

Diel rhythms in shallow Mediterranean rocky-reef fishes: a chronobiological approach with the help of trained volunteers

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Behavioural rhythms in marine species have been mostly investigated in laboratory organisms and their expression within the animals’ natural environments remains largely unknown. Here, we studied diel (i.e. 24-hours-based) and intra-diel (i.e. 12-hours-based) rhythmic variations in the abundance of seven shallow rocky-reef fish species, namely Coris julis, Epinephelus marginatus, Sarpa salpa, Serranus cabrilla, Serranus scriba, Sparisoma cretense and Thalassoma pavo, along the rocky shores of Linosa Island (Mediterranean Sea). Data were visually collected by trained volunteers along fixed transects at 3-hourly intervals throughout six consecutive 24-hours periods. Density estimates can vary greatly between consecutive days and during 24-hours periods according not only to the major day–night changeover but also to minor intra-diel variations at the daylight hours. In the case of T. pavo, C. julis, S. cabrilla and S. salpa waveform analyses showed midday troughs in abundance within the 24-hours period but significant variation within the hours of daylight was highlighted only for T. pavo. Although results were not conclusive at the intra-diel level, the employment of volunteers represented a valuable tool for chronobiology, suitable to improve our understanding of fish behaviour in natural systems.

Keywords: rocky-reef fishes, activity rhythms, visual census, waveform analysis, Fourier analysis, Mediterranean Sea, Citizen Science

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INTRODUCTION

All living organisms studied thus far exhibit rhythms at different levels of their biological organization provoked by the rotation of the Earth on its axis and the relative position of the sun and moon (i.e. geophysical cycles: Aschoff, 1981). In oceans, these cycles take the form of day–night light intensity variations or currents speed variations, as the product of tidal pulls (reviewed by Aguzzi *et al.*, 2010). Accordingly, marine animals vary their activity with a diel (i.e. 24-hours-based) periodicity. That rhythmic activity may result in massive displacements and migrations of organisms within substrata or between depth zones of the water column and the continental margin (Naylor, 2005). Rhythms can also manifest as increases and decreases in the rate of swimming (e.g. fishes: Reeb, 2002), locomotion (e.g. crabs and lobsters: Palmer, 2000; Chiesa *et al.*, 2010), or crawling (e.g. polychaetes: Last *et al.*, 2009).

Chronobiology is the field of biology that examines cyclic phenomena in living organisms (Reeb, 2002). To date, this science has been seldom applied in field studies of both land and marine species (Naylor, 2005). In fact, behavioural rhythms are largely analysed in laboratory organisms, so that their expression within the animals’ natural environments

remains largely unknown (Mrosovsky & Hattar, 2005). Conversely, studies on these rhythms in the field do not often comply with daily variability (Aguzzi & Bahamon, 2009) with some exception (e.g. Curley *et al.*, 2002; Gladstone, 2007). Therefore, 24-hours-based investigations in wild populations can be of importance when estimating the potential effects of diel variability of fish communities.

Fishes are excellent models for activity rhythm studies and the rhythmicity of fish behaviour is well known under controlled conditions (as reviewed by Helfman, 1993; Kasai *et al.*, 2009; Yammouni *et al.*, 2011). However, relatively little information exists regarding fish biorhythms in the wild with the exception of gross day–night changes (Thompson & Mapstone, 1997; Willis *et al.*, 2006; Azzurro *et al.*, 2007). Among Mediterranean fish communities day–night changeover has been evidenced using beach seines, trawl surveys and hydroacoustic techniques (see Azzurro *et al.*, 2007 for a review) with documented changes in feeding or habitat types occupation from day to night (Harmelin-Vivien, 1982; Bell & Harmelin-Vivien, 1983). Nevertheless, few studies have conducted repeated sampling at different times of the day (Spyker & van den Berghe, 1995; Letourneur *et al.*, 2001; Willis *et al.*, 2006) and scant information on 24-hours cycles is available. Although the understanding of these rhythms is the goal of chronobiology, poor cross-communication, if any, exists between this science and ecology (Marques & Waterhouse, 2004; Morgan, 2004). Indeed, laboratory-based chronobiology commonly deals with time-series gathered at minute and hourly frequency

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over several consecutive days (Aguzzi *et al.*, 2010) whereas field behavioural studies are particularly difficult to perform at such frequency.

Visual census is the method used most often to analyse littoral fish communities (Harmelin-Vivien *et al.*, 1985). This technique has been occasionally used to study diel variation in Mediterranean species (Azzurro *et al.*, 2007) but some obvious complexities have emerged that make it impossible to demonstrate the existence of uniform patterns (Willis *et al.*, 2006). Large amounts of data and intensive sampling designs are required to overcome this constraint. In this context, we considered collaborating with scientists and volunteers as an efficient means to perform extensive temporally scheduled surveys (Greenwood, 1994; Pattengill-Semmens & Semmens, 2003; Bonney *et al.*, 2009). This synergy, which is termed 'Citizen Science', has recently emerged as a new environmental monitoring technique (Silvertown, 2009). Here we carried out a novel and interdisciplinary effort using a team of trained volunteers who collected data at multiple sampling cycles with intra-diel frequency. Our aim was to test for the existence of rhythmic patterns in the abundances of rocky fish species and to describe this variation. Specifically, we wanted to: (1) test for the presence of significant variability at the diel (i.e. 24-hours based) and intra-diel (i.e. 12-hours based) level; (2) gain an overview on the general character and robustness of the species rhythms; and (3) to statistically assess their phasing.

