

Sex Differences in the Dorsolateral Telencephalon Correlate with Home Range Size in Blenniid Fish

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Key Words

Blennies · Spatial behavior · Sexual dimorphism · Hippocampal pallium · Teleost fish

Abstract

Blenniid fish exhibit a polygynandric mating system with parental care restricted to males. Nest-holder males defend a breeding territory centered on their nest, usually a crevice or hole in a rocky substrate, to which they attract females to spawn. Females, on the other hand, must search for nests in order to spawn and usually are the choosy sex, producing several sequential egg batches and broods during the breeding season. Therefore, male blennies are more site-attached than females. This situation offers an opportunity to investigate potential neural correlates of intraspecific differences in selective pressures for different spatial abilities in these species. Since the dorsolateral telencephalon has been considered a teleost homologue of the mammalian hippocampus, we predicted that the spatial abilities required for females to locate and return accurately to nests of males may have produced a sex difference in the size of the telencephalic nuclei involved in spatial abilities, biased towards females. To test this hypothesis, we assessed the home ranges and measured the size of the dorsolateral telencephalon of both sexes

during the breeding season in two blenniid species, the shanny (*Lipophrys pholis*) and the Azorean rock-pool blenny (*Parablennius parvicornis*). We chose these two species because they differ in the degree of chemical communication they use, and this could also lead to differences in telencephalic areas. As predicted, in both species females present considerably larger home ranges paralleled by larger dorsolateral ventral telencephalic nuclei (DLv) than males. Other telencephalic nuclei that were measured did not show any sex difference in size. These results suggest that the DLv is involved in spatial abilities in blenniid fish and that sexual selection may be promoting this divergence as already described for mammals and birds.

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Introduction

Ecological conditions and navigational demands are known to reflect the spatial strategies used by animals [Odling-Smee et al., 2006] and to shape the underlying neuroanatomy of the brain [Kotrschal and Palzenberger, 1992; Kotrschal et al., 1998; Shumway, 2008]. One of the best documented cases in neuroecology is the relationship between spatial ability requirements and the relative

Abbreviations used in this paper

BO	bulbus olfactorius
CA	commissura anterior
Dc	area dorsalis telencephali pars centralis
Dd	area dorsalis telencephali pars dorsalis
DLd	dorsal subdivision of area dorsalis telencephali lateralis
DLv	ventral subdivision of area dorsalis telencephali lateralis
Dm	area dorsalis telencephali pars medialis
Dp	area dorsalis telencephali pars posterior
E	nucleus entoptenduncularis
N Olf Ant	nucleus olfactorius anterior
Nt	nervus terminalis (nucleus olfactorectinalis)
POA	preoptic area
Vc	area ventralis telencephali pars centralis
Vd	area ventralis telencephali pars dorsalis
Vi	area ventralis telencephali pars intermedia
Vl	area ventralis telencephali pars lateralis
Vp	area ventralis telencephali pars postcommissuralis
Vs	area ventralis telencephali pars supracommissuralis
Vv	area ventralis telencephali pars ventralis

development of specific neural substrates for spatial cognition, such as the hippocampus in mammals, or other medial pallium homologues in other vertebrate taxa [Jacobs, 2009]. Examples come from mammals [Jacobs et al., 1990; Galea et al., 1996], birds [Sherry et al., 1993; Reboreda et al., 1996; Hoshoooley and Sherry, 2007], reptiles [Day et al., 1999a, b, 2001], and fish [Sovrano et al., 2003; Pollen et al., 2007] revealing striking patterns of sex, season, species and/or population differences [Jacobs, 2009].

Sex differences in spatial cognition have been detected both in vertebrates and in invertebrates [e.g. Gaulin and FitzGerald, 1986, 1989; Jozet-Alves et al., 2008] and can be explained by sexual selection if increments in spatial ability had a greater effect on the mating success of one sex than the other [Puts et al., 2007]. Gaulin and FitzGerald [1986, 1989] were the first to predict the link between mating system and spatial cognition. Their work in voles (genus *Microtus*) showed that breeding males of polygamous species have home range sizes 4–5 times larger than those of females, while monogamous species lack such sex differences in ranging behavior, since males and females defend a joint territory. Later Jacobs et al. [1990] made the link between mating system type and the relative volume of the hippocampus in voles, where only in polygamous species the hippocampus of males is enlarged relative to the entire brain. Similar findings were observed in birds. In the brown-headed cowbird, a nest parasite where females must find host nests, females have

relatively larger hippocampi than males, whereas in polygynous species, where males and females do not differ in space use, the hippocampal volume was not dimorphic [Sherry et al., 1993]. Therefore, the direction of dimorphism could be predicted by the sex-specific mating behavior.

