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Actividade e comportamento alimentar do xarroco *Halobatrachus didactylus* (Bloch & Schneider, 1801).

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Tese orientada por:

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Resumo

O xarroco, *Halobatrachus didactylus* (Bloch & Schneider, 1801), é um peixe teleósteo bentónico marinho, que se encontra secundariamente adaptado a sistemas aquáticos salobros no limite norte da sua distribuição, onde os adultos ocupam uma posição cimeira na teia trófica. O presente trabalho incidiu no estudo de padrões de actividade e movimento de espécimes de xarroco do estuário do Mira com recurso a equipamento de telemetria de registo de movimentos e de localização, e a sua relação com comportamentos tróficos. Habitualmente considerada uma espécie sedentária detentora de um comportamento predador de emboscada, os resultados revelaram uma elevada capacidade em realizar importantes e frequentes deslocações ao longo do estuário, e padrões de actividade nocturnos e indicativos de uma elevada aptidão na busca activa de alimento. Experiências em laboratório revelaram uma capacidade diferencial em capturar camarões e caranguejos, estando o sucesso de captura dependente do tipo, tamanho e comportamento da presa.

Palavras-chave: xarroco, telemetria, actividade e movimento, comportamento trófico, sucesso de captura de presas.

Em memória de meu pai.

Activity and feeding behaviour of the Lusitanian toadfish *Halobatrachus didactylus* (Bloch & Schneider, 1801).

Abstract

The Lusitanian toadfish, *Halobatrachus didactylus* (Bloch & Schneider, 1801), is a benthic marine teleost that in its northern distribution is secondary adapted to brackish water systems, where it occupies a top position on trophic webs. *H. didactylus* specimens were studied in the Mira estuary with data archival and acoustic tags in order to investigate their movements and activity patterns, and their hypothetic association with feeding activities. Being traditionally considered a sedentary species with an essentially ambush predation behaviour, the present telemetry study revealed it's ability to frequently perform large displacements and a nocturnal activity pattern, with insights of a predatory behaviour that probably include periods of an active search for food. Laboratory experiment revealed differences in its ability to capture different crustaceans prey, indicating that prey type and behaviour, and relative size of predator/prey are important in determining predator's choice and the success of capture attempts.

Keywords: Lusitanian toadfish, telemetry, activity and movement, feeding behaviour, prey capture success.

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Chapter 1

Introduction

1. *Halobatrachus didactylus*: general description and ecological aspects

The Lusitanian toadfish, *Halobatrachus didactylus* (Bloch & Schneider, 1801) (Pisces: Batrachoididae) (Fig. 1), is a teleost found in the continental shelf from the Gulf of Guinea to the Gulf of Biscay, including the western part of the Mediterranean (Roux, 1981, 1986; Bauchot, 1987), and in Atlantic islands relatively close to the continental masses like Madeira (Roux, 1986; Muzavor *et al.*, 1993; Santos *et al.*, 2000), Canaries (Fowler, 1936) and Cape Verde islands (Fowler, 1936; Reiner, 1996). Nevertheless, it only presents significant populations between the Liberian coast and the south of Portugal (Costa, 2004). In Portugal it occurs in regions to the south of Cape Carvoeiro and essentially in brackish water systems, like estuaries and coastal lagoons (Costa & Costa, 2002; Costa *et al.*, 2003). According to Costa (2004), *H. didactylus* is clearly a marine species that is secondarily adapted to brackish systems only on the northern limit of its distribution range, due to environmental constraints that affect the initial stages of development of this species.

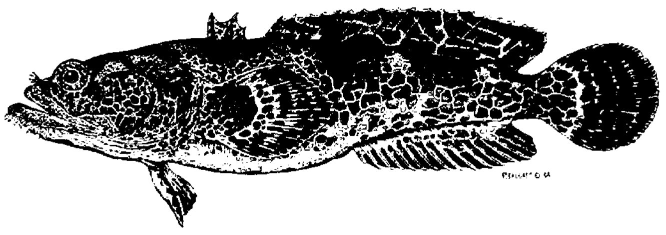


Figure 1. Adult Lusitanian toadfish, *Halobatrachus didactylus*.

Like other members of the Batrachoididae family, the Lusitanian toadfish is a benthic, solitary and relatively sedentary fish (Costa, 2004) and is usually associated with soft sand, muddy bottoms, but is also found in hard bottoms, under stones or sheltered in rocky crevices (Roux, 1986; Bauchot, 1987; Santos *et al.*, 2000). In coastal waters it can be found down to 50 m deep, with reports of catches by bottom trawling at 250 m depths (Roux, 1981).

H. didactylus is a gonocoric species with external fertilization, being attributed to males the building of nests and the provision of parental care to the eggs, embryos and larvae, which is sometimes long-standing until the newborns juvenile phase (Costa, 2004). Males attract females using an extensive vocal repertoire produced by the swimbladder, which is especially well developed (Santos *et al.*, 2000; Amorim *et al.*, 2006). This species shows the particularity of possessing two distinct male morphotypes (I and II) and therefore two alternative reproductive tactics in the male specimens (Modesto & Canário, 2003).

H. didactylus is the only recognized species of the *Halobatrachus* genus (Collette *et al.*, 2006), and like the majority of the species belonging to the Batrachoididae family it possesses a very robust body. It can reach up to 50 cm in body length, although most of the specimens do not exceed 35 cm (Roux, 1986; Bauchot, 1987). The head is very large, with a big, slightly protractile and terminal mouth. It is a voracious predator by nature and has a wide feeding range (Cárdenas, 1977; Sobral, 1981; Costa *et al.*, 2000), placing it in the top position in estuarine and coastal lagoons trophic webs, where it plays an important role in the structure and balance of the existing biological communities (Costa, 2004).

The Lusitanian toadfish is considered a relatively sedentary species, but mark-recapture experiments in the Mira estuary revealed that about 15% of the individuals could indeed perform important displacements (more than 13 km), even if not very frequently (Costa, 2004). The activity of the Lusitanian toadfish seems mainly influenced by nycthemeral and tidal cycles, with the former especially important in brackish water environments (Costa, 2004). In the Mira estuary, *H. didactylus* feeding activity increases during the dark periods and also during the flood, due to an increase in the vulnerability and availability of the prey (Costa, 2004).

Adult Lusitanian toadfish feed mainly on decapod crustaceans and teleost fish (Costa *et al.*, 2000; Costa, 2004). In the Mira estuary (Portugal), the Lusitanian toadfish analysed by Costa (2004) revealed more than 80 different prey in their gut contents, but fed particularly on crabs *Carcinus maenas* (Linnaeus, 1758), shrimps *Crangon crangon* (Linnaeus, 1758) and the teleosts *Pomatoschistus* spp., with the high abundance of these organisms and its benthic lifestyle contributing to this pattern. Throughout the year, a high degree of variation in the feeding activity of adult *H. didactylus* was found by Costa *et al.* (2000) and Costa (2004), largely associated with the reproductive process and its' associated high energetic demand. Cannibalism seems also to be a generalized behaviour in the Batrachoididae family (Cárdenas, 1977; Costa, 2004), and in *H. didactylus* this is mainly a function of animal size.

Regarding prey capture behaviour in fish and its complexity, Montgomery *et al.* (2002) pointed out the importance of the integration of hydrodynamic, chemosensory, tactile and visual information for the multimodal guidance of this type of behaviour. Hydrodynamic sense is mediated by the lateral line system, ubiquitous in fish, which detects displacements in the local water field, and is of fundamental importance in a fluid environment. The auditory and lateral line systems of teleosts are complementary in their ability to detect mechanical stimuli in the water, but in benthic fish it seems that the contact of the fish with the substrate reduces the inner ear range, and expands the lateral line distance range (Braun & Coombs, 2000). Nevertheless, some experiments have indicated the ability of some sensory systems on independently guiding prey capture behaviour, and therefore predators must adapt their predation strategies to optimize their chances of detection, approach and capture of the prey (Montgomery *et al.*, 2002).

When investigating these matters, Palmer *et al.* (2005) measured the sensitivity of the lateral line system in the oyster toadfish *Opsanus tau* (Linnaeus, 1766) and revealed that this species was able to detect mobile prey that approached within approximately 40% of their body length. In juvenile *O. tau*, Price & Mensinger (1999) observed that the success of the attacks during day light trials was strictly related with the distance to the prey, with successful attempts initiated at an average distance of 1.26 cm, and unsuccessful strike attempts initiated at 2.43 cm. These attacks were probably mediated by both visual and mechanical cues, and under low light conditions they would be even shorter and could indicate accurately the range of the lateral line (Palmer *et al.*, 2005). Therefore, although the lateral line can contribute significantly to the prey localization, its' short range indicates that other stimuli are definitely important in guiding successfully to prey capture (Montgomery *et al.*, 2002; Palmer *et al.*, 2005). Consequently, toadfish, that are not particularly fast swimmers, frequently adopt an ambush predation tactic, where they remain completely immobile but alert, attacking all the adequate preys that pass sufficiently close to them, and where the visual stimuli may play an important role (Philips & Swears, 1979; Price & Mensinger, 1999). Moreover, their chemosensitive barbels must also be an important prey detecting mechanism during the periods where they undertake an active search for food, bottom dwelling the substrata (Philips & Swears, 1979; Gosline, 1996).

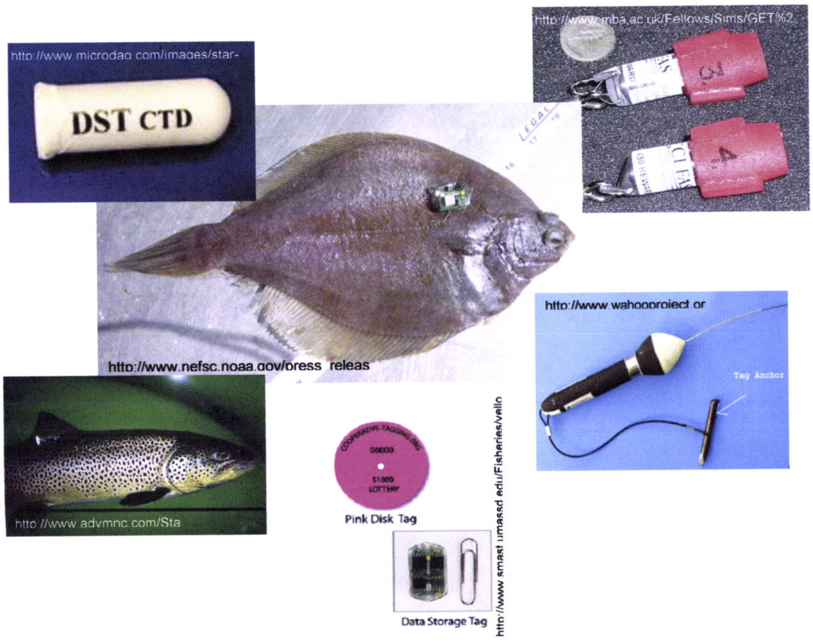
The Lusitanian toadfish seems to adopt different trophic strategies, which include feeding essentially on benthic prey (crabs, isopods and amphipods), or on water column associated prey (shrimps, fishes and mysidacea), according to diverse environmental conditionings like water circulation patterns and substrata characteristics, or by different predatory tactics along the day (Costa, 2004). Each one of these feeding tactics certainly involves different sensory systems to a different extent, in order to maximize capture success. Moreover, in an estuarine environment where biotic and abiotic alterations during one day may be quite severe, their influence on *H. didactylus* daily feeding activity and in its use of different sensory organs is likely to be great.