MATERIALS AND METHODS

Study area and the studied species

The study was performed along the rocky shores of Linosa (35°85'N 12°85'E), a small volcanic island located in the middle of Sicily Strait (Mediterranean Sea), 165 km from the African coast and 167 km off the coast of Sicily (Italy). Linosa was declared a Marine Protected Area in 2002. Two different locations at Linosa Island (Figure 1) were surveyed between 27 June and 2 July 2010. At that time, sunset and sunrise were at 5:47 and at 20:30 h, respectively.

We selected 7 target species on the basis of: (1) their ecological relevance; (2) their abundance in the study area; and

(3) the feasibility of their visual identification (during both day and night). The species were: the dusky grouper *Epinephelus marginatus* (Linnaeus, 1758); the ornate wrasse, *Thalassoma pavo* (Linnaeus, 1758); the parrotfish, *Sparisoma cretense* (Linnaeus, 1758); the rainbow-wrasse, *Coris julis* (Linnaeus, 1758); the painted comber, *Serranus scriba* (Linnaeus, 1758); the comber, *Serranus cabrilla* (Linnaeus, 1785); and the salema, *Sarpa salpa* (Linnaeus, 1758). *Epinephelus marginatus*, *T. pavo* and *S. cretense* have recently widened their distribution, a fact that may be linked to their thermophilic habit (Azzurro *et al.*, 2011). *Coris julis*, *S. scriba*, *S. cabrilla* and *S. salpa* are common and widespread throughout the Mediterranean, although *S. salpa* is now becoming rare in many areas of the Levant Basin (Bariche *et al.*, 2004).

Trained volunteers and sampling procedures

Thirty zoology graduates (Bachelors in Biology and Natural Sciences of the University of Pavia, Italy) divided into two groups of 15, volunteered to collect the field data. Operators were intensively trained in fish recognition for two days before sampling. Sessions included species identification, examinations and visual trials based on established practices of Citizen Science (e.g. Pattengill-Semmens & Semmens, 2003). The efficiency of the volunteers in species identification and counting was corroborated by an experienced scientist, co-authoring the present paper, who carried out preliminary trials, in tandem with each volunteer. After the surveys, the collected data were verified to test for possible outliers.

In each sampling area, 6 randomly chosen transects approximately parallel to the coast were permanently marked with a coloured tape 50 m in length, fixed on the bottom at 3 m depth. Fish censuses were performed by swimming along the centre line of each transect and counting all individuals for each targeted species within 2.5 m on either side (i.e. approximately 250 m² of total area) for a maximum time of 5 minutes. Transects were surveyed every three hours by observers who were randomly chosen using the random number generator application of Minitab 12.0 Student Edition Software.

In each location, sampling was performed over 8 temporal windows of 30 minutes each centred around the following times: 5:30, 8:30, 11:30, 14:30, 17:30, 19:30, 22:30 and 2:30 h. Nocturnal observations were carried out using a 50 W torch, following the method of Azzurro *et al.* (2007). Temporal censuses were repeated over three consecutive days at each location. Overall, 12 permanent transects were surveyed corresponding to a seafloor area of approximately 3000 m². The total number of replicated trials was 288, corresponding to the sum of 72,000 m².

Statistical analyses

The data were square root transformed overall and an adjusted Bray–Curtis measure of similarity plus an added dummy variable (=1) was used to calculate the resemblance matrix as is appropriate for datasets containing many zeros. A non-metric multidimensional scaling (nMDS) (Clarke, 1993) based on the Bray–Curtis similarity matrix was used to visualize the ordination of samples within a three-dimensional space.

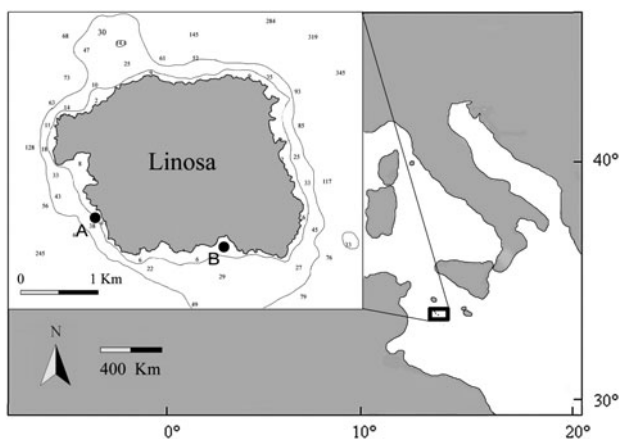


Fig. 1. Study locations (A, B) at Linosa Island (35°85'N 12°85'E) within the Central Mediterranean (Sicily Strait).

