Elasmobranch medicine: history, clinical exams and diagnostics

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What is an elasmobranch?

- Jawed fish (gnathostoma)
- Cartilaginous skeleton (chondrichthyes)
- Maxilla not fused to skull
What is an elasmobranch?

- **1,125 extant species** *(Compagno 2005)*

- **Shark orders**
  - Fusiform, lateral gill slits

- **Batoid orders** – skates, rays, guitarfish, sawfish
  - DV flattened, ventral gill slits
Elasmobranchs

• Respiratory system
  – Gills for gaseous exchange
    • 5 (or 6-7) paired gill arches
  – Use pressure changes to move water over gills
    • In through mouth or spiracles
    • Out through gill slits
  – Some ram ventilators
Elasmobranchs

- Cardiovascular system
  - 2 chambered heart
  - Hematopoiesis in spleen, epigonal and Leydig organs
  - Innate and learned immunity
Elasmobranchs

- **GI tract**
  - Oral cavity, esophagus, stomach, intestine with spiral valve, rectum, rectal gland, cloaca

- **Liver**
  - Two large lobes ± gall bladder
  - Rich in fats

- **Spleen + pancreas**
Elasmobranchs

• Urogenital system
  – Male: Paired testes, deferent duct, claspers
  – Female: Paired ovaries, only one is active, oviduct + glands, bicornate uterus, cervix

  – Mating is extremely aggressive

  – Various reproductive strategies
    • Oviparous e.g., zebra sharks, skates
    • Ovoviviparous e.g., sting rays
    • Viviparous e.g., hammerheads
Elasmobranchs

- **Musculoskeletal**
  - Cartilaginous skeleton

- **Integument**
  - Denticles
  - Fins: dorsals, pectoral, pelvic, anal, caudal
Elasmobranchs

• Sensory system
  – Vision
  – Chemoreception: nares, taste
  – Vibration: inner ear, lateral line
  – Electrorception: ampullae of Lorenzini

  – Electric organs in electric rays
Now, for the water...

- Fish are completely dependent on the water for
  - Oxygen
  - Temperature
  - Waste excretion ($H^+$, $CO_2$, $N_2$)
  - Ionic balance

- These can all be measured in the water

- Abrupt changes in these parameters are very detrimental
Water parameters

• Oxygen
  – Dissolved oxygen (DO) is provided by water flow, air pumps and photosynthesis
  – Measured using O2 meters
    • Should be > 6-15 ppm (mg/L) or > 90 %
  – Assumed to be low with
    • Low water flow
    • High temps
    • High organic load
Water parameters

• Temperature
  – Ectotherms
  – Evolved to live within specific temp ranges
  – Temp affects
    • Food intake + digestion
    • Immunity, pathogen load and drug Pk
    • Water chemistry (DO, toxicities)

– Appropriate temp provided by heat exchangers
Water parameters

- Waste excretion
  - Proteins are broken down to toxic urea, NH\textsubscript{3} and TMAOs
  - Urea
    - Stays in plasma at levels toxic to all other vertebrates
    - Commensal ureolytic bacteria (\textit{Clostridium} and \textit{Vibrio}) in tissue
    - TMAOs in blood to protect proteins
  - NH\textsubscript{3}
    - Excreted across the gills
    - Oxidized by bacteria in environment
    - These essential bacteria are cultivated in biological filters
Water parameters

Fish

Plants + algae

urea → $\text{NH}_3/\text{NH}_4^+$ → $\text{NO}_2^-$ → $\text{NO}_3^-$

$\text{NH}_3$, $\text{NO}_2^-$, and $\text{NO}_3^{2-}$ measured using commercial assays

Ideal: <0.02 ppm
Not: >0.2 ppm

$\text{Nitrosomonas} + ?$ $\text{Nitrobacter} + ?$

bacteria
fungi

$\text{O}_2$
Water parameters

![Graph showing water parameters over time with peaks for ammonia (NH3), nitrite (NO2), and nitrate (NO3).](image)
Water parameters

- Ions
  - Salinity = all salts
    - Most sp are marine (25-35 ppt or g/L); some freshwater; some euryhaline (bull sharks!)
  - pH = - log [H⁺]
  - Alkalinity = anions (buffering capacity) e.g., HCO₃⁻
  - Hardness = cations (essential minerals) e.g., Ca²⁺, Mg²⁺

- Measured using conductivity meters + commercial assays
So, onto the actual case...

