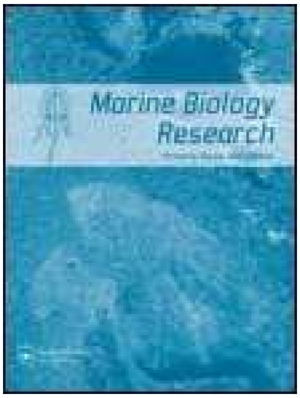

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Anomalous otoliths in juveniles of common sole, Solea solea, and Senegal sole, Solea senegalensis

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ORIGINAL ARTICLE

Anomalous otoliths in juveniles of common sole, *Solea solea*, and Senegal sole, *Solea senegalensis*

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Abstract
The otoliths of wild common sole, *Solea solea*, and Senegal sole, *Solea senegalensis*, from the Tagus and the Douro estuaries, and captive *S. senegalensis* were examined for the detection of anomalies. The anomalies detected were granules of crystals, a dark coloration over the entire otolith, a dark mark concentric to the nucleus and multiple nuclei. A higher proportion of anomalies was found in wild individuals of these species (16–63%) than is usually reported for other species. Captive *S. senegalensis* exhibited an incidence of anomalies within the range previously reported for other species also reared in captivity. The oceanographic–climatic conditions of the Portuguese coast, which cause strong and abrupt changes in water temperature, salinity and mineral composition, may be an important factor contributing to or causing otolith anomalies. Heatwaves, intense solar radiation and anthropogenic pollution affecting the estuarine nursery grounds may also play an important role. However, more experimental studies are needed to elucidate what causes otolith anomalies.

Key words: *Aquaculture, daily increments, estuarine nurseries, flatfish, otolith deformities, soles*

Introduction

Otoliths are small calcium carbonate structures of biogenic origin. They are located in the inner ear of vertebrates, immersed in endolymph. Teleost fish have three pairs of otoliths: *lapilli*, *asterisci* and *sagittae* (Moyle & Cech 1996). They play an important role in balance and hearing, sensing gravity, linear acceleration and sounds (Popper & Lu 2000).

In a three-dimensional environment, such as the aquatic environment, spatial awareness and postural equilibrium are fundamental for locomotion. Sound perception in the water is also crucial for the detection of congeners, prey and predators. Thus, functional otoliths appear to be essential for fish survival.

However, anomalous otoliths have been known for decades in various fish species, having been reported for several families, namely Engraulidae, Clupeidae, Salmonidae, Ophidiidae, Macrouridae, Gadidae, Moronidae, Pleuronectidae, Soleidae and Scianidae (Palmork et al. 1963; Collins & Spratt 1969; Mugiya 1972; Blacker 1974; Morales-Nin 1985; Wilson 1985; Strong et al. 1986; Gauldie 1993; Tomás & Geffen 2003; Béarez et al. 2005; Dierking et al. 2012). Their occurrence in wild populations generally varies between 1.0% and 5.5% (Blacker 1974; Morales-Nin 1985; Strong et al. 1986; Tomás & Geffen 2003; Béarez et al. 2005). However, higher values have been found for salmonids, between 18% and 45% (Morat et al. 2008), and 66% for *Solea solea* (Linnaeus, 1758) (Dierking et al. 2012). In captive fish these values are usually high. Tomás & Geffen (2003) reported values as high as 14% for herring, *Clupea harengus* Linnaeus, 1758, raised in the laboratory. Gauldie (1986, 1996) reported values of 34% for captive juvenile chinook salmon and Bowen II et al. (1999) reported values of 26–41% for stocked lake trout.

Most works on otolith anomalies report on polymorphs of calcium carbonate: calcite, aragonite and vaterite (Carlström 1963). They differ in the geometry of the crystal: calcite is trigonal, aragonite is

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orthorhombic and vaterite is hexagonal. Normal otoliths are aragonitic, while anomalous otoliths display overgrowths of translucent vaterite and/or calcite crystals (e.g. Strong et al. 1986; Gauldie 1993; Tomás & Geffen 2003; Béarez et al. 2005).

