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Experimental study on substratum preferences of early juvenile shanny, *Lipophrys pholis* (Pisces: Blenniidae)

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Substratum choice tests conducted on early juveniles of *Lipophrys pholis* show that they clearly prefer rocky to sandy substrata. The cues involved are still unknown. It is likely that this early preference for the habitat of the adults affords them a greater protection from both predators and wave action. At this early stage agonistic behaviour, although in simple forms, is already present and may play a role in competition for the best shelters.

There is a growing body of evidence indicating that larvae and early metamorphosed juveniles of coral reef and rocky intertidal fish, play a very active role in the process of finding adequate habitats (e.g. Marliave, 1986; Victor, 1991; Henriques & Almada, 1998).

Experiments and observations of substratum choice by young recruits indicate that they can use a variety of cues ranging from light levels and water depth, to tactile and chemical stimuli from organisms of the benthic habitats, to the topography of the substratum including in some cases the availability of hiding places of adequate size. They may even actively seek or avoid larger conspecifics or fish of other species (Marliave, 1977; Sweatman, 1983; Behrents, 1987; Sweatman & St John, 1990).

This paper presents experimental data on substratum choice by newly settled *Lipophrys pholis* (Linnaeus, 1758) and provides evidence on early occurrence of agonistic behaviour.

Fish were collected in January and February 1999 in the morning on spring tides, in tide-pools using hand-nets at Parede, west coast of Portugal, near the mouth of Tagus River (38°41′N 09°22′W).

Fish begin to settle in pools when they are 15.0 mm total length (TL) or slightly larger (mean ±SD=17.4 ±0.124 mm, range=15.0–19.0 mm, N=60), and their body pigmentation is still becoming apparent (unpublished data).

Two groups of 12 juveniles were placed in glass tanks of 118×28×27 cm. In each group, mean sizes were 18.7 ±0.750 mm TL (range=17.0–19.0 mm, N=12) and 21.1 ±0.640 mm TL (range=20.0–22.0 mm, N=12), which means that they had settled only a few days or weeks before capture (Faria et al., 1996).

The tank bottom was divided in two halves, one covered by a layer of sand and the other covered by a layer of rock pieces detached from the natural habitat of the adults. Sand was chosen as an alternative to rock, since it is extremely common on the shoreline between rock stretches and outcrops, and forms the bottom of many tide-pools and channels. Thus, it was assumed that for rocky intertidal fish arriving at the shore or recently settled there, discriminating between rocky and sandy habitats must be of prime importance to the success of the organisms. The rock pieces were rinsed in seawater and all visible organisms were removed before placing them in the tanks. Two stones, also collected from the fish habitat, were placed in the two halves of each tank.

For each group, observations started 24 h after the introduction of the fish, lasted 1 h per day and were continued for 6 d. They were made in the afternoon, from 1400 to 1900 hours, and were concentrated around the time of high tide, when the activity of *L. pholis* is known to be greatest (Gibson, 1976). In each observation period, 12 visual scans were conducted (every 5 min), and the locations of all fish were recorded according to the substratum type where they were located (sand, stone on sand, rock or stone on rock). The agonistic interactions observed during the observation period and their constituent behaviour patterns were also recorded, using the behavioural descriptions provided by Gibson (1968) and Almada et al. (1990).

The results obtained were analysed using a χ^2 goodness-of-fit test, assuming that the expected frequencies were proportional to the areas occupied by each substratum type (1/6 for each stone group, 1/3 for rock, and 1/3 for sand). The significance of the χ^2 was tested using a simulation procedure Adersim (see Almada & Oliveira, 1997 for details).

In Table 1 the statistical analysis for the fish distribution by substratum type at day one is shown, while the data for subsequent days are presented in Table 2.

There is a very clear substratum preference for rock over sand, already highly significant in the first day of observations and remaining consistent throughout the observation period (the occurrences on stones did not differ significantly from the values expected by chance).

A mean of 36.58 ±12.298 (range=12–60, N=12 h) agonistic interactions per hour was recorded, showing that an agonistic repertoire is already present in this species at this early developmental stage. The behavioural patterns observed correspond to the most common and unritualized elements seen in older fish (Gibson, 1968; Almada et al., 1990), including only advancing, charging, retreating and fleeing (9.25 ±4.437 advancing/retreating per hour, range=2–18, N=12 h; 27.17 ±10.495 charging/fleeing per hour, range=9–48, N=12 h).

The results presented above provide experimental evidence for an active substratum preference in these early juveniles, although the cues involving this choice need to be investigated in subsequent studies. Field observations are needed to confirm these results, though after many hundreds of hours of shore inspection no *L. pholis* were collected from purely sandy habitats suggesting that these findings are not laboratory artefacts.

Table 1. *Statistical analysis of the distribution of fish by substratum type for day one, using Adersim. Only the significant cells are presented. (A) Cases in which the observed values did not exceed the simulated values (observed values higher than simulated values). (B) Cases in which the simulated values did not exceed the observed values (observed values lower than simulated values). Group 1:* $\chi^2=232.5$, *df=47*, $P<0.001$. *Group 2:* $\chi^2=192.75$, *df=47*, $P<0.001$.

