

## Diel variability in counts of reef fishes and its implications for monitoring

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**Abstract** Studies of reef fish assemblages in space rarely consider the effects of temporal variability on spatial comparisons, and when they do, usually examine timescales of months to years. The nature of fish monitoring surveys is such that particular locations may be surveyed at one time of day, and surveys designed to establish the degree of spatial variability in assemblages may be confounded if the order of sampling within treatments is not randomised with respect to time of day. In this study, we tested the degree of temporal variability in temperate reef fish counts at the same sites in New Zealand and Italy, within and between days. Repeated counts separated by months returned quite different assemblages, whereas counts separated by days did not. Although overall assemblage structure did not generally differ significantly with time of day, at some locations there was evidence of changes in the relative density of certain species in the afternoon relative to the morning. Care must be taken not to introduce systematic bias to spatial comparisons due to behavioural variability in fishes at differing times of day.

*Keywords:* survey bias, marine reserve, monitoring, reef fishes, spatial pattern, temperate reef, temporal variability

## 1. Introduction

In surveys of reef fishes, it has not been often acknowledged that differences in the behaviour of fishes at different times of day may potentially influence relative density estimates (Colton & Alevizon 1981; Spyker & van den Berghe 1995). While some aspects of temporal variability in abundance or behaviour that operate over annual or seasonal time scales, such as recruitment, the formation of spawning aggregations or seasonal migration (Williams 1983, Tolimieri et al. 1998, Hyndes et al. 1999) may be more obvious, others may be subtle and operate over shorter time scales of hours to days. Reproductive behaviour can cause fish to aggregate and disperse at specific localities within a day (e.g. Samoily 1997), and the timing of such aggregations can vary considerably between species and mating systems (Colin & Bell 1991). Peaks in feeding activity may vary diurnally (Polunin & Klumpp 1989) and affect spatial distributions (Ogden & Buckman 1973; Warner 1995; Milazzo et al. 2005), or distributions of fishes may be influenced by the state of the tide. In particular, reef-associated planktivores tend to distribute themselves according to the availability of their food supply, and changing tidal or wind-driven currents can have marked effects (Kingsford & MacDiarmid 1988, Badalamenti 1990). It is well known that most marine habitats contain fish species that exhibit marked crepuscular peaks in activity or changes in distribution (e.g. Hobson 1965, Ebeling & Bray 1976, Colton & Alevizon 1981, Doherty 1983).

The nature of fish monitoring surveys designed to detect spatial pattern at medium to large spatial scales is such that particular locations are generally surveyed one by one by a diver or group of divers, and therefore counts from any particular site are usually available from only one time of day (e.g. Choat et al. 1988; Willis et al. 2000; Gust et al. 2001; Anderson & Millar 2004; and practically all other studies ever published). Replicating counts at the same location at a different time within the same survey is generally beyond the scope of most studies, as it means sacrificing spatial replication and stretching the resources available to complete the survey.

If different sites within treatments (e.g. location, habitat type, protection status) are randomised with respect to time-of-day, any effect of differing behaviour or activity level in the fish would not be expected to influence between-treatment comparisons. However, changing weather conditions or logistic considerations may mean that complete randomisation of sites with respect to time may not always be possible. Factoring out bias

related to the time of day may require high site-level replication within treatments, given the normally high between-transect variability seen in reef fish counts. Certainly, differences in the time of day when transects are conducted may confound between-location comparisons within particular treatments, or increase within-treatment variability such that the statistical power of between-treatment comparisons is significantly reduced.

Few published studies to date have conducted repeated counts of fish assemblages at the same sites over short time scales with the specific aim of assessing within-day variability (but see Colton & Alevizon 1981; Spyker & Vanden Berghe 1995; De Girolamo & Mazzoldi 2001; Thompson & Mapstone 2002). These studies did not detect significant short-term temporal variability in assemblages of temperate and coral-reef fishes on time scales of days to weeks. However, there was evidence of systematic within-day variability in the detectability of certain species. In this study, done on temperate reefs in New Zealand and Italy, sites were surveyed two or three times during the same day by divers using visual transect counts, to determine whether time-of-day influenced estimates of density and assemblage structure for making spatial comparisons. In New Zealand, two of the four sites were surveyed three days prior, to determine if there was significant between-day variability within the same survey period. In Italy, counts were repeated after 3 months to determine the extent of medium-term temporal variability.

## **2. Materials and methods**

### *2.1 New Zealand*

Fish counts were conducted at the Poor Knights Islands, New Zealand (35°26'S, 174°45'E), at the beginning (September 1998) of a long-term monitoring study to assess the effects of implementing no-take marine reserve protection (Denny et al. 2004). Two teams of 2 divers estimated the density of reef-associated fishes using 25 × 5 m strip transects at two “shallow” (Nursery Cove, 6-10 m; Aladdins Cave, 7-14 m) and two “deep” (Labyrinth, 18-26 m; Labrid Channel, 10-24 m) sites located at the south-western part of the islands. Transects were restricted to 25 m in length so as to reduce the likelihood of crossing habitat boundaries. Each of the two teams surveyed the same two of the four sites within each time period. Six replicate transects (three transects per diver) were done at each site for each time, generally over kelp (*Ecklonia radiata* (C. Agardh) J. Agardh) forest. The same divers surveyed the same transects

on each occasion to remove the potential for observer-specific bias to confound temporal comparisons. Counts were conducted at c. 0730-0830 hours, and repeated at 1030-1130 and 1400-1500 hours. To estimate between-day variability in fish counts, two of the sites (Labyrinth and Nursery Cove) were surveyed once (at 0900 and 1200 hours, respectively) three days prior to the within-day comparisons by the same divers. Water visibility exceeded 15 m for all counts. Time of sunrise during the sampling period was c. 0610 hours, and time of sunset was c. 1820 hours. Transects were conducted by swimming out a tape attached to the substratum. Counts began after a 5 m lead-in to avoid counting fish attracted to the diver while the tape was affixed.

Very common schooling species that require more specialised census methods (the pomacentrid *Chromis dispilus*, the scorpid *Scorpius violaceus*, and trachurid *Trachurus spp.*) were omitted from the counts to avoid biasing estimates (from observer error) of demersal species that were the focus of the study.

## 2.2 Italy

Reef-associated fishes were counted at Punta Galera, Ustica Island (38°42'N, 10°43'E) in June and September of 2002 using 25 × 5 m transects on rocky reef within two depth ranges: one “shallow” (1-8 m) and one “deep” (12-20 m). Transect methodology was identical to the New Zealand part of the study. Each team conducted four replicate transects at each depth. In June (sunrise c. 0550 hours, sunset c. 2045 hours), counts were conducted in the morning from 0900 to 1100 hours and repeated in the afternoon (1630-1830 hours). In September (sunrise c. 0700 hours, sunset c. 1925 hours), counts were performed at 0830-1030 and 1600-1800 hours. Water visibility exceeded 20 m for all counts.

