Intertidal and subtidal blennies: assessment of their habitat through individual and nest distribution

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ABSTRACT: Resident and transitory intertidal fish species are expected to differ in their utilization of space in the intertidal zone. Here we assessed fish zonation patterns on 2 rocky shores of the north-western Adriatic Sea (where tides can range up to 1.5 m) using blennies as study species and distribution of both individuals and nests as methodological tools. Of the 12 species observed, 5 were found exclusively in the intertidal zone: *Aidablennius sphynx*, *Coryphoblennius galerita*, *Lipophrys adriaticus*, *L. canevae* and *L. dalmatinus*. Two species, *Parablennius rouxi* and *P. tentacularis*, were found only in the subtidal zone, whilst *P. gattorugine*, *P. incognitus*, *P. sanguinolentus*, *P. zvonimiri* and *Salaria pavo* were observed in both environments. Nest distribution mirrored individual distribution, strongly indicating that some blenny species can be considered true intertidal residents, spending the main part of their lives in this area. Intertidal and subtidal species showed different patterns of space utilization. Indeed, the former nested higher in the vertical distribution than the average depth at which individuals were recorded, and the latter, despite visiting the intertidal area at high tide, always nested below the minimum water line. These results show that, contrary to theoretical predictions, intertidal-resident fish may fully exploit intertidal habitats, for foraging and for reproduction, occupying even the sectors most distal from the subtidal zone, i.e. the most variable in terms of physical conditions. Moreover, nest distribution was found to be an effective measure of residence and thus a useful referent to evaluate space use in intertidal fish.

KEY WORDS: Blenniidae · Nest · Eggs · Intertidal fish · Vertical zonation · Adriatic Sea

INTRODUCTION

The intertidal zone represents a challenging environment for marine organisms since they need to cope with predictable and unpredictable temporal variability related to emersion, wave action, heating, and predation by terrestrial animals (Newell 1970, Sayer & Davenport 1991). Nevertheless, intertidal environments also provide high productivity, and consequently food availability, variability in microhabitat (possibly decreasing competition), and low predation pressure from strictly marine organisms (Newell 1970, Sayer & Davenport 1991, Gibson 2003). Several marine species successfully exploit this zone, most of them spending their entire life cycle in it, with the exception of the planktonic larval period, whilst others remain during some stages only (Newell 1970, Gibson 1982).

For sessile organisms the colonization of the intertidal is a definitive choice, while mobile marine animals may utilize this zone seasonally or only when it is submerged, exploiting or avoiding it for reproduction (Gibson 1982, 2003).

Those fish species that spend a part of their lives in the intertidal are defined as residents or transients/visitors on the basis of their utilization of this zone (Gibson 1982, Gibson & Yoshiyama 1999). The former comprise those species that after settlement live and breed in the intertidal zone, whereas the latter mainly include sandy shore or pelagic fish that regularly visit the intertidal at high tide (Thomson & Lehner 1976, Gibson 1982). These categories, however, do not have clear boundaries, and should be considered as extremes of a continuum (Gibson & Yoshiyama 1999). Intertidal fish zonation patterns are predicted to be similar to those

observed among sessile individuals (Gibson 1982, Horn & Gibson, 1988, Zander et al. 1999), being primarily associated with physical conditions (Newell 1970, Connell 1972). In particular, resident fishes are expected to occupy the lowest sectors of the intertidal zone and thus the habitats that are the least variable in terms of physical conditions. Evidence supporting this hypothesis comes from studies on the fish zonation patterns of rocky tidepools (Gibson 1972, Thomson & Lehner 1976, Pulgar et al. 2005), whilst information on rocky shores is scanty (Zander et al. 1999). In rocky tidepools, sampling can be performed easily and efficiently at low tide with the aid of anaesthetics (Gibson 1999), whereas along rocky shores, where underwater visual census techniques are used (Gibson 1999), the quantitative assessment of fish distribution at low tide is difficult since fish usually hide in small crevices and holes or beneath algae (Martin 1995, Faria & Almada 2006). Species usually present different activity levels in different tidal phases (Horn & Gibson 1988, Martin 1995) and the niche a species occupies may actually differ among phases (Faria & Almada 2006), with the result that high tide sampling provides only a partial picture of their distribution.

The vertical position of an individual profoundly affects its exposure time, with differences of a few cm altering emersion time considerably and hence shaping the variability in temperature, oxygen concentration and salinity the individual has to face (Doty 1946). Fish, however, are mobile and may adjust their habitat according to their physiological performance. For this reason intertidal zonation and, consequently, level of adaptation to intertidal life, are not easily accurately attributed to fish species. Nevertheless, considering that resident species are expected to breed in the intertidal, that they lay eggs firmly attached where deposited (demersal eggs) (Gibson 1982, Almada & Santos 1995, De Martini 1999), and in several cases exhibit parental care (Almada & Santos 1995, De Martini 1999), a possibly useful tool to study fish zonation patterns might be the record of nest distribution.

Mediterranean coasts are microtidal (Cooper 1994), however the Adriatic arm, where tides can range up to 1.5 m, is considered an ideal site to study zonation patterns directly, rather than through tidepool position, since the height of the intertidal zone is expected to have led to a distinguishable vertical distribution of fish, comparable to that observed for sessile intertidal organisms (Zander et al. 1999). Among the Mediterranean intertidal fishes, Blenniidae is the most speciose family, being represented by 16 species mainly occupying shallow coastal rocky substrates (Patzner 1985, Illich & Kotrschal 1990, La Mesa & Vacchi 2005, Orlando-Bonaca & Lipej 2007, Santin & Willis 2007, Froese & Pauly 2008, Pizzolon et al. 2008). Blennies are

benthic fish, often showing markedly strict depth preferences (Gibson 1972, Patzner 1985, Illich & Kotrschal 1990, Zander et al. 1999, Faria & Almada 2006, Orlando-Bonaca & Lipej 2007), with intertidal species presenting morphological, physiological and behavioural adaptations to this environment (Laming et al. 1982, Gonçalves & Almada 1998, Sayer 2005). They release demersal eggs laid in a single layer inside a nest defended by a parental male that cares for the eggs until they hatch (Breder & Rosen 1966, Almada & Santos 1995). Given their high abundance and the occurrence of the 'sessile nest phase,' Mediterranean blenny species represent an ideal group for the study of zonation patterns not only in terms of individuals, but also of nests.

