Pain and Sentience in Aquatic Animals

LYNNE SNEDDON

MONDAY 7TH FEB - 11:10 AEDT

Decisions made regarding which species to protect under legislation and recommendations are intrinsically based upon whether that animal is sentient and is capable of suffering. Growing scientific evidence has been gathered to understand whether fishes and aquatic invertebrates are sentient and further whether they experience poor welfare states such as pain. The definitions of sentience and pain will be explored and research findings presented to demonstrate the capacity for sentience and the ability to detect, react to and experience the negative affective state of pain in fishes, crustaceans and cephalopds. This has important implications on the treatment and welfare of aquatic animals used by humans.
Presently, fish species are underrepresented in scientific welfare literature compared to other taxa. These gaps in knowledge are hindering efforts in husbandry, education, conservation and welfare science. We know fish species are capable of complex cognition, interacting within their own social networks, displaying choice and motivation, stress responses and pain perception. Zoos and aquariums are underused environments when it comes to research, yet are valuable for a number of reasons including ease of observations and detailed knowledge of species and individual animals by aquarium professionals.

At Sea World, we have continued our existing enrichment program and added data collection and analysis to objectively record the behaviours of eight elasmobranch species within our care. Through a collaboration between Macquarie University, Griffith University and Sea World Australia, we have collected hundreds of hours of behavioural observation data and have drawn objective conclusions regarding various enrichment techniques while also assessing baseline behaviour levels for multiple elasmobranch species and individuals. The aims of the project were to determine baseline activity levels and record behaviours of eight individual elasmobranch species to quantify effects of environmental enrichment across three multi-species exhibits. We measured activity budgets, depth- and space-use, directional swimming, environmental changes/weather events, husbandry events and interactions with other individuals. From the statistical analyses of these observations we have found significant outcomes on a number of factors.

Most notably, the process of regimented behavioural observations has allowed for more insight into individual animal behaviours with implications for both husbandry and welfare in the context of enrichment and inter-species interactions. Assessment of the impact of distinct environmental enrichment types has resulted in better understanding of species
sensory biology and relative importance of enrichment types. One clear outcome is the ability to begin considering positive and negative welfare indicators for certain aquarium species based on welfare events. The future development of these indicators and a framework to consider welfare in unique elasmobranch species is a part of our future project designs. The methods we have developed here to assess welfare and environmental enrichment through behavioural observations are both low-cost and highly effective with the potential to be applied across institutions.
Identity matters – The Importance of Taxonomy in the Husbandry of Sea Jellies

The exhibition of jellyfish in aquariums has been dated from the earliest 1900’s with the first husbandry approaches in 1932 from the Berlin Aquarium and then, in 1968, from Aquamarine Fukushima aquarium from where, most of the husbandry protocols known nowadays came from, particularly for the cosmopolitan sea jelly species *Aurelia aurita*. Sea Jellies Illuminated at Sea World-Gold Coast is the largest jellyfish exhibit in Australia and it is a unique exhibit with a research laboratory open to public, in a partnership with a local University specialised in research of sea jellies.

In the early stages of our exhibit, the main animals for display were moon jellies, which were locally acquired from other aquarium facilities but also collected from South Australia. By then, it was assumed that the Australian moon jellyfish and its husbandry protocols were linked to *A. aurita* until, for scientific purposes, the identity of our moon jellyfish had to be confirmed for publication reasons and all the husbandry protocols had to be re-assessed due to our findings.

In this presentation, we will highlight the importance of taxonomy in the husbandry of sea jellies and how advances in technology, as molecular taxonomy, can help to elucidate the identity of cryptic species as moon jellyfish, from which, several species have been described in the last decade and most of them presenting important differences in their husbandry protocols.
The aquarium fish hobby is a global industry comprised of hundreds of freshwater and marine fish species originating from farms and wild-capture fisheries world-wide. With so many species mixing and traveling through a variety of channels prior to reaching their end market, emerging and re-emerging infectious diseases are not unexpected. Advances in fish disease diagnostics; increased awareness and surveillance; and enhanced research into pathogenesis and management help inform prevention, mitigation, and treatment. Important emerging and re-emerging ornamental fish diseases to be discussed include Megalocytiviral infections; several bacterial diseases including Edwardsiellosis, Francisellosis, and *Erysipelothrix* spp. infection; as well as the recently described “fungal” disease, Tetra Disseminated Microsporidiosis. General clinical and pathological findings as well as potential management strategies will be covered.
Incorporating Lidocaine as Analgesic during Fin Clipping using Tecniplast Gene - S Rack System