Sex differences are known to have seasonal patterns for several species of birds and mammals, in which larger dispersal areas are associated with larger hippocampus in a specific sex only during the breeding season [Galea et al., 1996; Jacobs, 1996; Clayton et al., 1997; Galea and McEwen, 1999; Lavenex et al., 2000a, b]. In fish correlations between environmental complexity, social organization (mating system) and brain structures were recently reported for African cichlids [Pollen et al., 2007]. In a cichlid fish, sexually distinct performances have also been observed using a spatial maze, with males outperforming females in a cichlid fish [Sovrano et al., 2003]. However, data on the relationship between the occurrence of sexually distinct dispersal patterns and sexual dimorphisms in putative areas of the central nervous system involved in spatial cognition are still lacking for teleosts.

Blenniids provide a good model for the study of sex differences in spatial cognition in fish since during the breeding season the spatial behavior is distinct between the sexes. Parental males are known to be highly site-attached to a nest waiting for females to spawn and providing parental care to the eggs [Neat and Lengkeek, 2009]. Females, on the other hand, visit several nest sites during the breeding season having higher mobility rates than males and exploring larger areas [Santos, 1986; Santos et al., 1989, 1995]. Since blenniid females do not establish territories and explore larger areas than males in search for mates, one can hypothesize the occurrence of sex differences in brain regions involved in spatial memory was biased towards females. Studies in goldfish revealed that the dorsolateral ventral region of the telencephalon (DLv) is involved in the processing of spatial learning and memory [Vargas et al., 2000; Portavella et al., 2002; Rodríguez et al., 2002; Vargas et al., 2006] and considered homologous to the hippocampus of amniotes [Bradford, 1995; Wullimann and Mueller, 2004; Northcutt, 2006, 2008]. Thus, it is expected that sex differences in spatial behavior in blennies should be paralleled by sex differences in the size of the ventral area of the dorsolateral telencephalon.

We tested this hypothesis in two closely related blenniid species [Almada et al., 2005] (see fig. 1) which live in the same habitat (the intertidal zone of exposed rocky

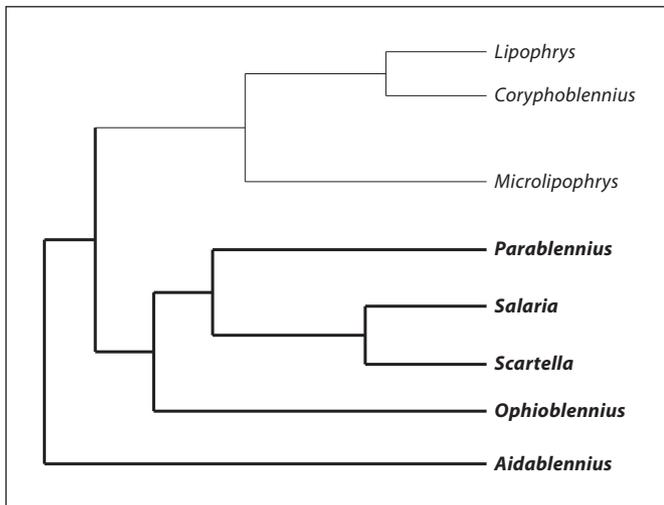


Fig. 1. Phylogenetic tree of the north-eastern Atlantic and Mediterranean blenniids (genera). Thick lines indicate the presence of anal glands [adapted from Almada et al., 2005].

shores): the Azorean rock-pool blenny (*Parablennius parvicornis*) and the shanny (*Lipophrys pholis*). The rationale for the use of this pair of species is that sex differences in the size of the ventral area of the dorsolateral telencephalon could also be explained as an adaptation for the processing of sex pheromones, since sex pheromone-producing anal glands are present in some blenniid lineages [Laumen et al., 1974; Zander, 1975; Barata et al., 2008] and secondary olfactory projections throughout the whole telencephalon are known to occur in teleosts [Finger, 1980; Davis et al., 1981; Rooney et al., 1992; Eisthen, 1997]. The DLv specifically receives olfactory input in several species, namely in goldfish [von Bartheld et al., 1984], cod [Rooney et al., 1992] and channel catfish [Bass, 1981]. Moreover, several lines of evidence suggest that this area is one of the targets of the secondary olfactory tracts that mediates reproductive behavior in goldfish [Weltzien et al., 2003 and references therein].