2. Fish tagging and movement description

Fish exhibit a diverse array of movement behaviour, which is associated with diverse aspects of the biology and ecology of each species. There can be predictable movement patterns associated with ontogenetic shifts and spawning migrations, or with food availability or predation pressure (Pittman & McAlpine, 2001). Mark-recapture is nowadays a standard method used to record the movements of individual fish, and tagging has proved to be a valuable tool in fish ecological studies. Although the first attempts to mark an animal occurred sometime between 218 and 201 B.C., it is not clear when fish were first marked (McFarlane *et al.*, 1990). Since the late 1800s, numerous fish tagging experiments have been conducted on salmonids followed by successful attempts at tagging flatfish and cod (Latour, 2005). Pelagic species, namely Pacific herring *Clupea harengus pallasi* (Valenciennes, 1847) and bluefin tuna *Thunnus thynnus* (Linnaeus, 1758), were successfully tagged in the early 1900s. As an attempt to study the biology and ecology of fish populations, large-scale tagging programmes have been initiated since 1945 (McFarlane *et al.*, 1990).

Conventional tag and recapture techniques are considered to be cost effective for monitoring the movements of fish (Childs, 2005). However, they do not provide the precise temporal and spatial data that is essential for behavioural ecology (Baldwin *et al.*, 2002). As a result, advances in telemetry were crucial to study fish daily movements, migration, behaviour, reproduction and basic temporal patterns of space and habitat use.

The study of fish behaviour and its connection with environmental variables has advanced with the advent of electronic data storage tags (DSTs) (Arnold & Dewar, 2001). Numerous types of DSTs had been developed for different sizes of fish, with different memory capacity and designed to record different types of information (pressure, temperature, salinity, and light intensity). Tags are usually attached to several fish, and recapture and tag return is generally due to commercial fisheries (Fig. 2).



http://www.microdaq.com/images/star-

http://www.mba.ac.uk/Fellows/Sims/GET%2

http://www.nefsc.noaa.gov/press_releas

http://www.wahooproject.or

http://www.advmnc.com/Sta

http://www.smast.umassd.edu/Fisheries/yello

Figure 2. Different types of data storage tags.

DSTs have been successfully used in a variety of fish species: plaice *Pleuronectes platessa* (Linnaeus, 1758) (Hunter *et al.*, 2004), yellowtail flounder *Limanda ferruginea* (Storer, 1839) (Cadrin & Westwood, 2004), cod *Gadus morhua* (Linnaeus, 1758) (Steingrund, 1999; Stensholt, 2001; Heffernan *et al.*, 2004), thornback ray *Raja clavata* (Linnaeus, 1758) (Hunter *et al.*, 2005), redfish *Sebastes mentella* (Travin, 1951) (Sigurdsson *et al.*, 2006), sea trout *Salmo trutta* (Linnaeus, 1758) (Sturlaugsson & Johansson, 1996; Rikardsen & Thorstad, 2006), salmon *Salmo salar* (Linnaeus, 1758) (Karlsson *et al.*, 1996; Sturlaugsson *et al.*, 2003; Reddin *et al.*, 2006), Atlantic goosefish *Lophius americanus* (Valenciennes, 1837) (Rountree *et al.*, 2006) and saithe *Pollachius virens* (Linnaeus, 1758) (Armannsson *et al.*, 2006). Crustaceans like spider crab *Maja squinado* (Herbst, 1788) (González-Gurriarán *et al.*, 2002) have also been subjected to tagging with DSTs.

One of the most important aims of fish tagging with DSTs is mapping the behavioural ecology of a given species. Profiles of seasonal and daily activity can be obtained, and these new data can be applied to conservation/management projects. Another important part of fish ecology studies involves the use of tracking devices, like ultrasonic, radio and satellite telemetry tags. Acoustic tags transmit a sound signal or "ping" that sends location information about the tagged fish to the hydrophone receiver, and have been used recently to study the behaviour and passage routes of migrating juvenile salmonids *Onchonrynchus* spp. (Steig & Timko, 2000), movements and distribution of common bream *Abramis brama* (Linnaeus, 1758) (Lyons & Lucas, 2002), to understand the impact of cod farming for wild cod *G. morhua* and endangered salmon *S. salar* populations (Brooking *et al.*, 2006).

The existing information on *H. didactylus* movements and activities, plus the major importance of this species in brackish water systems and all the background knowledge on its biology and ecology lead to the idea of performing telemetry experiments using data archival tags and localization tags.

3. The Mira estuary

According to Costa *et al.* (1994), the River Mira is the only large river in Portugal with its estuary totally included in a nature protected area (Parque Natural do Sudoeste Alentejano e Costa Vicentina), and its basin one of the least polluted Portuguese watersheds. This entire region is located in the transition zone between hot and cold areas of the North Hemisphere, and encloses a high biological diversity (Henriques, 1996).

With an area of about 2 km^2, the Mira estuary is a tidal small brackish water system located in the southwest coast of Portugal, (37°40' N 8°45' W) (Fig. 3). It has a channel-like form along its entire course, and runs for about 32 km from Odemira to Vila Nova de Milfontes. The Mira estuary increases in width from upstream to downstream, reaching its maximum (about 400 m during high tides) near the mouth, which is permanently open to the sea (Andrade, 1986; Bettencourt *et al.*, 1993; Leitão, 1997). The depth increases gradually towards downstream, with a mean of about 6 m. With a semi-diurnal cycle, tides have a permanent delay between Vila Nova de Milfontes and Odemira, reaching 75 min in spring tides, and slack tides have a mean duration of 30 min (Andrade, 1986). The water column is well mixed during spring tides and partially stratified during neap tides (Andrade, 1986). Regarding the estuary organic matter production and circulation, the Mira estuary presents a relatively oligotrophic water, but a high production of saltmarsh vegetation, where the majority of the organic matter is produced (Catarino & Serôdio, 1992; Bettencourt *et al.*, 1993). Therefore, water chemical composition of the estuary is mainly dependent on the flux that occurs between the saltmarshes and the circulating water (Bettencourt *et al.*, 1993).

The number of fish species that uses this brackish water system as a nursery ground (preferentially or not) undoubtedly represent the major part of the fish community present in the Mira estuary, with more than 40% of the total number of species. The marine and resident species include about 30% and 20% of the total, respectively. In contrast, the assemblage of diadromous migrating species and freshwater species represent only 5% of the fish species in the Mira estuary (Costa, 2004).

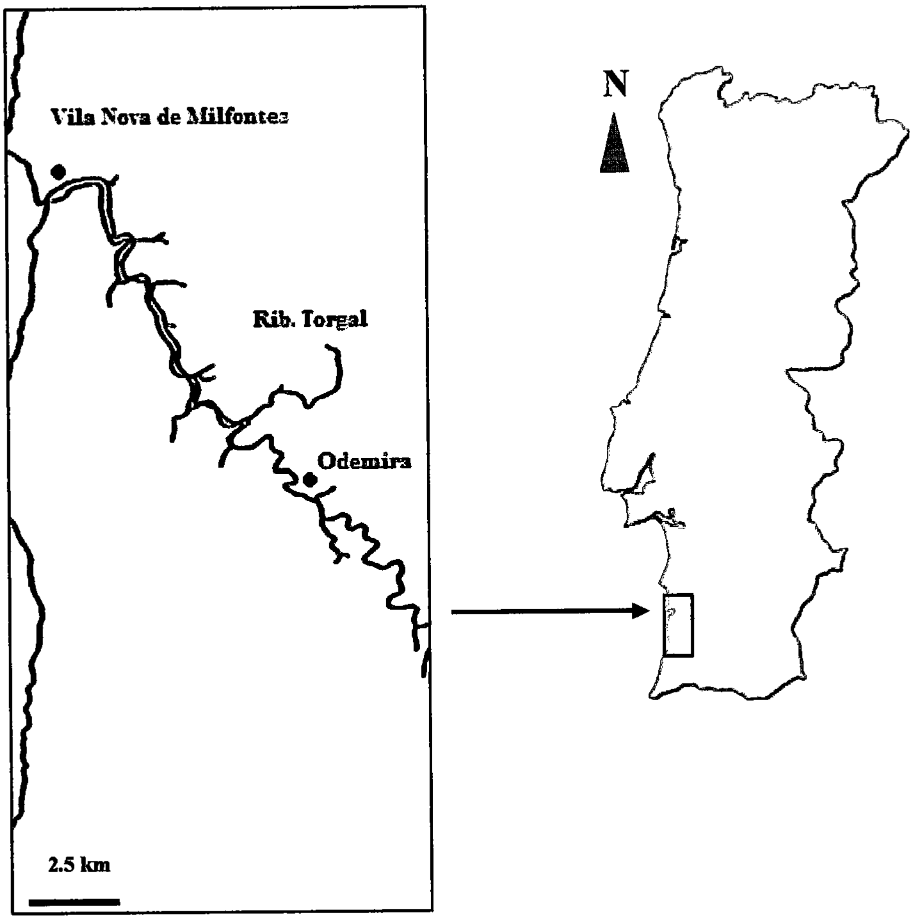


Figure 3. Location of the Mira estuary in the southwest of Portugal, between Vila Nova de Milfontes and Odemira.

According to Costa *et al.* (1994) the estuary can be divided in three distinct zones according to some biotic and abiotic characteristics: upper, middle and lower estuary. The upper zone shows the greatest temperature range and an important influence of fresh water; the low number of fish species, with the dominance of those that use the estuary as nursery grounds, is a marked characteristic of this area. Species like *Dicentrarchus labrax* (Linnaeus, 1758), and *Liza ramado* (Risso, 1826) are very common in this zone. *Diplodus vulgaris* (E.G. Saint-Hilaire, 1817), *Solea vulgaris* (Quensel, 1806), *H. didactylus* and *Anguilla anguilla* (Linnaeus, 1758) are also commonly found. The middle estuary has a lower temperature variation, but the highest salinity range, and is bordered by saltmarsh vegetation, like *Spartina maritima* (Curtis). The fish community in this area is more diverse than the upstream one and is dominated by *H. didactylus* and *S. vulgaris*, with *D. labrax*, *Diplodus sargus* (Linnaeus, 1758), *D. vulgaris* and *Chelon labrosus* (Risso, 1826) also common in this estuary trench (Costa, 2004). The lower estuary has essentially marine characteristics, including lowest temperature and saline ranges, and presents important eel grass beds used as nursery areas by several marine species. Fish diversity is maximal in this area, compared with the rest of the estuary, and *Engraulis encrasicolus* (Linnaeus, 1758), *H. didactylus*, *Liza aurata* (Risso, 1810), *D. sargus*, *D. vulgaris*, *Callionymus lyra* (Linnaeus, 1758), *Gobius niger* (Linnaeus, 1758), *Pomatoschistus microps* (Krøyer, 1838), *Pomatoschistus minutus* (Pallas, 1770), *S. vulgaris*, *Atherina presbyter* (Cuvier, 1829) and *C. labrosus* (Costa, 2004) are among the species commonly found is this area.

