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Abnormal swimming is associated with inner ear damage and improves with meclizine treatment and increased habitat complexity: A case study in *Urobatis jamaicensis*

Lauren Puishys[1,2], Natalie Mylniczenko[1,3], Ryan De Voe[1,3], Alisha Fredrickson[1], Dani Salles[1], Todd Harmon[1], Scott Martin[1], Leah Maurer[1] and Austin Leeds[1,3]

[1]Disney's Animals, Science and Environment, The Seas with Nemo and Friends®, Walt Disney World® Resort, Lake Buena Vista, FL 32830, USA
[2]New College of Florida, 5800 Bay Shore Road, Sarasota, FL 34243, USA
[3]Disney's Animals, Science and Environment, Disney's Animal Kingdom®, Lake Buena Vista, FL, 32830-1000, USA

Correspondence: Lauren Puishys, email; Lauren.Puishys@disney.com

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Abstract
At the Seas with Nemo and Friends® exhibit (Lake Buena Vista, Florida, USA), a yellow stingray *Urobatis jamaicensis* was diagnosed with a *Mycobacterium chelonae* infection of the inner ear. The animal was moved to an isolated environment for treatment. One element of therapy included meclizine which was administered in an attempt to reduce the occurrence of abnormal swimming hypothesised to be occurring due to inner ear damage caused by the mycobacteria. Behavioural observations were conducted throughout the medical treatment to monitor response. Following the administration of meclizine, a significant reduction in abnormal swimming behaviour and an increase in normal swimming and resting were observed. Subsequent to the stingray's move back to its home environment, resting further increased, while abnormal swimming behaviour significantly decreased, even from already reduced levels during the meclizine treatment. These data highlight the unique relationship between veterinary diagnosis/treatment and behaviour in an elasmobranch with vestibular damage as well as demonstrate the effectiveness of the drug meclizine in treating the symptoms of infection.

Background

Abnormal neurological behaviour in elasmobranchs has been reported and is usually attributed to disease of the central nervous or endolymphatic/vestibular system (Grimes et al. 1984; Li et al. 2017; Pereira et al. 2002; Rich et al. 2021; Schaffer et al. 2013). This is often associated with bacterial infection (Borucinska 2016). One such bacteria, mycobacteria, are found in natural biofilms and on the skin of healthy elasmobranchs (Anderson et al. 2012; Modzelesky 2018), but as with teleosts, high organic loads, immunosuppression and population density are suspected to exacerbate the replication of these bacteria (Francis-Floyd 2011). Once an animal is infected, treatment is unlikely to be successful (Pereira et al. 2018). Additionally, due to anatomical complexity (Maisey 2001), when the deep structures of the inner ear are affected, treatment is very challenging as tissue penetration from systemic medication is not simple.

In addition to sound detection and sourcing, the inner ear of elasmobranchs is responsible for equilibrium, motion and balance (Maisey 2001). Damage to the central nervous and vestibular systems following bacterial infection has been reported in elasmobranchs (Li et al. 2017; Pereira et al. 2002, 2018; Rich et al. 2021; Schaffer et al. 2013). Various clinical signs have been documented in these case studies including agitated and circular/spiral swimming patterns (Pereira et al. 2002, 2018; Rich et al. 2021), discoloured skin (Pereira et al. 2002), skin nodules (Pereira et al. 2002, 2018), and lethargy and anorexia (Rich et al. 2021). In each of the aforementioned cases, diagnosis was subsequently followed by death or

euthanasia of the infected individual. It is worth highlighting that these case studies focused on clinical behavioural indicators of health status versus an overall description of broader animal behaviour.

In zoos and aquariums, behaviour is often associated with the welfare state of an animal (Hart et al. 2021; Martins et al. 2012; Miller et al. 2021) but can also be an indicator of the onset of illness (Khalil and Emeash 2018) or a sign of an underlying medical condition (Tate et al. 2013). Outside of medical diagnosis, aquarium-housed elasmobranchs can exhibit various abnormal swimming behaviours, including both quick/jerky and slow movements, deviation from the animal's typical horizontal plane, looping behaviour and poor navigation (Charbeneau 2004). Here, the onset of abnormal swimming behaviour in a yellow stingray *Urobatis jamaicensis* following a diagnosis of a mycobacterial infection (*Mycobacterium chelonae*) and subsequent inner ear damage is described, providing insights into the relationship between health status and abnormal swimming in elasmobranchs.