The causal mechanism behind anomalous otoliths is still unknown. Several hypotheses have been put forward. Gauldie (1986) suggested that otolith malformations may be the expression of malfunctioning genes. However, they may also reflect stressful conditions. That may be especially true for reared animals (Gauldie 1986, 1996; Bowen II et al. 1999; Tomás & Geffen 2003) and wild fish living in highly variable oceanographic conditions like those characteristic of upwelling systems (Béarez et al. 2005).

There is little evidence of negative effects of these anomalies on the survival and development of fish. They seem to appear, behave and grow like fish with normal otoliths (Tomás & Geffen 2003; Béarez et al. 2005). However, experimental studies are still scarce. Because vaterite is less dense than aragonite, it is thought that fish with anomalous otoliths may have their balance and hearing ability affected, particularly when only one otolith is affected and the other is normal (Popper & Lu 2000).

In addition to structural anomalies, Berghahn (2000) reported on coloration alterations in juvenile flatfish inhabiting tidal pools exposed to intense ultraviolet solar radiation. These alterations consisted of hyaline zones concentric to the nucleus.

In 2005 several surveys were undertaken to study growth of the 0-group soles, *Solea solea* and *S. senegalensis* Kaup, 1858, in the Douro and Tagus estuaries, Portugal. An additional investigation was carried out with reared *S. senegalensis* in order to validate otolith daily increment deposition. The occurrence of anomalies in the otoliths of these juveniles was higher than had been reported previously for wild and captive fish. The aim of the present study is to describe the types and incidence of otolith anomalies in wild and captive juveniles of *S. solea* and *S. senegalensis*.

Materials and methods

Sampling was carried out in the spring and summer of 2005, in the Douro estuary and at two nursery areas in the Tagus estuary (Figure 1). Isotopic studies have shown that the juvenile cohorts of *Solea senegalensis* that colonize the two nursery areas of the Tagus, which have different timings of