4. Objectives

The most important aim of this study is to provide information on the movement patterns and activity rhythms of adult *H. didactylus* in the Mira estuary (Portugal), and its possible relation with feeding behaviour, using electronic data-storage tags. The confrontation of this new data with data from conventional mark-recapture experiments will serve also to validate the applicability of this procedure on this kind of studies.

Regarding the feeding behaviour of *H. didactylus*, a laboratory experiment with different preys was carried out, in order to understand the dynamics and success of the attacks.

5. Thesis outline

This thesis is divided into four chapters:

Chapter 1 – Provides a general introduction to the biology of the Lusitanian toadfish, some aspects of fish movements and tagging importance, a description of the study area, and the objectives of this work.

Chapter 2 – Comprises a scientific paper that resulted from this study, to be published in the scientific journal *Fisheries Management and Ecology* (in press). This study focus on the movements and activity rhythms of the Lusitanian toadfish in the Mira estuary.

Chapter 3 – Describes a laboratory experiment concerning the feeding behaviour of *H. didactylus*, specifically the success of the attacks regarding different prey types.

Chapter 4 – The general conclusions of this work are presented, and some future research initiatives are pointed out.

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Chapter 2

Activity and movement patterns of the Lusitanian toadfish inferred from pressure sensitive data-loggers in the Mira estuary (Portugal).

(Fisheries Management and Ecology)

Activity and movement patterns of the Lusitanian toadfish inferred from pressure sensitive data-loggers in the Mira estuary (Portugal).

Maria Catarina Campos [1],

José Lino Costa[1]

Bernardo Ruivo Quintella[1, 2],

Maria José Costa[1,3]

Pedro Raposo de Almeida[1,2, §]

1. Institute of Oceanography, Faculty of Sciences, University of Lisbon, Campo Grande, 1749-016 Lisbon, Portugal.
2. Department of Biology, University of Évora, Apartado 94, 7002-554 Évora, Portugal. E-mail: pmra@uevora.pt; Tel. +351266760800; Fax. +351266760914
3. Department of Animal Biology, Faculty of Sciences, University of Lisbon, Campo Grande, 1749-016 Lisbon Portugal.

§ - Author for correspondence.

Key words: *Halobatrachus didactylus*; biotelemetry; activity rhythms; circadian cycle; tidal cycles.

Running title: Activity of *H. didactylus* in the Mira estuary.

Abstract

Activity rhythms and movement patterns of ten adult specimens of the Lusitanian toadfish *Halobatrachus didactylus* were studied in the River Mira estuary (Portugal) using pressure sensitive data-loggers and acoustic transmitters. Laboratory experiments were performed to assess the applicability of the sampling methodology to track the Lusitanian toadfish movements. Field results confirmed that the species' displays usually a sedentary behaviour, although revealing a stronger disposition to perform greater longitudinal movements than it was formerly believed. Frequency of movements was influenced by the circadian cycle, tidal stage and type of tide. Lomb-Scargle periodograms revealed significant daily periodicity in all the studied specimens. Two fishes displayed intercalary significant rhythms that could also be related with tidal cycle. This study also seems to indicate that these species do not adopt exclusively a sit and wait predation behaviour, but probably undertakes an active search for food.

Introduction

The Lusitanian toadfish *Halobatrachus didactylus* (Bloch & Schneider) is a solitary benthic fish belonging to the Batrachoididae family (Roux 1986). It occurs in the North-eastern Atlantic Ocean, ranging from the Gulf of Guinea to Portugal, including western Mediterranean (Bauchot 1987). This species inhabits marine coastal waters, estuaries and open coastal lagoons living at depths up to 50 m or more (Roux 1981). The offspring development is constrained by low water temperature in the northern limit of the species distribution range (Costa & Costa 2002). Hence, in Portugal it is restricted to the south region of Cape Carvoeiro and lives mainly in brackish water systems (Costa, Almeida & Costa 2003). These brackish water systems include the Tagus, Sado, Mira, Arade and Guadiana estuaries, and Alvor and Formosa coastal lagoons (Costa & Costa 2002).

Like most batrachoidids, *H. didactylus* lives partially buried in muddy or sandy bottoms, but is also found concealed in hard substrata, using stones or rock crevices as shelters and nesting sites (Roux 1986; Bauchot 1987). In brackish water systems, this species appears mainly in the middle and lower reaches (Costa & Costa 2002), where it is frequently associated with oyster banks (Costa 1999). Because it is a voracious predator (Cárdenas 1977; Costa, Silva, Almeida & Costa 2000) these brackish water populations promote major changes in biological communities, especially in small systems (Costa 2004). *H. didactylus* presents two distinct male morphotypes with alternative reproductive tactics (Modesto & Canário 2003).

The Lusitanian toadfish was considered by Roux (1986) as showing sedentary behaviour, but Costa (2004) revealed that some individuals might perform important displacements (more than 10 km). Adult specimens show an increased activity during the reproductive period, which occurs in spring and early summer (Palazón-Fernández *et al.* 2001; Costa & Costa 2002). In contrast, they become almost inactive during winter as a result of a drastic decrease in the water temperature (Costa *et al.* 2000). Some studies based on stomach contents analysis have reported significant differences in the species daily feeding activity in the Mira estuary (Costa 2004), with an increase of feeding during the day-night transition and close to the end of flooding periods.

Nevertheless, there is still a serious lack of knowledge on the movement patterns and activity of the Lusitanian toadfish.

The main objective of this study was to investigate the activity rhythms and movement patterns of adult *H. didactylus* in the Mira estuary using pressure sensitive data-loggers and acoustic transmitters. This was accomplished in two steps: (i) a laboratory experiment to assess the applicability of the sampling methodology in determining the activity of the tagged fish, particularly to determine if distinct behaviours are converted in different pressure records; and (ii) a field study with tagged fishes to obtain information on their individual behaviour, regarding the activity and movement patterns.

Material and Methods

Study site

The Mira estuary is a small tidal estuary located on the southwest coast of Portugal (37°40' N - 8°45' W). It runs for 32 km, and is about 400 m wide near the mouth during high tides, and about 30 m in the upper part, with a medium depth of 6 m. Tides have a semi-diurnal cycle, with a permanent delay between the river mouth and the estuary upper limit, reaching 75 min in spring tides, and slack tides have a mean duration of 30 min. The water column is well mixed during spring tides and partially stratified during neap tides. Daily water temperature variations due to the tide range between 0° C during winter and-7° C during summer (Andrade 1986).

According to Costa, Costa, Almeida & Assis (1994), the estuary can be divided in three distinct zones according to some biotic and abiotic characteristics: upper, middle and lower estuary (Fig. 1). The upper zone shows the greatest temperature range (8 °C – 26.5 °C) and an important influence of fresh water (salinity range: 0 psu – 23 psu), with a low number of fish species and the dominance of those that use the estuary as nursery grounds as marked characteristics of this area. The middle estuary has a lower temperature variation (9 °C – 26 °C), but the highest salinity range (7 psu – 35 psu), and is bordered by saltmarsh vegetation, like *Spartina maritima* (Curtis). The fish community in this area is more diverse than the upstream one and is dominated by *H. didactylus*. The lower estuary has essentially marine characteristics, including lowest temperature (12 °C – 22.5 °C) and saline ranges (27 psu – 35 psu), with important eel grass beds used as nursery areas by several marine species. Fish diversity is maximal in this area, comparatively to the rest of the estuary.

In Portugal, the Mira is the only large river with its estuary entirely included in a nature protected area, and one of the lesser polluted watersheds (Costa *et al.* 1994).

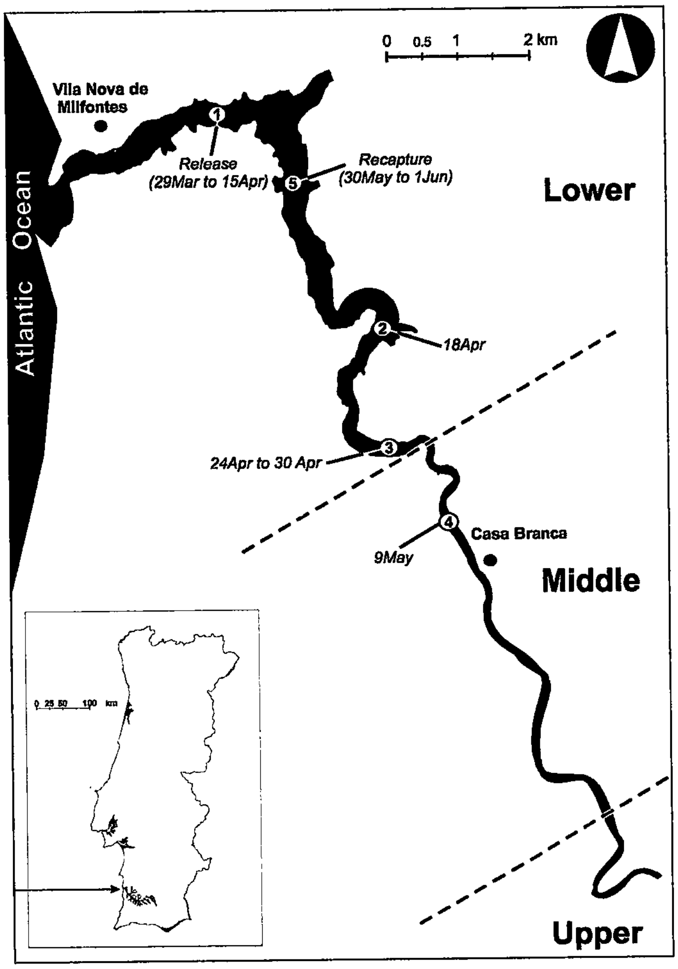

Figure 1. Map of the River Mira estuary, showing the division in three zones proposed by Costa *et al.* (1994). Also represented the tracking of specimen *Hd* 8 in the Mira estuary, with the release and recapture locations and date, and some manual tracking surveys.

Capture, telemetry equipment and tagging

All the specimens of *H. didactylus* used in this study were collected by bottom trawling, trammel nets or baited traps in the lower zone of the Mira estuary.