On 13 June 2019, 'Scooby', an approximately nine-year-old male yellow stingray, presented with a small abscess on his cranial dorsal midline. A fine needle (22G) aspirate of the material collected tested positive (Ziehl-Neelsen cytology, N. Stacy) for the presence of *M. chelonae,* as well as a mycobacterial culture (IDEXX Laboratories). Two months after this diagnosis, Scooby began engaging in a previously unobserved abnormal swimming behaviour where his ventral surface was oriented perpendicular to the bottom of the habitat, described as a side dorsal spin (see ethogram in Table 1). A cone beam computed tomography (CBCT) (Vimago, Epica International, USA) scan of Scooby revealed asymmetry of the labyrinth with marked anatomical changes to the right inner ear structure. Recheck evaluation of the abscess revealed continued accumulation of infectious material and a fistula extending from an opening in the abscess through to the right side of the inner ear. A 22G needle inserted into the deepest area aspirated the material and the resulting cultures confirmed the mycobacterial infection. Scooby was then moved to an off-exhibit holding environment to facilitate medical treatment. Relevant medical treatment consisted of parenteral and oral antibiotics, flushing the abscess and packing with handmade plaster of Paris antibacterial beads and silver-sulfadiazine impregnated cream to manage the infection. Four months into the disease process with worsening behavioural signs, 2.5 mg/kg daily oral meclizine was initiated. This drug, traditionally used to treat motion sickness, was expected to help with treating the behavioural symptoms. Meclizine serum levels were tested and verified to ensure appropriate absorption (NMS Labs, USA). Desired absorption levels were extrapolated from relevant human literature (Wang et al. 2012) and scaled down to achieve the minimal amount needed for a therapeutic effect. Before the onset of the meclizine treatment, behavioural observations focused on swimming and resting were initiated to better understand the relationship between diagnosis, treatment and behaviour.

Action

This study occurred at the Seas with Nemo and Friends® aquarium (the Seas), Lake Buena Vista, Florida, USA. Scooby was housed with other small elasmobranchs and teleosts in an open top aquarium approximately 4.79 m^3 in size. The aquarium substrates were gravel and artificial rock and coral structures were located throughout

Table 1. Ethogram of yellow stingray behaviour

Behaviour	Description
Resting	Subject is stationary and not traveling any distance.
Normal	Subject is swimming with ventral surface oriented parallel to the bottom of the environment.
Top Spin	The subject is oriented with its ventral surface parallel to the bottom of the environment. Behaviour consists of small tight circles where little to no distance is covered. Often in short spurts or as a precursor to foraging behaviours.
Engaged	Subject is physically interacting with another animal in the environment.
Side Dorsal Spin	Subject is swimming with its ventral surface perpendicular to the bottom of the habitat. Ventral surface not against a wall.
Wall Riding	Subject has its ventral surface perpendicular to the bottom of the habitat. Ventral surface against a wall.
Surface Riding	Subject is travelling with ventral side against surface of water (upside down).
Flips	The subject is performing a 360-degree flip in the water column.
Coral Riding	The subject is swimming with its ventral surface against a structure while horizontal to the benthic environment.
Not Visible	The subject is outside the scope of the video footage.
Directionality	Description
Clockwise	Subject is moving in a clockwise circular swimming pattern (applies to normal, top spin, side dorsal spin, wall riding and coral riding).
Anti-Clockwise	Subject is moving in an anti-clockwise circular swimming pattern (applies to normal, top spin, side dorsal spin, wall riding and coral riding).
Roaming	Subject is moving throughout the exhibit without a defined pattern or direction (applies to normal, engaged, side dorsal spin, wall riding, surface riding, flips and coral riding).
N/A	Subject is engaged in a stationary behaviour (applies to resting and not visible).

the exhibit. Water quality parameters were consistent throughout the monitoring period. Swimming and resting behaviour was monitored during a three-month study period, 21 September to 28 December 2019, which spanned the course of initial medical treatment. Behavioural data were collected by video using a Sony Handycam HDR-CX440 camera mounted on a tripod for off-exhibit observations and a GoPro Hero 4 camera for observations in the stingray's home environment. Both cameras were positioned to capture an aerial view of the environment. From video, Scooby was observed during 15-minute focal observations once per hour (0800–1600). Observations did not occur within 15 minutes of any husbandry or veterinary procedures (e.g., feeding, medical treatment). Scooby's behaviour was recorded using a point sampling method at 10-second intervals (see ethogram in Table 1).