To test for the occurrence of temporal variation in the entire set of species at the scale of days and hours we used a two-way permutational multivariate analysis of variance (PERMANOVA: Anderson, 2001) considering the 3-hour time periods (i.e. the term 'Time') as fixed with 8 levels. The 24-hours cycle (i.e. the term 'Day') was considered as random with 3 levels. As the participation of single volunteers was randomized with respect to the terms 'Time' and 'Day', we assumed that data variability regarding the subjectivity of species recognition had no effect on between-treatment comparisons.

To gain an overview on the general character and robustness of the species rhythms, visual observations for all selected species were represented over time. Data sets were plotted as the average number of observations of all transects for each corresponding 3-hours period, standardized over the total transect surface (250 m²). In the graphing, the timing of sunset and sunrise were also considered.

Population behavioural rhythms are intrinsically noisy, due to the variable synchronic activity of all constituting individuals (e.g. Jadot *et al.*, 2002; Aguzzi *et al.*, 2010). To statistically quantify the consistency (i.e. repeatability) of any putative diel fluctuation, we used periodogram analysis. Significant periodicities were detected using the Lomb–Scargle periodogram, based on the least-square fitting of sine waves to the data in the time-range from 0 to 30-hours. This method provided consistent period estimation for time-series of short duration (Schimmel, 2001), such as those typically obtained in the field. In periodograms, the highest significant ($P = 0.05$) peak represented the maximum percentage of total data variance fitted by the corresponding periodicity. The peak value was chosen for period attribution of the analysed time-series.

To statistically assess the phasing of biorhythms in relation to day–night alternation we used waveform analysis, a methodology currently used in laboratory chronobiology (Fernández *et al.*, 2009). Time-series were subdivided into sub-sets of 24-hours duration (i.e. 8 time-interval values in each). The data for all subsets were averaged based on corresponding time periods. Averages and their standard deviations were represented as a consensus plot over 24-hours (i.e. the waveform). A daily mean was estimated by re-averaging all waveform values. The resulting estimate was represented on the waveform plot as a threshold line. All waveform values above this line indicated a significant increment in population activity rhythm. The threshold line also indicated the temporal limits of peaks allowing the activity timings of different species to be compared with each other (Aguzzi *et al.*, 2009). This procedure was carried out similarly to the Mean Estimate Statistic Of Rhythm procedure (MESOR: Aguzzi *et al.*, 2006). MESOR is the value midway between the highest and the lowest values of a cosine function best fitting rhythmic time-series.

The percentage of activity occurring during daylight in relation to the total activity that each animal carried out throughout the 24-hours period was calculated based on individual waveforms and used to assess either the diurnal or nocturnal activity distribution. Behavioural patterns were identified as diurnal when photophase activity was greater than 60% and as nocturnal when less than 40% (Chiesa *et al.*, 2010).

To explore the occurrence of intra-diel activity patterns we used Fourier analysis because it can detect the 12-hours peak patterns in rhythms that show certain variability over

consecutive days (Díez-Noguera, 2006). Time-series were analysed with that technique by setting both a fundamental harmonic with a period of 24-hours to study the diel variation and setting its submultiple at 12-hours to study the intra-diel variation. The minimum square fitting of these cosine functions onto consecutive 24-hours data sections of species time-series was estimated. For regularly sampled series, the quadratic power of the amplitude of each cosenoidal function (i.e. the harmonic) obtained from the Fourier decomposition is defined as the harmonic power content (PC). This value can be expressed as the percentage of the total variance in the time-series explained by the least-squares fitting of each harmonic (Díez-Noguera, 2006). The PC was obtained as the percentage of variance of the time series segment explained by these harmonics (PC₂₄ and PC₁₂). A repeated-measures analysis of variance (ANOVA) was designed to study differences in PC values between days and species as factors after having verified the assumptions of this test. Two-way ANOVA was also designed to study the PC harmonics and the species as factors. Multivariate analyses were performed with the PRIMER 6 + PERMANOVA software package from Plymouth Marine Laboratory, UK. The software package STATISTICA 8.0, from StatSoft, Inc, was used for univariate analyses.

RESULTS

The mean number of individuals per species at each transect of the two sampling areas is listed in Figure 2. The most abundant species were, in order of abundance, *T. pavo*, *C. julis*, *S. cabrilla*, *S. scriba*, *S. salpa*, *S. cretense* and *E. marginatus*.