This study combined the use of data storage tags and acoustic transmitters. The G5 are pressure (depth) and temperature data storage tags (DST) (8 mm diameter, 30 mm length, 1.0 g in water, Cefas, Lowestoft, UK), and were programmed to register the pressure at one second intervals. This design guaranteed 7 days of continuous data recording. The data were downloaded to a laptop computer using a G5 Host Windows

Interface. The location of the fish was accomplished using acoustic coded pingers (V8SC-6L, 2.0 g in water; and V7-4L, 0.8 g in water, Vemco, Halifax, Nova Scotia, Canada) with 20-60 seconds delay between code transmissions.

The acoustic transmitters (V8 or V7) and the data storage tags (G5) were surgically implanted in the body cavity of the fish, with the two tags not exceeding 1.75% of the fish's total body weight (rule of 2%, see Jepsen, Koed, Thorstad & Baras 2002). Toadfish were anaesthetized in 0.4 ml 2-phenoxyethanol L^{-1} water, and the anaesthesia induction took between 5-8 minutes. The abdominal region was disinfected with an iodine solution (Betadine®), and an incision of *c.* 1 cm was made 2 cm anteriorly to the anal orifice. The incision was closed with two or three independent monofilament sutures (Monosyn DS19, B. Braun®), and disinfected with Betadine. The complete procedure took 10-15 min.

In the field, toadfishes were manually tracked by boat using a VR100 acoustic receiver (Vemco, Halifax, Nova Scotia, Canada).

Laboratory experiments

Laboratory experiments with tagged fishes were essential to determine if the different behaviours observed in captivity were converted in distinct pressure records associated with specific activity patterns. It was also assessed if that kind of information can support the interpretation of the field results. Additionally, these experiments were also conducted to evaluate the response of the specimens to the tagging procedure.

Ten adult specimens of *H. didactylus* (total length range: 150-218 mm) were captured at the Mira estuary and kept in a holding tank (146 L capacity, 18ºC water temperature, salinity 16 psu) until the trials. The fish were tagged according to the procedure described previously. All the toadfishes were successfully revived in a holding tank and then placed in the experimental tank. To permit the complete recover of the animals, G5 data-loggers were previously programmed with a start logging time delay of one week after the surgery.

The experimental aquarium (dimensions L×W×D: 76×56×40 cm) was set with a 12 hour light-dark cycle (to mimic summer L:D cycle) and with a water temperature close to the average water temperature during summer period (*c.* 21ºC) (Andrade 1986). Specimens were left starving for two days before the beginning of the experiments. Several feeding trials were performed using the Green crab *Carcinus maenas* (L.), and shrimps *Crangon crangon* (L.) and *Palaemon serratus* (Pennant) as potential prey. All the experiments were recorded with a digital video camera. After one week the animals were euthanized by controlled doses of the anaesthetic and dissected. Retrieved data from G5 were analysed and compared with video recorded movements during different activity periods, in particular the movements performed to capture the prey.

Field experiments

Ten toadfish were tagged and released in the Mira estuary, three during August 2006 and seven in March-April 2007. The specimens' total length ranged from 190 mm to 340 mm (Tab. 1).

All the fish were tagged with one G5 data-logger and one acoustic transmitter following the procedure described previously. After surgery, all animals were left recovering in a net cage suspended from the boat for a minimum period of 2 hours, before being released. Additionally, the fish were tagged with an external tag (T-bar) to facilitate identification during recapture attempts.

Table 1. Data on *H. didactylus* specimens used in the field experiment

ID	SRD	TL (mm)	M (g)	Sex	TTT (days)	TDM (m)
Hd **1**	09.08.06	191	105	F	31	4525
Hd **2**	09.08.06	194	125	F	16	6067
Hd **3**	09.08.06	238	239	F	97	7133
Hd **4**	25.03.07	220	180	M	24	2301
Hd **5**	25.03.07	226	160	M	25	10
Hd **6**	25.03.07	190	120	?	69*	130
Hd **7**	29.03.07	249	240	M	42	17853
Hd **8**	29.03.07	224	190	F	64	17238
Hd **9**	19.04.07	340	675	?	24*	8881
Hd **10**	20.04.07	277	325	F	41	15095

ID, animal identification; SRD, surgery and release date; TL, total length; M, total mass; TTT, total tracking time; TDM, total distance moved (minimum); * – not recaptured.

G5 data-loggers were programmed to start recording data one week after being implanted in the animals to allow the recover of the tagged fishes from the surgical procedure and restoration of their natural behaviour. Tag deployment had a pressure (equivalent to depth) time series interval of 1 second for a total period of 1 week. Tagged fish were located several times (i.e. 24 h after release, 7 days after release and before all the recapture attempts) until their recapture, and each position was registered using a GPS receiver. A number of different fishing techniques were used to recapture animals, namely beam trawl, trammel nets, baited traps and spearfishing with scuba diving equipment. After recaptured, the animals were euthanized (anaesthetic) and dissected. Data from the G5 DSTs was downloaded to a lap top computer. Specimens' sex was determined by macroscopic observation.

Data analysis

Laboratory experiments confirmed the G5 depth sensing resolution, meaning that all the variations smaller than 4 cm are mostly due to the sensor adjustment to small changes in pressure, and therefore cannot be attributed to movements made by the fish. Moreover, the continuous variation of the depth in the estuary due to the tidal cycle induced the G5 to perform typical 3-4 cm jumps between two consecutive readings.

Video observations also confirmed that most of the small movements presented depth variations up to 9 cm between two consecutive pressure records (one second interval). As a result, only the 5-9 cm depth changes in two consecutive depth readings by the G5 were considered as fish movements. For simplicity, it was assumed that the working unit would be one minute, meaning that this time interval was labelled as an activity or inactivity event, depending upon the fact that it presented at least one second of activity (i.e. a change in two consecutive depth readings between 5 cm and 9 cm), or no activity at all (i.e. no depth variation between 5 cm and 9 cm during the working unit), respectively. Therefore, the minutes of activity and the minutes of inactivity were counted and used to calculate proportion of movement during a certain period.

Activity and stationary moments were observed directly from the depth graphics, and their duration was scrutinized.

Some fish revealed only short movements, but longer directional longitudinal movements were also performed along the estuary by the remaining tagged individuals.

This former type of record was identified for its great depth variations in a relatively short period of time, and therefore its occurrence could be easily distinguishable from other activity patterns. Due to the different nature of these two kinds of movements they were then analysed separately, whenever possible. For each of them, the proportion of 5-9 cm movements (proportion of movements - PM) was then calculated for tidal (ebb, flood, low tide, high tide) and daily (dawn, day, dusk and night) cycles. Dawn and dusk periods were defined as 60 min before plus 60 min after the sunrise or sunset, respectively. Movement proportion was also calculated in separate for spring and neap tides. Using the BIOMstat statistical package, the G-test of independence with the Williams' correction (Sokal & Rohlf 1995) was applied to detect if the frequency of movements was independent of the day period and state or type of tide.

In order to detect eventual periodicity in *H. didactylus* activity, complete time-series data of the tagged specimens were analysed via the Circadian Rhythm Laboratory suite of time series data analysis programs (© R. Refinetti). Significance of rhythmicity was determined by the method of Lomb-Scargle periodogram (Ruf 1999). This method is a least-squares power spectrum analysis procedure that can be used to analyse unequally spaced data sets. When applied to equally spaced data, it yields the same results as the classical power spectrum, although Lomb-Scargle periodograms allow a much higher frequency resolution, and show higher efficiency and accuracy if periodic components are obscured by noise (Ruf 1999; Van Dongen, Olofsen, VanHartevelt & Kruyt 1999). For each individual, the period (Tau) in the circadian range was determined by recording the highest significant peak on the Lomb-Scargle periodogram between 22 hours and 26 hours, and between 10 hours and 14 hours for the circatidal rhythms. *PN* values give the normalized (referent to the total variance of the data set) power as a function of angular frequency for all periods tested, which allows determining the statistical significance ($P < 0.01$) of peaks in the periodogram (Ruf 1999). Robustness of the rhythms can be used as an index of the stationarity of the rhythm (Refinetti 2004) and was calculated as the ratio between *PN* and half of the number of data points (R. Refinetti pers. comm.). The 12 hours rhythmicity associated with the estuary tidal cycle (and not with activity of the fish) was firstly removed using the moving averages procedure of filter software Clean 5.0 (© R. Refinetti).

To measure the longitudinal movements of the fish during the studied period, a map with tracking locations of each tagged toadfish was produced using ArcGIS software. Because tracking surveys were not continuous, the calculated distances represent the minimum travelled distances for that period.

Results

Laboratory experiments

The 10 Lusitanian toadfish that were tagged survived the surgical procedure. No visible infection or other internal damage was observed by the *post mortem* examination of the tagged animals. Laboratory trials also indicated that the *H. didactylus* behaviour was apparently not altered with the surgical implantation of the tags, by comparing the behaviour of tagged specimens with the behaviour of untagged individuals that were kept in the same conditions.

The feeding experiments with *H. didactylus* revealed that the attack movements and prey capture were not all recorded as pressure variation by the G5 DSTs. Several attacks were performed in contact with the aquarium bottom, and consequently the pressure variation was inferior to the pressure sensor resolution (better than 3 cm). Therefore, some of the attacks were not distinguishable and their total number was underestimated.

Observations of Lusitanian toadfish feeding behaviour revealed a furtive approach to the prey, and most of the strikes were performed near the bottom of the aquarium, very close to the prey (for both crabs and shrimps). As a result, in these trials no differences were noticed between the DST's records in the attacks (i.e. depth variation patterns) to the different prey types.

Field experiments

Only seven of the 10 tagged specimens produced usable DST data. Two were not recaptured (*Hd* 6 and *Hd* 9) due to exhaustion of the transmitters' battery before a successful attempt, and the pressure data of the other (*Hd* 3) was corrupted. The recaptures occurred between 16 days and 3 months after releasing (Tab. 1). The percentage of time devoted to movement by the seven specimens of *H. didactylus* in the Mira estuary during the studied period was 2.6%.

Most of the tagged animals (80%) made important longitudinal movements along the estuary (between 2-17 km) (Tab. 1), whether upstream or downstream (see example in Fig. 1), and four of these animals (*Hd* 1, *Hd* 2, *Hd* 3, and *Hd* 10) were recaptured near the capture location (within 100-150 m). Two fish (*Hd* 5 and *Hd* 6) displayed only short movements (Tab. 1).

The *G*-test of independence revealed significant variations in the frequency of short movements during the night (PM = 1.7%) and all the other periods (Fig. 2a). Directional longitudinal movements showed differential frequency in all daily periods, and also occurred mostly during the night (PM = 3.3%).