Since in some blenniid species, males present anal glands, females of these species could also have acquired brain specializations for pheromone processing (i.e. a co-evolution between the production of the pheromone signal by males and its processing by females). Therefore, we chose one species (the rock-pool blenny) with anal glands and one species that lacks these glands (the shanny) in order to determine the relative contribution of chemical communication and spatial cognition for the expected sex differences in the size of the ventral area of the dorsolateral telencephalon.

The following alternative hypotheses can be expected:
 (1) If we observe a female-biased sex difference in the ventral area of the dorsolateral telencephalon of the rock-pool blenny that is not present in the shanny, it should be due to a specialization in pheromone processing.

(2) If we detect a female-biased sex difference in the ventral area of the dorsolateral telencephalon in both species, then it should be seen as a specialization for spatial cognition, and pheromone processing should not be invoked as the causal explanation.

To test these hypotheses, we have not only measured telencephalic nuclei but also the size of the nucleus olfactorius anterior of the olfactory bulb that is predicted to be more developed in females of the species with pheromone communication.

Materials and Methods

Animals and Study Sites

We have studied the rock-pool blenny *P. parvicornis* at Faial Island, Azores, Portugal. The home range observations for this species were conducted in a 400-meter line shore of the tidal platform of Feteira at the south coast of Faial. To avoid interference with the behavioral observations, the collection of specimens to be killed for brain measurements was done at a similar rocky area 2 km east from Feteira. The shanny (*L. pholis*) was studied in mainland Portugal at two different sites: (a) specimens for brain measurements were collected at the tidal platforms of Mexilhoeira and Cabo Raso (Cascais, Portugal), and (b) observations for the calculation of home ranges were performed at the Arrábida Marine Park between the beaches of Alpertuche and Pilotos (Setúbal, Portugal). During the experimental procedure the 'Association for the Study of Animal Behaviour (ASAB) guidelines for the use of animals in research' were applied.

Home Ranges

We used two different methods for the computation of home ranges in *P. parvicornis* and in *L. pholis*, and therefore the data cannot be compared between the two species.

We caught and marked 73 individuals of *P. parvicornis* (60 females and 13 males) during low tide. In addition, 44 males recognized individually using a combination of individual marks (e.g. scars), relative size differences and nest location were also used in this study. Animals were followed on a daily basis in subsequent low tides for 60 days during two consecutive breeding seasons (June/July 1998 and 1999). Individuals were tagged with a combination of three plastic color beads inserted at the base of the dorsal fin following the method described by Patzner [1984], and previously proved successful in this species [Taborsky and Limberger, 1980; Santos et al., 1989]. All the tide pools and the nests inside them present in the study area, a 400-meter rocky shore front (transect), were mapped and followed on a daily basis during the 2 months of the field study. Every day during low tide we conducted scan observations during which the location of each individual was recorded in a map of the area. Distances between refer-

ence points were taken to the nearest centimeter. Home ranges are expressed as the maximum distance between two points where the same individual has been spotted. Because males are more site-attached than females, we collected home range data for 34 males but only for 6 females.

In *L. pholis* we performed underwater animal focal observations while scuba diving during high tide and the maximum distance where the same individual was spotted was recorded. Individual nest-holder males were recognized using a combination of individual marks (e.g. scars), relative size differences and nest location. Females were identified by size and distinctive features (e.g. scars). These observations were done during the peak of the breeding season of *L. pholis* (males: $n = 22$, during March and April 1986, 1987 and 1993; females: $n = 24$, during March 1993). Observation time ranged from 6 to 20 min for females and from 13 and 120 min for males. No correlation was found between observation time and recorded distance for each sex (males: $r = 0.22$; females: $r = -0.05$). Therefore, comparisons of the distances were used to further analyze differences between the sexes, independently of the time of observation.

Measurement of Brain Regions

Brain measurements were taken from sexually active adult males [$n = 5$; standard length (SL) = 12.46 ± 0.68 cm; weight = 27.99 ± 5.18 g] and females ($n = 5$; SL = 10.26 ± 0.52 cm; weight = 16.54 ± 3.2 g) of the rock-pool blenny *P. parvicornis* and for sexually mature males ($n = 5$; SL = 10.9 ± 0.79 cm; weight = 23.36 ± 5.04 g) and females ($n = 5$; SL = 11.93 ± 1.35 cm; weight = 29.99 ± 8.74 g) of *L. pholis*. We opted for histology on frozen sections similar to Kotrschal and Palzenberger [1992], because brain surface measurements would not have revealed the details required in the present context.