## 2.3 Data Analyses

New Zealand analyses were done using a mixed-model permutational multivariate analysis of variance (PerMANOVA, Anderson 2001a), a method which allows multivariate data to be analysed on the basis of any distance measure of choice, with P-values obtained using permutations. Since PerMANOVA is sensitive to differences in multivariate dispersion between groups (which can inflate Type 1 error even when centroids have identical locations), the same models were tested for differences in dispersion using the programme PERMDISP

(Anderson in press). Since reef topography at the surveyed locations is complex and temperate reef fish assemblages generally have strong relationships with water depth (Kingsford 1989; Vacchi et al. 1998; Hyndes et al. 1999; Anderson & Millar 2004), the effects of site and time of day on counts were tested with depth included in the model as a covariate. Site was treated as a random effect, and time of day as a fixed effect. There were 28 species variables, transformed to  $y' = \ln(y+1)$  in all analyses to retain information on relative abundances but reduce differences in scale among the variables. The Bray-Curtis dissimilarity measure was used for all analyses, and all P-values were calculated using 9999 permutations of the residuals under a reduced model (Anderson 2001b).

Data from Italy were collected at one site only, but the within-day counts were repeated after 3 months. The same model was used as for the New Zealand data, with transect depth included in the model as a covariable, but in this case Month was treated as the random factor, while Time (morning and afternoon) was fixed. There were 26 species variables, transformed to  $y' = \ln(y+1)$ . Because of a limited number of unique values in the permutation distribution for Time of day, the P-value for this factor was estimated using Monte Carlo random draws from the asymptotic permutation distribution (Anderson & Robinson 2003). In the case of a significant interaction term, pairwise comparisons for all combinations of Time of day and Site, or Time of day and Month, were conducted using t-tests. P-values for these were corrected for multiple comparisons using the Bonferroni adjustment, which increases the probability of Type II error for post-hoc tests.

Differences between times and sites were examined in more detail using canonical analysis of principal coordinates (CAP, Anderson & Willis 2003). This analysis performs a canonical discriminant analysis on a subset of principal coordinate (PCO) axes from a metric (classical) multidimensional scaling (Gower 1966). A major advantage of this method is that, through the use of PCO, one can choose to base the constrained (discriminant) analysis on any distance measure that is appropriate for the type of dataset and hypotheses being addressed. In addition, species responsible for differences among groups can be identified by the strength of their correlation with the canonical discriminant axis (Anderson & Willis 2003, Willis & Anderson 2003). Only species correlations of  $|r| > 0.4$  from species with frequency of occurrence of  $> 30\%$  were considered significant.

### 3. Results

#### 3.1 New Zealand

In the between-day comparison, there were significant differences between the two sites surveyed, but not between the two days, and no significant interaction between them (Table 1). Tests of multivariate dispersion were not significant for any of the three terms ( $p > 0.2$  in all cases).

For the within-day comparisons, the effect of the covariable depth was significant, as was the random effect of site. However, the three times did not differ, and the interaction term of Time  $\times$  Site was also not significant (Table 2). Multivariate dispersions differed significantly for the effect of Site, but not Time (Table 3) which reinforces the non-significant effect of Time, but raises the possibility that the significant effect of Site seen in the PerMANOVA was an artefact of variable dispersions. *A posteriori* pairwise tests of dispersion between sites (Bonferroni-adjusted  $\alpha = 0.008$ ) showed that three of the six possible pairwise comparisons of the four sites were significantly different: counts at one of the shallower sites (Aladdins) exhibited relatively little variability with an average within-group dissimilarity (WGD) of 41.1, and was significantly different from Labrid ( $t = 4.62$ ,  $P < 0.001$ ) and Labyrinth ( $t = 4.81$ ,  $P < 0.001$ ). Counts from Aladdins may also have been less variable than from Nursery, but were not significant using the adjusted P value ( $t = 2.73$ ,  $P = 0.013$ ). Counts from the deepest site (Labyrinth) were considerably more variable (WGD = 62.5), and was significantly more dispersed than from Nursery ( $t = 2.72$ ,  $P = 0.008$ ).

An unconstrained (metric MDS) ordination of the data did not show any clear separation between any of the groups (Fig. 1a), despite the significant effect of site in the PerMANOVA. The Aladdins site did appear to be less dispersed on the plot than the others (in accordance with its low average WGD), but there was no obvious explanation for the site differences, except that the Labyrinth counts were ordinated slightly further to the right of the plot. The CAP ordination clarified this considerably (Fig 1b). The axis for Time of day could not separate any of the three times, and confirmed the lack of significant difference between them. The overall leave-one-out correct allocation success in the discriminant analysis was 34.72% (Table 4), where with three groups a correct allocation success rate of 33% would suggest a random distribution. The axis for Site, however, successfully separated the counts at

Labyrinth from the other three sites, with 16 of the 18 transect counts (88.9%) correctly allocated (Table 4). Labyrinth was the deepest of the four sites, with a mean transect depth of  $18.9 \pm 5.3$  (sd) m, which was reflected in the depth distributions of the species correlated with the Site axis. For example, the pomacentrid *Parma alboscapularis*, generally found only in depths of <10 m, was negatively correlated with the axis, whereas the latrid *Nemadactylus douglasii*, usually seen on or adjacent to deeper reefs, was positively correlated with the axis (Table 5).

Since the large amount of variability associated with site effects may have masked time-of-day effects in the PerMANOVA model, we conducted individual CAP analysis with time as the discriminant for each site. There was no difference between any of the times at the Nursery Cove and Labyrinth sites, as shown by the mixing of points in the metric MDS plots (Fig. 2, left-hand side) and similar distributions along the canonical axis (Fig. 2, right-hand side). At Labrid Channel and Aladdins Reef, however, the CAP plots indicated that the third set of counts for the day (at 14:00) differed somewhat from the two counts done earlier. At Aladdins Reef, this is also reflected by separation of these transects in the metric MDS plot (Fig. 2). Correlations of the individual species with the canonical axes indicated that the result at Aladdins Reef was primarily due to the presence of *N. douglasii* in the third count whereas it was absent in the first two, a decrease in density of *P. alboscapularis*, and the disappearance of the schooling planktivore *Scorpius violaceus* (Scorpidae).

Plots of the mean densities individual common species that had impacts on the canonical analysis illustrated how temporal variability in relative density was inconsistent between sites (Fig. 3). For example, although the goatfish *Upeneichthys lineatus* appeared to steadily increase in density with time at Labyrinth, densities were stable at the other three sites. *Coris sandageri* density increased between the first and second counts at three sites, but decreased at Nursery Cove.

A list of species included in the counts and their frequency of occurrence in each time period is given Appendix A. Background information on the biology of the individual species may be obtained from Francis (2001).