In this study, with the aim of attributing intertidal residence status to northern Adriatic blennies and analysing their vertical zonation, we (1) compared the depth distributions of nests and individuals, and (2) calculated the emersion time per day of nests.

MATERIALS AND METHODS

Study site. The study locations were the jetties of Ca' Roman (45.240° N, 12.309° E) and Sottomarina (45.232° N, 12.309° E), Italy, in the northwestern Adriatic sea. The 2 parallel jetties run at right angles to the shore and delimit a channel (500 m wide) connecting the Venetian lagoon to the open Adriatic sea (Fig. 1). They are ~1.3 km long and mainly consist of stone blocks and artificial concrete acropods, giving rise to a heterogeneous habitat, rich in shelters, crevices and holes. The subtidal is densely covered with algae, like *Dictyota dichotoma*, *Dictyopteris polypodioides* and *Gracilaria* sp., particularly in Ca' Roman' jetty, while the snakelocks anemone *Anemonia viridis* and the black mussel *Mytilus* sp. can represent up to 50% of the rocky cover at Sottomarina. The intertidal appears poorly covered, mainly by the green alga *Ulva* sp. and *Mytilus* sp. (N. Chimento & C. Mazzoldi unpubl. data).

The jetties are surrounded by a sandy-muddy bottom and the maximum depth of the hard substrates is reached at the seaward end (8 m in Ca' Roman, 6 m in Sottomarina), progressively decreasing toward the beach. The study was done only on the seaward (external) sides of the jetties.

Sampling design and techniques. Blenniid fauna was surveyed during spring and summer 2005 (from 16 May to 24 June) and 2006 (from 10 May to 30 July). A survey was conducted in each of 2005 and 2006 to record the location of blenny nests in terms of depth (see below, this section and 'Nest sampling'), while in 2006 two surveys were performed, respectively, focused on evaluating species richness and abun-
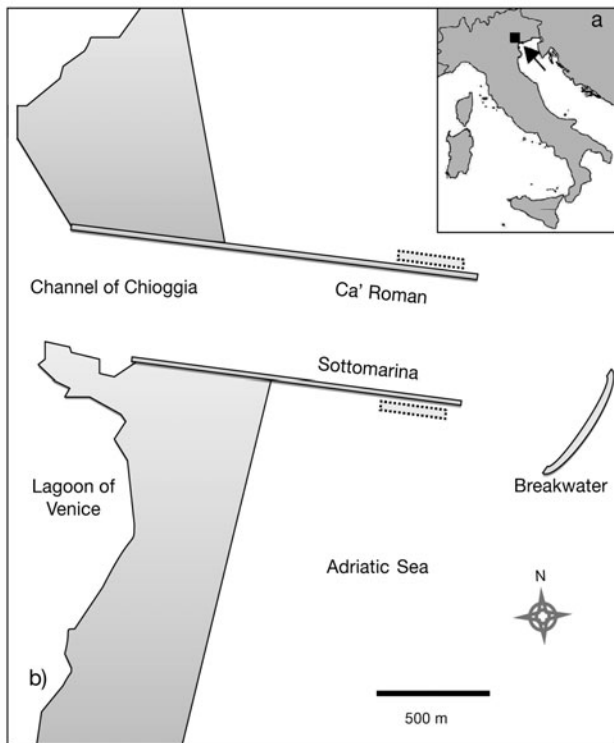
Fig. 1. (a) Location of the Venetian lagoon; (b) study sites, dotted rectangles indicate position of transects along Sottomarina and Ca' Roman jetties

dance, and on species depth distribution, (see below, this section and 'Horizontal transects' and 'Vertical transects'). Fish distribution was studied using visual census techniques along horizontal transects to quantitatively assess species distribution and down vertical transects to record mean, minimum and maximum depths for every species (Harmelin-Vivien et al. 1985). Nest vertical distribution was assessed in both years, recording the depth of all nests encountered. All counts were conducted during daylight (10:00 to 16:00 h) in calm weather conditions where visibility was > 1.5 m. At the beginning of each survey, time and water temperature were recorded with a dive computer. Water temperature over the sampling period ranged from 18°C at the beginning of May to 29°C at the end of July. We used the time to correct depth measures (for horizontal and vertical transects and for nest locations, taken with a dive computer) to the actual measured tide level (actual tide level for Chioggia channel at hourly intervals; available from the municipality of Venice, www.comune.venezia.it/flex/cm/pages/ServeBLOB.php/L/ IT/IDPagina/1729). As a consequence, all reported depths are relative to the mean water level (MWL).

Horizontal transects: We considered 4 bathymetric areas on each jetty: high intertidal (HI), from +0.5 to −0.5 m depth, characterized by constant air exposure at low tide; low intertidal (LI), from −0.5 to −1 m depth, a transitional zone exposed to air only during exceptionally low tides but still influenced by tides due to the turbulence that extends beyond the HI (Faria & Almada 2006); high subtidal (HS) from −1 to −3 m depth; and low subtidal (LS), from −3 to −6 m depth. Each area was surveyed using strip transects, 25 m long and 0.6 m wide, delimited along 1 side by a rope kept close to the bottom by weights. The transect width was chosen in order to sample a narrow depth range on the steep substrate of the jetties. Each transect was surveyed at a mean speed of 1.5 m min^{-1}, with holes, crevices and areas under algal cover searched carefully by a trained observer who classified and counted all blennies. Blenny species were recognized by unique characteristics such as supraorbital tentacles (presence/absence, shape), head crest (presence/absence), and colour pattern (Zander 1986, Abel 1993). Data were recorded (on a PVC slate) by snorkelling in the HI and by SCUBA diving in the 3 deeper areas. A total of 4 surveys per area were conducted at each jetty.