TUESDAY 8TH FEB - 11:00 AEDT

Work between the Aquatics team, veterinary services and researchers lead the Francis Crick Institute to develop a novel refinement, analgesia, to the fin clipping procedure. The developed methodology provided the analgesia at the correct dosage and exposure time without significantly impacting the time required for sampling. In addition, the protocol avoided fish experiencing repeated exposure to Lidocaine while housed on a recirculating system (Gene-S).
Histology testing is a powerful tool to investigate a variety of causes of fish diseases. Histopathology allows Veterinary Pathologists to examine for pathological changes in multiple organs microscopically. We also present simple ways to preserve specimens for laboratory testing.
Tropical ornamental aquaculture is increasingly used for conservation efforts focused on coral reef species, restoration projects, and mitigating the pressures of wild collection. The College of the Florida Keys offers a Tropical Ornamental Mariculture Technician Certificate, which focuses on marine ornamental aquaculture for the following reasons: (1) the facility requirements are less than those focused on food producing organisms, (2) aquaculture technology for several marine ornamental species are well developed (e.g., clownfish, dottybacks, gobies) and excellent for training purposes, (3) the tremendous potential for undergraduate research to develop aquaculture techniques for new marine ornamental species, (4) the potential to transfer technology to the overarching marine aquaculture industry, and ultimately (5) to reduce the demand for wild caught marine ornamental species for the aquarium trade. The certificate is a 30-credit hour program comprised of the following courses: Introduction to Marine Biology, Survey of Mariculture, Introduction to Business, Mariculture Systems and Design, Aquaculture Best Management Practices, Nutrition of Mariculture Species, Disease and Parasites in Marine Aquaculture, Tropical Ornamental Mariculture, and culminates with a 300-hour internship in situ at a local facility. Students gain hands-on husbandry skills incorporating biology, chemistry, physics and maths using the campus’s indoor recirculating and outdoor flow-through systems. This program provides an option for graduating high school students who may not be interested or ready for a 4-year academic Bachelors program, but rather thrive in environments focused on applied, hands-on skills. The one-year Certificate offers the option to feed into a 2-year Associate of Science in Marine Environmental Technology degree, where students’ technician-level skills are enhanced with courses such as Marine Data Collection, Basic Research Diving, and Basic Seamanship. With an additional 2 years of
training, students can progress into the Bachelors of Science in Marine Resource Management program, which is the culminating pathway designed to provide not only a strong academic foundation in the marine sciences, but also real-world, applied skills to make them marketable in the job-force when they graduate. Students who have completed the program and/or taken Tropical Ornamental Mariculture Technician classes as electives have successfully landed jobs in the marine ornamental aquaculture, aquatic animal husbandry, and public aquarium fields. In addition, students acquire small-business marketing and management skills, as they sell fish produced during their studies to local fish distributors, pet shops, and community members. We view this program as a model to promote successful completion via progressive end points while reducing the tendency for students to get overwhelmed during career training.
CAROL LEE

Optimising Laboratory Zebrafish Health and Well-being for Improving Scientific Research

WEDNESDAY 9TH FEB - 11:00 AEDT

Globally, millions of zebrafish (Danio rerio) are used in wide-ranging biological and medical research spanning understanding genetic variation, unpicking mechanisms of diseases, studies on infection, inflammation and cancer, developing and testing new drugs, and investigating epigenetics, neurobehaviour and ecotoxicology. Considering the growing use of the zebrafish as a vertebrate model for addressing such a diverse range of scientific questions, optimising their laboratory conditions is of major importance for both welfare and improving scientific research. However, most guidelines for the care and breeding of zebrafish for research are concerned primarily with maximising production and minimising costs. These guidelines pay little attention to the effects on welfare of the environments in which the fish are maintained, or the implications of these maintenance conditions for scientific research. Also very few studies consider how the differences in environment between wild and captive fish may affect their physiological and wider biological responses. This talk begins with a description of attributes that make zebrafish popular for scientific research. It will then illustrate the environment, diet and behaviour of wild zebrafish in their native environment of the Indian subcontinent and compare and contrast these conditions with those experienced by many laboratory zebrafish. The implications for differences in environment between wild and captive fish for their health and well-being will then be discussed. The presentation will describe studies we have undertaken to measure the effects of laboratory housing conditions on the welfare of zebrafish. In these analyses the advantages and disadvantages of different indicators of fish welfare, including survivorship, growth, reproductive performance, levels of the stress hormone, cortisol, and alterations in normal physiology and behaviour, will be outlined, and the challenges of interpreting findings with regard to welfare discussed. In the final analysis we identify the fundamental lack of knowledge of how zebrafish interact with many biotic and abiotic features in their
natural environment, illustrate how we can optimise zebrafish health and well-being in the laboratory, and, in turn, how this will improve the quality of scientific data produced. The talk concludes with an evidenced argument that better welfare leads to better science.
JOLENE HANNA & JEN HAZERES

