Common clinical signs of fish diseases- Quick reference tool for laboratory animal research veterinarians

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Abstract

There is a wide range of clinical signs of disease in aquatic animals, unfortunately not many of them pathognomonic. Presented in this short review are examples of the clinical signs associated with selection of pathologies related to most common infectious and non-infectious noxic agents. Discussed information is aimed at veterinarians and paraveterinarians that are involved in aquatic laboratory animal care and use, with examples of clinical observation score sheets to be used in evaluation of clinical condition of aquatic animals used in institutionally approved disease experiments during which animals are expected to develop pathologies related to experimental challenges.

Keywords: Clinical disease, Aquatic animals, Laboratory animal experiments, Score sheet

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Introduction

Fishes show a range of clinical signs during disease progression. There are some clinical signs that are pathognomonic, however, majority are non-specific, and a systematic approach to work up a disease case is necessary (Noga, 2010). It is therefore very important to familiarize oneself with what is "normal" in terms of appearance and behavior for the species you are dealing with. For example, common normal behaviors across different species are that healthy fish should have a good appetite; have clean, clear, vibrant body coloration; hold their fins erect; and have bright red gills. Healthy fish should be active and not display abnormal behavior, swimming patterns, or loss of buoyancy control (Loh and Landos, 2011).

However, there are always exceptions to these rules as there are over 35,000 different fish species, out of which there are dozens that are used in research laboratories in the areas of biomedicine, biology, aquaculture or others. Even though zebrafish (*Danio rerio*) are most commonly found in the research laboratories, many other species are used as well in different venues, including invertebrates and vertebrates alike (Powell, 2000). For example, some fishes (e.g. wrasses) normally lie on the bottom or on the side; there are "upside down" catfishes that literally swim upside down; and some labs work with goldfish strains with apparent deformities (wens), all of the examples being "normal" for that particular species. Therefore, veterinarians and paraveterinarians involved in laboratory aquatic animal care, where different fish research models are used, are required to have at least basic awareness and knowledge about their patients (Kent *et al.*, 2009).

To assist the personnel in charge of daily evaluations of aquatic laboratory animals, this review offers a brief look into clinical presentations and their interpretations in terms of pathobiology and causatives. Common etiologies as they relate to the general syndromes that are discussed are presented below (Table 1). Additionally, example score sheets for evaluating health status of aquatic animals (vertebrates – fish, and invertebrates – shrimp) used in animal experiments is presented (Appendices 1 and 2) to assist responsible veterinary and paraveterinary personnel in aquatic animal disease research facilities compliance with regulatory requirements associated with animal care and use in research.

Most common clinical signs and their frequent etiologies

Skin conditions can present in different ways, such as erosions on the mouth or fins (usually due to Flavobacterium infections) or as tissue destruction along the lateral line system (e.g. hole-in-the-head disease). Hyperemia of fins or body is a common sign of stress and/or bacterial infection. Among multiple conditions, ulcerations are probably the most common skin lesion that is easily observable during routine health checks. Ulcers can originate from external lesions (e.g. invasion of

opportunistic pathogens in areas of the broken integrity of skin/integument due to various causes), or present with internal etiology (e.g. during bacteriemia due to furunculosis, or mycobacterial origin). Ulcerative changes may present as discrete lesions anywhere on the body including the fins, flank and operculum, frequently circumscribed and with advancing border with corresponding hemorrhages (Fig. 1). Color of the ulcers may vary from pale/pink to red, depending on the depth of the ulcer. The pale ulcers (Fig 1, arrowhead) are more superficial, can indicate edema or swelling due to a recent injury, and the redness may be due to hyperemia. However, a dull or dark red appearance is an evidence of deeper ulcerations usually with exposed muscle tissue (Fig 1, arrow). Such deep ulcers may be a consequence of a fungal infection (e.g. *Aphanomyces invadans*), protozoa (e.g. Tetrahymena), or simply from a predator attack or aggressive behavior of tank mates (as presented in Fig. 1). It is more common that the advanced skin ulcers are observed in relation to secondary bacterial and fungal infections, with the primary pathogens being skin flukes or fish lice. Thus, it is very important to investigate primary cause of the ulcerations (Law, 2001; Smith *et al.*, 2019) (Fig. 1).