Vertical transects: During the 2006 survey, 8 vertical transects were established on each jetty. Each transect consisted of a 40 m long weighted rope laid (following the bottom profile) from the sea surface to the end of the hard substrate, perpendicular to the jetty (transect width 0.6 m). The transects were surveyed in SCUBA diving gear, at a speed of 1.5 m min^{-1}, from the deepest end to the sea surface by a trained observer who recorded the species and depth of each blenny encountered.

Nest sampling: Nest data were collected through surveys conducted using snorkelling equipment to a depth of 1 m and by SCUBA diving, and consisted of the careful search for blenniid nests in the whole depth range. Nests were recognized by the presence of a male with breeding colouration exhibiting behaviour typical of nest ventilation and, when visible, by the presence of eggs. We recorded on a PVC slate species, depth, type (hole in the rock, black mussel shell, oyster shell, barnacle), and nest entrance size (hole diameter or the largest distance between the 2 valves of the shell, measured with a calliper to the nearest mm) for each nest. To check for male presence when nests were exposed to air, we chose 3 d presenting very low tides, 24 June 2005 (tide level: −0.32 m), 26 May (tide level: −0.41 m) and 11 July (tide level: −0.41 m) 2006 and performed the following observations each time. We marked the position of the highest nests observed in the high intertidal zone with a sign on the emerged rock during the preceding high tide, and recorded the species and the type of nests. We then verified the presence of the male in each marked nest during the low tide.

Data analysis. For horizontal transects, the richness (number of species) and abundance of individuals for each species (ind. transect^{-1}, 15 m^2) were calculated. The results were compared among the 4 areas to detect a bathymetric pattern, and between the Ca′ Roman and Sottomarina sites, in order to compare blenniid distribution. We performed a non-metric multidimensional scaling (NMDS) on a Bray-Curtis similarity matrix to examine overall patterns across the entire data set (Clarke 1993). Permutational multivariate ANOVA (PERMANOVA) was then applied to verify differences among groups (Anderson 2001). Simper analysis was applied to select species responsible for differences between Sites and similarities within Areas (Clarke & Gorley 2006). Only the species presenting the highest contribution to dissimilarities or similarities — on the basis of their percentage of contribution (>15%) and value of dissimilarity/SD or similarity/SD (>1) — will be discussed.

To highlight biotic interactions among the most abundant and ubiquitous species, Pearson′s correlation analyses were performed on log transformed data from horizontal transects, applying the Bonferroni correction for multiple correlations (significance value: $\alpha'' = \alpha/k$, with k = number of correlations performed).

Depth distribution of nests for each species was compared between years, and depth distributions of individuals (from vertical transects) and nests for each species were compared, using a t-test or Mann-Whitney U-test, according to data distribution and test assumption, and applying the Bonferroni correction for multiple comparisons (significance value: $\alpha'' = \alpha/k$, with k = 6 for comparison between years and between nests

and individuals). The relation between nest entrance size and species size was calculated using Spearman′s non-parametric correlation coefficient, using the maximum species size (Froese & Pauly 2008).

For each nest, we calculated air exposure time, divided into day- and night-time exposure, over 2 discrete weeks, 1 preceding and 1 following the collection of nest data, using the tide tables available from the municipality of Venice (see ′Materials and methods: Sampling design and techniques′). The mean and maximum times of air exposure during night- and day-time were then calculated for each nest and for each species. A rank of intertidal species on the basis of nest exposure was calculated, summing the rank attributed to each species for mean and maximum time of air exposure during day- and night-time, and the percentage of nests that emerged from the water. Statistical analyses were performed using STATISTICA 7.1 software, PRIMER 6 and the PERMANOVA computer program (Anderson 2005).

RESULTS

A total of 12 species of blenny were recorded in the 2 sampling years (Table 1). Total abundance and richness were significantly different among bathymetric areas (abundance: $F_{3,30} = 8.51$, p < 0.001, richness: $F_{3,30} = 7.86$, p < 0.001), but not between sites (all p > 0.1), with values decreasing from the HI to the LS (Fig. 2).

The NMDS ordination highlighted a separation of transects on the basis of Area and Site (Fig. 3). These results were confirmed by PERMANOVA analysis with

Table 1. List of species: abundance (mean ± SD of ind. 15 m^{-2}) per site and per area. HI: high intertidal; LI: low intertidal; HS: high subtidal; LS: low subtidal; see ′Materials and methods′ for detailed information on sites and areas

Species	Sottomarina				Ca′ Roman			
	HI	LI	HS	LS	HI	LI	HS	LS
Aidablennius sphynx	4.0 ± 3.2	1.2 ± 1.9	0	0	3.5 ± 3.1	1.2 ± 1.9	0	0
Coryphoblennius galerita	0.2 ± 0.5	0	0	0	9.0 ± 5.1	0	0	0
Lipophrys adriaticus	9.2 ± 6.2	0	0	0	3.7 ± 3.1	0	0	0
L. canevae	0.5 ± 1.0	0.2 ± 0.5	0	0	1.7 ± 1.5	0.5 ± 0.6	0	0
L. dalmatinus	0	0.7 ± 1.0	0	0	0	3.7 ± 3.5	0	0
Parablennius gattorugine	0	0.2 ± 0.5	0.2 ± 0.5	0	0	0	0.2 ± 0.5	0
P. incognitus	0.2 ± 0.5	0.7 ± 1.0	2.0 ± 1.8	3.0 ± 3.8	0.2 ± 0.5	5.0 ± 4.4	2.0 ± 1.4	0
P. rouxi	0	0	0	0.7 ± 1.0	0	0	0	0.5 ± 0.6
P. sanguinolentus	7.5 ± 5.7	10.7 ± 6.1	1.2 ± 1.0	0	0.5 ± 0.6	5.0 ± 2.4	0	0
P. tentacularis	0	0	3.2 ± 1.9	7.0 ± 5.6	0	0	0	3.2 ± 2.1
P. zvonimiri	0.2 ± 0.5	2.0 ± 2.8	5.0 ± 3.4	3.0 ± 3.4	0	1.2 ± 2.5	2.5 ± 3.0	0
Salaria pavo	20.5 ± 14.2	7.0 ± 1.8	0.7 ± 1.5	0	8.5 ± 6.0	9.5 ± 5.4	7.0 ± 6.4	0.5 ± 0.6
Total	42.3 ± 27.4	22.7 ± 7.1	12.3 ± 1.7	13.7 ± 7.9	27.1 ± 13.5	26.1 ± 13.8	11.7 ± 8.8	4.2 ± 2.1