Multivariate analyses

The day–night changeover was well represented by the nMDS time periods (Figure 3) which showed a clear-cut separation among diurnal and nocturnal surveys and PERMANOVA outputs (Table 1) showed significant differences for the factor 'Time' in both locations A and B. On the other side, 12-hours based patterns were not apparent in the multivariate ordination with no clear separation between within-night nor

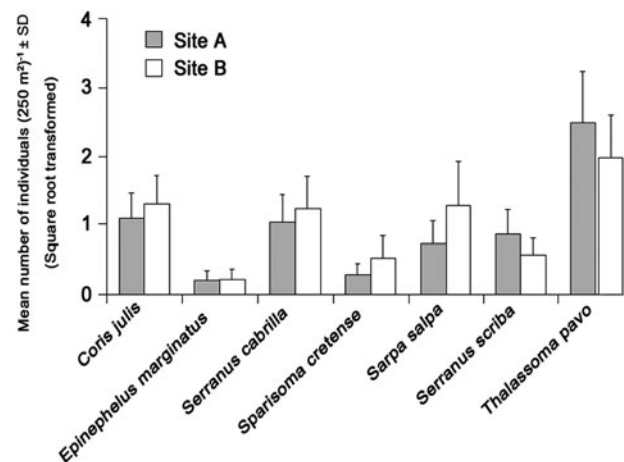


Fig. 2. Square root-transformed mean abundances (number of individuals in 250 m²) of target fish species registered at locations A and B (see Figure 1).

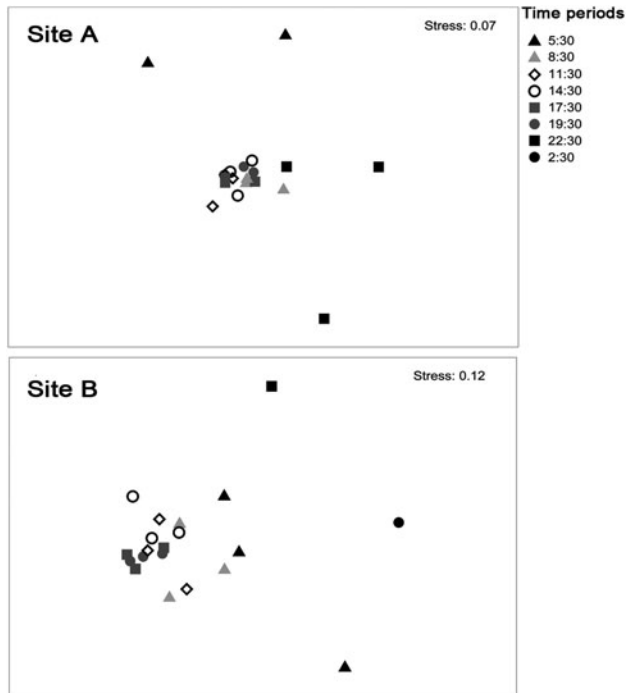


Fig. 3. Non-metric multi-dimensional scaling three-dimensional ordination of the site's centroids comparing the species recorded during the 8 periods. The replicate symbols indicate the 3 days for each time period.

within-day trials. Significant between-days differences were observed only at location A, and a significant interaction between 'Time' and 'Day' was highlighted at both sites.

Chronobiological analyses

As expected, all the targeted fish species showed a sharp day–night fluctuation in their counts (Figure 4). Higher abundances were apparent during daylight hours, with minima commonly recorded during the central darkness hours. Lomb–Scargle periodogram analysis detected significant diel periods (i.e. close to 24-hours) in three of the studied species. *Coris julis* presented a peak corresponding to a period of 24.9 hours (i.e. 24-hours, 54 minutes). *Serranus cabrilla* exhibited a peak with a period of 23.30 hours (i.e. 23 hours, 27 minutes). *Thalassoma pavo* showed the highest peak of all species, with a period of 23.60 hours (i.e. 23

Table 1. Permutational multivariate analysis of variance based on the Bray–Curtis dissimilarity measure for square root-transformed abundance data in locations A and B. The test was performed using 9999 permutations under the reduced model. Values for different levels of significance are reported as follows: *, $P < 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

| Source | df | A | | B | | Pseudo-F |
|------------|-----|--------|----------|--------|----------|----------|
| | | MS | Pseudo-F | MS | Pseudo-F | |
| Time | 7 | 19.147 | 10.91*** | 20.440 | 20.82*** | |
| Day | 2 | 2.215 | 4.77*** | 215.1 | 0.39 | |
| Time × Day | 14 | 1.755 | 3.78*** | 981.5 | 1.80*** | |
| Residuals | 120 | 464.1 | | 544.8 | | |
| Total | 143 | | | | | |

hours, 36 minutes). No significant peak was found in the periodogram of the remaining species given the low levels in visual counts (see Figure 4). Waveform analysis reported the phase onset and phase offset of significant (i.e. above the daily mean) increases in visual count for all targeted species (Figure 5). These coincided with the sampling time intervals that included sunset and sunrise.