### 3.2 Italy

The depth covariable and random effect of Month were both significant in the PerMANOVA (Table 6). The main effect of Time (in this case, morning versus afternoon) was not, but there was a significant interaction between Time and Month. Pairwise t-tests for the effect of Time within Month were both significant however with a Bonferroni-adjusted significance level of  $\alpha = 0.025$  (June:  $t = 1.973$ ,  $P = 0.0056$ ; September:  $t = 1.663$ ,  $P = 0.0172$ ), indicating that the differences between morning and afternoon were caused by different species in the two months. This variability was reflected in the PCO and CAP ordinations (Fig. 4), which both indicated differences between months (Table 7), but did not separate morning and afternoon counts. Indeed the total allocation success rate of the discriminant analysis was not significantly different from random (Table 7).

The species primarily responsible for differences between months (ie significantly correlated with the axis for Month in Fig. 4) were *Sarpa salpa* (Sparidae,  $r = -0.503$ ) which occurred only in June, *Diplodus annularis* (Sparidae,  $r = -0.438$ ) and *Coris julis* (Labridae,  $r = -0.411$ ). The only species with a significant positive correlation with the axis (ie with September counts) was *Thalassoma pavo* ( $r = 0.679$ ).

CAP analyses done individually for each month supported the result of the t-test, with significant differences between the distributions of the canonical scores of morning and afternoon (Fig. 5, right hand side). However, as indicated by the significant interaction in the PerMANOVA, different species were correlated with the canonical axis in different months. In June, *Chromis chromis* and *Coris julis* increased significantly in density from morning to afternoon, whereas *Symphodus mediterraneus* and *Symphodus tinca* decreased (Fig. 6). In September, *Thalassoma pavo* increased dramatically in density from morning to afternoon, *S. mediterraneus* also increased, but *Serranus scriba* decreased (Fig. 6).

#### 4. Discussion

This study did not indicate significant systematic bias in the enumeration of assemblages of demersal temperate reef fishes counted at the same locations at differing times during daylight hours, and thus agrees with previous work from both temperate and tropical environments (Colton & Alevizon 1981; Kingsford 1989; Rooker & Dennis 1991; Spyker & van den Berghe 1995; Thompson & Mapstone 2002). However, the acknowledged high variability that exists between individual transect counts of mobile reef fishes in space (Edgar et al.



2004) is also manifest in time, even over scales of hours. No doubt the mobility of many fish species means that this variability may be just as great over temporal scales of minutes. Given that fish move, some of them continuously, it is highly unlikely that repetition of the same transect will ever yield the same data as the original count. Visual counts of fishes are therefore not truly “repeatable”, and must always be regarded as a snapshot indication of the real composition and relative density of a given assemblage (Sale 1997). This is likely to be especially true for counts of planktivorous fishes, which are very mobile, known to change distribution with tidal currents (Kingsford & MacDiarmid 1988), and have very high variances due to the aggregation of individuals in schools.

The sources of small-scale temporal bias in fish counts may arise from the fish, or from the observer. Because of logistic constraints and safety considerations, our studies were limited to daylight hours, and we were not able to repeat the counts at early dawn and dusk when feeding activity often peaks for some species, and fish distributions may change markedly as diurnally and nocturnally active species exchange places (Hobson 1965; Ebeling & Bray 1976; Galzin 1987). That variability in fish distribution and behaviour between night and day can influence relative abundance estimates is well accepted by both fishery biologists (e.g. Francis & Williams 1995; Casey & Myers 1998) and ecologists (Hobson 1965; Doherty 1983; Galzin 1987; Thompson & Mapstone 2002). Some existing large-scale monitoring programmes explicitly require that fish counts are conducted only during daylight hours to avoid this source of bias (e.g. long term monitoring of the Great Barrier Reef, Halford & Thompson 1994). The efficiency of the observer may be impeded at dawn and dusk by low light levels, or efficiency may decrease during the day as the fatigue induced by multiple dives increases.

The diel variability in the apparent abundance of some species did not have significant effects on the multivariate analysis, for three reasons. First, the high between-transect (ie within-group) variability common in fish transect counts has the effect of reducing the statistical power of tests for between-group differences. Second, although some species may vary systematically in density during the day, other common members of the assemblage either do not vary, or vary randomly, which means that in the multivariate case, species exhibiting consistent temporal pattern must have large and consistent effects to avoid being masked by multivariate “noise”. Third, when diel patterns existed within species, they were often inconsistent between sites. These factors add up to mean that systematic bias generated at

small temporal scales in a small subset of the species in a given assemblage is unlikely to significantly influence the conclusions from large spatial scale surveys of assemblages of temperate reef fishes. However, where very common species exhibit strong systematic variability in activity patterns during the day (that may vary between habitats), or tidal changes cause redistribution of species (Kingsford & MacDiarmid 1989, Thompson & Mapstone 2002), sampling a particular treatment only in the morning, for example, could cause significant bias.

It is generally not practicable to randomise replicate transects within sites with respect to time in large-scale fish monitoring surveys. Such surveys are generally heavily constrained with respect to time, funding, availability of trained personnel, and weather conditions. However, workers should attempt to randomise or intersperse sites in time with respect to treatments, whether these be water depth, habitat type, marine reserve status, or some other *a priori*-defined factor depending on the hypothesis being tested. Surveys of territorial species should be relatively temporally consistent, but chance variation in the movements of species that habitually move over spatial scales greater than metres can confound comparisons of treatments, or the relationship of fishes to measured habitat variables. Thompson & Mapstone (2002) suggested that one solution is to use sampling units that are sufficiently large as to encompass the home range size of target species, however pointed out that such measures are likely to increase sampling bias and logistic difficulties. Our study used relatively short transects (25 m) so that sampling units could fit within habitat patches, and to enable greater levels of replication of the sampling unit within sites. We concur with Thompson & Mapstone (2002) that increasing replication at the site level (to better describe patterns of spatial variability) is probably preferable to taking extreme measures to account for small-scale temporal variability.

Obviously, the degree to which temporal variability in fish behaviour may influence spatial comparisons should depend largely on the relative mobility of the species under investigation. Counts of strongly site-attached or territorial species should vary relatively little, whereas counts of schooling planktivores may change by an order of magnitude or more. Despite this expectation, we obtained significant within-day variability in the density of the black angelfish *Parma alboscapularis* – a strongly site-attached species - at two of the four New Zealand sites. We cannot determine if this was due to real changes in the density of this species during the day, to sampling errors such as slight inconsistencies in the estimation of

transect width between times (Harvey et al. 2004), or whether individual fish might have changed their behavioural response to the diver after repeated visits. Behavioural responses to divers occur frequently where fish are either fed (Cole 1994; Milazzo et al. in press) or hunted (Kulbicki 1998), but in the absence of these stimuli, repeated visits to a site by a diver should not normally be expected to cause significant changes in fish behaviour (De Girolamo & Mazzoldi 2001). An exception to this generalisation might occur when individual fish lose fear of divers after repeated visits, with no stimulus other than habituation. In this case, later density estimates will be higher than those made initially.

