

# **The 2009 Bermuda Reef Fish Dieoff: Evidence for the Role of an Infectious Disease Process**

## **Appendix A**

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Specimen #	Liver	Spleen	Heart
BFK-09-02	widespread diffuse single cell (hepatocyte) necrosis, ceroidosis	numerous MAs, autolysis/bact along organ edge	foci of myolysis, numerous pigment containing cells in blood
BFK-09-06	liver cells rounded, vacuol, sep, pyknotic (autolysis?) WBCs in vessels destroyed		
BFK-09-14	liver is lobular, inflammation, exocrine acinar cell lysis, single cell hepatocyte necrosis/ceroid, autolysis	numerous MAs, autolysis/bact along organ edge	normal
BFK-09-15	advanced autolysis along edge, hepatocytes rounded, vacuolated, condensed nuclei, abundant MAs		digene (metacerc) infection
BFK-09-17	elevated Mas, ceroidosis, widespread single cell necrosis	few granulomas, MAs	Numerous MAs
BFK-09-19	fatty change, increased Mas, focal inflammation, mild ceroidosis	MAs, normal, bordering autolysis	Normal, some leukocyte accumulations
BFK-09-22	Increased Mas, very high ceroidosis	MAs, normal	
BFK-09-29	Hepatic parench normal, widespread pancreatic acinar cell degran and hemorrh hepatic portal veins	normal, autolytic, hypertrophied ellipsoids	normal
BFK-09-40	architect normal, Mas, early stage of autolysis, advanced autolysis around edge, mild ceroidosis	normal architect, autolytic at periph, single cell dropout, starry sky	normal, accum of pale cell in sinus venosus
BFK-09-42	hydropic change, ceroidosis, extensive rounding of cells with pyknosis, bact colonies (order Gram stain), endothel necrosis????		numerous pigmented leukocytes, normal
BFK-09-43	Huge MAs, severe ceroidosis,	Huge MAs, (occupying 80%), lymphocyte depletion?	normal
BFK-09-44	hepatocyte necrosis, fatty change, mild ceroidosis, vasc endothel destruction, signif debris in blood vessels, diffuse leukocytic infiltr		
BFK-09-45	Mas increased, mild ceroidosis, hepatocytes contain ground glass eosinophilic lamellar inclusions	normal	
BFK-09-50	periph autolysis? Exocrine acinar cell lysis? Picture is unclear	normal	normal, few metacercarial cysts
BFK-09-52	very fatty, otherwise normal	normal	normal
BFK-09-55	normal hepatic parench, exocrine pancreat acinar cell lysis, bacterial colonies assoc (order gram stain)	normal	normal, mild pericardial inflamm
BFK-09-56	very fatty, exocrine pancreatic acinar cell necrosis/autolysis??? Numerous degran EGCs (order Gram stain)	normal, numerous Mas	

**Table 5. Histopathological diagnostic notes of Bermuda reef fishes: Liver, spleen and heart.**

Specimen #	Liver	Spleen	Heart
BFK-09-59	diffuse pancreatitis with acinar cell necrosis/lysis (order Gram stain), ceroidosis, bile duct necrosis, foreign bodies	normal, autolytic at periphery? Bacterial colonies? (order Gram stain)	normal, mild pericardal inflamm
BFK-09-62	diffuse pancreatitis (order Gram stain), ceroidosis, bile duct necrosis (bile necrogenesis????)	normal Mas numerous	normal, metacercaria
BFK-09-71	diffuse pancreatitis (order Gram stain), ceroidosis	normal	normal
BFK09-76	no liver	normal	normal
BFK09-80	liver normal, Mas	normal	normal
BFK09-102	normal hepatic parenchyma, same pancreatic lysis (autolysis?)		normal
BFK09-111	normal hepatic parenchyma, same pancreatic lysis (autolysis?)	normal	normal
BFK09-113	focal hepatocyte necrosis, bile necrogenesis, acinar cell lysis	normal	normal
BFK09-114	normal parench, acinar cell rounding lysis mild	normal	normal