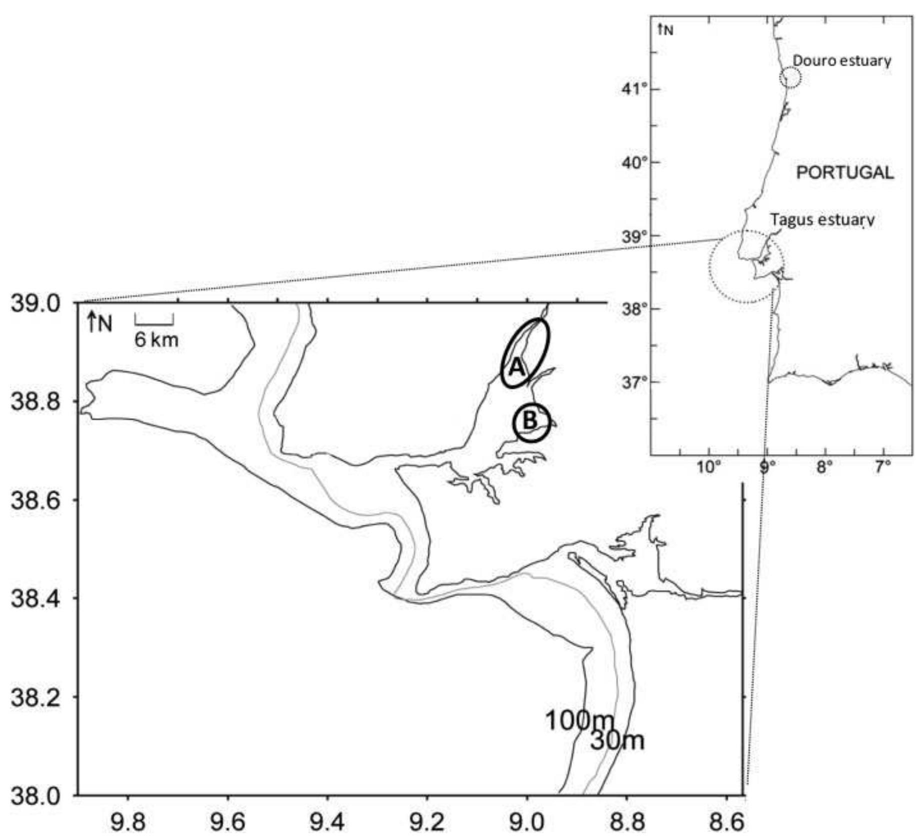

Figure 1. Location of the sampling areas, the Douro and Tagus estuaries (insert shows the location of the two nurseries of the Tagus estuary, A and B).

recruitment, present high site fidelity and do not migrate between nurseries (Vinagre et al. 2008, 2011). *Solea solea* only colonizes nursery A in the Tagus estuary (Figure 1). *S. senegalensis* was not present in the Douro estuary. Trawls were conducted with a 2.5 m beam trawl with 10 mm mesh size (stretched mesh) and a 5 mm cod end. All samples were immediately frozen.

In wild sole juveniles *lapilli* otoliths were used because they are relatively thin and have well-defined increments that are spatially more uniform than in *sagittal* otoliths, which have accessory primordia (Amara et al. 1994). In *S. senegalensis* born and raised in captivity, 28 fish were removed for analysis from day 1 to day 28 of their lives. *Sagittal* otoliths were used in this case because these fish were too young to have accessory primordia, which appear during metamorphosis. The otoliths were removed from the cranium, cleaned and mounted with cyanoacrylate glue on microscope slides. They were polished in the sagittal plane to the central primordia with a polishing bar of aluminium oxide (Amara et al. 1994, 2000). They were examined using a light microscope at 400 × or 1000 × magnification.

Results

The anomalies found were granules of crystals (Figure 2), similar to those reported previously for various fish, a dark coloration over the entire otolith, a dark band concentric to the nucleus (Figure 3) and multiple nuclei (Table I, Figure 4). Most of the otoliths were still readable for age determination based on the microincrements. Often, crystal granulations did not affect most of the otolith and a reading pathway was still available. Some of the dark otoliths were readable, others were not. The dark band concentric to the nucleus (Figure 3) did not affect the readability of the otoliths, similar to multiple nuclei.

The percentage of crystal granulations (Figure 2) was highest in *Solea senegalensis* from nursery B (Tagus estuary) (Table I). The amount of otoliths with its surface 100% granulated was highest in