Sampling errors may contribute as much or more to the misinterpretation of field studies as errors brought about by statistical errors or experimental design problems (Andrew & Mapstone 1987). Many of the methodological problems encountered in estimation of relative density of fishes are well documented elsewhere (e.g. Lincoln Smith 1989; Sale 1997; Thompson & Mapstone 1997, Willis & Babcock 2000; De Girolamo & Mazzoldi 2001; Edgar et al. 2004). Here, we are dealing with a potential sampling problem that can be obviated by putting a little thought into survey design. In many situations, variability in fish densities at small temporal scales will not influence the assessment of larger-scale spatial pattern in assemblage structure. However, the relative density of individual fish species can vary within locations at different times of day, so the randomisation of sampling order in time with respect to treatment should be standard practice.

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## Appendix A

Lists of the 28 New Zealand species and 26 Mediterranean species recorded in the study, with their frequency of occurrence in the measured time periods.

Poor Knights Islands, New Zealand. n = 24 time period<sup>-1</sup>

Family	Species	Time period		
		0730-0830	1030-1130	1400-1500
Blenniidae	<i>Plagiotremus tapeinosoma</i>	1	0	0
Carangidae	<i>Seriola lalandi</i>	0	0	1
Chironemidae	<i>Chironemus marmoratus</i>	0	2	1
Kyphosidae	<i>Kyphosus bigibbus</i>	0	0	1
	<i>Kyphosus sydneyanus</i>	1	0	0
Labridae	<i>Bodianus unimaculatus</i>	13	12	9
	<i>Coris sandageri</i>	22	24	22
	<i>Notolabrus celidotus</i>	3	6	3
	<i>Notolabrus fucicola</i>	12	12	12
	<i>Pseudolabrus inscriptus</i>	4	1	3
	<i>Pseudolabrus luculentus</i>	7	6	6
	<i>Pseudolabrus miles</i>	12	11	9
	<i>Suezichthys arquatus</i>	0	2	0
	<i>Suezichthys aylingi</i>	10	10	9
Latridae	<i>Cheilodactylus spectabilis</i>	4	2	3
	<i>Nemadactylus douglasii</i>	8	8	7
Monacanthidae	<i>Parika scaber</i>	13	12	8
Mullidae	<i>Upeneichthys lineatus</i>	10	11	12
Muraenidae	<i>Gymnothorax prasinus</i>	1	1	1
Odacidae	<i>Odax pullus</i>	1	3	3
Pomacentridae	<i>Parma alboscapularis</i>	16	16	15
Scorpaenidae	<i>Scorpaena cardinalis</i>	0	0	4
Scorpidae	<i>Scorpis violaceus</i>	2	6	0
Serranidae	<i>Caesioperca lepidoptera</i>	3	2	3
	<i>Caprodon longimanus</i>	1	0	0
	<i>Hypoplectrodes sp B</i>	8	4	2
Sparidae	<i>Pagrus auratus</i>	5	7	2
Tetraodontidae	<i>Canthigaster callisterna</i>	3	1	2

(continued)

Ustica Island, Italy. n = 16 time period<sup>-1</sup> month<sup>-1</sup>

Family	Species	June		September	
		0900-1100	1630-1830	0830-1030	1600-1800
Apogonidae	<i>Apogon imberbis</i>	0	0	1	3
Carangidae	<i>Seriola dumerili</i>	0	0	0	1
Labridae	<i>Coris julis</i>	16	10	6	7
	<i>Labrus viridis</i>	0	2	1	1
	<i>Labrus merula</i>	0	0	1	1
	<i>Symphodus mediterraneus</i>	4	12	10	8
	<i>Symphodus melanocercus</i>	1	1	0	0
	<i>Symphodus ocellatus</i>	7	8	2	0
	<i>Symphodus roissali</i>	2	1	0	0
	<i>Symphodus rostratus</i>	0	2	2	0
	<i>Symphodus tinca</i>	6	13	10	12
	<i>Thalassoma pavo</i>	8	11	16	16
Centracanthidae	<i>Spicara maena</i>	0	1	0	0
	<i>Spicara flexuosa</i>	1	0	0	0
Mugilidae	<i>Mugil spp</i>	0	0	1	1
Mullidae	<i>Mullus surmuletus</i>	2	2	0	0
Pomacentridae	<i>Chromis chromis</i>	16	14	12	12
Scorpaenidae	<i>Scorpaena scrofa</i>	0	1	0	0
Serranidae	<i>Epinephelus marginatus</i>	4	0	1	1
	<i>Serranus cabrilla</i>	0	6	3	6
	<i>Serranus scriba</i>	13	11	8	10
Sparidae	<i>Diplodus annularis</i>	7	8	2	2
	<i>Diplodus puntazzo</i>	0	0	1	0
	<i>Diplodus vulgaris</i>	0	2	0	3
	<i>Oblada melanura</i>	0	0	0	1
	<i>Sarpa salpa</i>	6	3	0	0

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Table 1

Mixed-model permutational multivariate analysis of variance (PerMANOVA) on the basis of Bray-Curtis dissimilarities for northern New Zealand reef fish assemblages (28 species) at two sites surveyed three days apart.

Source	df	SS	MS	F	P
Day	1	3362.8	3362.8	3.26	0.2102
Site	1	14776.1	14776.1	9.68	0.0001
Day × Site	1	1029.8	1029.8	0.67	0.6342
Residual	20	30520.5	1526.0		
Total	23	49689.2			

Table 2

Mixed-model permutational multivariate analysis of variance (PerMANOVA) on the basis of Bray-Curtis dissimilarities for northern New Zealand reef fish assemblages (28 species) at four sites and three times of day.

Source	df	SS	MS	F	P
Covariate = depth	1	2.24	2.24	16.44	0.0001
Time	2	0.26	0.13	0.95	0.5180
Site	3	2.48	0.83	6.05	0.0001
Time × Site	6	0.79	0.13	0.96	0.5434
Residual	59	8.05	0.14		
Total	71	13.82			

Table 3

Mixed-model permutational tests of multivariate dispersion (PermDisp) on the basis of Bray-Curtis dissimilarities for northern New Zealand reef fish assemblages (28 species) at four sites and three times of day.

Source	df	SS	MS	F	P
Time	2	480.62	240.31	3.04	0.1200
Site	3	2212.00	737.33	11.13	0.0001
Time $\times$ Site	6	473.46	78.91	1.19	0.3241
Residual	60	3975.68	66.26		
Total	71	7141.76			

Table 4

Results of canonical analysis of principal coordinates (CAP) examining the effects of Site and Time on reef fish assemblages in New Zealand. %Var = percentage of the total variation explained by the first  $m$  principal coordinate axes. Allocation success = percentage of points correctly allocated into each group.  $\delta^2$  = squared canonical correlation.