Figure 1: Ulceration and surrounding hemorrhage on tilapia skin attributed to aggressive behavior of tank mates. Arrowhead: pale ulceration indicating superficial injury and missing scales. Arrow: deep ulcerative injury with disrupted integument with marked lesion edge and dark red color indicating exposed muscle tissue (Photo credit: Palić, D, Wojnarowski, K).

Common proliferative skin lesions in fish can present as raised and smooth (e.g. carp pox and neoplastic conditions) or be fine and granular (e.g. lymphocystis). Most frequent presentations include fine white spots (e.g. white spot disease), hazed appearance (e.g. velvet disease), or

larger spots (e.g. digenetic trematodes). Fish may also display tuft-like off-white growths that can be due to fungi (Saprolegnia) or bacteria (Flavobacteria); however, the color may vary considerably as well, especially when combined with ulcerations. Excessive slime production can be seen due to diverse issues, ranging from an indication of ectoparasitism to poor water quality issues (e.g. low pH) and other stressful factors, and in some cases is actually normal behavior related to reproductive cycle status. A change in body color patterns, whether it be pale or dark, is non-specific and, when considered abnormal, often assigned to general stress. Often, fish with skin lesions may present with flashing behavior (scraping against substrate or tank walls), have clamped fins and separate from the group. If they are infested with particularly irritant parasites (e.g. Argulus), the fish may increase frequency of jumping out of the water in an attempt to dislodge the parasites (Roberts *et al*., 2009; Smith *et al*., 2019).

As gills are in intimate contact with aquatic organism external environment, many pathogenic organisms that colonize the skin can also be found in or on the gills. Generally, individuals with clinical picture of severe epidermal injury frequently are also lethargic and present with symptoms of respiratory distress in conjunction with skin issues. Of course, there are frequent gill-specific pathologies, and it is always beneficial to clinically inspect the gills and confirm that the gill color is a healthy bright red. Some common findings during visual checkup of the gills include pale "washed out" appearance and pink/rosy coloration (indicating possible anemic status of the patient), whereas dark gills can be associated with methemoglobin formation during increased nitrite (NO_2) concentrations, the cause of the "brown blood disease" (nitrite poisoning). Excessive mucus observed on the gills indicates irritation (mechanical or chemical), frequently due to ectoparasitism, recent, or current water quality problems (Smith, 2019).

Damaged gills have a limited range of histological responses, including formations of synechiae (secondary lamellae that 'stick' to each other), epithelial hyperplasia, secondary lamellar fusion, and if given sufficient time, mucus cell hyperplasia. All of the above can and will decrease the efficacy of gill function and fish will quickly develop respiratory distress during these pathologies. As most frequent signs of respiratory distress, fish may congregate at water inlets and 'pipe' or 'gasp' at the water surface. The opercular movement rate may initially be increased as the fish try to respire through inefficient gills, but as fish become moribund, the opercular movements will become irregular, decrease or stop completely (Roberts, 2012) (Table 1).

Table 1: Summary of clinical signs and their association of most frequently observed etiologies (adapted from Loh and Landos, 2011).