A weaker bimodal phase with a drop in correspondence with central hours of the day was apparent, although not statistically significant, in waveform profiles of *C. julis*, *S. cabrilla*, *S. scriba*, and finally *T. pavo* for which PERMANOVA showed instead significant variation within the daylight for the term 'Time period' (Pseudo $F = 2.223$, $P \text{ perm} < 0.05$) and a significant interaction between the terms 'Day' and 'Time' period (Pseudo $F = 2.5047$, $P \text{ perm} < 0.01$). The term 'Day' was not significant for this species (Pseudo $F = 1.0985$, $P \text{ perm} < 0.05$).

Waveforms featured high activity percentages during daylight hours in all species: *C. julis*, 78%; *E. marginatus*, 75%; *S. cabrilla*, 80%; *S. cretense*, 82%; *S. salpa*, 74%; *S. scriba*, 73%; and *T. pavo*, 82%.

Fourier analysis provided the percentage of variance explained by both 24-hours and 12-hours harmonics (i.e. the PC) in time-series sections of one-day duration (Figure 6). ANOVA indicated the occurrence of similar values of PC24 and PC12 for all days in all species. In contrast, comparisons among different species presented significant differences (PC24-hours: $P < 0.05$ for the repeated measures, $P < 0.0001$ for the species; PC12: $P < 0.05$ for the repeated measures, $P < 0.001$). Significant differences were found considering the harmonics and the species as factors in a two-way ANOVA (for the harmonics: $P < 0.0001$; for the species: $P < 0.0001$).

DISCUSSION

Diel variability

Multivariate analysis showed an expected sharp day–night changeover in the group of chosen species. Waveform analysis was used to evaluate both the phase and the amplitude stability of the 24-hours activity pattern in our selected species based on the variability of the averaged visual count bins in relation to the daily mean (i.e. as significant increments or decrements).

First, assuming that our result is more accurate for those species showing higher abundances, a diurnal temporal niche was observed with high activity percentages (i.e. greater than 70%) during daytime in all species. Waveform profiles depicted sharp 24-hours cycles for *C. julis*, *S. cabrilla* and *T. pavo*, the 24-hours rhythms of which were statistically proven by periodogram analysis. This was also confirmed by the Fourier analysis, where the 24-hours harmonic component showed better time-series fits for these species. In some cases, these results confirm previously published behavioural observations. For example, *C. julis* leave just before dusk and return to the foraging area around dawn; in aquaria, it buries itself within the sand at night (Videler, 1986). Similarly, *S. scriba*, seems to decrease in activity at night, when many individuals were observed remaining in hiding under *Posidonia oceanica* leaves (March *et al.*, 2010). A decrease in the nocturnal abundance of *T. pavo*, *C. julis*, *S. scriba*, *S. cretense* and many other