The individuals were deeply anesthetized with MS-222, their SL and body weight were measured and they were then perfused transcardially via the bulbus arteriosus with a marine teleost Ringer solution to clear the vasculature, followed by a solution of 4% paraformaldehyde in 0.1 M phosphate buffer to fix the tissues. The brains were removed from the neurocranium and postfixed overnight at 4°C using the same fixative solution, and transferred to 0.1 M phosphate buffer for storage at 4°C. Brains were embedded in gelatine and coronally sectioned at 20 μm on a Microm HM 500 M cryostat at -22°C. The sections were mounted in series and stained with cresyl violet.

All slides were coded so that the observer who quantified the different brain areas did not know the identity of the fish to which each slide corresponded. Size of brain nuclei was measured by capturing images from an Olympus Stereomicroscope (Olympus BX 50) with a digital camera (Camedia C-2020 Z). The outlines of the regions in question were then traced using ImageTool 5.0 (Texas Health Centre, San Antonio, Tex., USA). After calibrating for magnification, the imaging program provided a measure of the area of each region. We measured all the sections of each brain from its most rostral part until the end of the telencephalon (*P. parvicornis*: 86 ± 5.2 sections; *L. pholis*: 110.2 ± 3.5 sections). The rostral margin of the anterior commissure was used as a reference point.

In this study, we concentrated on the dorsolateral areas of the telencephalon since recent evidence suggests an involvement of these regions in the resolution of spatial tasks [Vargas et al., 2000]. Thus, we measured the pars dorsalis (DLd), the DLv and the pars

posterior (Dp) of the area dorsalis of the telencephalon [nomenclature according to Northcutt and Braford, 1980]. For reasons explained above, we also measured the nucleus olfactorius anterior of the olfactory bulb. Left- and right-side measurements were averaged for each structure.

The volume of each region was calculated by adding the measured areas which were multiplied by the thickness of each section (20 μm) and expressed as μm^3 . The volume of the whole telencephalon was also computed. Since we found a positive correlation between body size and telencephalon volume (*P. parvicornis*: $r = 0.87$; *L. pholis*: $r = 0.71$), we expressed all volume measurements of the different regions as a percentage of total telencephalon size, with the exception of the nucleus olfactorius anterior, in which case the volume was expressed as a percentage of the SL³.

Results

Home Ranges

During the breeding season, *P. parvicornis* and *L. pholis* males presented significantly smaller home ranges than females (Mann-Whitney U test: *P. parvicornis*: $Z = 3.693$, $p < 0.001$; *L. pholis*: $Z = 4.189$, $p < 0.001$, fig. 2). Males of *P. parvicornis* presented a maximal distance that ranged from 0 to 0.47 m, with one outlier that reached 216.1 m (mean \pm SE: 6.52 ± 6.35 m, $n = 34$), while maximal distance for females ranged from 2.7 to 328.1 m (mean \pm SE: 128.63 ± 64.98 m, $n = 6$). In *L. pholis*, the range of maximum distance presented by the males is between 0 and 1 m (mean \pm SE: 0.32 ± 0.07 m, $n = 22$) and females present maximum distances between 0.3 and 3 m (mean \pm SE: 1.01 ± 0.12 m, $n = 24$).

The Blenniid Telencephalon

Since there are no available descriptions of the forebrain of these two species in the literature, a short description is provided before presenting the quantitative results. The telencephalon of *P. parvicornis* and *L. pholis* has many features in common with other teleosts [Kotrschal et al., 1998], and all major divisions are clearly recognizable. The telencephalic areas were determined based only on the cytoarchitectural analysis. Since no connectional or immunohistochemical data are available for these species, no further subdivision of the main areas was established. Both species present a dorsal area more developed than the ventral area, with some dorsal areas positioned ventrally to the ventral areas (fig. 3). The most rostral part of the brain is composed of sessile olfactory bulbs, i.e. fused in the posterior area with the rostromedial telencephalon, and connected to the olfactory epithelium by a long olfactory nerve. The nucleus olfactorius