General disease cause	Skin conditions	Respiratory	Distended abdomen	Buoyancy	Wasting	Sudden death	Innapetance
Bacterial							
	Flavobacteria *Aeromonas* spp. *Pseudomonas* spp. *Citrobacter* spp.	Flavobaceria	*Aeromonas* spp. *Pseudomonas* spp.	*Aeromonas* spp. *Pseudomonas* spp. Mycobacteria Nocardia	Mycobacteria		
Fungal							
	Saprolegnia Aphanomyces						
Algae/Protozoa							
	Ichthyophthirius Ichthyobodo Trichodina Chilodonella Oodinium Tetrahymena Hexamita	Ichthyophthirius Ichthyobodo Trichodina Chilodonella Oodinium Amoeba	Hexamita		Spironucleus Hexamita	Toxic algae Algal bloom crash	
Metazoa							
	Gyrodactylus Dactylogyrus Learnea Argulus Predators	Gyrodactylus Dactylogyrus Learnea Argulus					
Viral							
	Iridovirus Herpesvirus	Herpesvirus	Rhabdovirus				
Toxic/Environmental							
	Low pH	Nitrite toxicosis Hypoxia			Hypoxia Hypercapnia Overdose medications Pesticide/ Herbicide spray drift	Hypothermia Hypoxia Poor water quality	
Nutritional							
	Micro/macro nutrient deficiencies				Nutrient deficiencies	Rancid feed	
Physical/other							
	burns (heater/sun)	Hyperthermia	Hyperthermia Neoplasia Ingested large meal			Intestinal blockage Neoplasia	
Genetics							
				Upside down catfish			

Various etiologies can be associated with a clinical sign of a distended abdomen. Different proliferative (e.g. ovarian neoplasia as commonly observed in koi variety of *Cyprinus carpio*) or cystic (polycystic kidney disease common in goldfish, *Carassius auratus*) conditions, as well as

infectious agents, can lead to development of "bloat" and/or "dropsy" presentations. Occurrence of bloated abdomen can typically be observed in certain cichlid fishes, and is commonly referred to as "Malawi cichlid bloat disease". This syndrome is often caused by Hexamita (intestinal flagellated protozoa), however, enteric infections with bacteria such as Pseudomonas can also present in a similar fashion (Densmore, 2019).

"Dropsy", another common term among fish keepers, is often used when in a "pine cone" appearance is observed in addition to bloating. Such appearance comes from protrusion of the scales due to subcutaneous edema. This symptom is obvious in fish with larger scales, but is difficult to appreciate in species with fine or no scales (e.g. catfishes or angelfishes). Dropsy is often accompanied by exophthalmia ("pop-eye"). Both clinical presentations (dropsy and pop-eye) are commonly observed because of inflammation and vascular damage during primary or secondary bacterial infections, especially when such damage is present in rich vascular beds of the kidney and in choroid rete network located behind the eyes. The inflammatory and vascular insult to the kidney interferes with fluid balance, causing the 'dropsy' appearance, while inflammation behind the eye(s) causes the 'pop-eye' appearance (Densmore, 2019). It should be noted that in some breeds of goldfish exophthalmia is a selected trait, such fish being known as "telescope moors" or "telescopes" therefore not considered a pathology (Omori and Kon, 2019).

Fish in advanced disease stages can frequently present with buoyancy disorders. They may either become negatively buoyant and sink to the tank floor, or become positively buoyant, floating to the surface (Smith, 2019a). However, buoyancy problems without other obvious disease signs are commonly observed condition in highly selected goldfish breeds with rotund body shape such as the ryukin, pearl scale and orandas (affected fish frequently have twin tails) (Omori and Kon, 2019). In experimental facilities, fish are frequently fed to satiation, and this overabundant diet can further exacerbate underlying buoyancy problems with fat deposits in liver or around intestines, leading to further obstructive enteritis situations and possible development of intestinal gas. Fish should not be overfed and that they should be given adequate fiber in their diet to combat these situations. In case when association with known genetic selection or dietary issues is excluded or unlikely, next disease differential for fish with clinical buoyancy problem should be a systemic bacterial infection, also a common cause of buoyancy disorders. Less common, but still frequent causes include coccidiosis or fungal infections of the swim bladder (Smith, 2019a).