	Scan 1	Scan 2	Scan 3	Scan 4	Scan 5	Scan 6	Scan 7	Scan 8	Scan 9	Scan 10	Scan 11	Scan 12
A. Rock (group 1)	1**	7**	1**	4**	1**	2**	1**	5**	4**	3**	0***	1**
Rock (group 2)	0***	3**	23*	4**	26*	3**	1**	8**	10*	9**	3**	8**
B. Sand (group 1)	23*	23*	14*	14*	17*	22*	100	253	248	21*	19*	12*
Sand (group 2)	16*	18*	221	89	432	98	97	227	216	96	18*	91

*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$ (one-tailed).

Table 2. *Statistical analysis of the distribution of fish by substratum type for days 2–6, using Adersim. The observed frequencies are averaged for the 12 scans of each day. Only the significant cells are presented. (A) Cases in which the observed values did not exceed the simulated values (observed values higher than simulated values). (B) Cases in which the simulated values did not exceed the observed values (observed values lower than simulated values). Group 1:* $\chi^2=84.923$, *df=19*, $P<0.001$. *Group 2:* $\chi^2=86.239$, *df=19*, $P<0.001$.

	Day 2	Day 3	Day 4	Day 5	Day 6
A. Rock (group 1)	2**	2**	1**	2**	0***
Rock (group 2)	0***	1**	2**	1**	2**
B. Sand (group 1)	23*	232	8**	95	18*
Sand (group 2)	12*	25*	12*	92	86

*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$ (one-tailed).

As the fish were not laboratory reared the effects of experience on preference development cannot be ruled out. Thus, further ontogenetic investigations with inexperienced individuals are needed.

This study does not address the possibility of active habitat selection during settlement. It suggests that after settlement, habitat choice may play a role in driving the fish to the substratum preferred by larger individuals (Faria & Almada, in press), rich in complexity and with intersticies of various sizes likely to provide shelter from predators, wave action and desiccation.

In larger individuals, agonistic behaviour is functionally linked to shelter access (Faria et al., 1998), and the presence of incipient agonistic behaviour in small juveniles suggests the possibility of an early form of such a mechanism.

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REFERENCES

Almada, V.C. & Oliveira, R.F., 1997. Sobre o uso de estatística de simulação em estudos de comportamento. *Análise Psicológica*, **15**, 97–109.

Almada, V.C., Oliveira, R.F. de, Barata, E.N., Gonçalves, E.J. & Rito, A.P., 1990. Field observations on the behaviour of the breeding males of *Lipophrys pholis* (Pisces: Blenniidae). *Portugaliae Zoologica*, **1**, 27–36.

Behrents, K.C., 1987. The influence of shelter availability on recruitment and early juvenile survivorship of *Lythrypnus dalli* Gilbert (Pisces: Gobiidae). *Journal of Experimental Marine Biology and Ecology*, **107**, 45–59.

Faria, C. & Almada, V., in press. Microhabitat segregation in three rocky intertidal species in Portugal: does it reflect interspecific competition? *Journal of Fish Biology*.

Faria, C., Almada, V.C. & Gonçalves, E.J., 1996. Juvenile recruitment, growth and maturation of *Lipophrys pholis* (Pisces: Blenniidae), from the west coast of Portugal. *Journal of Fish Biology*, **49**, 727–730.

Faria, C., Almada, V. & Nunes, M.C., 1998. Patterns of agonistic behaviour, shelter occupation and habitat preference in juvenile *Lipophrys pholis, Coryphoblennius galerita and Gobius cobitis*. *Journal of Fish Biology*, **53**, 1263–1273.

Gibson, R.N., 1968. The agonistic behaviour of juvenile *Blennius pholis* L. (Teleostei). *Behaviour*, **30**, 192–217.

Gibson, R.N., 1976. Experiments on the tidal rhythm of *Blennius pholis* L. *Journal of the Marine Biological Association of the United Kingdom*, **47**, 97–111.

Henriques, M. & Almada, V.C., 1998. Juveniles of non-resident fish found in sheltered rocky subtidal areas. *Journal of Fish Biology*, **52**, 1301–1304.

Marliave, J.B., 1977. Substratum preferences of settling larvae of marine fishes reared in the laboratory. *Journal of Experimental Marine Biology and Ecology*, **27**, 47–60.

Marliave, J.B., 1986. Lack of planktonic dispersal of rocky intertidal fish larvae. *Transactions of the American Fisheries Society*, **115**, 149–154.

Sweatman, H.P.A., 1983. Influence of conspecifics on choice of settlement sites by larvae of two pomacentrid fishes (*Dascyllus aruanus* and *D. reticulatus*) on coral reefs. *Marine Biology*, **75**, 225–229.

Sweatman, H.P.A. & St John, J., 1990. Effects of selective settlement and of aggression by residents on distribution of young recruits of two tropical damselfishes. *Marine Biology*, **105**, 247–252.

Victor, B.C., 1991. Settlement strategies and biogeography of reef fishes. In *The ecology on coral reefs* (ed. P.F. Sale), pp. 231–260. San Diego: Academic Press, Inc.

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