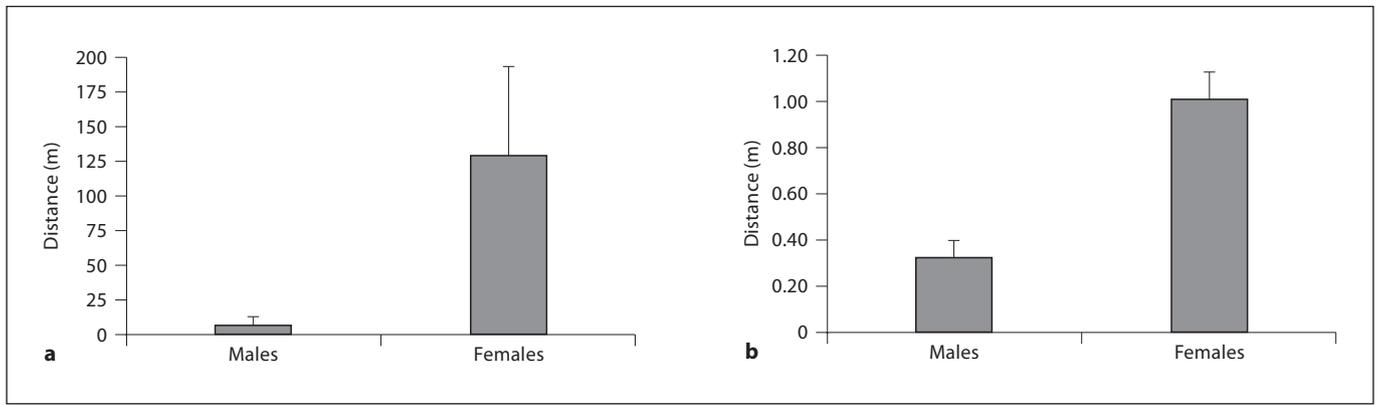


Fig. 2. Home ranges of *P. parvicornis* (a) and *L. pholis* (b). Bars represent mean values; error bars represent standard error. See Results for statistical comparisons.

anterior is positioned in the dorsal part of the olfactory bulb (fig. 3a). The medial ventral and dorsal lateral regions of this structure correspond respectively to the medial and lateral olfactory tracts, which in other species project to the telencephalon and posterior regions of the brain. The ventral caudal region of the olfactory bulb is designated nervus terminalis, a nucleus of cells with neurosecretory functions.

The dorsal telencephalon of actinopterygians is generally believed to correspond to the pallium, while the ventral telencephalon is generally considered to be homologous to the subpallium of tetrapods [Nieuwenhuys and Meek, 1990]. The pallium (dorsal telencephalon) of both species is characterized by extensive lateral, medial (Dm), and central (Dc) divisions with many distinct cell groups, and large, but more uniform dorsal and Dp divisions. The Dm area presents high cell density structured in columns. The DLv present low cell density, while the DLd present an intermediate cell density. Dc is characterized by its larger and more dispersed neurons (fig. 3). Since a clear distinction between dorsal division and Dc is not evident in these species, they were considered as a whole. At the level of the anterior commissure, composed of bundles of decussating axons between the two telencephalic hemispheres, Dc presents the maximal density of fibers (fig. 3c). At this level, the division between the dorsal and ventral telencephalon is clear through a fiber sheet, the lamina terminalis. At the level of the anterior commissure, the DLv and DLd areas present their cells arranged in cortical columns. It was not possible to clearly distinguish the nucleus taenia, and since it is located in the border of both DLv and Dp, it is necessarily integrat-

ed in one of these areas. At the caudal region of the telencephalon, the two species acquire distinct features: while in the shanny the precommissural pattern is maintained, in the rock-pool blenny the hemispheres become coalescent and the Dc and Dm areas of each hemisphere become fused (fig. 3d, e). In both species a new area emerges, the area Dp located ventrally between the DLv and lateral nuclei (Vl) (fig. 3d).

In the ventral telencephalon, it is possible to distinguish four main cell groups in the periventricular region, the ventral (Vv), dorsal (Vd), supracommissural, and postcommissural (Vp) nuclei. Rostral to the anterior commissure, the ventral telencephalon consists of Vd, Vv, Vl, and central/commissural (Vc). In both species the Vc, Vl, and intermediate nuclei are also observed (fig. 3b). Near the anterior commissure, ventrally to area ventralis, the preoptic area (POA) starts to emerge. At the level of the anterior commissure, the Vd is replaced by the area ventralis supracommissuralis (fig. 3c), which is then replaced more caudally by the area Vp. At the level of the anterior commissure, both Vc and Vl are replaced by the area ventralis intermedia located ventrally to the Vp (fig. 3d).