Bowmouth Guitarfish – An Evolution of Species Management from Local to International Applications within the Aquarium and Zoo Industry

WEDNESDAY 9TH FEB - 12:00 AEDT

The species *Rhina ancylostoma* has been an engaging ambassador in public aquaria for over a decade. The evolution of its husbandry management and subsequent AZA SAFE Candidate Program are evidence of the success that can be attained through professional care. IUCN states that adequate research of this species in their natural habitat has yet to be documented and currently lists the bowmouth as Critically Endangered. These two items together provide strong stimulus for our future collaboration in research and conservation. Newport Aquarium has been graced with the opportunity to work with *R. ancylostoma* from acquisition through disposition of aquarium bred specimens. Advancing our knowledge of successful breeding under professional care will prove to be vital to sustaining this species in the future.
Broodstock condition, inclusive of physiological, nutritional and stress aspects, is one of the key issues for the success of any aquatic organism hatchery. High quality larvae and, therefore, optimal growth and high survival is dependent on the broodstock nutrition and their health. In general, there are no standard feeding protocols for marine and freshwater fish broodstock and the nutrition and feeding protocols can vary significantly between hatcheries and species. The use of fresh and frozen seafood as a partial or complete diet for broodstock is still the standard with many aquatic species. While there are commercial broodstock diets that can be used solely, aquatic organisms such as Artemia, polychaetes, sardines, squid, mussels are considered to be essential for many aquatic broodstock. Natural additives such as herbal and yeast extracts found to support the hormonal cycle and leads to better gonadal development and higher fecundity. This is especially true for out-of-season and multi-spawning broodstock groups. Moreover, these compounds may reduce stress and act as immune system and digestive system boosters. There is no ‘one-size fits all’ formulation, hence, the need for ‘tailor-made’ broodstock nutrition to specific species and conditions. The presentation will review the current developments in aquatic broodstock diets and nutrition.
TIM MILLER MORGAN

Protecting Your Fish From a Dangerous World – The Basic Principles of Biosecurity

WEDNESDAY 9TH FEB - 13:10 AEDT

High quality health management within the aquarium fish industries is based upon the concept of prevention. Prevention of disease is always cheaper than dealing with a disease outbreak once it has occurred. Disease prevention is also a key factor when developing a sustainable aquarium fish industry.

Biosecurity, is the term used to describe all the policies and procedures utilized within a facility to prevent the introduction of disease to the facility and to prevent the spread of disease within a facility. A high quality biosecurity program typically has four key elements:

Facility design, policies and procedures that focus on the exclusion of pathogens.
Facility design, policies and procedures that focus on the containment of pathogens
Excellent record keeping and an active quality Control/Quality assurance program
Ongoing and regular staff training

Successful implementation of these four principles will ensure that a facility has a biosecurity program that optimizes the potential for mitigating ongoing and emerging disease risks.
CARRIE BARTON

Cutting Through the Static: An Assessment of Spawning Methodology and Facility Design in Relation to Welfare and Breeding Performance

THURSDAY 10TH FEB - 11:00 AEDT

In recent years, major advancements have been achieved with regards to facility design and equipment availability for post procedure on flow housing options, but a majority of facilities still perform static spawning and facilities are designed around the need for bench and shelf space to accomplish this. This will undoubtedly become a focus area for improving animal welfare in our field.
LAUREN PANDOLFO

A 30 Day Cleanse for Your Fish Systems?

THURSDAY 10TH FEB - 11:50 AEDT

This talk will walk through a zebrafish life support system decontamination utilizing hydrogen peroxide and system restart with rapid biofilm re-establishment without the use of sentinel fish.
In this presentation, we will examine the inter-relationship of the Host, Pathogen and Environment and the development of disease. The concept of the three Circles of Disease was developed many years ago but is still very applicable today.