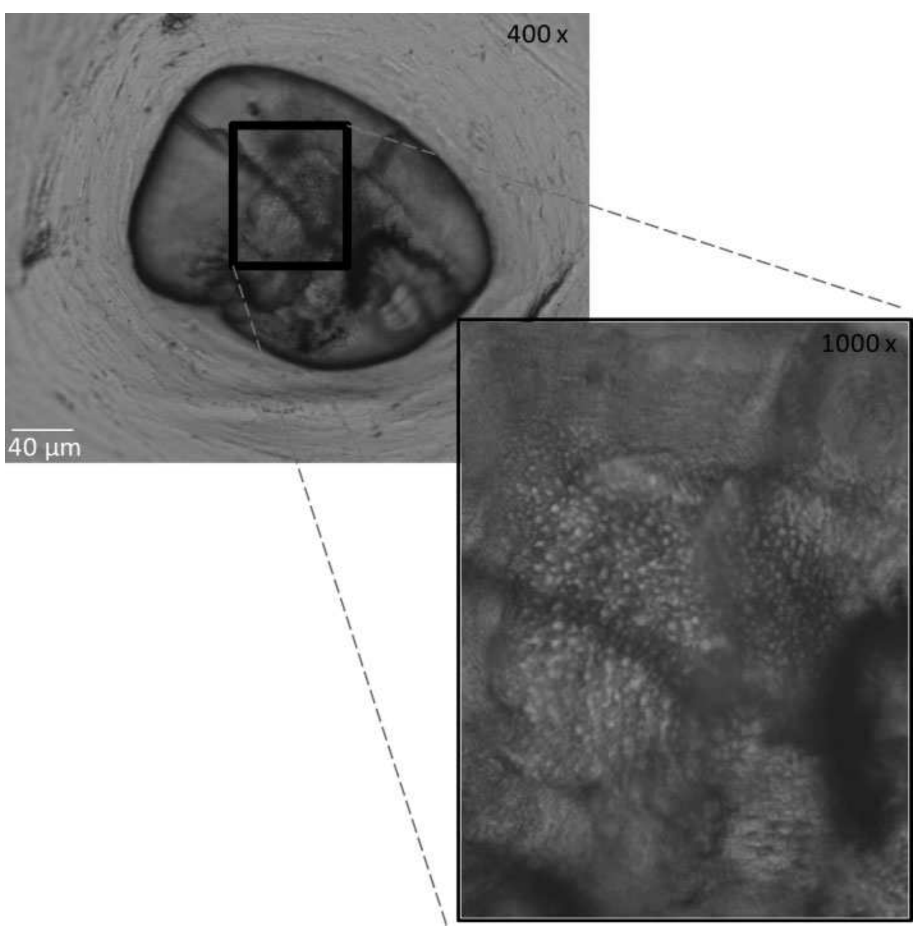

Figure 2. Photograph of an otolith with structure granulations (insert shows a magnification of the affected area). Lapillus of a juvenile *Solea senegalensis* captured in nursery A of the Tagus estuary in July 2005.

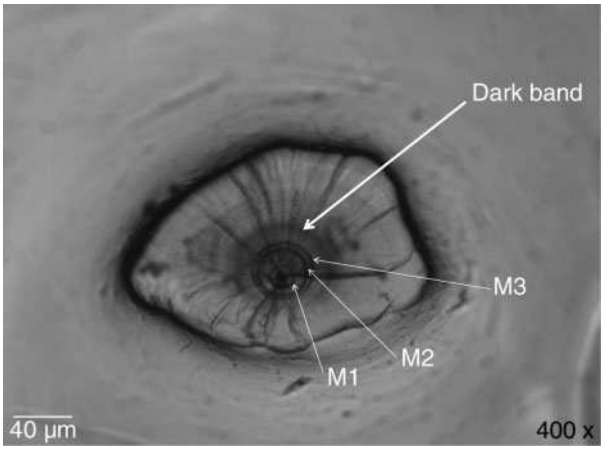

Figure 3. Photograph of an otolith with a dark band. Lapillus of a juvenile *Solea solea* captured in nursery A of the Tagus estuary in July 2005. M1, M2 and M3 are life-history marks, probably marking hatching, mouth-opening and an unidentified event, respectively.

S. senegalensis, both wild and captive (24–25%), while in *Solea solea* the amount of affected otoliths was lower (0–19%) (Table II). *Solea solea* from the Douro estuary seem to be the least affected by anomalies, with the exception of the dark band concentric to the nucleus (Tables I, II, Figure 3).

Overall dark coloration was prevalent in captive *S. senegalensis* (Table I). The dark band concentric to the nucleus did not occur in captive *S. senegalensis*. This anomaly was prevalent in *S. solea* from the Douro estuary, with 83% of individuals affected (Table I). It was also more common in *S. solea* (57–83%) than in *S. senegalensis* (32–42%) (Table I). Multiple nuclei were not observed in wild *S. senegalensis*, only in wild *S. solea* (2–3%) and captive *S. senegalensis* (4%) (Table I, Figure 4).

Discussion

The present work revealed that *Solea solea* and *Solea senegalensis* juveniles from the Tagus and the Douro estuaries present a variety of otolith anomalies and a higher percentage of crystal granulations than those found for wild fish of other species. In fact, the values found for wild *S. solea* and *S. senegalensis* in this work are similar to those reported for captive fish of other species, with the exception of those reported by Dierking et al (2012), who observed 66% of anomalous otoliths in wild *S. solea*.