Factor	$m$	%Var	Allocation success (%)					$\delta^2$	$P$
			Group 1	Group 2	Group 3	Group 4	Total		
Time	5	76.56	50.00 (07:30)	8.33 (10:30)	45.83 (14:00)		34.72	0.049	0.919
Site	4	68.35	66.67 (Aladdins)	66.67 (Labrid)	88.89 (Labyrinth)	66.67 (Nursery)	72.22	0.757	< 0.001

Table 5

Correlation coefficients for individual species ( $|r| > 0.4$ ) with the canonical axis for effects of Site (see Fig. 2).

Negative correlation		Positive correlation	
<i>Parma alboscapularis</i>	-0.75	<i>Upeneichthys lineatus</i>	0.49
<i>Pseudolabrus miles</i>	-0.63	<i>Nemadactylus douglasii</i>	0.48
<i>Notolabrus fucicola</i>	-0.59		
<i>Coris sandageri</i>	-0.54		
<i>Parika scaber</i>	-0.42		

Table 6

Mixed-model permutational multivariate analysis of variance (PerMANOVA) on the basis of Bray-Curtis dissimilarities for Ustica Island reef fish assemblages (26 species) at two times of day surveyed in two different months.

Source	df	SS	MS	F	P
Covariate = depth	1	184.15	184.15	15.70	0.0001
Time	1	27.44	27.44	0.57	0.7330
Month	1	119.98	119.98	10.23	0.0001
Time $\times$ Month	1	47.98	47.98	4.09	0.0022
Residual	59	691.86	11.73		
Total	63	1071.41			

Table 7

Results of canonical analysis of principal coordinates (CAP) examining the effects of Month and Time on reef fish assemblages in Italy. %Var = percentage of the total variation explained by the first  $m$  principal coordinate axes. Allocation success = percentage of points correctly allocated into each group.  $\delta^2$  = squared canonical correlation.

Factor	$m$	%Var	Allocation success (%)			$\delta^2$	$P$
			Group 1	Group 2	Total		
Time	5	85.40	46.87 (am)	68.75 (pm)	57.81	0.165	0.055
Month	5	85.40	75.00 (June)	93.75 (Sept)	84.37	0.526	<0.001



**Figure captions:**

Fig. 1. Principal coordinates (metric MDS) ordination (a) and CAP analysis (b) of New Zealand reef fish transect counts taken at three times of day at four sites. Percentage variability explained by the PC axes are given on the plot.

Fig. 2. Principal coordinates (metric MDS) ordinations (left) and CAP analyses (right) of New Zealand reef fish transect counts taken at three times of day for each site. Percentage variability explained by the PC axes are given on the plot.

Fig. 3. Changes in mean density of key fish species with time of day on northern New Zealand reefs.

Fig. 4. Principal coordinates (metric MDS) ordination (a) and CAP analysis (b) of Italian reef fish transect counts taken at two times of day in two months. Percentage variability explained by the PC axes are given on the plot.

Fig. 5. Principal coordinates (metric MDS) ordinations (left) and CAP analyses (right) of Italian reef fish transect counts taken in the morning and afternoon for each of June and September. Percentage variability explained by the PC axes are given on the plot.

Fig. 6. Changes in mean density of key fish species with time of day on southern Italian reefs.

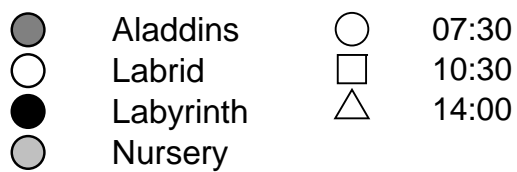
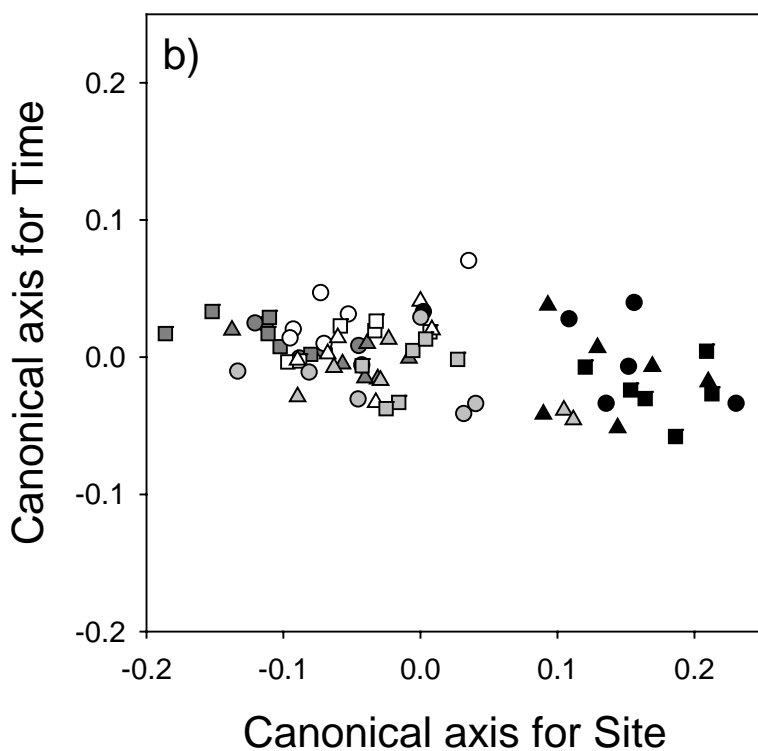
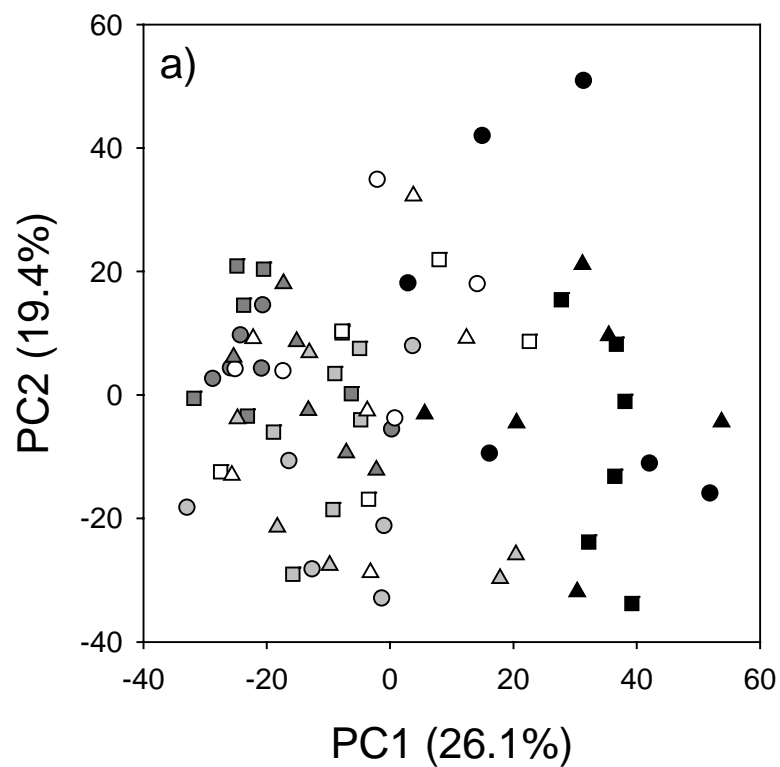


Fig.1. Willis et al.