1. Signalment and history
   - Animals affected and other species
   - System, water and diet information

2. Clinical exam
   - Observation of animals and environment
   - Hands-on exam where necessary

3. Further diagnostics

4. Differential list

5. Treatment plan
History

- **Animals affected**
  - Species, size, date of acquisition, medical history, clinical signs, onset and progression (acute/chronic)

- **Other species in system**
  - Fish, plants, invertebrates
  - Most recent introductions
  - Biosecurity (cleaning, disinfection, vaccination, quarantine)
History

• **System information**
  – Type (open, flow-through, closed)
  – Size
  – Time in operation
  – ‘Life support’ - these ALL require maintenance
    • Air pumps, water pumps
    • Mechanical filters e.g., protein skimmers, sand filters
    • Biological filters e.g., canister
    • Chemical and other filters e.g., ozone, UV sterilizers
    • Heaters/chillers
    • Lights
History

• Water
  – Source (tap-water, sea water)
  – Additions (conditioners, salts, meds), contaminants
  – Water turnover (volume + frequency of changes)
  – Water parameters (target + actual)
    • Temperature
    • Dissolved oxygen
    • Salinity
    • pH
    • $\text{NH}_3$, $\text{NO}_2^-$, $\text{NO}_3^-$
    • Alkalinity, hardness
    • $\text{Cu}^{2+}$, $\text{Zn}^{2+}$
History

• **Diet**
  - Food fed (historic and recent)
  - Frequency and weight
  - Storage and thawing
  - Supplements (MUST add vit B₁, C, E, iodide)

• **Dietary deficiencies are very common**

• **Food intake is essential for hydration**
Clinical exam - observation

- Observation of the animals
  - Alertness, responsiveness (‘BAR’)
  - Ventilation
  - Swimming behavior and position
  - Body condition
  - External lesions
    - Eyes, mouth, skin, fins and claspers, cloaca

- Always compare to the species normals
Clinical exam - observation

- **Observation of the environment**
  - Tank and décor
  - Tank mates
  - Life support systems
  - Water

- **Are these all suitable?**

- **Aggression, trauma and poor water quality are extremely common**
Clinical exam - restraint

• First, do you really need to get hands on?
  – Risk of trauma
  – Risk of acidosis

• Second, do you need manual or chemical restraint?
  – Manual:
    • Sufficient personnel
    • Calm species (‘tonic immobility’ in VD recumbency)
    • Non-invasive
  – Chemical:
    • Fractious species (e.g., reef sharks, pelagic rays)
    • Invasive procedure
Clinical exam - restraint

Some general principals:

- ALWAYS have all the equipment ready
- Use original source water
- Keep the gills and skin wet
- Consider temp, NH₃, DO
  - Aerate well
  - Obligate ram ventilators
  - Monitor if procedure is long
Clinical exam - manual restraint

Some hazards!

Cut the spines from stingrays

Hold sharks + sawfish behind the head (avoiding gill slits)
Clinical exam - chemical restraint

- **MS-222** bath (tricaine methane sulfonate, Finquel)
  - 40-50 ppm or mg/L for sedation
  - Up to 100 ppm for anesthesia
  - Licensed in fin fish in US + UK, not Canada
  - Wear gloves

- Eugenol (in clove oil) bath
- Propofol IV or ICe
- Ketamine ± medetomidine or xylazine IM
Clinical exam - chemical restraint

• **Stages of anesthesia**
  - Early: Ataxia, excitement
  - Surgical: Loss of responses, slow and shallow resps
  - Excessive depth: Loss of resps, cardiovascular collapse

• **Monitor**
  - Aeration (flow of water, gilling rate, gill color)
  - Heart rate (Doppler or B-mode US)
  - Temperature

• **Allow full recovery before re-introducing tank mates**
Clinical exam

- Examine eyes, nares, oral cavity, spiracles, gill slits
- Examine entire skin surface
- Examine claspers, cloaca
- Palpate coelom, M/S system
Diagnostics

• The list...
  – Morphometrics
  – Skin scrapes
  – Fecal analysis
  – Diagnostic imaging
  – Biopsies/aspirates
    • Gills, masses
  – Blood sampling
  – Necropsy
Morphometrics

- Always obtain a weight
- **Sharks**
  - Total length
  - Precaudal length
  - Clasper length
- **Batoids**
  - Disc width
  - Clasper length
Skin scrapes

- **Method**
  - Use scalpel blade
  - Add drop of tank water and a coverslip
  - Examine under direct microscopy ASAP at x40-400

- **Abnormalities include monogene trematodes** (flukes) e.g., *Dermophthirius* and *Dermophthiodes*
Fecal analysis

• **Method**
  - Obtain by cloacal wash
  - Direct, float, Dif-Quik stain, Gram stain

• **Abnormalities include**
  - Coccidia
  - Nematodes
  - Trematodes
  - Cestodes
  - White blood cells
  - Significant Gram positive flora
Diagnostic imaging

- **Ultrasonography**
  - Excellent soft tissue detail (cardiac, GIT, liver and gall bladder, pancreas, spleen, UG tract)
  - Good for dystocia, pyometra, neoplasia, goiter
Diagnostic imaging

- **Radiography**
  - Good cartilage detail and gaseous detail (e.g., necrotic pups)
  - Contrast studies provide good GIT detail
Tissue sampling

- Biopsy gill filaments

- Aspirate or biopsy any masses or fluid accumulations
  - Cytologies
  - Fluid analysis
  - Cultures
  - Histology
  - Viral isolation
  - Electron microscopy
Blood sampling