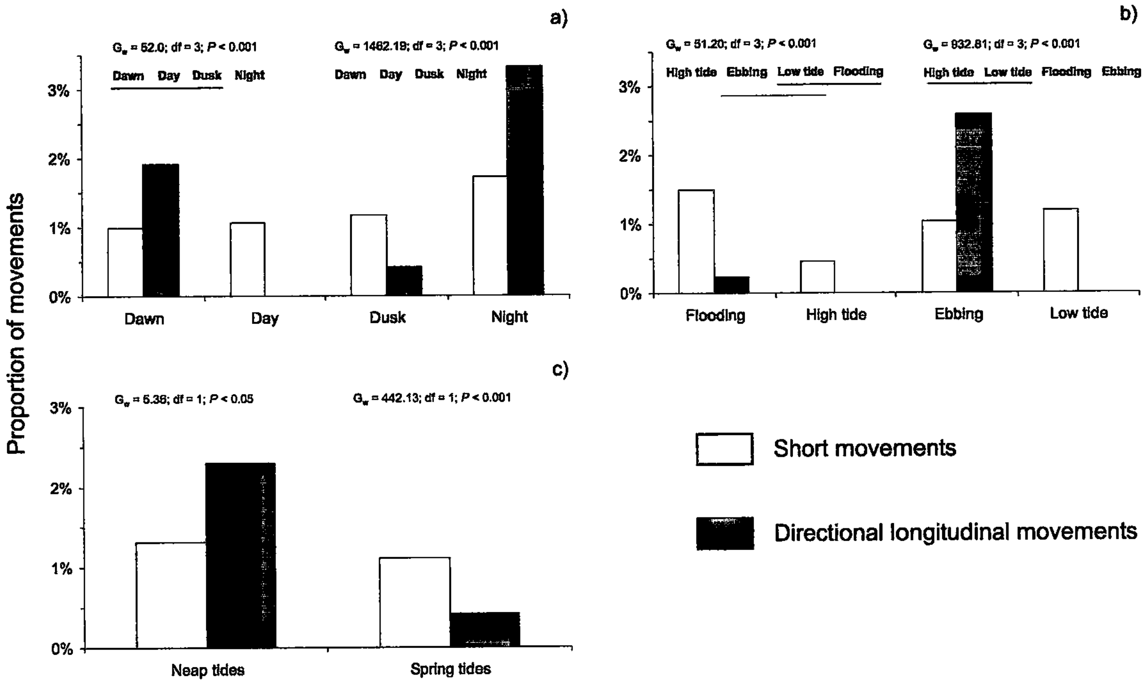

Figure 2. Proportion of short movements and directional longitudinal movements (PM) of *H. didactylus* specimens throughout the day (a), throughout the tidal cycle (b), and during spring and neap tides (c). Results of the *G*-tests performed to determine if the frequency of those movements was independent of the type of tide are also presented. N (short movements) = 69590; N (directional longitudinal movements) = 69669.

The proportion of short movements throughout the tidal cycle was significantly lower during the slack of high tides (PM = 0.5%), and the proportion of these movements during flooding (PM = 1.5%) was significantly higher than during ebbing (PM = 1.0%) (Fig. 2b). The analysis of directional longitudinal movements revealed a significantly higher occurrence of those movements during the ebbs (PM = 2.6%) than during the floods (PM = 0.2%). No directional longitudinal movements were detected during slacks of high or low tides.

Significant variation in the frequency of short movements was also observed between neap and spring tides, with the sum of movements during the neap tides (PM = 1.3%) higher than during the spring tides (PM = 1.1%) (Fig. 2c). Regarding the directional longitudinal movements, variations were once again significant, with the proportion of movements during neap tides greatly increased, up to 2.3%.

The duration of short movements of *H. didactylus* was more commonly between 1 minute and 4 minutes long (74.7% of the movements) and clearly identifiable in the depth records. These short duration movements were sometimes isolated in time, or could be grouped and form periods of higher activity levels, with a mean duration of 78.8 min (median = 50 min, percentile$_{25}$ = 28 min; percentile$_{75}$ = 116 min; range: 14-310 min). The stationary periods within these episodes of activity were mostly between 1-12 min (66.8%). Very long stationary periods were observed between short movements, ranging between 1-42 h long.

The continuous periods of directional longitudinal movements had an average duration of 150.7 min (median = 158 min; percentile$_{25}$ = 141.5 min; percentile$_{75}$ = 184 min; range: 72-264 min). These periods were composed of short movements and pauses, with movements ranging between 1-10 min, and stationary periods between 1-9 min. Its occurrence was also easily distinguishable in the depth graphics (not shown).

Data analysis with the Lomb-Scargle periodogram revealed that all the tagged toadfish displayed significant activity rhythms close to 24 h ($P < 0.01$). Tau values had an average of 23.7 ± 1.2 h, being all within the circadian range (Fig. 3).

Values of rhythms' robustness (index of stationarity) were somewhat low (10.5% in the higher case) indicating that *H. didactylus* rhythmicity must suffer slight changes every day, but is always very close to 24 h. Two fish (*Hd* 1 e *Hd* 7) displayed significant rhythms close to 12 h (compatible with tidal cycles) but associated *PN* values (89 and 48, respectively) were lower than those related with daily rhythms. Specimens *Hd* 1, *Hd* 4, *Hd* 7 and *Hd* 8 showed other peaks close to the circadian one, but because robustness of data was low, it was difficult to conclude if these events were due to noise or to real activity.

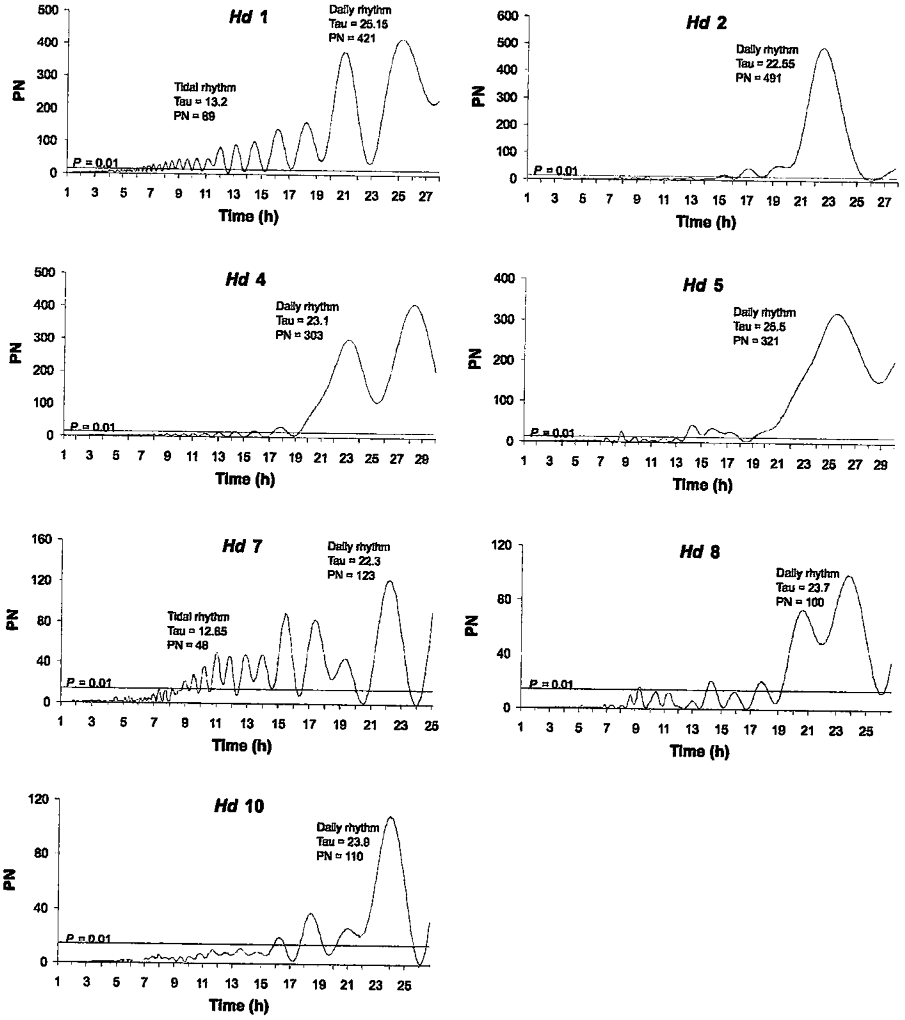

Figure 3. Lomb-Scargle periodograms of daily activity of the seven *H. didactylus* specimens. PN is the value for the significance level, with *P* = 0.01. The values of PN and Tau are referent to the highest significant peak in each graphic.

Discussion

This study provides the first results concerning activity of *H. didactylus* inferred from pressure sensitive data-loggers. Although the data and analysis presented here are by no means exhaustive, it is clear that data storage tags (DSTs) can be used in this type of study, and are particularly valuable when confronted with results of more conventional tagging experiments.

The Lusitanian toadfish is usually considered a sedentary species, but acoustic tracking data obtained from this study revealed a greater amount of long directional movements than it was formerly believed. In fact, with mark-recapture samplings, Costa (2004) detected that only 15% of the Lusitanian toadfish performed important longitudinal movements along the estuary. Important displacements are also reported in other Batrachoididae species. Conventional tagging studies with the oyster toadfish *Opsanus tau* (L.) in Maryland indicated that it could perform extensive movements (up to 8.9 km) and return to the original place many days after release (until 1134 days later). In this species these extensive movements seem to be strongly correlated with spawning (Schwartz 1974). Mature specimens of *Porichthys notatus* (Girard) are also known to display inshore migrations to spawn in the intertidal area (Feder 1974). Costa (2004) reported longitudinal movements of *H. didactylus* in the Mira estuary, with an average distance of 8.4 km, and a maximum extension of 13.3 km. These movements were thought to be predominantly towards downstream areas as toadfish individuals grow, but tracking data of the present study revealed great displacements both in the upstream and downstream directions, and sometimes a return to near the original capture location. The spawning season could be associated with some of these movements. However, more research on the relation of sex and gonad maturation with movements is needed to investigate the association of reproductive activity with longitudinal movements. Nevertheless, although being able to perform long displacements under the influence of environmental and/or intrinsic behavioural phases, regarding the total proportion of movements and stationary periods, *H. didactylus* displays usually a sedentary behaviour.

Data storage tags have already been used to quantify vertical movements of the cod *Gadus morhua* (L.) (Heffernan, Righton & Michalsen 2004) and adult plaice *Pleuronectes platessa* (L.) (Hunter, Metcalfe, O'Brien, Arnold & Reynolds 2004), and to identify patterns of on/off bottom movements of the yellowtail flounder *Limanda ferruginea* (Storer) (Cadrin & Westood 2004; Cadrin & Moser 2006). Due to Lusitanian toadfish benthic and sedentary nature, the majority of short movements could be clearly identified in the depth records downloaded from the DSTs. The fact that they could last for a significant time period indicates active exploratory actions that must have essentially feeding purposes. These findings seem to confirm that this species does not adopt exclusively a sit and wait predation behaviour, but probably undertake an active search for food (Costa 2004).

