Biosecurity New Zealand
Tiakitanga Pūtaiao Aotearoa

Heads-up notification

Date: 10 November 2022

Summary statement for communication:

Biosecurity New Zealand (BNZ) is investigating reports of snapper and trevally with white, soft, and mushy flesh from the east coast of Northland and Auckland. The issue was brought to the attention of BNZ on the 1st of September and an investigation was opened. Sampling commenced on the week of the 12th of September and the collected samples were analysed at the MPI Animal Health Laboratory (AHL). The results of this analysis have now been finalised.

Six whole, fresh fish (5 snapper and 1 trevally) were received at AHL. These fish were subject to macroscopic and histological examination and general aquatic bacteriology was carried out. The results from the laboratory analysis has not indicated any biosecurity concern regarding exotic diseases or infectious agents. This includes bacteria, virus, protists, and metazoans.

There were, however, two key observations from the laboratory analysis: evidence of nutritional deficiencies, and evidence of iron deposits within the livers of the snapper.

Evidence of nutritional deficiencies was observed in the fish, indicated by:

- Early degenerative changes in skeletal muscle relating to muscle atrophy and a loss of polysaccharides within muscle tissue.
- Liver atrophy with an absence of polysaccharides suggests a complete loss of glycogen stores.
- Aggregates of macrophages within viscera and the brain, associated with tissue breakdown of fat and connective tissues cells and intake of polysaccharides.
- Evidence of nematode (round worm) parasitism was noted in most fish and this may have contributed to the poor body condition seen.

Additional samples are being collected to undertake confirmatory testing and to compare healthy and affected fish.

It is difficult to correlate these findings to the white flesh seen but the change in colour and consistency may be associated with changes in glycogen storage and tissue fluid in the muscle.

BNZ is working closely with Fisheries New Zealand on further sampling to help confirm findings. A second round of sampling that includes healthy fish as a comparison is underway.

BNZ is also concurrently coordinating samples for investigations to be led by Food Safety New Zealand.

Heads-up notification Date: 31 January 2023

Investigation into Snapper Milky-White Flesh Syndrome on the east coast of Northland and Auckland.

Summarised results from the second round of sampling. Affected and unaffected fish from the Hauraki Gulf.

Summary statement for communication:

Biosecurity New Zealand (BNZ) are investigating reports of snapper and trevally with white, soft, and mushy flesh from the east coast of Northland and Auckland. The investigation has been ongoing since 1st of September 2022 and the results from initial sampling efforts were released in November 2022.

Additional trevally samples have proven to be logistically difficult to obtain due to the populations current position in deeper oceans. These have therefore been placed on hold and snapper have become the focus of the investigation. Additional snapper samples have been collected and analysed at the MPI Animal Health Laboratory (AHL), and results of this second round of analysis have now been finalised.

Six whole, fresh snapper were received at AHL. Four showing characteristic macroscopic signs of the syndrome were submitted as affected fish, and two were submitted as healthy controls. All fish were subject to macroscopic and histological examination, and general aquatic bacteriology.

The results from the laboratory analysis did not indicate any biosecurity concern regarding exotic diseases or infectious agents. No clear evidence of a septic or infectious (viral, bacterial, fungal, or protozoal) cause for the muscle changes was diagnosed.

The four affected snapper show similar evidence of nutritional deficiencies to what was seen in previous samples:

- Poor condition factor.
- Muscle degeneration seen macroscopically and histologically.
- Liver atrophy and abdominal cavities lacking healthy viscera.
- Accumulation of macrophage aggregates in multiple body organs and brain, supporting the process of tissue breakdown.

Similarly, the four affected fish showed the same iron deposits within livers as was seen in previous affected fish samples.

These common symptoms were more advanced and significant in these fish when compared with the fish from the previous round of sampling.