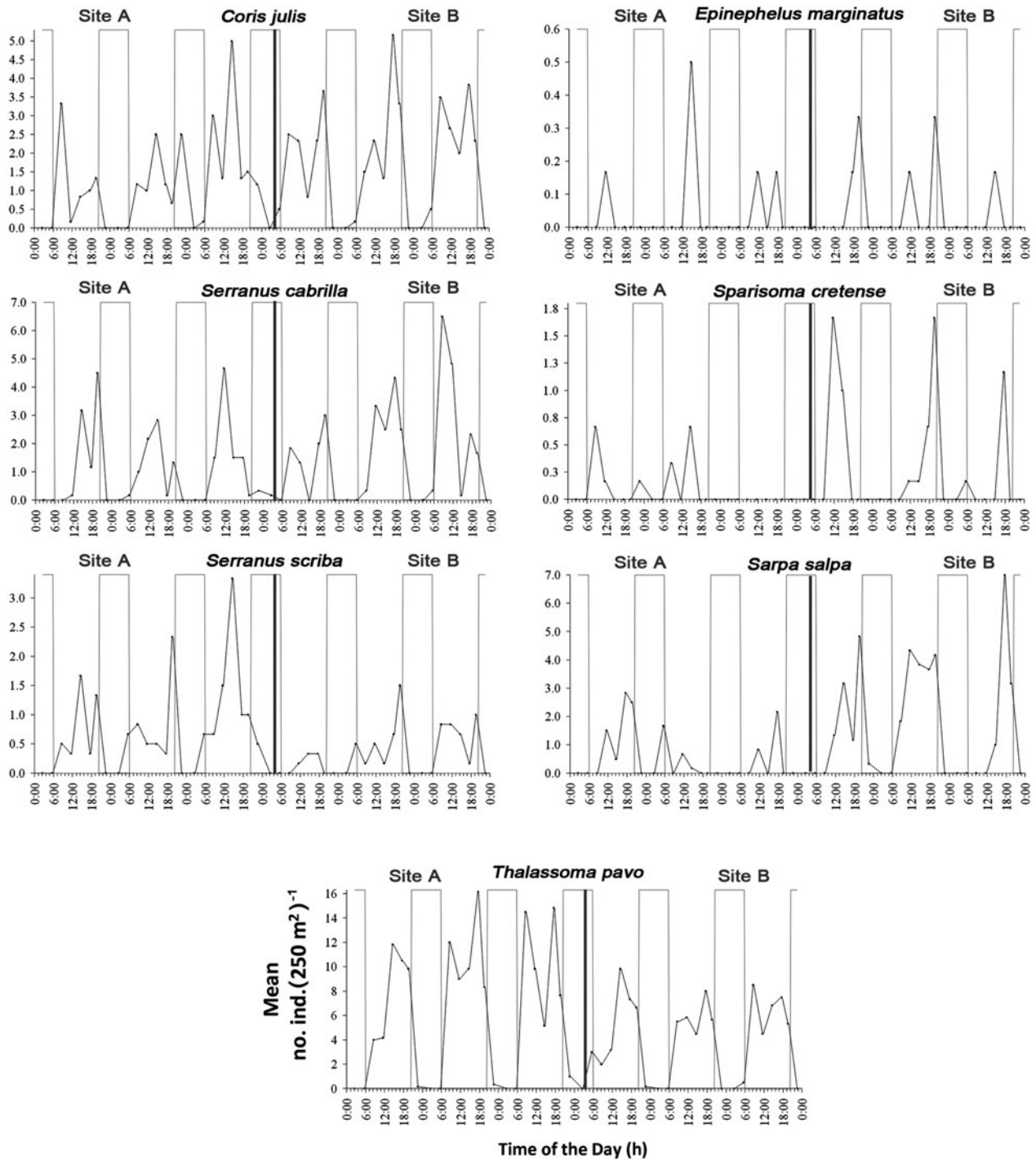


Fig. 4. Time-series of visual observations (standardized for transect total area) for all selected species for the two study locations (A and B; see Figure 1). Vertical dashed lines delimit visual counts for the two locations. Grey vertical rectangles represent night.

rocky-reef fishes was observed by Azzurro *et al.* (2007), but without any further observations regarding their rhythmic activity.

Periodogram analysis of *S. scriba* and *S. cretense* failed to show any significant diel rhythm. Regardless, a diurnal niche is also evident based on waveform analysis for these species. The non-significant periodogram outputs are reasonably explained by the interaction between the terms 'Day' and 'Time' in our design (see Table 1), whereas for *E. marginatus*,

lower count values are responsible for the non-significant periodogram results (Schimmel, 2001).

Diel fluctuations in the counted fishes reported in this study can be explained in terms of changes in the behaviour of the constitutive individuals, which depend on the day–night alternation. Even though the observed nocturnal drop in counts is mostly related to the inactivity and sheltering of the studied species in response to darkness (Azzurro *et al.*, 2007), these variations could also occur in relation to habitat