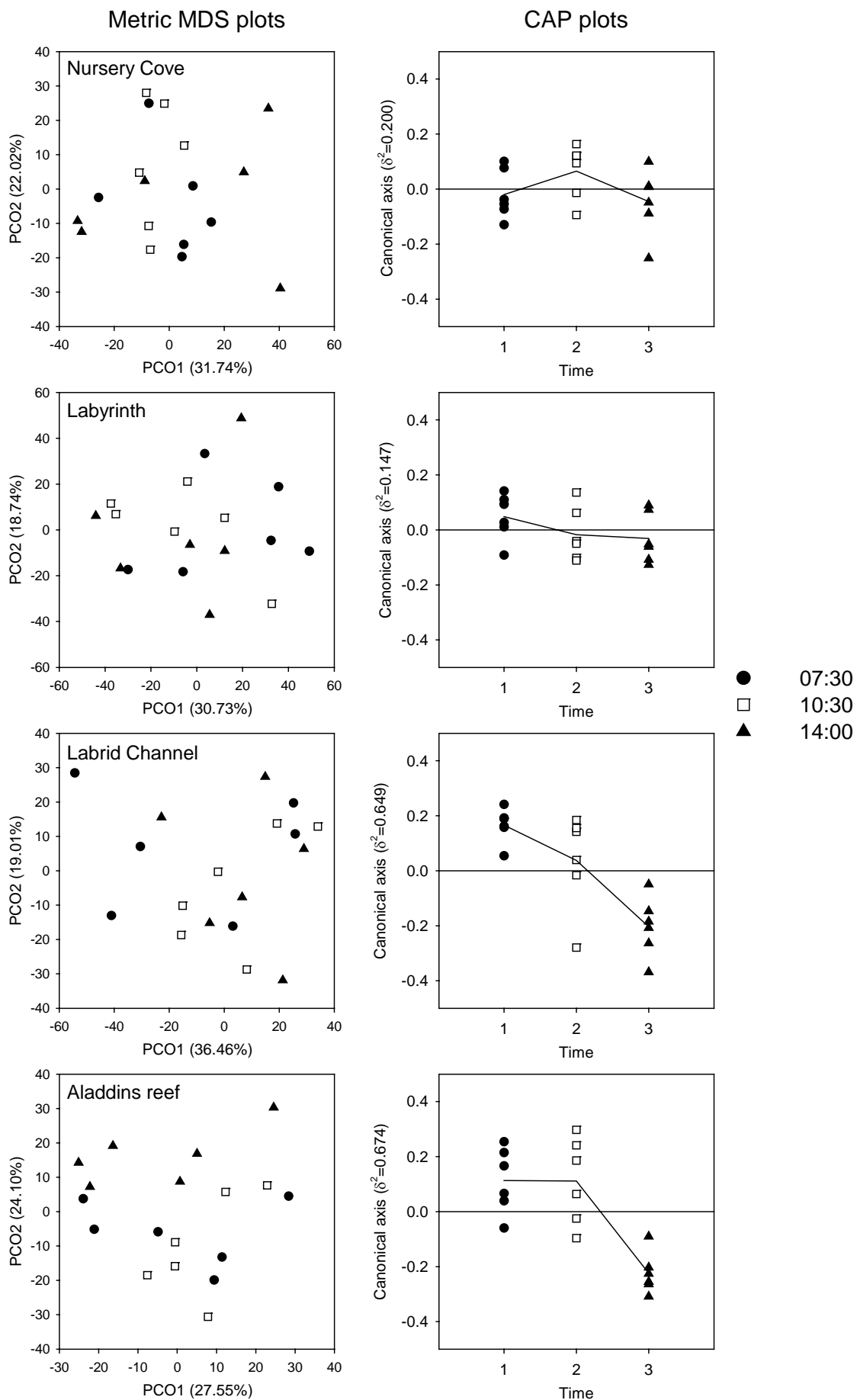


Fig. 2. Willis et al.