Quantification of Telencephalic Nuclei

The nucleus olfactorius anterior of the olfactory bulb is significantly larger in females than in males of the rock-pool blenny, but there is no significant difference between the sexes in the shanny (table 1). Although sex differences in body size (i.e. SL) are only detected in the rock-pool blenny, and the total telencephalon is larger in males of rock-pool blenny and in females of shanny, no sex differ-

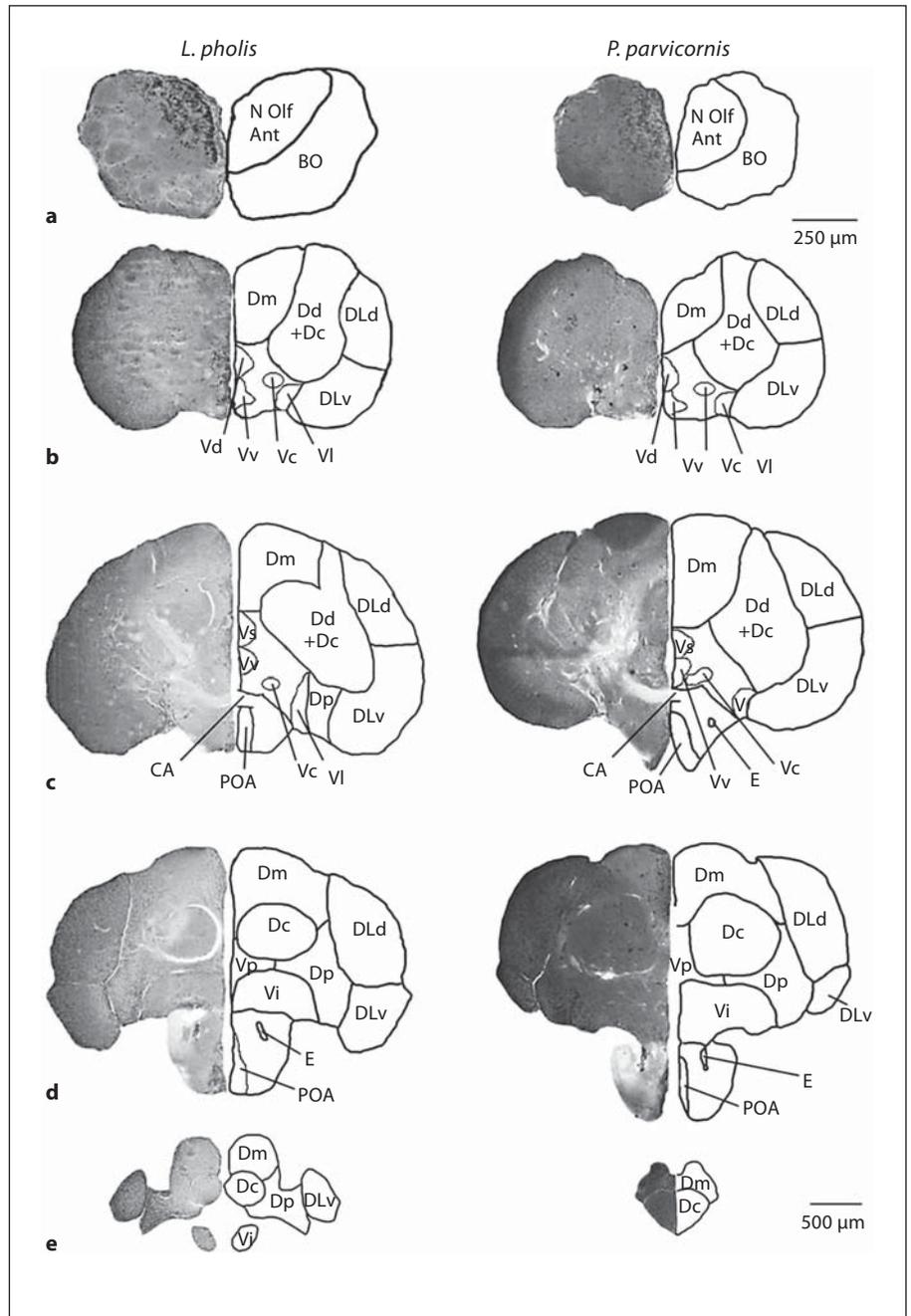


Fig. 3. Cross sections of the telencephalon and olfactory bulb from *P. parvicornis* (right) and *L. pholis* (left). Cresyl violet staining is shown on the left and a schematic illustration of the figure is drawn on the right. The rostral-caudal distance of each section from the rostral margin of the anterior commissure is the following: *L. pholis*: -1,080 µm; *P. parvicornis*: -1,120 µm (a); -400 µm (b); *L. pholis*: +80 µm; *P. parvicornis*: 0 µm (c); +500 µm (d); *L. pholis*: +740 µm; *P. parvicornis*: +900 µm (e).

ences are observed in the volume of the telencephalon when corrected for body size, i.e. SL^3 (table 1).