The data-logger analysis performed in the present study revealed the preferential periods of movements displayed by *H. didactylus*. Short movements were clearly predominant during the night and flooding tide. Costa (2004) also described a higher feeding activity of *H. didactylus* in the Mira estuary during the beginning of the night and the dusk period, and related it with the increase of vulnerability of the prey in this transition period. Furthermore, these higher activity periods are certainly not independent of the activity of some of the preferential prey of the Lusitanian toadfish, like the green crab *C. maenas* or the shrimp *C. crangon*, that display a higher activity during the night and high tide periods (Polet *et al.* 2004; Baeta *et al.* 2006). Greater activity during the night period was also observed for other species of the Batrachoididae family, like *P. notatus,* which is a planktonic and nocturnal fish predator, ascending in the water column at night to feed (Mensinger & Case 1991). Also laboratory studies with *O. tau* revealed a peak in its activity in the 3-h period following dusk (Phillips & Swears 1981).

Lomb-Scargle periodogram analysis revealed significant close to 24-h rhythms in movements of *H. didactylus,* and by proportion of movements throughout the day it is clear that is mainly a nocturnal activity pattern. Although rhythmicity was not highly statistically significant, the fact that it was present in all the fish studied is clearly an indicator that diel activity in the Lusitanian toadfish follows a pattern close to 24-h, which must be mainly related to the light-dark cycle. Refinetti (2004) referred to the robustness of circadian rhythms as an index of the stationarity of the rhythm. The fact that during the entire 24-h period the toadfish displayed short movements, although in significantly lower frequencies outside the night period, could be the cause of some weakness in the observed daily patterns. Consistency of activity patterns is found in many fish species, but not in all (Helfman 1993), and in a considerable number of fish species some individuals are diurnal while others are nocturnal. Additionally, the same individual can be diurnal and switch to nocturnalism in a few days, so activity patterns in fishes are frequently said to be plastic (Reebs 2002). *H. didactylus* must not differ greatly from what is observed in other fish species. Slight rhythm changes should occur daily in its activity, which may be related to previous satiation by the ingestion of prey captured in the vicinity of the refuges outside the periods of more intense activity. Nevertheless, the dark phase certainly triggered movement in this species.

Maximum feeding activity (minimum in vacuity index) of the Lusitanian toadfish is referred to be found close to the end of flooding tides (Costa 2004). The low proportion of movements during the high tide verified in the present study is probably related to the prior foraging activity during the flooding tide, and the resulting satiation. Lomb-Scargle periodograms apparently revealed a weak but significant tidal related activity pattern in two fishes (*Hd* 1 e *Hd* 7). The fact that 12-h noise was previously filtered to remove the marked estuary tidal cycle may have influenced these results and lead to some loss of information (Ruf 1999). Because *H. didactylus* is a benthic and relative sedentary species, if this procedure was not performed, this peak would conceal all the possible activity associated with the tidal cycle. Hunter *et al.* (2004) also revealed significant 12 h periodicity in the vertical activity of *P. platessa* but with lower values than the 24 h rhythmicity. The interaction of both tide and circadian cycles can possibly have different effects in the daily activity of *H. didactylus*, but the light-dark cycle probably has a major influence. Apparently, specimens living in the sea have a marked light-dark rhythm, and are not influenced greatly by tides (Costa 2004). The interaction between the tidal stage and the light-dark cycle should be more thoroughly examined in the future.

Short movements were also more frequent during the neap tides than during the spring tides. According to Andrade (1996), the water turbidity in the Mira estuary depends mainly on the tidal amplitude, being maximal during spring tides. Diminished visual acuity could be associated with the observed decrease in the toadfish activity. In fact, it is known that in spring tides water turbidity induces a decrease in the rate of chasing prey and in the probability of success in prey capture (De Robertis, Ryer, Veloza & Brodeur 2003). Moreover, turbidity also reduces the distance at which predator-prey interactions may occur (Abrahams & Kattenfeld 1997). Powerful currents during spring tides may also make *H. didactylus* rapid movements harder and feeding can become energetically more demanding, even if the fish is successful on capturing the prey (Mussi, McFarland & Domenici 2005).

H. didactylus displayed directional longitudinal movements mostly during the night, the ebb tides and the neap tides period. These movements may be associated with the need to change habitat, and preferential use of night period related to lower visibility and consequent predator avoidance. The teleost *Dicentrarchus labrax* (L.), the otter *Lutra lutra* (L.), and the Grey Heron *Ardea cinerea* (L.) are some of the potential predators of *H. didactylus* in the Mira estuary, as well large marine fish that may enter in the lower estuary (Trigo 1994; Costa 2004). The choice of neap tides for longitudinal movements may be connected with weaker currents and resultant less energy expenditure if moving against the flow, but more tracking data are needed to clarify this point. Concerning the tidal cycle, the higher frequency of longitudinal movements during the ebb may be related with downstream displacements, similarly to what happens in other species, like the Atlantic salmon *Salmo salar* (L.) (Aprahamian, Jones & Gough 1998); but with the present data it was not possible to confirm precisely the direction of those movements. Numerous upstream movements of *H. didactylus* specimens were performed after the data-logger stopped recording the depth information, and because tracking surveys were not performed on a regular basis, some downstream movements may have not been identified.

The voracity and ability of *H. didactylus* in performing some important displacements may lead to the need of some control and register of its moves, particularly if moving into nursery places of commercially important species, like *Solea solea* (L.) and *D. labrax*. Rising temperatures caused by climatic changes can lead to the spread of the Lusitanian toadfish northward, and species like the European eel *Anguilla anguilla* (L.) can be subjected to a loss of habitat due to the competition and predation exerted by *H. didactylus* (Costa *et al.* in press). More precise management measures could therefore be taken in communities where the Lusitanian toadfish is present, after the implementation of extensive tagging studies.

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Chapter 3

Predatory behaviour of the Lusitanian Toadfish *Halobatrachus didactylus* (Bloch & Schneider, 1801).

Predatory behaviour of the Lusitanian Toadfish *Halobatrachus didactylus* (Bloch & Schneider, 1801)

Abstract

The feeding behaviour of an organism is influenced by morphological and behavioural traits of both predator and prey, and its purpose is to provide a positive energetic balance to the predator. Lusitanian toadfish predatory behaviour seems to be modulated towards different crustacean prey (shrimps and crabs), and capture success was also different, being higher for the crabs. Handling times were superior for crab prey than for shrimps, and prey/predator size ratio seems an important factor influencing this variable and prey selection as well. Toadfish strike success seems to be closely related with the distance to the prey, especially in the attacks on shrimps, with successful strikes being initiated very close to the prey. The different sensory systems of Lusitanian toadfish must play an important role in the successful guidance to the prey, but the input of each one of them is unknown.

Introduction

The feeding behaviour in fish is influenced by environmental factors like temperature, photoperiod, and habitat structure, but also by the presence of predators, and by energy associated with a given food type (Volkoff & Peter, 2006). Feeding behaviour is directly related to food intake, and in any case the energy balance between feeding effort and energetic gain from food must be positive for fish metabolic needs. Food intake can be indirectly quantified using different approaches (Volkoff & Peter, 2006), like feeding fish with a known amount of food and weighing the remaining food in tanks (Boujard *et al.*, 2002), but the analysis of stomach contents gives the most insights on the feeding ecology of a given species in its natural environment. However, gut content analysis cannot tell when and how quickly prey items were ingested, and the capture success rate of the predatory attempts. For these reasons, behavioural observations have been conducted for decades to study feeding behaviour of fish under experimental conditions (Jobling *et al.*, 2001). Direct laboratory observations also allow detecting other feeding-related behaviours than intake like chewing, bumping, or ejection of a pellet (Volkoff & Peter, 2006), or even swimming and aggressive behaviour (Andrew *et al.*, 2004).

The feeding performance of a predator is strongly influenced by morphological design and behavioural aspects of both predator and prey, and is an important mechanism in the structuring of aquatic communities (Nemeth, 1997; Scharf *et al.*, 2003). Prey anti-capture abilities will influence their susceptibility to capture, and the ability of a predator to use a combination of feeding methods will increase the range of prey it can efficiently handle (Nemeth, 1997). Prey that use rapid locomotion to evade a predator necessarily require different predatory approaches from a prey that defend itself by means of self-protective morphological traits and aggressive behaviour, and probably the predator capture success on these two prey will differ as well. This modulation of the attack is thought to reflect adaptive behavioural plasticity of the predator (Bolnick & Ferry-Graham, 2002). Additionally, relative body size of predator and prey is also of great importance in the outcome of predator-prey interactions. Evidences that morphological aspects can influence the handling process of a prey and therefore the energetic balance were suggested, namely by behavioural observations in juvenile *Pomatomus saltatrix* (Linnaeus, 1766) where shrimp prey were more difficult to handle and ingest than fish prey (Juanes *et al.*, 2001). Moreover, interspecific differences in capture success can be influenced by factors like predator mouth morphology and attack kinematics, as revealed by Norton (1991, 1995) in the predation of different cottid fish of shrimp and crab prey.

Several studies were made regarding the trophic relations in this species (Cárdenas, 1977; Sobral, 1981; Costa *et al.*, 2000; Costa, 2004), but investigations on other aspects of its feeding behaviour, such as predator-prey interactions, are scarce. Philips & Swears (1979) showed that the oyster toadfish *Opsanus tau* (Linnaeus, 1766) could more effectively capture blennies in darkness than in light. Concerning toadfish's approaches to prey, Price & Mensinger (1999) examined the interactions between the toadfish *O. tau* and guppies prey in the laboratory, and found that *O. tau* are effective short-range ambush predators. Strike success was mostly dependent upon the distance to the prey, declining with increasing distance. Palmer *et al.* (2005) studied the sensitivity of the lateral line in *O. tau* and revealed that this species is able to detect mobile prey that approached within approximately 40% of their body length. Therefore, although the lateral line can contribute significantly to the prey localization, its' short range of detection indicates that other stimuli are also important in guiding successfully to prey capture (Hanke *et al.*, 2000; Montgomery *et al.*, 2002; Palmer *et al.*, 2005). The toadfish *O. tau* revealed a peak in activity in the 3-h period following dusk (Phillips & Swears, 1981), and Costa (2004) and the work described in the Chapter 2 of the present thesis found a higher feeding activity of *H. didactylus* in the Mira estuary during the dusk period and beginning of the night, so it is possible that *H. didactylus* integrates visual, chemosensory and mechanosensory systems to detect prey during darkness. However, the contribution of each one of these systems to a successful orientation towards the prey it is not known. Studies with juvenile toadfish revealed that reactions distance and attack range are less during the night (Palmer *et al.*, 2005), and Richmond *et al.* (2004) found that predatory fish without visual cues reacted to prey at distances of less than 50% of the predator's body length. *H. didactylus* chemosensitive barbels must also play an important role in prey detection during the periods where they undertake an active search for food (Philips & Swears, 1979; Gosline, 1996). Barbels possess a high density of taste buds (aggregations of chemosensory receptor cells), that are the specialized end organs of gustation (Kinnamon & Cummings, 1992), and can be used to find prey that live associated with the substrate.