Data were collected throughout the course of medical treatment in three incremental phases. During the baseline phase, data were collected from 21–28 September 2019, while Scooby was undergoing initial antibacterial treatment. The next phase, labelled the meclizine phase, occurred from 2–9 October 2019, during which the drug meclizine was administered to treat the presumed behavioural symptoms of the infection. Both baseline and meclizine study periods occurred while Scooby was off-exhibit in a 1.83 m, 1,600 L circular shaped holding aquarium. On 20 November 2019, Scooby was moved back to his home environment while continuing meclizine treatment. After one month of acclimation, observations were made from 19–28 December 2019, labelled the habitat change phase. A summary timeline of events can be found in Figure 1. The baseline, meclizine and habitat change phases consisted of 25, 23 and 18 observations respectively, totalling 66 observations (16.5 hours). Videos were coded by two observers (LP and LM). Five observations were chosen at random for interrater reliability testing following initial training. Overall, the two observers had an interrater agreement of 94.15%.

Behaviour data were analysed as a percentage of visible time engaged in each swimming behaviour, or the ratio of scans for each behaviour to total visible scans multiplied by 100. Summary statistics are presented with standard error (SE). All statistics were run using RStudio 3.6.1 (R Core Team 2019). Kruskal-Wallis tests for each swimming behaviour were used to determine significance between phases. Post hoc comparisons were observed with Mann Whitney U tests using a Benjamin Hochberg correction for multiple comparisons. Significance was denoted as P≤0.05.

Consequences

Side dorsal spin swimming behaviour was first observed following bacterial infection diagnosis and in the baseline phase accounted for 69.3% (SE=4.5%) of activity. This behaviour saw a significant 40% decrease in the meclizine phase (to 42%, SE=6%) and a further 95.5% decrease in the habitat change phase (to 2%, SE=1%) from the meclizine phase (Figure 2, Table 2). Top spin behaviour was often observed as a precursory behaviour to the side dorsal swimming behaviour and similarly significantly decreased between the baseline phase (4%, SE=1%) and habitat change phase (0.8%, SE=0.3%). However, unlike the side dorsal swim behaviour, top spin was seen before the onset of infection, typically associated with foraging. Flip swim behaviour significantly changed throughout treatment (Table 2), increasing from the baseline phase (0%) to habitat change phase (0.4%, SE=0.2%), however, this behaviour

Table 2. Statistical analyses by swim behaviour across three phases of treatment. Significant differences (α<0.05) are denoted by an asterisk (*). Post hoc comparisons for significant results are included. +Behaviour was only possible in the habitat change treatment given that the subject was housed alone and without hard structure during initial treatment, therefore no statistical analyses were performed

Behaviour	Chi-squared	df	P value	Comparison	P value	Mean ranks	U value
Side Dorsal Spin	42.206	2	<0.001*	Baseline (n=25) to Meclizine (n=23)	<0.001*	31.36 / 17.04	784.000
		2		Meclizine (n=23) to Habitat Change (n=18)	<0.001*	29.00 / 10.78	667.000
		2		Baseline (n=25) to Habitat Change (n=18)	<0.001*	30.92 / 9.61	773.000
Normal	8.8867	2	0.012*	Baseline (n=25) to Meclizine (n=23)	0.012*	19.18 / 30.28	479.500
		2		Baseline (n=25) to Habitat Change (n=18)	0.049*	18.32 / 27.11	458.000
Resting	15.038	2	<0.001*	Baseline (n=25) to Meclizine (n=23)	0.043*	20.60 / 28.74	515.000
		2		Meclizine (n=23) to Habitat Change (n=18)	0.036*	16.67 / 26.53	383.500
		2		Baseline (n=25) to Habitat Change (n=18)	0.006*	16.36 / 29.83	409.000
Wall Riding	1.3145	2	0.518	---	---	---	---
Top Spin	9.3111	2	0.010*	Baseline (n=25) to Habitat Change (n=18)	0.012*	26.52 / 15.72	663.000
Flips	6.0038	2	0.049*	Baseline (n=25) to Habitat Change (n=18)	0.045*	20.00 / 24.78	500.000
Surface Riding	1.8696	2	0.393	---	---	---	---
Other	5.0729	2	0.079	---	---	---	---
Engaged+	---	---	---	---	---	---	---
Coral Riding+	---	---	---	---	---	---	---