Among the dorsolateral areas of the telencephalon, only the DLv shows a significant sex difference in both species with females having relative larger areas than males (table 1). No significant differences were observed between the two species in the size of the DLv (Mann-Whitney U test: $Z = -0.302$; $p = 0.762$).

Discussion

Our results show that the anterior olfactory nucleus is only larger in females of the species with specialized pheromone production while the DLv telencephalic region is larger in females of both species. These results taken together suggest that the sex differences found in the nucleus olfactorius of the rock-pool blenny are prob-

Table 1. Sex differences (Z values from Mann-Whitney U tests) in body size and brain measurements in *P. parvicornis* and *L. pholis*

	<i>P. parvicornis</i>		Z value	p value	<i>L. pholis</i>		Z value	p value
	males	females			males	females		
SL	124.6 ± 6.8 (5)	102.6 ± 5.28 (5)	-2.19	0.03*	109.8 ± 6.5 (4)	127.8 ± 8.0 (4)	-1.16	0.24
Tel	5.10 ± 0.44 (5)	3.85 ± 0.28 (5)	-2.19	0.03*	5.13 ± 0.23 (5)	6.34 ± 0.42 (5)	-1.99	0.04*
Tel/SL ³	2.72 ± 0.29 (5)	3.66 ± 0.38 (5)	-1.36	0.18	4.09 ± 0.70 (4)	3.38 ± 0.59 (4)	-1.12	0.248
Nolf	21.34 ± 3.73 (3)	32.46 ± 3.69 (5)	-1.92	0.05*	36.83 ± 5.06 (4)	25.75 ± 4.09 (4)	-1.44	0.14
DLv	0.157 ± 0.006 (5)	0.186 ± 0.005 (5)	-2.40	0.02*	0.165 ± 0.007 (5)	0.184 ± 0.006 (5)	-1.99	0.04*
DLd	0.141 ± 0.014 (5)	0.117 ± 0.005 (5)	-1.56	0.11	0.160 ± 0.006 (5)	0.152 ± 0.014 (5)	-0.94	0.34
Dp	0.044 ± 0.004 (5)	0.035 ± 0.005 (5)	-1.77	0.075	0.045 ± 0.005 (5)	0.041 ± 0.004 (5)	-0.73	0.46

Values are expressed as mean ± SE. Figures in parentheses represent number. SL = Standard length (cm); Tel = volume of the telencephalon (E+9 μm³); Tel/SL³ = relative volume of the telencephalon corrected for SL³ (E+3 μm³ cm⁻³); Nolf = nucleus olfactorius anterior; DLv = ventral subdivision of area dorsalis telencephali lateralis; DLd = dorsal subdivision of area dorsalis telencephali lateralis; Dp = area dorsalis telencephali pars posterior. The volume of each nuclei is presented as the relative value of the total volume of the telencephalon (DLv, DLd and Dp) or of the (SL)³ (Nolf). Significant differences are indicated with *.

cephali lateralis; DLd = dorsal subdivision of area dorsalis telencephali lateralis; Dp = area dorsalis telencephali pars posterior. The volume of each nuclei is presented as the relative value of the total volume of the telencephalon (DLv, DLd and Dp) or of the (SL)³ (Nolf). Significant differences are indicated with *.

ably related to a specialization in chemical processing by females whereas the sex differences detected in the DLv of both species cannot be explained by the same factor alone, since in the shanny, in which males lack the anal gland, the DLv region is still larger in females.

We also documented a large sex difference in home range size in both species, and therefore the abovementioned sex difference in the DLv region of both species might be related to differences in spatial abilities between males and females. In *L. pholis*, the dispersal seems to be more restricted in both sexes when compared to the rock-pool blenny, but the different methods used to estimate home range sizes in these two species prevent a direct comparison of the estimates.