The present study focuses on the predatory behaviour of the Lusitanian toadfish *Halobatrachus didactylus* (Bloch & Schneider, 1801), a benthic, solitary and relatively sedentary teleost that in Portugal is found essentially in brackish waters systems, like estuaries and costal lagoons (Costa & Costa, 2002; Costa *et al.*, 2003). The adult Lusitanian toadfish feed mainly on decapod crustaceans and teleost fish (Costa *et al.*, 2000; Costa, 2004), and frequently adopt an ambush predation behaviour.

Our goal was to evaluate the attack success of *H. didactylus* concerning different prey types such as shrimps *Crangon crangon* (Linnaeus, 1758) and *Palaemon serratus* (Pennant, 1777), and the green crab *Carcinus maenas* (Linnaeus, 1758). Some parameters of the capture, like capture success, handling times and successful strikes distance will also be assessed, as well the toadfish predation behaviour when approaching the different prey.

Methods

Three adult Lusitanian toadfish (total body length: 218 mm female, 195 mm male, 178 mm male) were collected by bottom trawling or trammel nets in the lower zone of the Mira estuary during November of 2006. Fish were immediately transported to the laboratory and allowed to acclimate for at least 1 week prior to use in experiments. All fishes were maintained in a holding tank with 146 L capacity with a water temperature close to the average water temperature during summer period (*c.* 21°C), and salinity 16 psu, and fed daily with frozen shrimp. One specimen at a time was then placed in the experimental aquarium (56 cm × 76 cm × 40 cm) with a water temperature of 21°C. Specimens were left starving for two days before the beginning of the experiments. Grids (3 cm x 3 cm) were placed on the back side of the aquarium so that recorded measurements could be calibrated.

All the crustaceans used in the feeding trials were collected in the Mira estuary in November of 2006, and immediately transported to the laboratory, where they were maintained separately in holding aquariums (20 L capacity, 18°C water temperature, salinity 16 psu).

Each feeding trial consisted of: (i) one Lusitanian toadfish presented with one shrimp or one crab, (ii) the same prey with 2 sizes simultaneously, and (iii), similarly sized shrimp and crab prey (as much as possible, due to their great morphological differences).

Prey were put inside a transparent plexiglass tube placed vertically in the experimental aquarium, and left there for a few minutes to acclimate to tank water, after which the tube was removed. The size of the green crabs ranged between 2.5 cm and 4 cm (carapace width), and the shrimps between 3 cm and 5 cm total length (from rostrum to tail). Prey that were not captured by the fish were removed at the end of every trial. Each *H. didactylus* specimen was studied during one week. All feeding experiments were recorded with a digital video camera placed one meter in front of the tank, and latter on, the attacks were viewed frame by frame on a laptop computer using Microsoft® Windows® Media Player 11 (25 frames per second).

For each prey species, capture success was calculated as the proportion of prey capture attempts that resulted in prey consumption. A capture attempt was defined as a directed strike at a prey, in which the toadfish oriented toward a prey and displayed a burst in swimming speed culminating in an attempt to grab the given prey, or when it made a slow and quiet approach and then a rapid capture attempt.

Handling time, defined as the time from strike initiation of a given prey until swallowing activity ceased was estimated for each successful capture. Strike initiation was defined as the frame in which the pectoral fins begin to contract. The duration of the bite was also measured, beginning in the frame of the strike initiation and finishing in the frame where the toadfish snaps the jaws around the prey. The distances between predator strike initiation and prey during successful and unsuccessful attempts of capture were also measured.

Additional observations were made of the Lusitanian toadfish swimming behaviour towards the different prey, to examine possible modulations of the attack.

Results

Capture success and handling times

A total of 32 Lusitanian toadfish feeding trials were conducted, that generated 74 observed attacks resulting in 24 successful prey captures, corresponding to 32.4% of capture success. Capture success declined with decreasing in the *H. didactylus* body size (37.8%, 31.5% and 27.8% for 218 mm, 195 mm and 178 mm total body length, respectively).

Attacks on shrimps were less successful than attacks on crabs, with crabs with an adequate size relative to toadfish size more easily captured by *H. didactylus*. A total of 12 attacks were made against *C. maenas*, resulting in 5 captured animals (41.7%), but only the crabs between 2.5 - 3 cm carapace width were successfully caught. Attacks on shrimps (62) leaded to 30.6% of successful captures, with a difference between *C. crangon* (33.3%) and *P. serratus* (23.1%).

Interactions between Lusitanian toadfish and prey appeared to be influenced by the relative sizes and prey type. The larger *H. didactylus* specimen (218 mm total body length) struck shrimps from 3 cm to 5 cm (Tab. 1). The two smaller toadfish did not capture shrimps larger than 3 cm with the 195 mm animal missing all attempts and the 178 mm specimen not attacking these prey at all. Green crabs larger than 3 cm were only struck by the largest toadfish, although unsuccessfully. When given the choice of 2 shrimp sizes (3 cm and 5 cm), the two smaller toadfish they seemed to choose the smaller prey (Tab. 1). Between the smaller shrimps (~ 3 cm) and the crabs of 2 – 2.5 cm, the smaller toadfish chose the shrimp, but the largest animal captured both prey equally (Tab. 2).

Table 1. Total numbers of prey eaten by *H. didactylus* when given a choice of equal numbers of small (3 cm – 3.5 cm) and large (4 cm – 5 cm) shrimp, and small (2.5 cm – 3 cm) and large (3.5 cm – 4 cm) crabs

H. didactylus (mm)	N trials	N captured shrimp		N trials	N captured crab	
		Small	Large		Small	Large
218	3	3	2	2	2	0
195	3	3	0	1	not attacked	not attacked
178	2	2	not attacked	1	1	not attacked

Table 2. Total numbers of prey eaten by *H. didactylus* when given a choice of equal numbers of 2 prey types, crab and shrimp, of similar sizes (2 cm – 3 cm)

H. didactylus (mm)	N trials	N captured prey	
		Crab	Shrimp
218	3	2	2
195	1	not attacked	1
178	1	not attacked	1

Handling time was estimated for each successful capture where it was possible to visualize and measure the swallowing. Handling times ranged between 3 seconds and 1 minute and 30 seconds, being generally longer for crab than for shrimp prey. For the

218 mm *H. didactylus*, mean handling time for crabs was 56.67 s, 8.67 s for smaller shrimps and 9.00 s for larger shrimp. Lusitanian toadfish handling times seemed to increase with increasing prey length/predator length for shrimp (Tab. 3). Capture success for shrimp prey was lower in the smaller toadfish, but a relation in capture success proportion was not found.

Table 3. *H. didactylus* capture success (%) and mean handling time (s) for large and small shrimp prey

H. didactylus **(TL) (mm)**	**Shrimp 3 cm - 3.5 cm Predator/Prey size ratio**	**Capture Success (%)**	**Mean handling time (s)**
218	0.14 - 0.16	30.00	8.67
195	0.15 - 0.18	46.20	24.00
178	0.17 - 0.20	23.50	28.00
H. didactylus **(TL) (mm)**	**Shrimp 4 cm - 5 cm Predator/Prey size ratio**	**Capture Success (%)**	**Mean handling time (s)**
218	0.18 - 0.23	40.00	9.00
195	0.20 - 0.26	not captured	-
178	0.22 - 0.28	not attacked	-

Predator attack behaviour

Capture attempts of Lusitanian toadfish for both shrimps and green crabs involved (i) a cautious observation and a furtive approach to get very close to the prey, or (ii) an observation followed by rapid burst from a greater distance directed to the prey.

Slow approaches were frequent against crab prey, being normally preceded by a prolonged observation. Attacks on shrimp were often performed from about 10 cm to 12 cm distance with a rapid burst, but furtive approaches were also done. Frequently, the swimming movement of *H. didactylus* towards the shrimps formed an arc followed by a rapid dive, with the strike initiated very close to the prey. Prey movement seemed to be an important trigger in the predation behaviour, since for several occasions the toadfish only attacked prey that were in motion, either next to or distant to the fish.

Aggressive behaviour by *H. didactylus* was frequently observed almost immediately after the prey was placed in the aquarium, with the toadfish trying to capture it within the plexiglass tube. Several consecutives capture attempts directed at the same prey were also observed. Toadfish sometimes also undertook an active aquarium bottom prospection with its sensitive barbils.

Only when predator and prey were moving in a direction perpendicular or approximately perpendicular to the camera was it possible to measure the distances of successful and unsuccessful attacks and the strike's duration. All the strikes measured were completed in 3 to 4 film frames, which mean a duration of 0.12 s to 0.16 s (Fig. 1). All the successful attempts where it was possible to assess the distance to the prey (22 attempts) had strike initiations at a distance shorter than 3 cm (Fig. 1). The majority of failures had strike initiations between 3 cm to 6 cm (65.5%) and 27.6% of unsuccessful capture attempts were made when the toadfish were within 1 cm to 3 cm of the prey.

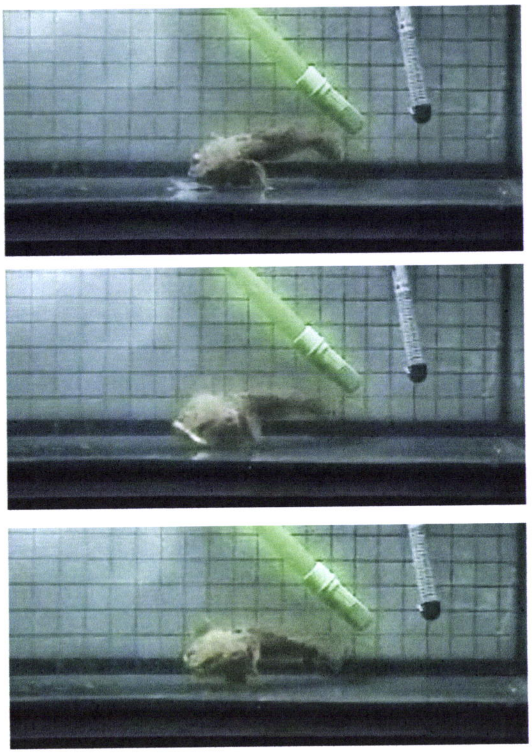

Figure 1. Attack sequence of a Lusitanian toadfish to a shrimp.

Discussion

Results of this laboratory experiment revealed differences in the ability of Lusitanian toadfish *H. didactylus* to capture different crustaceans prey. Differences between capture of shrimps and crabs could be directly attributed to prey behaviour. Our behavioural observations suggest that green crabs require more time to handle and ingest than shrimp prey. However, the crabs with an appropriate size relative to toadfish size were comparatively easier to capture than the shrimps. Normally, shrimps tended to stay on the tank bottom, but very quickly reacted to toadfish attacks by jumping up and away, and could remain swimming for some time, particularly *P. serratus*. As a result, in this study, the Lusitanian toadfish capture success was superior for crabs than for shrimp prey, and superior for *C. crangon* specimens (33.3%) than for *P. serratus*

(23.1%) ones. Since the former is more dependent of the water column than *C. crangon*, it probably has better swimming movements that allow it to easily escape to *H. didactylus* attempts. Attacks on larger crabs (3.5 – 4 cm) were quite unsuccessful, with the fishes not very interested in crabs proportionally too large for them. Morphological and behavioural traits of larger crabs must be considered serious antipredator attributes by smaller *H. didactylus*. A similar situation occurs with larger shrimps, that are not particularly appealing to the smaller toadfishes.