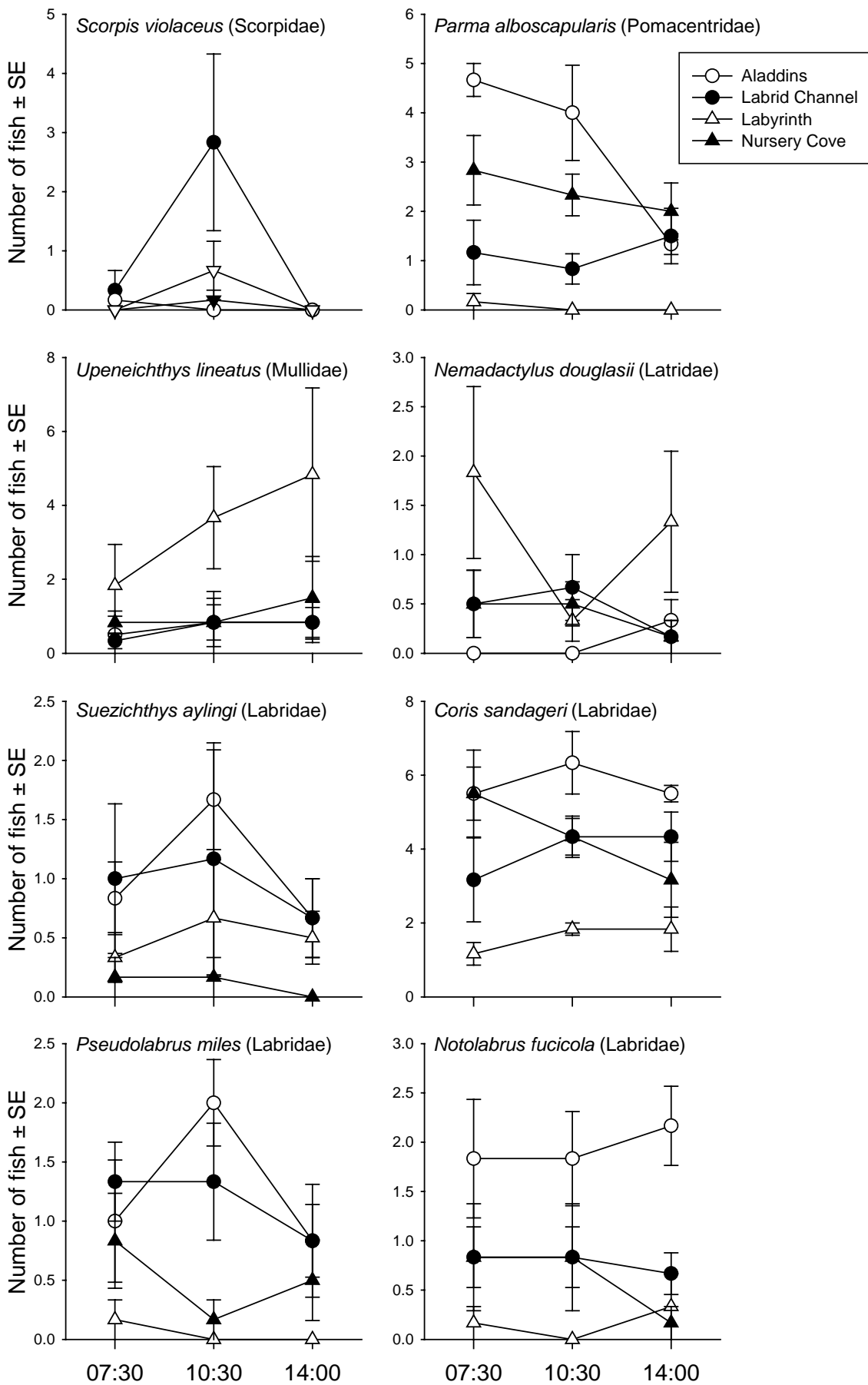


Fig. 3. Willis et al.

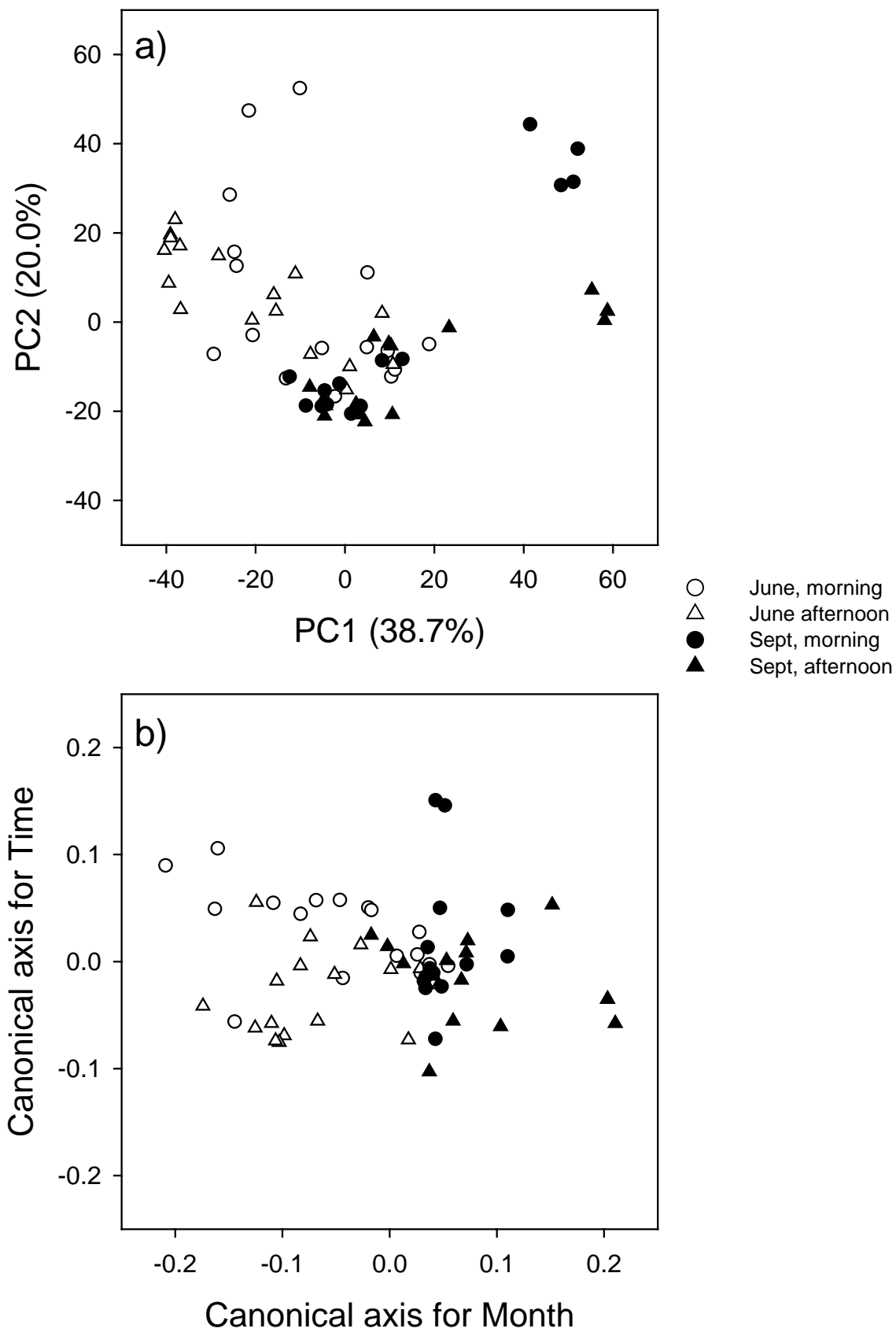


Fig. 4 Willis et al.