significant results for the interaction Area × Site ($F_{3,31}$ = 3.83, p < 0.001). The Sottomarina intertidal transects showed higher similarities between them than with subtidal transects, whilst in Ca′ Roman the transects presented high similarity only with those of the same area (Table 2). Simper analysis showed that several species were responsible for the dissimilarity between sites in the different areas (Table 3). In the intertidal areas, *Coryphoblennius galerita*, *Parablennius incognitus* and *Lipophrys dalmatinus* were more abundant in Ca′ Roman than in Sottomarina, where *P. sanguinolentus* and *Salaria pavo* were more abundant (Table 1). In the subtidal areas, all the species, with the exception of *S. pavo* in the HS, were more abundant in Sottomarina than in Ca′ Roman (Table 1). Considering the bathymetric areas, *S. pavo L. adriaticus* and *Aidablennius sphynx* characterized the HI in both sites, *P. sanguinolentus* and *C. galerita* characterizing Sottomarina and Ca′ Roman, respectively. *S. pavo* and *P. sanguinolentus* characterized the LI in both sites. The HS was characterized by *P. zvonimiri* and *P. tentacularis* in Sottomarina, and *P. incognitus* and *S. pavo* in Ca′ Roman. The LS was characterized by *P. tentacularis* in both sites, and *P. zvonimiri* in Sottomarina (Table 4). In general, *S. pavo*, *P. incognitus* and *P. zvonimiri* were observed in most of the 4 bathymetric areas, with *S. pavo* dominating the intertidal and HS samples and *P. zvonimiri* presenting low abundances. By contrast, *A. sphynx*, *C. galerita*, *L. adriaticus* and *L. dalmatinus* were found only in the intertidal samples, whilst *P. tentacularis* and *P. rouxi*, only in the subtidal ones, with the LS samples dominated by *P. tentacularis*. *P. gattorugine* was observed in only 3 transects, in the LI or HS.

Correlation analyses performed between the most abundant and ubiquitous species (*Parablennius incog-*
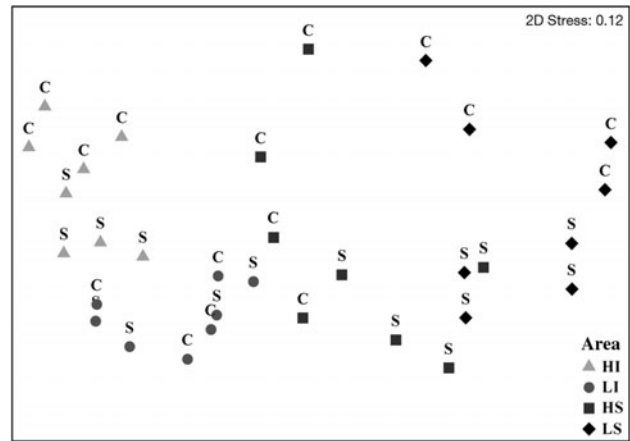
Fig 3. Non-metric multidimensional scaling (NMDS) ordination of horizontal transects. C: Ca′ Roman, S: Sottomarina. Other abbreviations as in Table 1
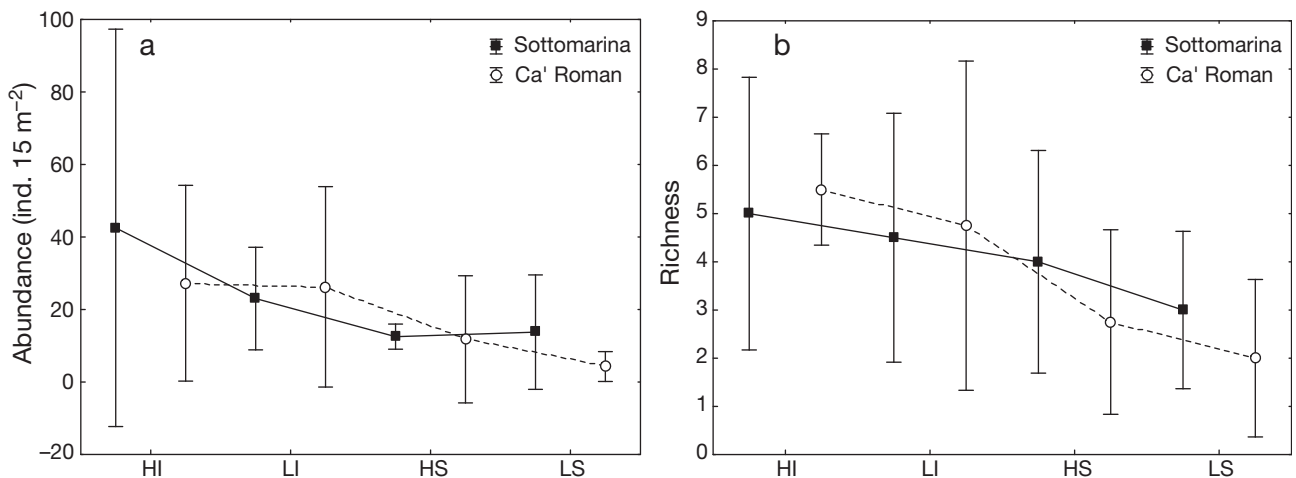
Fig. 2. (a) Mean ± SD abundances and (b) richness of the 2 sites and 4 bathymetric areas. Abbreviations as in Table 1