Fish that are wasting present with a concave abdomen. The differential diagnoses for poor body condition in fishes include chronic malnutrition, or infections by bacteria (e.g.

mycobacteriosis), protozoan organisms (e.g. Hexamita, Spironucleus, Cryptobia, Sporozoa, Ichthyobodo) and metazoan (e.g. Gyrodactylus, Dactylogyrus and a number of cestode species). Frequently, fish with enteropathies will have long fecal strings, that may contain bubbles and float, or they may be empty fecal strings (note: in most freshwater fishes, normal fecal casts should resemble a dark, relatively short string). If the fish are overfed, their fecal strings can take on the color of the food. One of prominent signs of enteritis in fish is a congested vent, often red/bloody in appearance (Mocho and Pereira, 2022). However, congested/bloody vent symptom can be overlooked during health assessment of the fish who are living in a pond or in a non-transparent tank, as their ventral sides are not normally exposed. Therefore, it is very important to net and observe ventral/abdominal sides of at least some fish in such enclosures, so that one can examine their vents in detail (Smith, 2019a).

Inappetence is probably the most frequent non-specific sign of distress, including illnesses, and usually occurs as the first clinical sign in fish. Among many causes, inappetence can also occur when water temperature deviates from the tolerance range of a species, and as such, inappetence observed in research facilities should prompt the personnel to check controlled environment parameters status and records for possible malfunction or water quality issues. One of immediate signs of inappetence is to notice remains of uneaten food in the tank or filter (Loh and Landos, 2011).

Lastly, it can happen that fish simply die without previously observed symptoms, and occurrence or increase in mortality numbers are the only clinical sign we can observe. Such "sudden death" situations are particularly difficult to diagnose because fish tissues rapidly degrade due to autolysis, leaving limited clues as to the cause of the death. Based on epidemiological principles, peracute or acute mortalities are most often caused by environmental problems related to water quality (Iaria *et al*., 2019). In such cases, collating a good history and water quality analyses are important, with assistance of disease score sheets as presented in the appendices (App. 1 – fish; and App. 2 decapods/shrimp) (Mocho *et al*., 2022).

Use of appropriate records is of utmost importance, including legal responsibility of the laboratory animal experiment and facility personnel (Directive 2010/63/EU, 2019). From the aquatic animal health perspective and daily health checks, qualified animal care personnel should be supported by prominently displayed species reference sheets (with "normal" characteristics for the species), stop criteria list (with most significant "abnormal" situations that require immediate action and/or euthanasia), and records kept in detail to support good laboratory practices in a research facility. A copy of a quick clinical disease symptoms, such as the above

text or similar, may be of use as a quick reference for personnel responsible for daily checks of animal health status.

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Appendices

Appendix 1: Aquatic animal disease score sheet example – finfish

SCORE SHEET for endpoint scoring of finfish

File number		
Project manager and veterinarian	NAME	Contact
Deputy-Project manager and veterinarian	NAME	Contact
Test planner	NAME	Contact

observations	score
apparition	
body weight	
Convex abdominal line, back muscles pronounced	0
Straight abdominal line	2
Easy sunken abdominal line	6
Severely sunken abdominal line and loss of back muscles	11
skin	
Pale appearance	
Up to 40% of the body surface	2
From 40% of the body surface	6
Darkening	
Up to 40% of the body surface	2
More than 40% body surface	6
Eyes	
Species specific size, shape and transparent cornea	0
Changes in color of the eyes	
Hemorrhages	2
Up to 5% of the cornea	4
More than 5% of the cornea	7
Loss of substance in the cornea	
Erosions or ulcers of the cornea	8
Size and position of the globe	

observations	score
skin	
Intact	0
Erosion/ Ulcers less than 5% of the body surface	3
Erosion between 5-20% of the body surface	7
Erosion greater than 20% of body surface	9
Skin ulcers	
Pinhead size	3
Larger than pinhead size	8
fins	
Intact	0
Erosion up to 10% of the fins	2
Erosion 10%-50% of the fins	6
gills	
Strong pink-red in full expression	0
Color of the gills	
paleness	
More than 20% of the gills discolored pale	7
Hyperemia	
Gills loss of substance	
More than 10% of the gills	11