Both control fish submitted as healthy had good condition factors. One of them showed early changes suggesting a progression towards an affected state: iron accumulation and early abdominal visceral and liver atrophy. The other showed a very well-developed gonad, large plump liver, viscera filled abdominal cavity and no iron accumulation, suggesting normal liver activity.

Inflammation of the intestinal tract with variable but frequently abundant parasitism was present in all six snapper, including the two submitted as healthy control fish. Evidence of parasite migration to the brain and further cysts within various body tissues, including the gonads, spleen, kidney, and brain was also present. These snapper show significant parasite

burden, which could contribute to, or exacerbate, poor body condition in affected fish. Intestinal intussusception was seen in three of the affected fish and is likely associated with heavy intestinal parasitism, inflammation, and a reflux effect in the intestine. This would have further affected feeding in these fish.

While a relatively heavy parasite load was observed in these fish and may have contributed to poor body condition, there is no evidence to suggest that any specific parasite is linked to the syndrome or that there are any biosecurity concerns.

Based on our examinations of snapper to date, BNZ has found no evidence to suggest an infectious cause for the white milky flesh, it appears that the syndrome is associated with starvation.

Additional sampling and analysis of healthy fish from the Bay of Plenty, an unaffected area, has been completed. Details of this analysis are presented in a third summary report.

Biosecurity New Zealand
Tiakitanga Pūtaiao Aotearoa

Heads-up notification Date: 31 January 2023

Investigation into Snapper Milky-White Flesh Syndrome on the east coast of Northland and Auckland.

Summarised results from the third round of sampling: unaffected fish from an unaffected area – Bay of Plenty.

Summary statement for communication:

Biosecurity New Zealand (BNZ) are investigating reports of snapper and trevally with white, soft, and mushy flesh from the east coast of Northland and Auckland. The investigation has been ongoing since 1st of September 2022 and results from initial sampling were released in November 2022.

A third round of sampling was completed for the purpose of collecting snapper samples from an unaffected area as healthy controls. These samples have been analysed at the MPI Animal Health Laboratory (AHL) and results of analysis have now been finalised.

Four whole, fresh snapper were received at AHL, all submitted as healthy controls from an unaffected area (Bay of Plenty). These fish were subject to macroscopic and histological examination, and general aquatic bacteriology.

All four fish showed good condition factors. They had no significant external abnormalities and appeared healthy. There was no evidence of infectious agents present (viral, bacterial, fungal, or protozoal) and histology showed healthy livers and no abnormalities of the other organs.

All four fish showed evidence of parasites. They had encysted and migrating nematode larvae and encysted helminths' present. Additionally, all fish showed significant inflammation in the intestinal tract associated with helminth parasites. This parasite burden was seen in both affected and unaffected snapper assessed in previous sampling in the Hauraki Gulf. There is no evidence to suggest that any specific parasite is linked to the syndrome or that there are any biosecurity concerns.

Biosecurity New Zealand

Tiakitanga Pūtaiao Aotearoa

Heads-up notification Date: 31 January 2023

Investigation into snapper with milky-white flesh on the east coast of Northland and Auckland.

Summarised results from samples submitted by commercial fishers – snapper fillets from the east coast of Auckland.

Summary statement for communication:

Biosecurity New Zealand (BNZ) are investigating reports of snapper and trevally with white, soft, and mushy flesh from the east coast of Northland and Auckland.

As part of ongoing sampling to investigate this issue, fillets from four snapper, obtained off the east coast of Auckland, were submitted by commercial fishers. These samples have been analysed at the MPI Animal Health Laboratory (AHL) and results of analysis have now been finalised.

Fillets from four snapper were received at AHL. Fillets from two of the fish were submitted as affected and fillets and the other two as healthy controls. These fish were subject to macroscopic and histological examination.

The fillets submitted from healthy fish did not show any abnormalities macroscopically or histologically.

The fillets submitted from affected fish show degenerative muscle changes. No significant inflammation, or infectious or parasitic agents were seen.