Captive *S. senegalensis* presented an amount of crystal granulations within the range reported for other species reared in captivity, such as herring, *Clupea harengus* (Tomás & Geffen 2003), chinook salmon (Gauldie 1986, 1996) and lake trout (Bowen II et al. 1999). Yet, in addition, *S. senegalensis* presented other anomalies, such as darkened otoliths and multiple nuclei. Captive fish are exposed to conditions that differ from those found in their natural environmental and although aquacultures try to mimic natural factors such as photoperiod, temperature and salinity, there may be unknown stressors affecting fish. High densities of fish and frequent human presence may be important stressors affecting captive fish and potentially contributing to the development of deformities, such as otolith anomalies. In the case of the aquaculture where these fish were reared, formaldehyde was used as a disinfectant. This chemical may have contributed to or caused the high amount of anomalies in these fish.

The reason for the high amount of granulations found in wild *S. solea* and *S. senegalensis* may be stressful environmental conditions during the larval stage in coastal waters or during the time spent in the estuary as young juveniles. Béarez et al. (2005) discussed the potential role of oceanic–climatic upheavals such as upwelling and El Niño/La Niña phenomena as the cause of otolith anomalies found in Scianidae from Peruvian coasts. Strong and abrupt variations in water temperature, salinity and mineral composition may affect otolith crystalline growth by influencing the calcification process. Calcification is dependent on endolymph chemistry and on the organic matrix, and thus alterations in its homeostasis may generate different forms of crystals (Gauldie 1986; Shivkumara et al. 2006).

The Portuguese coast is affected by upwelling. In this area, upwelling phenomena occur in summer, with offshore Ekman transport of surface water (Peliz et al. 2002). Although upwelling is more frequent in summer, it is generally considered that winds that favour this phenomenon are a recurrent feature of the Portuguese coast (Huthnance et al.

Table I. Percentage of otoliths affected by each anomaly.

	Location	*N*	Granulations (%)	Dark otoliths (%)	Dark band (%)	Multiple nucleus (%)
Solea solea	Tagus estuary - A	100	55	12	57	3
Solea solea	Douro estuary	58	16	3	83	2
Solea senegalensis	Tagus estuary - A	79	51	6	42	0
Solea senegalensis	Tagus estuary - B	72	63	4	32	0
Solea senegalensis	Aquaculture	28	29	43	0	4

Table II. Percentage of otoliths affected by various degrees of granulations.

		Otoliths affected by granulations		
	Location	< 50% of surface affected	> 50% of surface affected	100% of surface affected
Solea solea	Tagus estuary - A	22	14	19
Solea solea	Douro estuary	9	7	0
Solea senegalensis	Tagus estuary - A	11	15	24
Solea senegalensis	Tagus estuary - B	25	13	25
Solea senegalensis	Aquaculture	4	0	25

1995). In this way, the abiotic stress caused by recurrent upwelling phenomena may be the mechanism behind the high rate and variety of anomalies found in juvenile soles in the present study. In that case, these anomalies would have their origin during the larval stage, when sole inhabit coastal waters.