Eyes slightly enlarged without affecting behavior	4
Eyes enlarged and prominent	7
loss of the eyeball	11

observations	score
body orifices	
Present, species-typical	0
color of body orifices	
Pale	2
Darkening	4
Hyperemia	7
swelling of the orifices	
Swelling	5
Feces	
Firm consistency, brownish	0
blood in the feces	
Up to 10% of stool bloody red	4
More than 10% of the stool bloody red	6
abdominal region	
Typical for the species, slightly convex	0
Expansion	
Abdomen permanently slightly distended	3
Abdomen permanently severely distended (and signs of inflammation)	8th
Pinecone-shaped protruding scales	11

observations	score
spine	
Natural convex topline	0
deformation of the spine	
kyphosis	11
Lordosis	11
scoliosis	11
individual behavior	
orientation	
Natural swimming movements in a position typical of fish	0
movement and position	
Stay at the air source	3
Jump	7
Permanently resting at the bottom of the tank in a natural posture	5
Prolonged resting on the ground in an unnatural posture	11
Scrub	6
loss of buoyancy (unnaturally strenuous swimming)	11
Increased buoyancy (swimming effort towards the bottom)	11
Uncontrolled wandering	11
Persistently unnatural posture (lateral, vertical, tummy up)	11
activity	
Hyperactivity (sustained frantic swimming around)	4
Hypoactivity (staying in one position stoically)	4

breathing	
Without any special findings (up to 70 gill movements/min)	0
Persistent hyperventilation	8
consciousness	
Alert, responds appropriately to stimuli	0
Slightly delayed response to stimuli	3
Response only to vigorous repeated stimuli	8
None response to stimuli	11

Social behavior	
Free swimming, forming a group when approaching or begging for food	0
Permanent isolation from the group	7
feeding	11
entire amount of food will consume	0
Reduced feed intake (10-30% of the total left)	2
Reduced feed intake (31-50% of the total left)	5
Reduced feed intake (51-100% of the total left)	7

SCORE

0 = No distress 1 - 5 = Mild distress 6 - 10 = Moderate distress Score > 10 = Severe distress

The individual points are to be added up for each fish in order to react according to the action plan.

SCORE SHEET for endpoint scoring of Fish

Action plan depending on the score the observations take place daily at 9:00 and 16:00.

Score 0: No action necessary. Note in the record that the state of health is good.

Score 1-5: Record the score and observed changes in each fish.

It is important to check again carefully whether the water parameters and environmental parameters correspond to the stored specifications (posted in the fish holding room) in order to correct them if necessary.

During the next 6 health checks, more attention should be paid to whether the changes in intensity and severity are decreasing, staying the same or increasing. If the trend remains the same or increases, the investigator or deputy investigator (both of whom are veterinarians) should be informed to discuss and initiate further steps.

Score 6-10: Record the score and observed changes in each fish.

It is important to check again carefully whether the water parameters and environmental parameters correspond to the stored specifications in order to correct them if necessary.

The head of the experiment (veterinarian) or the deputy head of the experiment should be informed, and they will decide whether the animals can be given relief and stress reduction through improved water quality (increased water change rate), increased aeration or other measures such as dimming the light.

If the mean burden persists for 4 health checks (2 days) with no tendency to improve and/or is in the upper range of the score (9.10), one of the veterinarians will decide whether to redeem the animal to avoid cumulative burden.

Score >10: The score and the observed changes of the respective fish are to be noted.

The head of the experiment or the deputy head of the study (both veterinarians) are to be informed and the affected animals are to be euthanized as gently as possible immediately according to the protocol.

It is important to check again carefully whether the water parameters and environmental parameters correspond to the specified specifications (posted in the fish holding room) and correct them if necessary.