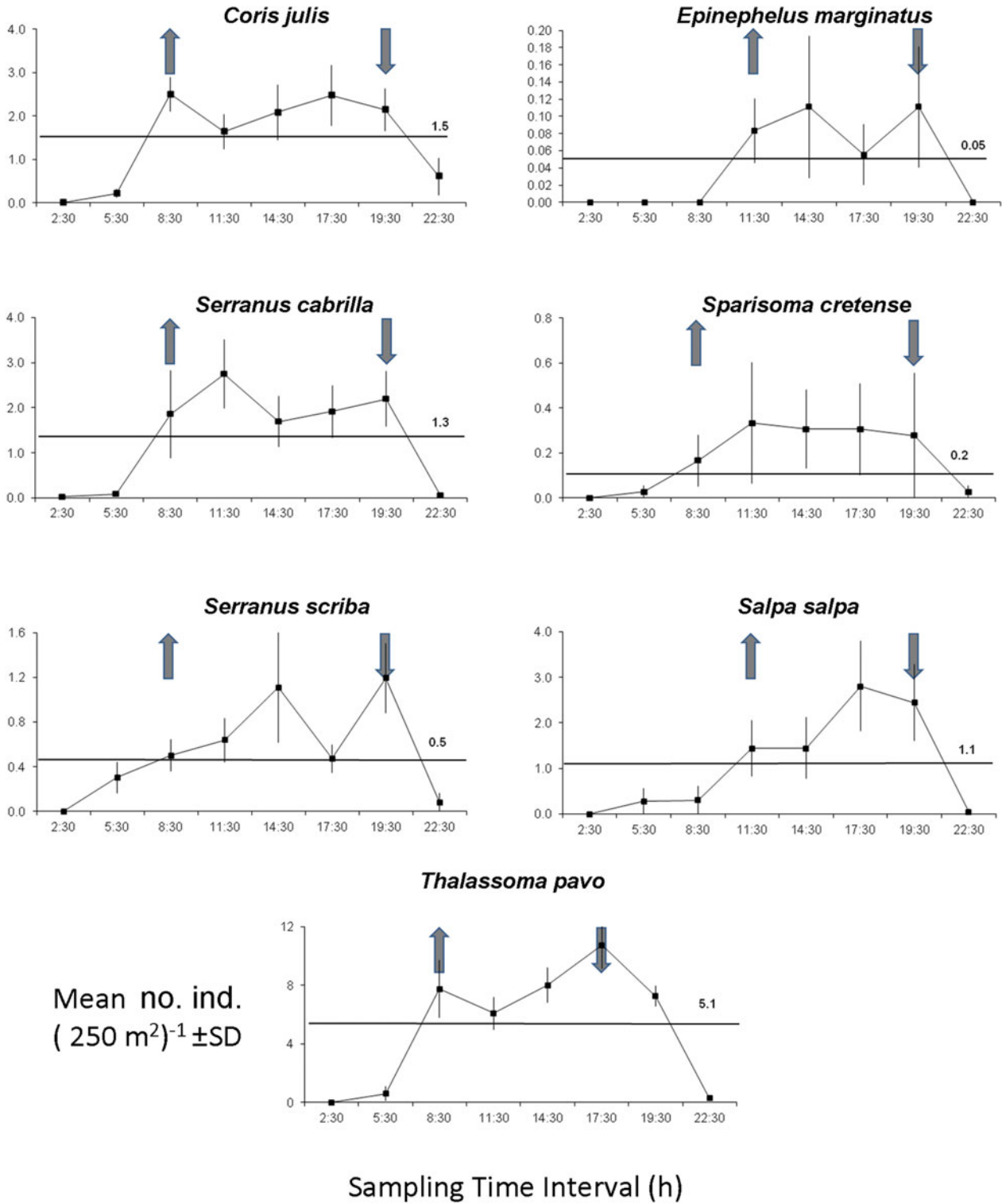


Fig. 5. Waveform analysis findings for the time-series of visual observations of the selected species. The horizontal line is the daily mean, and values above that line represent significant increases in visual counts (i.e. the phase). Upward and downward arrows indicate the first and the last activity value above the daily mean, representing the timings of the waveform peak onset and offset, respectively.

use. This includes bathymetric shifts (Spyker & van den Berghe, 1995; Colmenero *et al.*, 2010), which may depend on changes in feeding behaviour (Piet & Guruge, 1997; Letourneur *et al.*, 2001; Carpentieri *et al.*, 2006) and predator avoidance (Copp & Jurajda, 1993; Arrington & Winemiller, 2003).

Intra-diel variability

While both, multivariate and univariate analyses were highly effective in detecting day–night variation, our study provided only a weak evidence of the existence of an intra-diel pattern in the counts of rocky-reef fishes. This finding would confirm

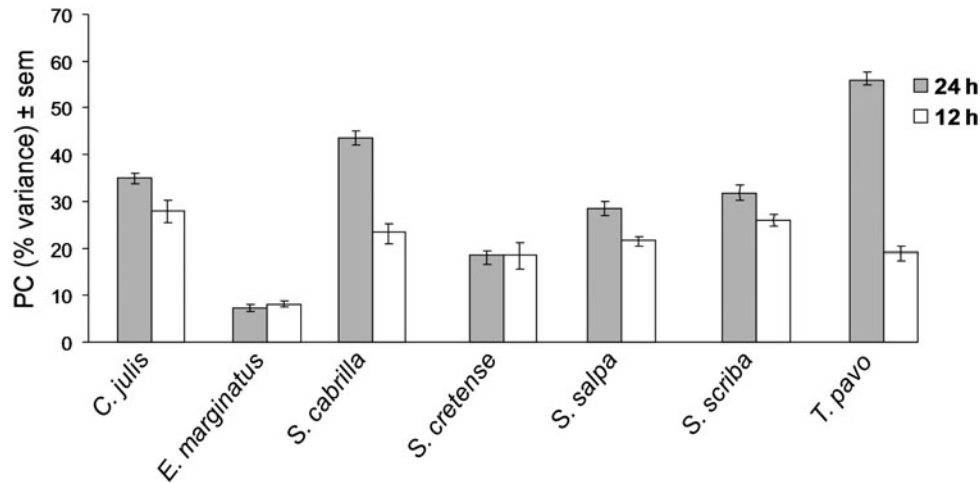


Fig. 6. Fourier analysis findings as the mean (\pm SEM) power content (PC) obtained for all species by the fitting of two harmonics of submultiple periods (i.e. 24-hours, PC₂₄ and 12-hours, PC₁₂) on visual count time-series segments of 24-hours duration.