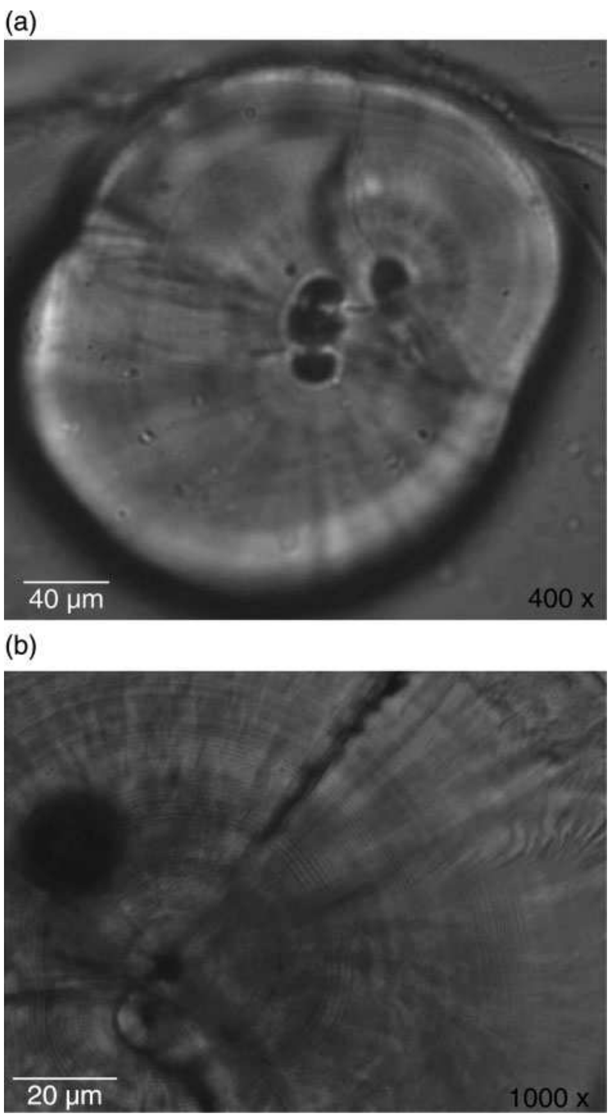

Figure 4. (**a**) Photograph of an otolith with multiple nuclei. Sagitta of a 15-day-old *Solea senegalensis* born and raised in captivity. (**b**) Photograph of an otolith with double nuclei. Lapillus of a juvenile *Solea solea* captured in nursery A of the Tagus estuary in July 2005.

However, conditions endured by juveniles in the estuarine nurseries are also stressful. In addition to the general abiotic variations that are characteristic of estuaries worldwide, Portuguese estuaries are subjected to heatwaves, usually in summer (Aschmann 1973). The Tagus estuary presents a mean water temperature in summer of 24°C, yet temperature values can rise steeply to 28°C during heatwaves, a stressful temperature for this and other fish species using this estuary as a nursery ground (Gauldie 1996; Madeira et al. 2012; Vinagre et al. 2012a,b,c). Thus, the possibility of these anomalies being instigated by stress in the estuary during the juvenile stage cannot be rejected at this point.

Another important potential source of stress in Portuguese estuaries such as the Tagus and the Douro is anthropogenic pollution. However, some authors argue that this factor is not important, because otolith anomalies, similar to the ones found in the present study, can also be found in palaeoecological studies in areas where environmental conditions have been stable for thousands of years (Béarez et al. 2005). Experimental studies are scarce. Van den Brandhof & Montforts (2010) found that medical drugs such as carbamazepine, diclofenac and metoprolol did not provoke otolith deformities in zebrafish, *Danio rerio* (Hamilton, 1822), yet studies are still lacking for common pollutants in estuaries, such as heavy metals, PCBs and PAHs.

The dark band concentric to the nucleus (Figure 3) observed only in wild individuals in the present study has similarities to the hyaline concentric zones reported by Berghahn (2000), and can be attributed to the effect of solar ultraviolet radiation. The fact that captive *S. senegalensis* did not present this anomaly is in accordance with this theory. The fact that this dark band was very frequent in the otoliths of wild fish in the present study is also consistent with the theory of Berghahn (2000), as Portugal has one of the highest rates of sunny days per year in Europe. This band is located in an area of the otolith consistent with settlement time. According to Berghahn

(2000), settlement would be the stage most exposed to ultraviolet radiation.

The study of anomalies in fish otoliths urgently requires experimental work. The manipulation of variables in a controlled environment would elucidate which factors control otolith calcification. Experiments with contaminants could also reveal if otolith anomalies reflect environmental pollution and can be used as a biological indicator for environmental monitoring. The functional implications of otolith deformities could also be assessed experimentally. Research in this direction would increase our knowledge on fish welfare in aquaculture and the natural environment.

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