**Table 5 contd. Histopathological diagnostic notes of Bermuda reef fishes: Liver, spleen and heart.**

Specimen #	Gills	Stomach	Intestine
BFK-09-02	severe damage to resp and osmoreg epithel, bact colonization (pathology/autolysis?) photo G stain		
BFK-09-06	severe damage to resp and osmoreg epithel, bact colonization (pathology/autolysis?) photo G stain	architecture intact. Sluffed mucosa in lumen, leukocytes lytic	autolytic bacterial colonies
BFK-09-14	severe damage to resp and osmoreg epithel, bact colonization, degen larger nucleated bodies ( <i>Brooklynella</i> ?)	architecture intact. Sluffed mucosa in lumen	advanced mucosal loss (autolysis?)
BFK-09-15	severe damage to resp and osmoreg epithel, bact colonization (pathology/autolysis?) few ciliate parasites seen	advanced autolysis, nematode infection, mucosa sloughed	advanced autolysis
BFK-09-17	severe damage to resp and osmoreg epithel, bact colonization, degen larger nucleated bodies (ciliate?)		
BFK-09-19	severe damage to resp and osmoreg epithel, bact colonization	ulceration with bact colonization (photo)	advanced mucosal sloughing
BFK-09-22	severe ciliate ( <i>Brooklynella</i> sp. ?) infection, extensive resp & osmoreg epithelial sloughing/necrosis. Chloride cell prolif	normal	normal but some autolysis, lamina inflamed
BFK-09-29	severe damage to resp and osmoreg epithel, hyperplasia, bact colonization, degen larger nucleated bodies (ciliate?)		widespread autolytic sloughing of mucosa
BFK-09-40	severe autolysis (use as example of autolysis of fairly intact 2ndary lamellae), some areas of necrosis/hyperplasia		autolytic, mucosal sloughing
BFK-09-42	severe necrosis/inflamm to resp and osmoreg epithel, bact colonization, degen larger nucleated bodies (ciliate?)		mucosa destroyed, bacterial colonies
BFK-09-43	severe ciliate ( <i>Brooklynella</i> sp. ?) infection, extensive resp & osmoreg epithelial sloughing/necrosis. Chloride cell prolif (order gram stain)		Mucosal disruption (sloughing??)
BFK-09-44	mild ciliate infection, mild fila tip hyperplasia, chloride cell hyperplasia? Mild resp epithel lifting/damage	mucosal necrosis complete loss	
BFK-09-45	mostly normal, some mild osmoreg epithel damage	normal	normal, leukocyt inflit of lamina
BFK-09-50	severe destruction of gill tissues necrosis/autolysis? No evidence of ciliate		autolysis?
BFK-09-52	widespread separation of resp epithel, necrosis/autolysis? Inflamm, no ciliates no clear bacterial colonies		
BFK-09-55	severe necrosis of resp and osmoreg epithel, severe hyperplasia, inflammation, bact colonization, Epitheliocystis, numerous degen larger nucleated bodies (ciliate)	mucosal autolysis, lamina propria inflamm mild	
BFK-09-56	widespread resp epithel lifting, chloride cell hyperplasia and sloughing, no ciliate infection		mucosal autolysis

**Table 6. Histopathological diagnostic notes of Bermuda reef fishes: Gills, stomach, intestine.**

Specimen #	Gills	Stomach	Intestine
BFK-09-59	widespread resp epithel lifting, chloride cell hyperplasia and sloughing, inflammation, lamellar necrosis no ciliate infection		mucosal autolysis
BFK-09-62	widespread resp epithel lifting, chloride cell hyperplasia and sloughing, intense inflammation, lamellar necrosis, one ciliate evident (gram stain)		mucosal sloughing, intense inflammm in lamina, trematode infection
BFK-09-71	Severe gill hyperplasia w lamellar fusion, ciliate infection, inflammation lamellar necrosis, sloughing	mucosal autolysisleukocytic infiltr of lamina	mucosal autolysis
BFK09-76	Severe gill hyperplasia w lamellar fusion, ciliate infection, inflammation lamellar necrosis, sloughing		mucosal autolysis
BFK09-80	severe damage to resp and osmoreg epithel, ciliate present	mucosal autolysis	mucosal autolysis
BFK09-102	moderate damage to lamellae , mild ciliate infection, hyperplasia, chloride cell prolif, inflammm	mucosal autolysis/necrosis? Intense leukocytic infiltrate in lamina	normal, intense leukocytic infiltr into lamina
BFK09-111	severe damage to resp and osmoreg epithel, ciliate present	mucosal autolysis, lamina inflammm	mucosal autolysis
BFK09-113	severe damage to resp and osmoreg epithel ciliate not apparent	mucosal autolysis	mucosal autolysis
BFK09-114	severe damage to resp and osmoreg epithel ciliate not apparent	mucosal autolysis	mucosal autolysis