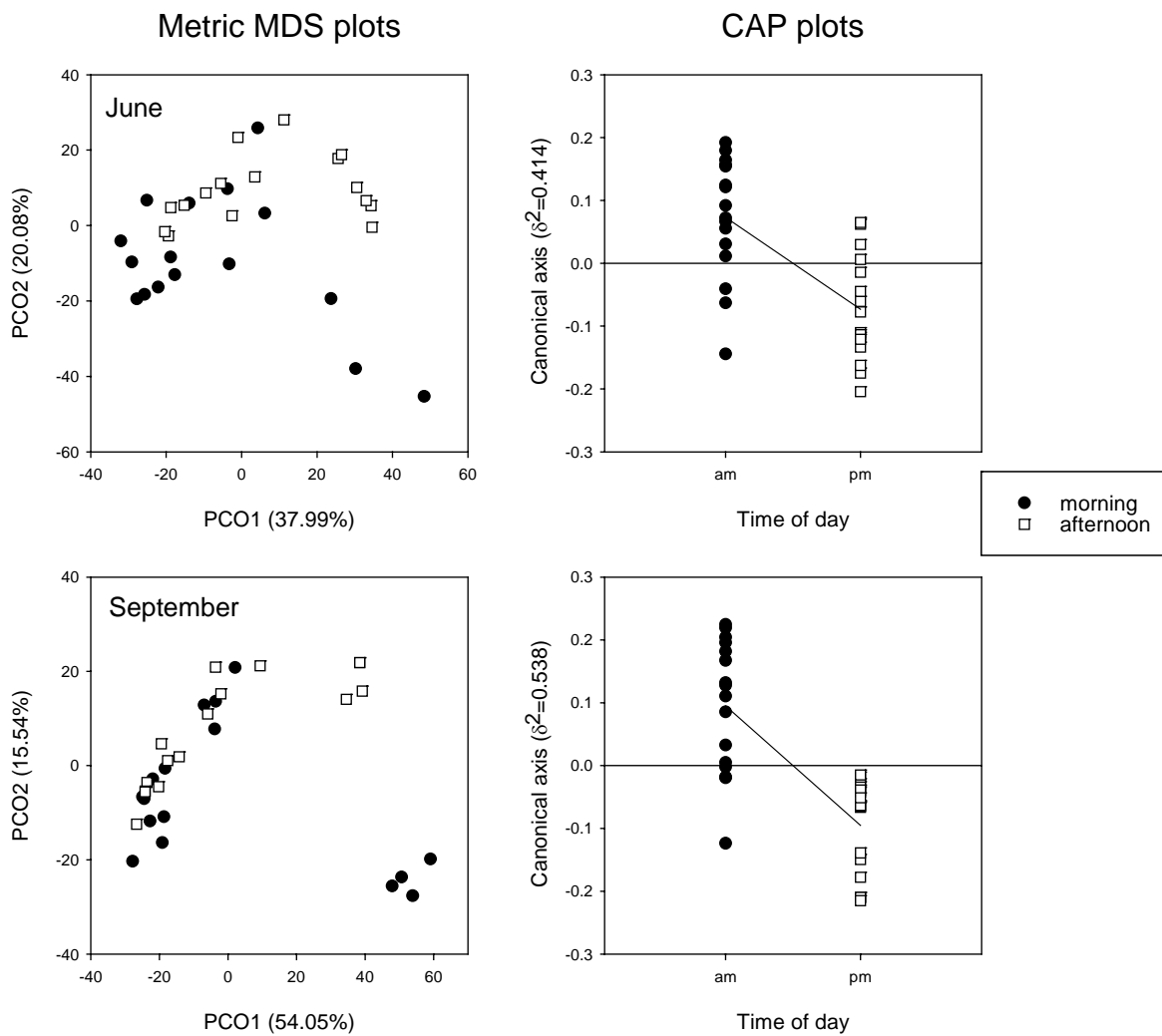


Fig. 5 Willis et al.

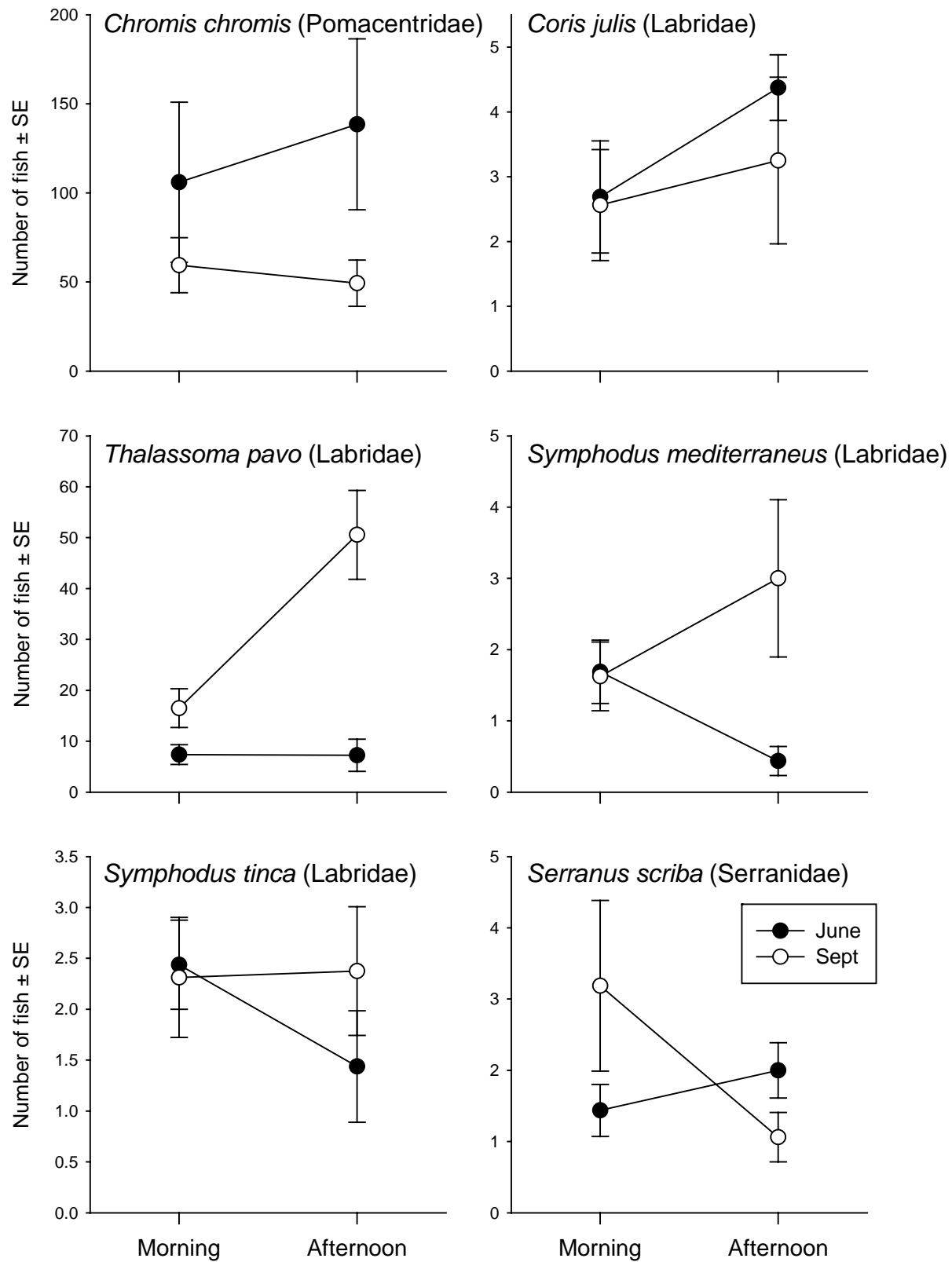


Figure 6. Willis et al.