Capture success declined with decrease in *H. didactylus* body size. Possibly, the prey presented to the toadfish were quite large for them. According to Costa (2004), the average size of the *C. maenas* ingested by *H. didactylus* in the Mira estuary was of 8.9 mm, clearly smaller than the crabs here presented to the toadfishes. The average size of the shrimps found by this author was 24.6 mm for *C. crangon* specimens, and 35.5 mm for *Palaemon* spp., sizes more like the ones presented in this study. However, green crabs of the appropriate size to perform this experience were not possible to capture and maintain, and the smallest possible were given to the toadfishes. In gut content studies, Costa (2004) revealed that the crabs were always positively selected in relation to shrimps, so it is possible that if smaller crabs were here presented to Lusitanian toadfishes, they would capture more specimens of this type.

Higher handling times in crabs observed in the largest toadfish (56.67 s) were certainly due to crab's morphology, but also to their large dimensions. Not accidentally, prey handling is a function of the risk of injury during ingestion (Forbes, 1989). For shrimp prey (3 – 3.5 cm), *H. didactylus* handling times increased linearly with increasing relative prey size. However, in the largest toadfish, the average handling time for larger shrimp (9.00 s) was quite similar to that on the smaller shrimp (8.67 s), being both rather shorter than the handling times in the two smaller toadfish. Perhaps the size of this Lusitanian toadfish raised it prey-size level in relation to the other two specimens to a greater extent than the size differences between the smaller ones. Moreover, it is known that at about 200 mm total body length, Lusitanian toadfish undergoes ontogenetic shifts that require additional energy, and modify its capacity to easily capture and process larger prey (Costa, 2004).

Although additional experiments are needed, results of the present work seem consistent with studies that demonstrate that relative sizes of predator and prey are important factors in interactions, like prey selection and the time needed to prey manipulation and ingestion (Werner, 1974; Scharf *et al.*, 1998; Keller & Moore, 2000; Vacchi *et al.*, 2000). Some studies concluded that predator attack on a given prey is independent of prey size and suggest that size-selective feeding patterns may result from differential size-based capture success rather than active prey choice (Juanes, Buckel & Conover, 1994). In the present study, Lusitanian toadfish, particularly the smaller specimens, seemed to show active prey-size selectivity, which may be a valuable spare-energy behaviour.

Regarding Lusitanian toadfish prey edibilities, Costa (2004) showed that crabs and shrimps had lower edibilities than fish prey due to its chitinous exoskeleton. However, crab's lower edibility was balanced with its higher predominance in the toadfish diet. Profitability of a prey item (ratio of energy content of a prey type to the mean handling time of the predator-prey combination) to *H. didactylus* is not yet well understood. The lower consumption of shrimp and fish prey possibly reflects additional foraging costs to

Lusitanian toadfish, and may explain it preference for crabs in the field, despite their lower edibility.

The behaviour of prey can influence the likelihood of being detected, being its movements very important in determining the outcome of predator–prey interactions (Meager *et al.*, 2006). Prey's movement in an aquatic environment can be detected visually, but also via hydrodynamic trails left by the prey, which are sensed by the fish's lateral line. Strike behavior in fishes is partially a consequence of these external stimuli (Hanke *et al.*, 2000; Higham, 2007). In fact, Lusitanian toadfishes were able to detect prey even if it was in the opposite side of the aquarium, with prey movement's (either distant or close to the toadfish), being an important trigger in the predation behaviour. Although it is known that the lateral line in the oyster toadfish *O. tau* is able to detect mobile prey that approached within approximately 40% of body length (Palmer *et al.*, 2005), visual stimuli are most certainly also important in detecting prey. Nevertheless, in a highly turbid environment like an estuary, it is likely that there is an integration of different sensory systems in *H. didactylus*, particularly mechanosensory, chemosensory and visual systems. As pointed out by Abrahams & Kattenfeld (1997), with increasing turbidity, predator-prey encounters will occur at shorter distances with the probability of escape by the prey ultimately being determined by the probability of detecting the predator before the predator detects them.

The behavioural observations of the *H. didactylus* attacks on shrimp and crab seem consistent to what was observed in other studies, where the predator modulate its prey capture behaviour in response to different prey type (Nemeth, 1997). Attacks on more elusive prey (shrimps) began at a greater distance than attacks on less elusive prey (crab), indicating a modulation of attack velocity and a functional versatility of the toadfish.

Toadfish strike success seemed to be closely related with the distance to the prey, particularly in the attacks to shrimp prey. All the successful strikes possible to measure were initiated closer to the prey (< 3 cm) than the unsuccessful attempts (≥ 3 cm). Similar results, although with fish prey, were revealed by Price & Mensinger (1999) in *O. tau*, revealing high effectiveness of ambush predation behaviour on toadfish. However, different predatory strategies on *H. didactylus* were proposed by Costa (2004), particularly the active search for food and not only the sit and wait behaviour. Aggressive predation behaviour and bottom prospection with chemosensitive barbels observed in the present study, along with the results presented in the Chapter 2 of this thesis, are additional indicators of this kind of behaviour, which increases the probability of predator/prey encounters.

Results from this study indicate that prey type, behaviour and size are important in determining predator's choice and the success of capture attempts. Future research on interactions predator/prey should include: larger number of feeding trials, with more *H. didactylus* specimens from varied size-classes, diverse feeding trials, with more adequate prey sizes and also fish prey (namely *Pomatoschistus* spp., significant species in Lusitanian toadfish diet), and importantly, the estimation of different prey profitabilities, together with accurate evaluation of predator selectivity.

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Chapter 4

Final Remarks

Movement patterns of fish are thought to reflect ecological and evolutionary responses to the environment, and are directed to maximise growth, survival and reproductive output. Therefore, the assessment of an animal's movement in time and space should be an essential first step of any attempt at scaling the environment from the organism perspective (Pittman & Alpine, 2001). Often movements cover several distances and times scales within a single life cycle, including ontogenetic shifts and migrations movements, for instance. Home range movements show that most animals don't move randomly, but instead they establish areas were they undertake routine activity such as feeding, resting and defending. Home range incursions include tidal and diel movements and a vast literature is found to focus on this matter (e.g. Gibson *et al.*, 1996; Pittman & Alpine, 2001; Krumme *et al.*, 2004; Annese & Kingsford, 2005).

Telemetry has allowed great advances in studies of movement pattern. Telemetry is a powerful tool for an ecologist because of its potential for providing unbiased data on how an animal utilises space in time (Pittman & Alpine, 2001). The lack of knowledge on the movement patterns and activity of the Lusitanian toadfish, and its ecological importance in brackish water systems such as the Mira estuary, made it a credible candidate to telemetry studies. This first study on its routine activities resulted in an amount of data important in a variety of forms: either in the applicability of telemetry tags to this species, in the new type of information possible to obtain from *H. didactylus* individuals, in the confrontation of new data with previous studies, and in the discovery of new possible and better applications that this procedures may have in this species, or in others where may be relevant to perform behaviour studies. And since the estuarine environment is profoundly affected by tidal cycles, it possesses ideal conditions to carry out important tide-related ecological studies. The tide determines the habitat accessibility for tidal migrating estuarine species, cyclically changing the probability of encounters between predators and prey, while the nycthemeral cycle controls the visual conditions and daily changes in the organisms' activities.

The present work revealed that *H. didactylus*, despite its sedentary nature, is able to perform longitudinal movements in a greater amount than it was formerly believed, and its daily activities showed indeed a clear routine, with more movements during the dark periods, and also during the flood tide. Feeding activity is thought to be in the origin of the majority of these movements. Active search for food in Lusitanian toadfish is now considered as an important strategy to promote encounters with possible prey. Observations from the laboratory experiment also indicated this kind of behaviour, with the toadfish sometimes exploring the bottom with its sensitive barbils during a period of time. Nevertheless, its much higher capture success when striking within 3 cm of the prey pointed out its effectiveness as an ambush predatory. Therefore, it's really possible that these two feeding strategies are used by Lusitanian toadfish whenever they have the higher probability of success, as it was prior suggested by Costa (2004).

Light-dark cycles, tidal cycles and patterns of food availability are between the causes of organism's activity patterns. Endogenous mechanisms may also be present, and if so, removing the external stimuli would not change the circadian activity. Interestingly, activity patterns close to 24 hours were found in *H. didactylus*, using the data retrieved from the archival tags, which come close to what happens in other fish species, where biological clocks are regulated by nycthemeral cycles (see Reebs, 1992; Helfman, 1993). The analysis of retrieved time-series data revealed that tidal-based activity patterns were not as patent as daily activity patterns. Bias during the data analysis, particularly the fact that 12-h rhythmicity had to be filtered from data (resulting in the fact that pressure tags in a benthic fish like *H. didactylus* clearly register the tidal cycle) may have influenced the results. However, according to the results of the short movement's proportions, tides had definitely a great importance in *H. didactylus* daily activities and along with dark periods, probably also in longer longitudinal displacements throughout the estuary.

Crabs, particularly *C. maenas* specimens, are the most important food item of adult Lusitanian toadfish in the Mira estuary (Costa, 2004). Their high consumption by *H. didactylus* individuals is strongly related with their epibenthic nature. However, the greater availability of nectobenthic prey existent in this brackish water system comparatively to those in marine systems, allows prey like shrimps to also assume an elevated importance in the diet of *H. didactylus*, as well as teleost fish, particularly species belonging to *Pomatoschistus* genus (Costa, 2004). Nevertheless, edibilities of these prey (crabs, shrimps, and fish) are quite different, and its profitabilities have not yet been assessed. In our laboratory experience, crabs had greater handling times but elevated capture success, and shrimp showed shorter handling times but lower capture success. Perhaps these two feeding options can balance each other in energetic terms, but *H. didactylus* prey profitabilities must be estimated in future studies.

The information obtained in this study has provided some background for future ecological and behavioural investigations. In the future it would be interesting to monitor behavioural aspects of *H. didactylus* and relate them with other biotic and abiotic parameters. More extensive electronic tagging studies should be performed (both in time and number of specimens), including different periods of the life cycle of this species. Tagging studies of several months with regular recordings could be a valuable help in the understanding of shifts on Lusitanian toadfish behaviour during different periods, which certainly requires different amount of food, and probably include changes in hydrological parameters like temperature and salinity. Being the Mira estuary an excellent water system to undertake ecological studies, it would be quite appropriate to develop more behavioural experiments there. And as the recovering of tagged fish is such an important step, a relatively small system like the Mira probably enhances the chances of its recovering.

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