Table 2 . Similarity (%) between areas in the 2 sites. Abbreviations as in Table 1

Species	Sottomarina				Ca′ Roman			
	HI	LI	HS	LS	HI	LI	HS	LS
HI	68.11				69.80			
LI	52.39	61.21			36.52	62.05		
HS	18.06	32.36	61.40		26.02	42.62	57.41	
LS	3.87	11.80	57.51	58.40	6.71	7.03	12.48	59.97

Table 3. SIMPER analysis: species responsible for dissimilarity between the 2 sites, Ca′ Roman and Sottomarina (Bray-Curtis dissimilarity), within the 4 areas. Av.Diss: average dissimilarity; Diss/SD: dissimilarity/standard deviation; Contrib%: percentage of contribution to the dissimilarity. Other abbreviations as in Table 1

Species	Av.Diss	Diss/SD	Contrib %
AREA HI Average dissimilarity: 43.74%			
Coryphoblennius galerita	12.22	2.88	27.94
Parablennius sanguinolentus	8.27	2.07	18.92
Salaria pavo	6.94	1.47	15.88
Lipophrys adriaticus	5.39	1.60	12.32
L. canevae	4.54	1.35	10.39
Aidablennius sphynx	3.85	1.39	8.81
AREA LI Average dissimilarity: 37.97%			
P. incognitus	8.29	1.57	21.85
L. dalmatinus	7.02	1.34	18.49
P. sanguinolentus	6.19	1.59	16.30
P. zvonimiri	5.45	1.04	14.35
A. sphynx	4.80	0.95	12.65
S. pavo	2.60	1.40	6.84
AREA HS Average dissimilarity: 65.33%			
S. pavo	17.24	2.01	26.39
P. tentacularis	16.33	2.39	25.00
P. zvonimiri	13.83	1.14	21.16
P. sanguinolentus	7.95	1.52	12.17
P. incognitus	7.32	1.30	11.21
AREA LS Average dissimilarity: 55.19%			
P. zvonimiri	17.13	3.44	31.03
P. incognitus	13.68	0.95	24.78
P. tentacularis	11.91	1.19	21.58
P. rouxi	7.05	1.08	12.77

nitus, *P. sanguinolentus*, *P. zvonimiri* and *Salaria pavo*) were not significant (all p > 0.009, α″ = 0.008) with the exception of a positive correlation between abundances of *P. sanguinolentus* and *S. pavo* (r = 0.582, N = 32, p < 0.001).

Overall 261 nests were found, belonging to 10 species: 104 in 2005 and 157 in 2006. No nests of *Coryphoblennius galerita* or *Parablennius sanguinolentus* were observed. Significant differences in nest depth were found between years only for *P. tentacularis*, with nests in 2006 at higher depths than nests in 2005 (2005: –3.59 ± 0.68 m [mean ± SD]; 2006: –4.64 ± 1.34 m; Mann-Whitney *U*-test: Z = 3.13, $N_1 = 20$, $N_2 = 31$, p = 0.002), however nests were found only in the subtidal zone in both years. Therefore, nests recorded in both years were pooled for the analyses for all species, with the exception of *P. tentacularis*, for which only the 2006 data was used. In addition, since only 3 ind. of *P. rouxi* and *P. gattorugine*, and 1 of *Lipophrys canevae* were found in the vertical transects, these 3 species were not considered for the analyses. The most

Table 4. SIMPER analysis: species responsible for similarity (Sim) within area (Bray-Curtis similarity) for the 2 sites. Abbreviations as in Table 3

	Sottomarina				Ca′ Roman		
Species	Av.Sim	Sim/SD	Contrib %	Species	Av.Sim	Sim/SD	Contrib %
Area HI							
Salaria pavo	23.52	3.70	34.53	*C. galerita*	22.94	4.54	32.86
Lipophrys adriaticus	18.97	7.10	27.84	*S. pavo*	16.92	1.95	24.23
Parablennius sanguinolentus	14.11	2.47	20.71	*L. adriaticus*	11.58	3.72	16.59
Aidablennius sphynx	11.52	2.01	16.91	*A. sphynx*	11.50	2.97	16.48
Area LI							
S. pavo	27.94	8.37	45.64	*S. pavo*	24.65	5.76	39.74
P. sanguinolentus	26.52	2.33	43.32	*P. sanguinolentus*	17.86	4.41	28.79
P. zvonimiri	2.44	0.41	3.99	*P. incognitus*	9.45	0.88	15.24
				L. dalmatinus	7.59	0.89	12.24
Area HS							
P. zvonimiri	23.02	2.27	37.48	*S. pavo*	31.73	3.10	55.27
P. tentacularis	22.37	6.92	36.43	*P. incognitus*	20.94	3.03	36.48
P. incognitus	8.57	0.78	13.96				
P. sanguinolentus	7.45	0.90	12.13				
Area LS							
P. tentacularis	33.84	3.24	57.95	*P. tentacularis*	49.85	2.83	83.12
P. zvonimiri	17.39	5.16	29.78	*S. pavo*	5.06	0.41	8.44
P. incognitus	5.22	0.41	8.94				

representative species were *Salaria pavo*, with 53 nests, and *P. tentacularis,* with 51 samples. Nest and individual depth distribution were not significantly different (all p > 0.05, Fig. 4). Nests of *Aidablennius sphynx*, *L. adriaticus*, *L. canevae* and *L. dalmatinus* were distributed in the intertidal, while those of *P. gattorugine*, *P. rouxi*, *P. tentacularis* and *P. zvonimiri* were recorded only subtidally. *S. pavo* and *P. incognitus* had nests distributed over the whole depth range. However, with the exceptions of *A. sphynx* and *P. incognitus*, mean nest depth was higher than mean individual depth for subtidal species, while nests of intertidal species were on average more superficial than were individuals (Fig. 4). Among the species breeding in the intertidal, *L. adriaticus* and *S. pavo* presented the highest intertidal nest rank, followed by *A. sphynx*, *L. dalmatinus*, *L. canevae* and *P. incognitus* (Table 5).