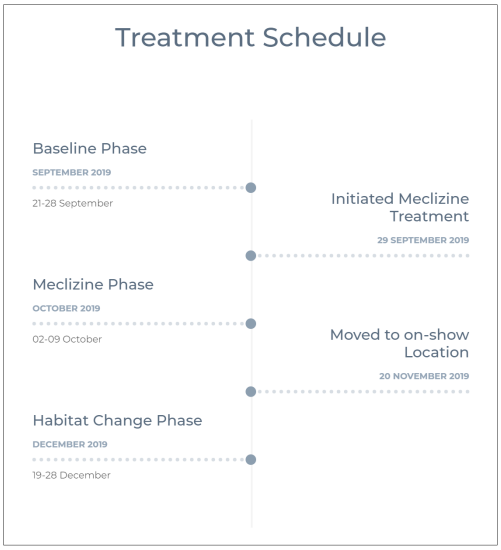

Figure 1. Timeline of treatment phases and major events

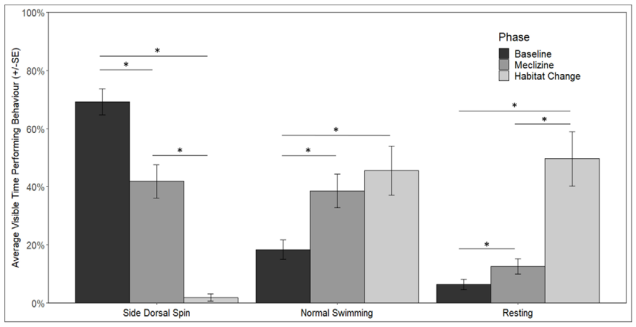

Figure 2. Percent of visible time spent performing swimming behaviours. Asterisks denote significance in post hoc testing (P<0.05)

was observed less than one percent of the time in all three phases and thus is difficult to interpret.

Normal swimming significantly increased in the two treatment phases (Figure 2, Table 2). From the baseline phase (18%, SE=3%) there was a two-fold increase in the meclizine phase (to 39%, SE=6%) and a 2.5-fold increase in the habitat change phase (to 46%, SE=8%). While an 18% increase was observed in the habitat change phase from the meclizine phase, the difference was not significant. Resting also significantly increased throughout treatment (Figure 2, Table 2). From the baseline phase (6%, SE=2%), a 96% increase was observed in the meclizine phase (to 13%, SE=3%) and a 7.7-fold increase in the habitat change phase (to 50%, SE=9%). A four-fold increase was also observed from the meclizine phase to the habitat change phase.

The decrease in abnormal swimming behaviour and increase in both normal swimming and resting suggests that meclizine could have been effective in treating abnormal locomotion from the mycobacterial inner ear infection and supports that the behaviour was related to the health condition. However, antibiotic treatment was only established a week prior to the meclizine phase and the effects of the antibiotic cannot be parsed out from the effects of the meclizine, thus it is not possible to ascertain that meclizine alone decreased the abnormal behaviours. Therefore, meclizine alone is not attributed to the normalisation of behaviour, instead as a combination of the entire medical treatment. Further supporting the link between health condition and behaviour, Scooby's side dorsal swimming was done almost exclusively (99.7%) in a clockwise direction where his left wing was elevated in the water column and his right wing touched the habitat substrate. Most of his inner ear damage occurred on the right side, which manifested as laterally biased abnormal swimming behaviour. This same pattern has been seen in both companion animals (Garosi et al. 2012) and lampreys (Deliagina 1997), with laterality occurring towards the direction of the affected side.

Antibiotics were administered during the baseline phase which may also have contributed to this change, though no decrease in abnormal swimming behaviour was observed until the initiation of meclizine treatment, suggesting that meclizine treatment seemed to be the major driver of this behaviour change. As meclizine commonly treats symptoms of motion sickness, and was successful at altering behaviour, it is most probable that the abnormal swimming behaviour was a side effect of the inner ear damage caused by the infection, and that meclizine paired with antibacterial treatment was able to reduce the effects of this damage.