**Table 6 contd. Histopathological diagnostic notes of Bermuda reef fishes: Gills, stomach, intestine.**

Specimen #	Mesenteries	Head	Gonad
BFK-09-02			
BFK-09-06	autolytic, bact colonies	odd cells (inflamm, protozoa??) infiltrating ct and neural tissues (gray granular cytoplasm, pyk nucleus) Gram stain	Testis: autolysis along edge with bact
BFK-09-14		brain mild vascular changes, diffuse neuronal death (autolytic?)	Testis: normal
BFK-09-15			Ovary immature, normal
BFK-09-17			Testis: normal
BFK-09-19		single neuronal necrosis, diffuse, oral mucosa sloughed	
BFK-09-22	multiple (foreign body) granulomas, also in liver and intest lamina	widespread loss of epiderm, oral mucosa intact, normal	
BFK-09-29		widespread loss of epiderm, focal inflamm meninges	Testis normal, gravid
BFK-09-40			ovary, normal
BFK-09-42	lytic, huge bacterial colonies		testis: normal some Mas, nematode
BFK-09-43		severe retinal degeneration	
BFK-09-44	inflammation, nematode		ovary immature, normal
BFK-09-45	normal		
BFK-09-50	inflammation, exocrine acinar cell lysis?	brain: mild leuko infiltr, Mas, focus of infection (unidentified), ganglion inflam, oral mucosa eroded necrotic, bacterial colonies	male normal gravid
BFK-09-52		widespread leuko necrosis, head kidney	
BFK-09-55		head: epidermal erosion/dermal inflammation	male: normal gravid
BFK-09-56			ovary: immature, Mas numerous infiltr by EGC
BFK-09-59			testis: normal, autolysis edge
BFK-09-62	pancreatitis, bacterial growth or bile necrogenesis???? (order Gram stain)		testis: normal
BFK-09-71		brain normal	ovary: immature
BFK09-76		brain normal	
BFK09-80		brain normal, epidermal erosion	testi: normal immature
BFK09-102	exocrine pancreat acinar cell lysis	brain normal, ganglion inflam, head kidney renal tubule degeneration	testis: normal
BFK09-111			
BFK09-113	acinar cell rounding lysis	brain normal	ovary: normal
BFK09-114	acinar cell rounding lysis		Testis normal, gravid

**Table7. Histopathological diagnostic notes of Bermuda reef fishes: Mesenteries, head, gonad.**

Specimen #	Skin
BFK-09-02	widespread loss of epidermis, underlying lysis, no to mild inflammation, bacterial infiltr (autolysis?)
BFK-09-06	
BFK-09-14	
BFK-09-15	
BFK-09-17	lesion: loss of epidermis widespread, bacterial colonization of dermis & muscle, myolysis, mild inflamm (photo)
BFK-09-19	Lesion: ulceration into dermis with intense bact growth, underlying inflammation, hemorrh, mild myonecrosis. Nematode
BFK-09-22	lepidophthosis, loss of epiderm, dermal inflamm, bact colonization, mild myolysis (order Gram stain)
BFK-09-29	
BFK-09-40	
BFK-09-42	
BFK-09-43	loss of large areas of epiderm, ulceration through dermis with bact colonization, no inflamm (order gram stain)
BFK-09-44	widespread epidermal loss, <i>Brooklynella</i> infection (?), hemorrhage into scale pockets /dermis
BFK-09-45	normal, much of epiderm lost in processing
BFK-09-50	
BFK-09-52	
BFK-09-55	opercular epidermal erosion bact colonization? (order Gram stain),
BFK-09-56	epidermal loss, no inflammation
BFK-09-59	epidermal erosion/necrosis, fungal hyphae (focal, minor)
BFK-09-62	
BFK-09-71	epidermal necrosis/sloping thinning dermal inflamm hemorrh (Gram stain)
BFK09-76	
BFK09-80	epiderm missing eroded, mild dermal inflamm
BFK09-102	epiderm eroded, intense dermal inflamm (order Gram stain)
BFK09-111	epiderm eroded, intense dermal inflamm (order Gram stain)
BFK09-113	epiderm eroded, intense dermal inflamm (order Gram stain)
BFK09-114	

**Table 8. Histopathological diagnostic notes of Bermuda reef fishes: Skin.**