The intertidal blennies generally nested inside rocky holes and black mussel shells while subtidal species primarily used oyster shells. Among intertidal species, *Lipophrys adriaticus,* *L. canevae* and *L. dalmatinus* nested mainly in small holes in the rocky substrate, while *Aidablennius sphynx* used both holes and black mussel shells. Subtidal species such as *Parablennius tentacularis*, *P. rouxi* and *P. zvonimiri* primarily used oyster shells, while nests of *P. gattorugine* were recorded only in large holes and crevices. *P. incognitus* generally nested in black mussel shells, but nests were also found in oyster shells and holes, while *Salaria pavo* used oyster shells as nests for the most part, but also black mussels shells and holes (Fig. 5). The mean nest entrance size positively correlated with species maximum size (r_S = 0.723, N = 10, p = 0.018).

During the surveys performed during very low tides, males were found inside the emerged nests (*Lipophrys adriaticus*: 3 nests, *L. canevae*: 1 nest, *L. dalmatinus*: 5 nests, *Salaria pavo*: 8 nests out of 13), with the exception of *A. sphynx*, whose nests (11) were empty. In 2 cases, the male was observed close to the nest, just below the water surface.
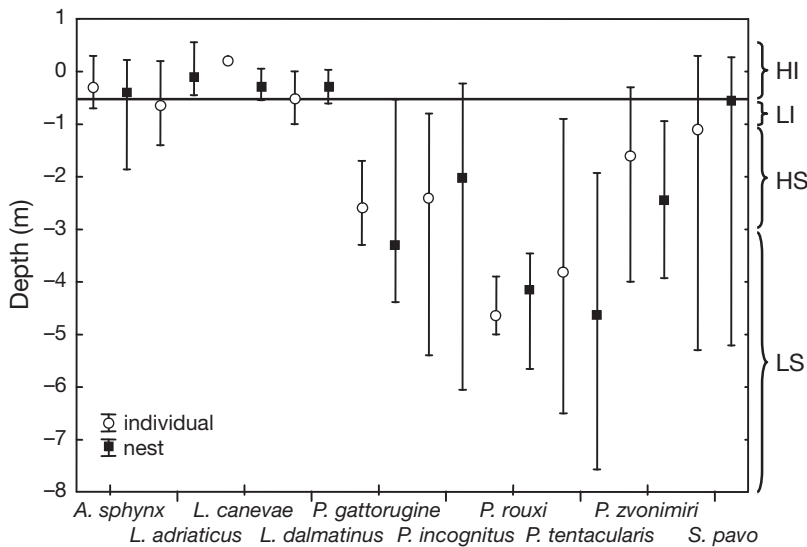

Fig. 4. Vertical distribution of nests and individuals. Bars: minimum and maximum depth values; horizontal line: minimum water level recorded during sampling period (−0.49 m in 2005, −0.52 m in 2006). Maximum water levels recorded were 0.97 m in 2005 and 1.07 m in 2006. The 4 bathymetric areas used for horizontal transect sampling are given on the right. Genera as in Table 5

DISCUSSION

The results of this study demonstrate that the Adriatic intertidal zone is highly exploited by blennies, which present a clear vertical distribution. The consistency in distribution between individuals and nests provides evidence that some species are true intertidal residents, able to exploit the highest zones of this environment.

The blenny assemblage presented the highest abundance and diversity in the intertidal, including the zone regularly subjected to tide flow (HI) and the transitional zone just below the average minimum water level (LI). This
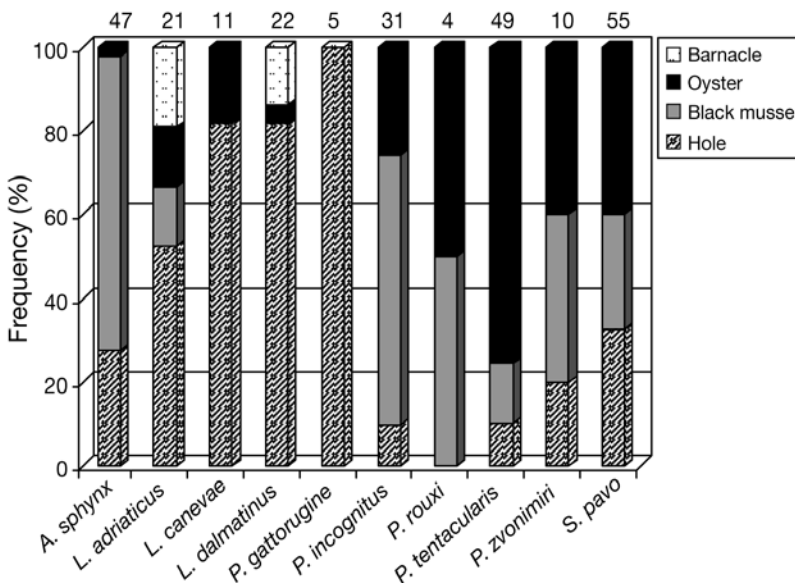

