Short Communication

**Occurrence of nephrolithiasis in a population of longsnout seahorse, *Hippocampus reidi* Ginsburg, and analysis of a nephrolith**

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The longsnout seahorse, *Hippocampus reidi* Ginsburg, lives in the tropical regions of the western Atlantic Ocean from Carolina to Brazil, the Indian Ocean and several other tropical regions (Hercos & Giarrizzo 2007). Adults reach a size of 8–19 cm and live in the wild for approximately 30 months (Mai & Velasco 2012).

*Hippocampus reidi* is a very common species in public aquaria and it has been successfully bred by professionals and hobbyists. This study reports the diagnosis and prevalence of nephrolithiasis in a tank reared population of longsnout seahorses in Austria.

A group of approximately 70 adult seahorses lived in a 210-L tank filled with artificial sea water, in a public aquarium in Vienna. Water was filtered with a modified canister filter. The tank did not have any decoration and plants, but it had enough sites for the animals to hold on. In addition to this breeding tank, there was a quarantine tank and several exhibition tanks. Most of the seahorses were born in September 2005 and were reared in a circulating water system. They were fed a diet of mysid shrimp, Artemia nauplii, and adult Artemia, which were commercial frozen products that were thawed and enriched with a mixture of vitamins prior to feeding. On a daily basis, 10–20% of the water was changed and the bottom of each tank was cleaned. The water was tested using a JBL test kit and a photometer (Lamotte ‘smart spectro’). The reason for this study was to investigate the acute onset of disease, which was found to be caused by a change in the feeding supply. Since after the correction of the feeding management, a constant low mortality rate remained, clinical, parasitological, bacteriological and histological investigations were undertaken from November 2006 to October 2008. Examined fish were either euthanised due to their bad condition (tricaine methane sulphonate, MS 222; Sigma Aldrich Handels-GmbH) or had been found dead in one of the tanks. Kidneys of 19 seahorses were sampled for native microscopic examination. Kidneys were dark red and measured in their cranial parts about 1 mm in diameter and in their caudal parts 1–5 mm in diameter. Some kidneys had pinpoint white or red foci, and in one case, there was adhesion to connective tissue. For histopathological examination, the kidneys were fixed in 10% neutral buffered formalin and were subjected to routine histological procedures and stained with haematoxylin and eosin (H & E). In two cases, nephroliths could be seen macroscopically as white, raised spots on the caudal part of the kidney. One of them was examined with a reflected-light microscope, embedded into methylmethacrylate, and uncalcified thin sections (120 μm) of the specimen were produced. The unstained sections were evaluated by light

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microscopy using a Nikon Microphot-FXA (Nikon Corp.) and polarized light and they were subsequently examined using an atmospheric Scanning Electron Microscope (LEO EVO 60VP; now Zeiss). This instrument contains an Energy Dispersive X-ray Spectroscope (EDX; Comp. Oxford) for the quantitative analysis of the chemical composition. A qualitative estimation of the distribution of chemical elements on the surface of the specimen’s cross-section was performed using the Quadrant Backscatter Detector (QBSD), which measures the amount of backscattered electrons.

Raman reflection spectroscopy (Labram HR-800, Horiba Jobin Yvon) and IR reflection spectroscopy (Vertex 70, Bruker Optics) were performed on the ground section to investigate the type of the mineral phase.

Clinical signs of nephrolithiasis associated with CO₂ supersaturation in fish include reduced growth (Foss, Røsnes & Øiestad 2003), as well as chronic low-level mortalities (Chen et al. 2001). The investigated population of seahorses showed signs of exophthalmia, gas bubble disease, neoplasia, dyspnoea, skin erosions, general weakness and anorexia. Some of these signs can easily be linked to impaired renal function. The analysis of a water sample revealed the following results: alkalinity 108 ppm measured as CaCO₃, ammonia 0.0 mg L⁻¹, nitrite 0.1 mg L⁻¹, nitrate 80 mg L⁻¹, phosphate 1.5–2.0 mg L⁻¹, iron 0.00–0.05 mg L⁻¹, dissolved oxygen 7.0 mg L⁻¹, temperature 26 °C and pH 8.6. The oxygen saturation was approximately 88%.

Wet mount examination of the kidney samples by light microscopy demonstrated black patches representing aggregations of melanomacrophages.

Figure 1 Conglomerates of mineral deposits in a wet mount of kidney tissue.

Figure 2 H & E stain of kidney tissue crystals protruding into the lumen of a tubule.

Figure 3 (a) Nephrolith, reflected-light microscopy, (b) section of nephrolith, transmitted light microscopy.
Many of the samples (8 of 19, prevalence 42%) showed mineral deposits in the form of rectangular structures which were aggregated into spheres (approximately 80 μm) that formed conglomerates (approximately 600 × 500 μm) (Fig. 1). Histopathological examination of the H & E-stained kidney samples revealed the extent of mineral deposits (Fig. 2) Dilatation and degeneration of tubules containing mineral deposits could be observed. The infiltration with connective tissue as well as the aggregation of mononuclear inflammatory cells and hyperaemia were signs both of chronic and acute inflammation.