Changes in wall riding and surface riding behaviour were unaffected by meclizine and were observed prior to the illness, further supporting that meclizine was effective at treating the symptoms of infection and did not just broadly affect abnormal swimming behaviour. As far as the authors know, this is the first study to track behavioural change during treatment of this species with meclizine. Meclizine's effectiveness at reducing abnormal swimming behaviour in this individual suggests it may also be effective in other contexts where an aquatic animal's ability to swim is altered due to a health condition.

The additive decrease in abnormal swimming and increase in resting following moving Scooby back to his home environment is worth highlighting. It is not entirely clear if this behavioural change was solely related to the environmental change, further time under treatment with meclizine and antibiotics, or some combination of both, however, these findings do underline the complex relationship between behaviour, health and welfare. It is important to note that drowsiness in humans is a known side-effect of meclizine (Houston and Chowdhury 2021) and may have manifested in Scooby as an increase in resting as he underwent treatment.

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It is of interest to note that Scooby spent between 73.5% (baseline phase) and 93% (meclizine phase) of his normal swimming time moving in a clockwise direction while in his treatment environment. When returning to his home environment (habitat change phase), Scooby spent almost 100% of his normal swim time roaming, with no laterality bias. This observed difference in directionality could be a result of a change in the shape and size of the environment, as well as added structural complexity including tank mates. Greater habitat complexity promotes exploratory behaviours such as roaming/meandering (Arechavala-Lopez et al. 2021; Lawrence et al. 2021), reducing the presence of a laterality bias here for Scooby. Since the inner ear disease was static throughout the study period with no improvement (based on repeat CBCT and cultures), the decrease in laterality during the habitat change phase suggests that the animal's environment played a strong role in this bias. These results suggest that increased habitat complexity may be beneficial in the treatment process when possible, as complexity promotes engagement in natural behaviours (Lawrence et al. 2021). It is important to note that any sort of animal transport, even within similar habitat and size, has the potential to lead to behavioural changes, at least temporarily (Dembiec et al. 2004; Eguizábal et al. 2022; Laws et al. 2007).

When animals in zoos and aquariums present clinical signs of illness it can be easy to focus entirely on physical health, however, these findings provide additional support to holistically consider an animal's care and welfare during treatment. Recent publications have provided an emphasis on risk analysis when making quarantine decisions while introducing newly acquired animals to a collection (McLean et al. 2021; Pye et al. 2018). Risk analysis includes four main steps: 1) hazard identification, 2) risk assessment, 3) risk management/mitigation and 4) risk communication (Pye et al. 2018). Focusing primarily on the first two steps, hazard identification and risk assessment, it is emphasised that identifying major transmissible pathogens and assessing how those pathogens would affect an established population are crucial in the determination of any quarantine process (Pye et al. 2018). Should the identified risks pose no additional threat to a population (e.g., the established population has already been exposed to a certain transmissible disease that was identified), then the decision to quarantine the animal may no longer be necessary (Pye et al. 2018) and may cause preventable stress on the individual as a result of social isolation (Cacioppo et al. 2011) and potentially reduced habitat complexity (Arechavala-Lopez et al. 2021; Lawrence et al. 2021). Relating to medical cases, allowing the animal to remain in its home environment may be beneficial to the welfare of the animal without compromising the health of the population (Pye et al. 2018) and should therefore be considered during decision making on a case-by-case basis. In this case there was a greatly reduced rate of abnormal swimming behaviour subsequent to the return to the individual's home habitat.

This case study provides evidence of the relationship between infection and abnormal swimming behaviour, and that medical treatment supplemented with meclizine can reduce this behaviour. As abnormal behaviours, such as Scooby's side dorsal spin, are often associated with suboptimal welfare, it is important to uncover their cause to increase understanding of these behaviours in aquatic animals. However, to strengthen this understanding, an increased focus on behaviour during medical treatments for aquarium animals should be established. This study highlights the value of veterinary treatment coupled with systematic behaviour observation. Together, they provide a comprehensive picture of treatment progress and patient welfare for stakeholders, ultimately allowing for more informed decisions related to an animal's care and welfare.

Conclusions

Data show a relationship between inner ear infection and abnormal swimming behaviour in an elasmobranch. Meclizine was effective at helping to reduce the abnormal behaviour associated with inner ear damage. There may be an added benefit to treating an illness in an animal's home environment, allowing identification of differences between medically onset symptoms and welfare related abnormalities.

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