- **Anticoagulants**
  - **Sharks**
    - Dry EDTA preferable
    - Dry heparin
  - **Batoids**
    - Dry heparin preferable

Remember these blood cells are fragile and the plasma is very concentrated
Venipuncture sites – all species

- Ventral tail vein
  - MUST be midline
Venipuncture sites - sharks

- Posterior cardinal veins
  - Caudolateral to either dorsal fin
1. Blood culture
2. Blood smears
   - Wright or Dif-Quik for differential
   - Gram stain for bacteria
3. PCV, TS, i-STAT
4. Total cell counts
5. Plasma/serum separated
   - Allow 2-4hrs to clot
   - Dilute for BUN, Na, Cl

Whole blood will not survive overnight
Blood cells

• Red blood cells
  – Large (2.5x mammals) and nucleated
  – Immature forms common
  – HCT lower than mammals
  – RBC very unreliable

• Thrombocytes
  – Some species have 2 types
White blood cells

- WBC count >> mammals, up to 40,000/mm³
- Leukocyte nomenclature very confusing
  - Species differences
  - Different stages of cell maturity
White blood cells

- **Lymphocytes**
  - Most common cell type

- **Coarse eosinophilic granulocytes (CEG)**
  - Major phagocytes

- **Fine eosinophilic granulocytes (FEG)**
  - Have been called heterophils

- **Neutrophils**

- **Monocytes**

- **Basophils**
  - Common in rays only
Chemistries

• **Plasma osmolality**
  - 800-1100 mOsm/kg

• **Proteins**
  - TS by refractometer ~ 2 x TP by colorimetric assay
  - Albumin very low

• **Glucose**
  - Lower than other vertebrates

• **Tissue enzymes vary by species**

• **pH ~7**
  - Acidosis common
Euthanasia

- **Recommended (AVMA, 2006)**
  - MS-222 bath, >500 mg/L for 15 mins following loss of gilling
  - Sedation followed by pentobarbital IV or intracardiac, >100 mg/kg
  - Cranial concussion, decapitation then pithing

- **NOT approved**
  - Eugenol (clove oil)
  - Cooling/freezing
  - Asphyxiation
Necropsy

• Must be SOON

• Plan samples before starting
  – Cultures (bacterial, fungal, VI)
    • Liver + CSF
  – Squash preps
  – Stained impression smears (DQ, GS)
  – Formalin-fixed and frozen tissue
Necropsy - shark
Necropsy - batoid
Necropsy - batoid
Necropsy – ‘squash preps’

- **Sample sites:** gills, skin, fin, liver, spleen, kidney, gonads, GIT, lesions

- **Method:**
  - Take a small 1-2mm diameter section
  - Squash under a coverslip with a drop of saline
  - Examine at x40-x100
Necropsy – ‘squash preps’

• Abnormalities - infectious agents:
  – Fungi
  – Bacteria (shape and motility)
  – ‘Sporozoa’, protozoa
  – Nematodes
  – Trematodes
  – Acanthocephalans
  – Pentasomids
Necropsy – ‘squash preps’

- Abnormalities:
  - Granulomas
  - Increased melanomacrophage centers (chronic stress)
  - Increased WBC

- Also used to identify tissues and confirm tissue architecture and sex
Necropsy – impression smears

- Sample sites: liver, spleen, kidney, gall bladder, lesions

- Methods:
  - Take a small 2mm section
  - Dab on tissue
  - Make 2-3 touch impressions
  - Gram stain, Dif-Quik, modified acid-fast, PAS
Necropsy – impression smears

• Abnormalities:
  – Fungi
  – Bacteria (size, shape, stain, group)
  – ‘Sporozoa’, protozoa
  – White blood cells
  – Hyperplastic/neoplastic cells
Necropsy – other samples

- **Histopath**
  - Formalin-fixed (10% NBF), Bouin’s, Davidson’s, alcohol
  - H+E, GS, AF
  - Where? NW Zoo path, U Connecticut
- **Structural tests**
  - SEM, TEM
- **Antibody tests**
  - ELISA
  - IFA
  - Virus neutralization
- **DNA tests**
  - PCR
  - Sequencing
- **Toxicology**
Summary

• Elasmobranch health is completely dependent on the environment, water and diet that is provided

• Trauma, poor water quality and dietary deficiencies are common, and can be determined from a thorough history and observation

• Hands-on diagnostics are possible in most clinical settings

• Where further diagnostics are required, normal values from a conspecific are useful
For more info...

- **Books**
  - Fish medicine (Stoskopf)
  - Fish disease (Noga)
  - Elasmobranch husbandry manual (Smith)
  - BSAVA manual of ornamental fish (Wildgoose)

- **Journals**
  - Diseases of aquatic organisms
  - Vet clinics of north America
  - Exotic pet medicine
  - Journal of zoo and wildlife medicine
  - Journal of aquatic animal health

- **Conferences**
  - Eastern fish health workshop
  - International association of aquatic animal medicine

- **Courses**
  - Aquavet and others (Cornell, Penn, Florida, Davis, N Carolina)