The isolated nephrolith was approximately 1.5 mm in length and yellow. It was ovoid and had a rough surface with a metallic shine (Fig. 3a). As it had first been embedded within methylmethacrylate, an EDX spectrum was collected from the surface. EDX measurements showed that, although the surface was polished, glue remained on it because the specimen was porous. Not surprisingly, carbon (C) and oxygen (O) were detected as major

![Figure 4](image.png)

**Figure 4** Distribution of elements in cross-section, Quadrant Back Scatter Detector mapping.
components of the specimen, and traces of magnesium (Mg) and phosphorus (P) were detected within the range of a few per millilitre.

Using the QBSD, the inner structure of the nephrolith was visualized to show the distribution of elements (Fig. 4). An overall quantitative analysis was problematic for this sample because the distribution of each element varied substantially, as seen in the QBSD picture. Nevertheless, in addition to C (48.3 ± 0.5%) and O (39.0 ± 0.5%) which originated from the embedding material, the main detected elements were P (5.65 ± 0.09%), Mg (4.38 ± 0.08%), Ca (1.33 ± 0.04%) and Na (0.59 ± 0.04%). Furthermore, traces of Cl (0.49 ± 0.03%), K (0.28 ± 0.03%) and S (0.09 ± 0.02%) were detected. The distribution of each element is very well represented by the mapping of the elements (Fig. 4). A spectrum could not be collected from the sample using the Raman spectrometer, which means the crystallinity of the specimen was low, and therefore, the kidney stone was amorphous. The IR analysis showed a typical reflectance spectrum for embedding material, and a spectral pattern that is consistent with the presence of struvite (see Fig. 5). After close consideration of the results, it can be assumed that the kidney stone of the seahorse most probably consisted of amorphous struvite.

In general, the composition of nephroliths in fish is not well-documented. In rainbow trout, Ca was found to be the main component (30%), followed by P (15%), CO3 (5%) and Mg (2.5%) (Gillespie & Evans 1979). The composition of nephroliths in rainbow trout was described as (Na, Mg, Ca)3P2O-H2O similar to the mineral collinsite (Groff et al. 1998). Smart found that deposits consist mainly of calcium phosphate (Smart et al. 1979). Tyrosine crystal deposits in kidneys and other organs have been described in gilt-head seabream and turbot (Paperna, Harrison & Kissil 1980; Messager et al. 1986).

In most cases, struvite forms typical crystals (i.e. ‘coffin lids’ or ‘prisms’). The wet mounts and the histopathological sections showed a clear crystalline structure of mineral deposits in tubules, although the typical ‘coffin lid’ form could not be identified (Figs 1 & 2). In contrast, struvite of the examined specimen was amorphous.

The finding of struvite crystals in terrestrial mammal urine is not uncommon. Formation of struvite uroliths occurs primarily in alkaline urine which is supersaturated with magnesium ammonium phosphate. In humans and dogs, such conditions are usually due to urease-producing bacteria and therefore to infection of the urinary tract (Griffith 1978; Osborne et al. 1986). Nevertheless, in dogs, genetic predisposition and a high-protein diet may also lead to struvite formation (Osborne et al. 1986).

In other animals like cats and ferrets, the formation of struvite uroliths is possible without infection. Species-specific proteins may enhance struvite crystal formation in cats (Buffington, Blaisdell & Sako 1994; Matsumoto & Funaba 2008).

In marine mammals, the finding of struvite urolithiasis has been reported (Harms et al. 2004; McFee & Osborne 2004). In the case of a pygmy sperm whale, Klebsiella oxytoca could be isolated (Harms et al. 2004), and in the case of a bottlenose dolphin, an infection of the urinary tract was strongly suspected (McFee & Osborne 2004).
Urogenital sinus calculi of a sand tiger shark consisted mainly of struvite, without evidence of microbiological infection (Walsh & Murru 1987).

As for the seahorses, several bacterial examinations, including Gram stains, of the kidney tissues were negative. Although, unfortunately, no microbiological examination of the specimen described here was performed, infection as the reason for the struvite urolithiasis seems unlikely.

Besides infectious reasons, precipitation and crystallization of struvite in urine is dependent on pH, the content of Mg$^{2+}$, NH$_4^+$ and PO$_4^{3-}$, the ionic strength, and the ratio of Ca/Mg (Elliot, Sharp & Lewis 1959). The urine of marine agleran species contains high levels of divalent ions (Ca$^{2+}$, Mg$^{2+}$, SO$_4^{2-}$) (Beyenbach 2004), ‘very often to the point of super saturation and precipitation’ (Marshall & Grosell 2006).

The causes for changes in urine composition and the resulting precipitation of minerals are diverse. Factors concerning the seahorse such as general disease, infection, inherited disease, metabolic and toxic damage and environmental factors, especially nutritional imbalance and artificial sea water composition, may play a role in these changes.

In the case of the seahorses, water parameters, especially the extremely high concentration of phosphate together with a relatively high pH, and dietary mineral imbalances could be the reason for renal struvite urolithiasis. According to our knowledge, this is the first report of struvite nephrolithiasis in teleost fish.

References