The DLv in fish seems to be involved in spatial learning and memory in a similar manner to the role of the hippocampus in birds and mammals [Vargas et al., 2000; Portavella et al., 2002; Vargas et al., 2006]. The size of the telencephalon of African cichlids is known to be correlated with the spatial complexity of the habitat [van Staaden et al., 1995] and to the species mating system [Pollen et al., 2007]. Moreover, telencephalon ablation impairs spatial learning [Salas et al., 1996; López et al., 2000; Durán et al., 2008] and short-term memory retention [Ohnishi, 1997] in goldfish. More specifically, lateral pallium ablations selectively impair the encoding of geometric spatial information of environmental space [Rodríguez et al., 2002; Vargas et al., 2006]. Finally, learning of a spatial task in goldfish induces protein synthesis in dorsolateral telencephalic neurons but not in other areas of the telencephalon [Vargas et al., 2000]. Interestingly, this

is the same area where we find the sexually dimorphic nuclei in both species of blennies.

In closely related species of voles, a similar situation has been described but with the reversed sex difference. In the polygynous meadow vole, males have larger home ranges than females [Gaulin and FitzGerald, 1986, 1989], and this difference in home ranges has neural and cognitive correlates: males have a larger hippocampus and are better at solving spatial tasks than females [Jacobs et al., 1990; Galea et al., 1996; Kavaliers et al., 1998]. In monogamous pine voles, no sex differences in home ranges are present and concomitantly there are no sex differences, either in spatial abilities or in hippocampus volume [Gaulin et al., 1990; Jacobs et al., 1990; Galea et al., 1996].

The reproductive strategy of blenniid species favors the existence of a sex-biased dimorphism in the DLv area and ultimately in spatial cognitive strategies similarly to the abovementioned example. Blenniids share several reproductive and ecological traits that predispose them to the action of sexual selection: (1) a promiscuous mating system, (2) a demersal spawning strategy (i.e. spawning occurs in a substratum with the establishment of a nest), with external fertilization, and (3) the establishment of breeding territories and the occurrence of paternal care of the eggs by males [Neat and Lengkeek, 2009]. Since male-male competition for nest sites and egg predation is usually high, male home ranges are restricted to regions close to their nest location. Their feeding intensity decreases significantly during the breeding season when compared to females [Santos and Almada, 1988; Almada et al., 1992; Santos et al., 1996; Gonçalves and Almada, 1997]. In con-

trast, females must locate suitable males with adequate nests to spawn and therefore utilize larger areas, presenting a more extensive home range. There are multiple pieces of evidence that females mate in a nonrandom fashion [Neat and Lengkeek, 2009] and this requires good navigation and spatial abilities. For example, in a related blenniid species that also breeds in the intertidal zone (the peacock blenny, *Salaria pavo*), ripe females visit several nests and in some cases return to previously visited males, and mate choice decisions are based on the quality of the males encountered [Fagundes et al., 2007]. These observations suggest that females can recall their male searching excursions, and have a spatial map of the location of suitable nest sites in their area. Moreover, in other blenniid species (*Ophioblennius atlanticus*), feeding territories of the females can be located at significant distances from the nest sites of the males [Reynolds and Côté, 1995], suggesting again that during the breeding season blenniid females are under higher selective pressure than males for spatial abilities. For these reasons, it seems reasonable to hypothesize that in blenniids the enlargement of the DLv in females may result from sexual selection.

A more definitive test of this hypothesis requires the collection of data on a larger number of species and in particular the comparison of phylogenetically closely related species that differ in the presence of sex difference (i.e. species with females with larger home ranges than males vs. species with no sex difference in their home range). To our knowledge, this contrast is not possible in blenniids since males are always the sex that provides parental care and therefore are always more site-attached than females. However, the occurrence of alternative reproductive tactics in some blenniid species [e.g. peacock blenny, *S. pavo*, reviewed in Oliveira et al., 2009] offers the

possibility of comparing parasitic (e.g. sneaker) males with nest holders to further test this hypothesis at the intraspecific level.

Another topic that needs to be clarified in future studies is the seasonal plasticity of the observed sex difference in the DLv, since the sex difference in home ranges between the sexes is only expected to occur during the breeding season. Moreover, in order to further corroborate the functional involvement of the DLv enlargement with an increased spatial ability, behavioral tests should be made in these blenniid species to determine if spatial performance of females and males in allocentric spatial tasks is correlated with the sexual dimorphism presented here.

In summary, we describe a sex difference in the DLv region of the telencephalon of blenniid fish that is compatible with a role of this region in spatial cognition. These results are in accordance with the sexual selection theory already tested in mammals and birds [for a review, see Jacobs, 2009], where the relative larger hippocampal structure occurs in the sex, in which successful competition required a more developed spatial orientation and enlarged home ranges.

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