a general and well-known difficulty in extrapolating intra-diel variability in fish assemblages globally (reviewed by Willis *et al.*, 2006). Certainly, the elevated between-transect variability of visual counts was the basis of this constraint. Even if the term ‘Time’ was highly replicated in our design and the sample unit area was doubled with respect to standard strip-transects (Harmelin-Vivien *et al.*, 1985), the statistical power of our tests was not sufficient to detect multivariate signals at the intra-diel level. Other unexplored sources of variation, including individual changes in mobility (Jadot *et al.*, 2002), could have masked the existence of a main effect at the intra-day level. Nevertheless some weak but apparent signals were detected in three of the study species. In fact, waveform analysis showed midday troughs in *C. julis*, *S. scribe* and *T. pavo* with a significant within-day variation for *T. pavo*. As a matter of fact, midday troughs in abundances have been highlighted for many marine organisms because of parallel decreases in their behavioural activity (as reviewed by Aguzzi *et al.*, 2010). These decreases in animal activity at central photophase hours could be associated with crepuscular peaks in activity rhythms, as documented for some marine fishes (Jadot *et al.*, 2002 and references therein). *Sarpa salpa* represents another example of within-day variability. Waveform analysis showed minimal abundance during the first diurnal time interval (Figure 4) possibly due to a late beginning of activity (Jadot *et al.*, 2006) or to regular migrations within the home range (Jadot *et al.*, 2002). For the remaining species, no intra-diel pattern could be established.

Certainly, this study should be replicated on a wider spatial scale to verify the coherence and stability of observed patterns and eventually to investigate the causative factors. Fourier analysis indicated an elevated value of PC₂₄ that was not significantly different from the PC₁₂ in all species (see Figure 6). In some cases, both values were similar, suggesting consistency of the bimodal fluctuation within the overall 24-hours fluctuation by peak splitting. The results of the 12-hours harmonic fitting suggest the occurrence of crepuscular peaks in the time-series for *C. julis*, which featured the highest PC₁₂ value and a clear 12-hours peak related to sunrise in the waveform.

Intra-diel fluctuations in visual counts could be the product of differential swimming activity at certain times of the day,

which rendered individuals more or less visible in key moments of the photophase (Hobson, 1965; Ebeling & Bray, 1976; Colton & Alevizon, 1981). Moreover, the disturbance created by the observer cannot be completely disregarded. In fact, the behavioural response of fish to the diver is expected to vary at different times of the day, and this might contribute to count variability (Thompson & Mapstone, 1997).

The contribution of trained volunteers

The engagement of trained volunteers was crucial to performing a high ‘Time’ level replication within treatments and to collect substantial amounts of data in a short period of time. The manpower provided by this group of observers served to satisfy chronobiological sampling requirements. Although the use of volunteers is not novel in biodiversity (Evans *et al.*, 2001) and ecological (Foster-Smith & Evans, 2003) studies their employment can be considered original for chronobiology. It allowed us to approach fish in their natural environment.

A common concern regarding Citizen Science is the quality of data. As a matter of fact, Citizen Scientists may vary in their skill, compared to professional observers. This source of bias can be reduced much after personalized training and with the adoption of simple methods, adequate sampling efforts, improving identification of species and comfortable habitats to sample (Dickinson *et al.*, 2010). On the other hand, a limited expertise of contributors and complicated tasks should be discouraged, since it can lead to the collection of poor quality or even misleading data (Fitzpatrick *et al.*, 2009). When simple and standardized protocols are used, trained volunteers can provide data of comparable quality to professionals (Gillett *et al.*, 2012 and references therein). In this respect, marine fish have received considerable attention by Citizen Science through the use of volunteers to collect data regarding species occurrence and distribution (reviewed by Stallings, 2009; Ward-Paige *et al.*, 2010, 2011). We engaged volunteers in a specific training and conceived a strict and easy protocol that guided them in their visual counts. Data were pooled from multiple observers with broadly similar levels of experience and this usually helps to guarantee data quality (Williams *et al.*, 2006). Certainly,

individual differences in the efficacy of observation (e.g. Lincoln-Smith, 1988) might still have contributed to augment the variability in counts, but the random selection of volunteers with respect to the design factors eliminated the possibility of bias related to this factor.

The characterization of 24-hours patterns in visual count data for wild rocky-reef fishes is a challenging research target. In this paper, we provide evidence that density estimates can vary greatly during 24-hours periods according not only to the major day–night changeover but also to minor intra-diel variations observed during daylight hours. Chronobiological analyses, although far from conclusive, helped us to illustrate bimodal cycles within the 24-hours period, at least for the most abundant species such as *C. julis*, *S. cabrilla* and *T. pavo*. These regular fluctuations are probably species specific and possibly related to different activity rhythms or to a different use of space with an effect on their abundance and visibility to divers. This must be taken into account for performing reliable fish visual census estimates. Nevertheless we showed that intra-diel temporal factors can be particularly weak with respect to other sources of variability of these species on a local scale, such as to the between-day variability. Together, these results show promise with regard to identifying significant intra-diel patterns in wild littoral fishes. The presence of such regulation deserves to be further investigated in field studies possibly for longer durations of time (>6 days). The present study shows that sophisticated laboratory chronobiology paradigms can be efficiently used in the context of field studies with natural populations. Hopefully, this methodological transfer from laboratory analysis to field sampling will help us to clarify diel patterns in littoral fishes, which remains one of the most neglected sources of variability in the assessment of natural populations. The future use of trained volunteers in chronobiology studies could contribute to improve our understanding of fish behaviour.

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