AHL chose not to perform bacteriology on these fillets as adequate bacteriology samples had been obtained during previous sampling. No bacteriology or other analysis to date has shown any evidence of infectious agent presence (viral, bacterial, fungal, or protozoal).

Biosecurity New Zealand
Tiakitanga Pūtaiao Aotearoa

Summary of Results Date: 17 February 2023

Investigation: Snapper Milky-White Flesh

Summary statement for communication:

Biosecurity New Zealand (BNZ) have completed an investigation into the occurrence of snapper and trevally with white, soft, and mushy flesh from the east coast of Northland and Auckland. This condition has been named Milky-White Flesh Syndrome.

The investigation was opened on 01/09/22 and results were finalised on 31/01/23.

BNZ did not find any evidence to indicate a biosecurity concern regarding exotic diseases or infectious agents.

A relatively heavy parasite load was observed in all fish analysed, including those submitted as healthy controls. There is no evidence to suggest any related biosecurity concerns regarding these parasites and a level of parasitism is expected in all wild caught fish.

Evidence of nutritional deficiencies was seen in affected fish. It is suspected that the Milky-White Flesh Syndrome seen in snapper is related to chronic malnutrition.

BNZ has closed this investigation since there was no evidence of a biosecurity risk,.

A summary of sampling, analysis and results is provided below.

Samples submitted

Three rounds of sampling and associated analysis at the Animal Health Laboratory (AHL) were performed to investigate if any exotic disease, or other infectious cause could be determined as driving the syndrome. Snapper fillets from a commercial fishers were also received. There were five submission in total:

Round 1

- **Accession W22_02965-167194.** Five formalin and five ethanol preserved snapper muscle samples, taken from affected fish in the Hauraki Gulf.
- **Accession W22_02933-168487.** Five whole affected snapper and one whole affected trevally taken from the Hauraki Gulf.

Round 2

- **Accession: W22_03660-169679.** Four affected whole snapper and two healthy whole snapper collected from the Hauraki Gulf.

Round 3

- **Accession: W22_03984-170652.** Four healthy whole snapper taken from the Bay of Plenty (an unaffected area).

Commercial submission

- **Accession W22_03990-170651.** Two affected snapper fillets and two healthy snapper fillets submitted from a commercial fisher, taken from the Hauraki Gulf.

Further trevally samples were not able to be collected because the populations were in deeper waters at the time. By the time samples were landed and transported to AHL they would have been unfit for analysis.

Summary of Results

Date: 17 February 2023

Analysis performed

AHL performed macroscopic assessment of the fish, including the calculation of condition factors, and dissection to assess the internal condition of the fish. General aquatic bacteriology was performed using swabs from the kidneys of each fish. Cultured bacteria colonies were identified via sequencing with results analysed at AHL or using biochemical characteristics. Histological examination was performed on the preserved muscle samples and from samples taken from multiple areas of the whole fish that were submitted: gills, skin, brain, kidneys, spleen, liver, heart, intestinal tract, spleen, gonad, and skeletal muscle.

Summarised results

The laboratory analysis did not find any evidence of exotic diseases or infectious agents. No clear evidence of a septic or infectious (viral, bacterial, fungal, or protozoal) cause for the muscle changes was detected.

A relatively heavy internal helminth parasite load was observed in all fish. However, there is no evidence to suggest that any specific parasite was linked to the syndrome. No evidence was found to suggest the presence of myxosporean parasites such as *Kudoa spp.*, which have been linked to white flesh in other fish species.

Overall, the laboratory analysis did not find any evidence of a biosecurity risk (associated with an exotic or emerging infectious pathogen).

Key laboratory observations

Affected fish

There were two key observations from affected fish samples.