Fig. 5. Nest types. Numbers: sample size for each species. Genera as in Table 5

Table 5. Time of nest exposure to air and percentage (%) of nests exposed for intertidal breeding species. Mean and maximum exposure time is reported for day- and night-time. Species rank has been calculated as sum of ranks of mean and maximum exposure, and percentage of nests subjected to air exposure

| Species | Night | | Day | | % | Rank |
	Mean ± SD	Max.	Mean ± SD	Max.		
Aidablennius sphynx	29 min ± 49 min	6:01 h	1:07 h ± 1:5 h	11:32 h	58.3	3
Lipophrys adriaticus	1:19 h ± 1:24 h	7:27 h	2:35 h ± 3:21 h	16:34 h	85.7	1
L. canevae	25 min ± 39 min	3:55 h	48 min ± 1:21h	8:03 h	63.6	5
L. dalmatinus	28 min ± 37 min	4:09 h	49 min ± 1:10 h	7:59 h	63.6	4
Parablennius incognitus	3 min ± 9 min	2:36 h	4 min ± 14 min	5:22 h	12.5	6
Salaria pavo	1:04 h ± 1:10 h	8:04 h	1:52 h ± 2:03 h	11:57 h	73.7	2

finding is in accord with similar studies concerning other Mediterranean sites (Illich & Kotrschal 1990, Zander et al. 1999, La Mesa & Vacchi 2005, Orlando-Bonaca & Lipej 2007). The 12 studied species exploit different tidal zones. Indeed, 5 species, *Aidablennius sphynx*, *Coryphoblennius galerita*, *Lipophrys adriaticus, L. canevae* and *L. dalmatinus*, were exclusively observed in the intertidal and, with the exception of *L. dalmatinus*, were more abundant in the HI than in the LI. We found 2 species only in the subtidal (*Parablennius rouxi* and *P. tentacularis*), whilst *P. gattorugine*, *P. incognitus*, *P. sanguinolentus*, *P. zvonimiri* and *Salaria pavo* were observed in both environments. Our results did not highlight biotic interactions between species. Indeed only a positive correlation between the abundances of *P. sanguinolentus* and *S. pavo* emerged, likely related to their higher abundances in the same areas. However, the spatial scale of our study could be inadequate to highlight competition for nesting sites, refuges, etc., factors that could be important when analysing the microhabitat used by the different species within areas.

The 2 study sites, the Sottomarina and Ca' Roman jetties, differed in the composition of their assemblages, likely due to differences in wind and wave exposure. Indeed Ca' Roman is more exposed to northeastern winds, while Sottomarina is more protected and presents a higher sedimentation rate, lower water transparency and higher influence of river-water flow (C. Mazzoldi unpubl. data). Direct and indirect influence of wave exposure on blennies has been reported in other areas (La Mesa & Vacchi 2005, Santin & Willis 2007) and on a breakwater near these study sites (Pizzolon et al., 2008), with a general tendency for higher abundance and richness on more exposed sites (La Mesa & Vacchi 2005, Pizzolon et al. 2008). We did not find any difference in richness and abundance between our sites, nonetheless the observed differences in species composition may reflect species preferences. Indeed different abundances of some species between the 2 sites closely reflects the results obtained on the nearby breakwater, where *Parablennius san-guinolentus*, *P. tentacularis* and *P. zvonimiri* were in fact more abundant on the landward side, presenting characteristics similar to Sottomarina, whilst *Lipophrys dalmatinus* was more abundant on the seaward side, which is characterized by higher wave exposure (Pizzolon et al. 2008).

Individual depth distribution closely reflected nest distribution. Despite the lack of data on individual position during ebb tide (e.g. to determine whether there are 'remainers,' Martin 1995), this result strongly indicates that some blenny species can be considered true residents of the intertidal, since they spend the different phases of their lives there (Gibson 1982). With the exception of *Salaria pavo*, which inhabited both intertidal and subtidal zones, all the blennies predominantly nesting in the intertidal were mainly or exclusively recorded in this zone, whilst species characterized by a low utilization of the intertidal for nesting (higher individual depths and low number of nests in the intertidal), such as *Parablennius incognitus*, were seldom observed in the HI zone. In general, our results are in accord with those reported in other northern Adriatic studies (Patzner 1985, Illich & Kotrschal 1990, Orlando-Bonaca & Lipej 2007), with intertidal species appearing more stenobathic than subtidal ones. In this study, *Lipophrys dalmatinus* presented a narrower depth distribution than that reported in other areas, where the species was found up to 4 m below high tide level (Illich & Kotrschal 1990, Orlando-Bonaca & Lipej 2007), and *S. pavo* individuals were observed throughout the depth range, while other studies indicated this species to be confined to ≤1 m (Illich & Kotrschal 1990, Orlando-Bonaca & Lipej 2007). Shelter and the availability of appropriate size nests for the different species (Kotrschal 1988, Orlando-Bonaca & Lipej 2007, Santin & Willis 2007), as well as preferred microhabitat (Illich & Kotrschal 1990, La Mesa & Vacchi 2005, Orlando-Bonaca & Lipej 2007, Santin & Willis 2007) may differ in depth ranges among sites, thus also influencing species distribution accordingly. Such differences aside, blenny species are consistent in their utilization of intertidal and subtidal zones, since they

show a similar vertical distribution in different Adriatic areas (Patzner 1985, Illich & Kotrschal 1990, Orlando-Bonaca & Lipej 2007, present study), despite the likely different distribution of preferred habitat and shelters.

The comparison of nest and individual distribution highlighted a different pattern of space utilization between intertidal and subtidal species. Indeed, the former tended to nest higher in the vertical distribution compared to individual average depth, while the reverse was observed for subtidal species. Intertidal species, specifically exploiting the intertidal for nesting, appear to move subtidally on occasion. In contrast, it seems mandatory for subtidal species to accurately position the 'sessile' nesting phase well below minimum water level, in order to avoid emersion and, possibly, the influence of wave motion. However, at high tide, subtidal adults and/or juveniles may actually visit the intertidal, as observed for *Parablennius zvonimiri*. Nest type (and consequently shape) differed among species, with a positive relationship between nest entrance size and species size, confirming Kotrschal's (1988) findings. Moreover, the nest types recorded in this study closely reflect those described for Mediterranean species in previous studies (Kotrschal 1988, Orlando-Bonaca & Lipej 2007, Santin & Willis 2007).

