have a significant influence on constants in TS-to-weight formulas, but the large changes observed for individual fish will tend to be cancelled when the TS-tilt functions of all the detected fish are combined (Fedotova and Shatoba, 1983; MacLennan *et al.*, 1989).

Finally, a considerable number of Target Strength experiments have been conducted to investigate its variation in relation to environmental and biological factors

Table 4. Results (probability levels and degrees of freedom in parentheses) of ANOVA conducted on the mean fish biomass (g/m^3) recorded by the upward-oriented transducers (TRA; T2,T3,T4) during the eight sampling intervals (INT: S1,S2,S3,S4,S5,S6,S7,S8). In the 3-ways ANOVA, the factor "hour of the day" (HOUR) was used in order to randomize unsuspected sources of variation in the effects of the other two predicted variables (TRA,INT).

3-ways ANOVA				1-way ANOVA			
				S1	(1,22)	0.096	
				S2	(2,33)	0.000**	T3>T4; T2>>T3,T4
				S3	(2,33)	0.000**	T2>>T3,T4
TRA	(2,55)	0.000**	T3>>T4 T2>>T3,T4	S4	(2,33)	0.000**	T2>>T3,T4
				S5	(2,33)	0.012*	T2>>T4
				S6	(2,33)	0.028*	T2>T4
				S7	(2,33)	0.000**	T3>T2>T4
				S8	(2,33)	0.304	
				T2	(7,88)	0.000**	S2,S4>>S5,S6,S7,S8
INT	(7,55)	0.000**	S1,S2,S4>>S3,S5,S6,S7,S8	T3	(7,88)	0.021*	
				T4	(6,77)	0.730	
HOUR	(11,55)	0.000**					
TRA*INT	(13,55)	0.000**					
TRA*HOUR	(22,55)	0.289					

*$0.01<p<0.05$.
**$p<0.01$.

(Orlowski, 2000). The hydrographic factors such as water pressure, oxygen level, salinity, tides, light and water temperature strongly regulate processes inside the fish body but there are important differences between species (MacLennan and Simmonds, 1992). Therefore when more species are found in mixed pools as on the artificial reef investigated in the present study, the Target Strength exhibits a great deal of variation. The implication is that it may be convenient to summarize the analysis by quoting the regression TS-weight parameters depending only on the mean size of the mixed-species pool of fish (MacLennan and Simmonds, 1992).

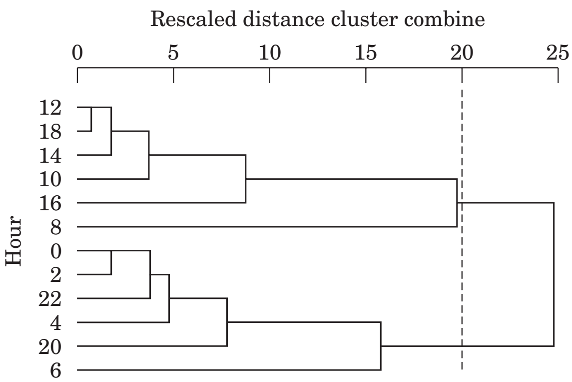

Figure 7. Group-average sorting dendrogram showing the measure of the linkage distances for clustering the Normalized values of the fish abundance (density and biomass) calculated at each hour of the day.

Suggestions for future studies

From a technical point of view several difficulties were found because the equipment had been previously used in areas (lakes, rivers and shallow-water sea sites) where a cable connection was possible. As most of these difficulties were solved the system used may be a suitable additional technique to evaluate fish biomass around artificial structures (artificial reefs, gas and oil platforms, etc.) located in open sea areas.

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