I have six steps that I use in the decision-making process to arrive at a diagnosis. Incorporating a holistic approach is essential to avoid a misdiagnosis. With a correct diagnosis, the appropriate husbandry changes and treatments (if required) can be commenced.
PAULA CARLSON

Syngnathids in Public Aquariums - Working together to turn challenges into opportunities

THURSDAY 10TH FEB - 13:10 AEDT

Seahorses and their relatives in aquarium collections serve as ambassadors of their fragile marine ecosystems. Successful husbandry and creative, educational exhibits allow zoological facilities to inspire visitors and increase awareness about the need to conserve these species and help to protect their natural habitats. Over the years important advances have occurred in the husbandry of Seahorses, Pipefish and Seadragons within Public Aquariums. Innovative research, detailed observations and professional collaborations have been the keys to success in maintaining, breeding, and rearing these unique animals. The publication of husbandry manuals, participation in symposia and other cooperative efforts have furthered our collective knowledge about the successes we have achieved and the challenges we still face in caring for Syngnathid fishes. Managed cooperative breeding programs, quality veterinary care and commitment to excellent welfare will ensure continued success into the future.
LYNNE SNEDDON

Big Brother is Watching You: Automated Monitoring of Fish Welfare

FRIDAY 11TH FEB - 11:00 AEDT

Detecting pain in many animals is a challenge and even more so when it comes to fishes. Fishes are now the second most popular experimental animal group across Europe and it is estimated half of the numbers used are the model species, zebrafish. We catch trillions of fish each year for food and many species are kept as companion animals or in public aquaria. There have been a plethora of published scientific studies demonstrating the capacity for pain in fishes and, since zebrafish are subject to invasive laboratory procedures, it is crucial to have a means of detecting pain but also to reduce it through pain management protocols. This presentation will showcase an automated intelligent software system that can accurately assess pain in individual and group held zebrafish and was further used to identify drugs that provide pain-relief. The tools could be employed in the assessment of welfare in other contexts such as aquaculture or public aquaria. Further the data can be used to inform the development of pain management protocols by fish veterinarians and researchers.
Experiments in a 4m high, 86mm internal diameter Tube Fishway have revealed the impacts of the vertical transport on a neutrally-buoyant fluid sensor and live fish. Ten repeat tests using the pressure and acceleration sensor and tests with seventy individual live juvenile fish demonstrated transport with 100% reliability. No ill effects due to transit were observed over a post-test monitoring period for two species of Australian native fish (Australian bass (*Percalates novemaculeata*) and Silver perch (*Bidyanus bidyanus*)). There may have been temporary bruising of a few of the largest Silver perch tested. The distributions of distance between the inert sensor and fish relative to the moving front during the transport process were quantified. Consequently, the volumes of water required during each operational cycle to ensure reliable delivery of fish over vertical distances less than 4m were determined. The sensor measurements indicated negligible interactions with straight pipe walls but exposure to significant accelerations at sharp bends.

To quantify the hydrodynamic effects on fish during transport more systematically, novel laboratory experiments will provide missing guidance on impacts of shear stresses, acceleration and pressure on live fish during transport through closed conduits. Preliminary experiments in steady pipe flows were conducted with sensor and juvenile Australian bass and Silver perch with length of ~120mm showing no observable injury for all flow conditions of present tests. Maximum values for brief exposure to stressors were 35Pa wall shear stress, acceleration of 16g and Ratio of Pressure Change of 1.25. Further experiments with sensors and live fish are presently being undertaken to quantify the possible adverse effects of wall stresses alternative pipe transition designs on animals transported through them.
Our understanding of the ability to increase the lifespan of biological material using low temperatures goes back to ancient times. In more recent years, the ability was developed to cryopreserve cells, tissues, or embryos at very low temperatures and retrieve those samples for future use. There are many benefits to maintaining lines in a cryopreserved state including critical back-ups in case of catastrophic colony loss and more efficient colony management through improved use of space and resources. This talk will touch on the history of cryopreservation and review the benefits gained from utilizing this technique.
MITCHELL BRENNAN

Breeding White’s Seahorses for Conservation

FRIDAY 11TH FEB - 13:30 AEDT

White’s Seahorse, *Hippocampus whitei*, is a medium-sized seahorse species endemic to the east coast of Australia. Anthropogenic impacts including loss of critical habitats have led to a significant decline in *H. whitei* populations. As a result, *H. whitei* was listed as an endangered species by the IUCN red list; the second species of seahorse to be classified as endangered globally.

A collaborative conservation effort was established to restock *H. whitei* populations through the provision of artificial habitat, known as “seahorse hotels”, and a novel breed-and-release program with juvenile seahorses reared at SEA LIFE Sydney Aquarium. To date, 27 seahorse hotels have been deployed into Sydney Harbour, and a total of 234 juvenile seahorses released throughout three sites. On-going *in-situ* monitoring will look to determine the survivorship of the juvenile seahorses, through mark-recapture techniques. The project aims to restore *H. whitei* populations and de-list the species, and inform conservation strategies for syngnathids and other marine fishes.