To judge the ability of a species to utilize the intertidal zone, nest emersion time is a crucial measure, allowing a comparison among species not exclusively based on individual vertical distribution. We found that eggs and, in some cases, parental males, can be out of the water for up to 5 h 22 min, for the least intertidal species, *Parablennius incognitus*, and 16 h 34 min during the daytime, for the most intertidal, *Lipophrys adriaticus*. Even if the range of time exposure is wide, such a period seems particularly long, since it occurs during late spring-summer daytime, when temperatures usually reach their highest values (in the study period, up to 31°C in 2005, 34°C in 2006, data from the Hydrobiological Station of Chioggia), further stressing desiccation problems. Splash from waves may reduce the time a nest is actually under threat of desiccation, while nest configuration (shape, orientation, position with respect to sun exposure and algal cover, etc.) may contribute to maintaining humidity for developing eggs (Gibson 1982, Shimizu et al. 2006). In this study, humidity inside nests or the contribution of splash to maintain wet eggs were not quantified and, consequently, the reported time period of emersion is likely an over-estimate of the period during which eggs actually suffer desiccation problems. Nonetheless, air exposure seems remarkably long for species such as *Aidablennius sphynx*, *L. adriaticus* and *Salaria pavo*. With the exception of *A. sphynx*, males of most intertidal species also remained in the nests during low tide, implying that the parental male suffers the same conditions as developing embryos. Up until now, a similarly long air exposure (c. 12 h d^{-1}) has been documented only in the blenny *Andamia tetradactyla*, a species considered to have terrestrial reproduction, since all the reproductive phases, from mating to egg development, occur consistently when nests are exposed, close to the limits of wave splash (Shimizu et al. 2006). Since the timing of mating with respect to tidal phases has not been investigated in our study species, we do not know if the intertidal species need to be submerged for spawning or if they perform a true terrestrial reproduction.

Intertidal residents are expected to occupy the zones closest to the subtidal, where physical conditions should be more stable. Our results are in contrast with such expectations and with the findings for tidepool species (Thomson & Lehner 1976, Pulgar et al. 2005), since resident blennies appear to fully exploit the HI, both as adults/juveniles and for nesting. According to Underwood (1981) 'pools do not represent an intertidal habitat' since organisms inside pools do not emerge at low tide. Even if such a statement is rather extreme, as conditions in tidepools are also determined by the tidal cycle, nonetheless tidepools and emergent rocky shores represent 2 highly different environments (Metaxas & Scheibling 1993, Martin & Bridges 1999). The degree of fluctuation in physical conditions with vertical distribution is considered larger in emergent rocky substrates than in tidepools. Nonetheless, tidepools high in vertical gradient may become stagnant with consequent pronounced changes in physical parameters such as pH, salinity, temperature, and the occurrence of hypoxic conditions (Metaxas & Scheibling 1993, Martin 1995, Martin & Bridges 1999). Consequently adaptations to tidepools and to rocky shores are expected to show some differences. In order to occupy the tidepools highest in the vertical gradient, species must be tolerant of the harsh conditions determined by the variation in physical parameters due to stagnant situations. For this reason the decrease in species diversity observed in tidepools with increasing intertidal height has been related to the low tolerance of such conditions (Metaxas & Scheibling 1993). Resident species of emergent rocky shores should not only be adapted to variations in temperature, but also capable of breathing air and overcoming desiccation problems (Gibson 1982, Metaxas & Scheibling 1993, Martin 1995, Martin & Bridges 1999). Species with such adaptations should consequently be more able to exploit the highest level of the vertical gradient. Blenny species are known to present several physiological and morphological adaptations to the intertidal, such as the ability to breathe air and to adjust heart rate in order to reduce oxygen demand (Laming et al. 1982). Moreover, their small size and compressed shape enables them to find shelters during low tide or in turbulent

conditions, while fin shape and characteristics help them to cling to rough surfaces (Gibson 1982).

Fish eggs attached to a substrate represent a 'sessile phase' for a mobile organism and are expected to face problems similar to sessile invertebrates. The upper and lower limits of intertidal exploitation by algae and invertebrates have been put in relation to physical factors and biological interactions, respectively (Connell 1972). In particular, the upper limits, for the most part, appear to be related to the species capacity to withstand environmental stresses due to emersion: desiccation threat, extreme temperatures and strong solar radiation (Newell 1970, Connell 1972). While general patterns of vertical distribution have been described since Stephenson & Stephenson (1949), the recently emerging picture appears more complex (Underwood & Chapman 2000). Indeed, besides biological interactions and recruitment variability (Underwood & Chapman 2000), small-scale environmental heterogeneity may also affect species distribution (Benedetti-Cecchi 2001), with factors such as rocky substrate orientation and complexity or wave action influencing the actual air and sun exposure an individual will face (Connell 1972). Similarly, the eggs of intertidal blenny species should present adaptations to desiccation, osmotic and temperature stress and, eventually, UV radiation, all of which have so far been poorly investigated (De Martini 1999, Martin et al. 2004). On the other hand, egg laying in the intertidal may confer advantages to the developing embryos in terms of higher oxygen availability, shorter incubation time and lower predation risk (Martin et al. 2004). A careful nest choice could allow deposition of eggs in a microhabitat particularly suitable for their development. Conversely, the observed nest vertical distribution might not necessarily represent the optimum for egg development but be the result of a trade-off between egg adaptations and other factors, such as nest availability, inter- and/or intra-specific competition for nesting sites.

CONCLUSIONS

The results of this study add an important piece to the analysis of fish vertical zonation in intertidal habitats. The assessment of both individual and nest distribution appear to be useful tools for studying fish vertical zonation, given that nests actually represent a 'sessile' phase. The comparison of nest and individual distribution with depths corrected for the actual tide level allowed the clear attribution of a residence status to some species, with criteria similar to those applicable to sessile invertebrates. Moreover, the observed vertical distribution, given the absence of tidepools, can be considered a real gradient of intertidality.

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