The experimenters (including veterinarians) decide whether it is necessary to prevent provide relief and stress reduction to the remaining animals through improved water quality (increased water change rate), increased aeration or other measures such as dimming the lights.

observation log

SCORE SHEET for endpoint scoring of Fish

Date	Experimental group	Unit No: Aquarium No:	Detailed description of the observed change	Score	Abbreviation/ signature

Appendix 2: Aquatic animal disease score sheet example – decapods (shrimp)

SCORE SHEET for endpoint scoring of decapods (Shrimp - *L. vannamei*)

File number		
Project manager and veterinarian	NAME	Contact Number
Deputy-Project manager and veterinarian	NAME	Contact Number
Test planner	NAME	Contact Number

observations	score
appearance	
Overall Body Condition	
well rounded, all limbs and antennae present, normal color	0
Thin, broken 1-3 limbs/antennae	4
Patched carapace, >3 limbs are missing (STOP CRITERIA)	11
Carapace	
Pale appearance, presence of white or dark/black spots	
Up to 40% of the body surface	6
From 40% of the body surface	8
Molting	
Accelerated (>3x week) or slowed (<1x week) molting	6
More than 60% body surface is not properly molted (STOP CRITERIA)	11
More than 40% body surface	6
Eyes	
Species specific size, shape and color	0
Size and position of the globe	
Eyes slightly enlarged without affecting behavior	4
loss of the eye	7

observations	score
Carapace/visible soft tissues	
Intact	0
Erosion/ Ulcers less than 5% of the carapace surface	3
Erosion between 5-20% of the carapace surface	7
Erosion greater than 20% of carapace surface	9
carapace ulcers	
pinhead size	3
Larger than pin button size	8
Appendages (antennae, limbs)	
Intact	0
Missing/broken one antenna or <2 limbs	2
Missing/broken both antennas and/or 10% – 50% of the limbs	6
Missing/broken more than 50% limbs/antennae	9
gills	
Clear, no visible damage	0
More than 40% of the gills damaged/with abnormal coloration	8

observations	score
feces	
Firm consistency, brownish	0
Discoloration in the feces	
Up to 30% of stool white	4
More than 30% of the stool white	6

observations	score
individual behavior	
orientation	
Natural swimming movements in a position typical for shrimp	0
movement and position	
Erratic swimming at the edges and surface of the tank	7
Permanently (>5 min) resting at the bottom of the tank in a natural posture, with feeding apparatus movement	5
Prolonged resting on the ground in an unnatural posture/no feeding apparatus movement (STOP CRITERIA)	11
loss of buoyancy (unnaturally strenuous swimming)	9
Increased buoyancy (swimming effort towards the bottom)	7
Uncontrolled wandering (STOP CRITERIA)	11
Persistently unnatural posture (lateral, vertical, tummy up) (STOP CRITERIA)	11
activity	
Hyperactivity (sustained frantic swimming around)	8
Hypoactivity (staying in one position stoically)	11

awareness	
Alert, responds appropriately to stimuli	0
Slightly delayed response to stimuli	3
Response only to vigorous epeated timuli	9
No response to stimuli (STOP CRITERIA)	11

Social behavior	
Free swimming, territorial behavior characteristic for species. Constant feeding movements	0
Overly submissive or aggressive behavior	7

feeding	
entire amount of food becomes consumed	0
Reduced feed intake (10-30% of the total left)	2
Reduced feed intake (31-50% of the total left)	5
Reduced feed intake (51-100% of the total left)	7

SCORE

0 = No distress 1 - 5 = Mild distress 6 - 10 = Moderate distress Score > 10 = Severe distress

The individual points are to be added up for each shrimp in order to react according to the action plan.

SCORE SHEET for endpoint scoring of decapods (Shrimp - *L. vannamei*)

Date	Experimental group	Unit No: Aquarium No:	Detailed description of the observed change	Score	Abbreviation / signature

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