Nutritional deficiencies were observed in the affected fish, indicated by:

- Poor condition factors calculated for affected fish. Affected fish condition factors ranged from 1.45 – 1.92 ($\bar{x}$ = 1.69). Factors > 1.95 are considered heathy and were seen in the healthy "control" fish.
- Degenerative changes in skeletal muscle relating to muscle atrophy and a loss of polysaccharides within muscle tissue, associated with tissue breakdown following a prolonged period of starvation.
- Liver atrophy with an absence of polysaccharides.
- Abdominal cavities lacking healthy viscera.
- Presence of PAS positive, ceroid/lipofuscin macrophage aggregates within viscera, multiple organs, and the brain. These aggregates are associated with the breakdown of fat and connective tissue cells, allowing the intake of polysaccharides stored within them.

Evidence of iron accumulation was seen in the livers of affected snapper and was also seen in the spleen of some affected snapper. These were observed as golden-brown pigments in the livers of the fish and confirmed by Perl's stain. The iron accumulation seen in the affected snapper is attributed to chronic starvation, tissue breakdown (releasing iron from cells) and poor haemostasis of iron in the body and tissue.

Summary of Results

Date: 17 February 2023

Normal fish

Snapper submitted as healthy controls in round two had good condition factors. One of them showed early changes suggesting a progression towards an affected state: iron accumulation and early abdominal visceral and liver atrophy. The other showed a very well-developed gonad, large plump liver, viscera filled abdominal cavity and no iron accumulation, suggesting normal liver activity.

All four snapper collected as healthy controls from the Bay of Plenty during round three showed good condition factors. They had no significant external abnormalities, appeared healthy, and histology showed healthy livers and no abnormalities of the other organs. No significant iron accumulation in the liver was seen in these fish.

Observations common to both affected and healthy fish

Variable but frequently abundant parasitism was present in all fish, including those submitted as healthy controls. Encysted and migrating nematode larvae and encysted helminths were present. All fish had at least one or more of the following signs of parasitism, evidence of parasite migration to the brain and further cysts within various body tissues, including the gonads, spleen, kidneys, heart, and brain was also present. This varied fish to fish. Anisakis spp was seen within the serosa of coelomic cavities in some fish. Inflammation in the intestinal tract (enteritis) associated with helminth parasites was commonly seen and intestinal intussusception was seen in three of the affected fish.

Parasites are common in wild caught fish, and even if not seen, are present in up to 90% of fish caught.

New Zealand
FOOD SAFETY SCIENCE
& RESEARCH CENTRE

Testing “milky” flesh fish – final report

Report Number: **23397**

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1. EXECUTIVE SUMMARY

In late 2022, fishers noticed that the flesh of snapper and trevally caught in Northland and the Hauraki Gulf had a “milky” appearance compared with its normal translucency. Concern was raised as to whether there were food safety risks from consuming such fish. Five freshly caught “milky” fleshed snapper were received and the skin and flesh assessed for the total number of bacteria present (aerobic plate count) and for *Escherichia coli*, an indicator of enteric pathogens, and *Vibrio parahaemolyticus* and *Vibrio vulnificus*, human pathogens that naturally occur in the marine environment.

Aerobic plate counts were as normally detected in the flesh of freshly caught fish, although counts on the snapper skin were lower than usual. No *E. coli* or *V. vulnificus* were detected. Concentrations of *V. parahaemolyticus* were low (maximum most probable number of 0.306/g) and no *V. parahaemolyticus* pathogenicity markers were detected. Thus, there was no evidence of increased microbiological food safety risk.

2. OBJECTIVES

In December 2022, fishers noticed that fish caught in Northland and the Hauraki Gulf had “milky” flesh (Rawling 2022) compared with its normal translucency. Concern was raised as to whether there might be food safety risks from consuming such fish. The aim of this project was to check for the presence of some foodborne pathogens and for microbiological food safety indicators in “milky” fish.

3. METHODS

3.1. Snapper

The National Institute of Water and Atmospheric Research (NIWA) supplied snapper (n=5) taken from a Danish seine trawl landed on board the vessel Da Vinci at 1600 h on 13 November 22. End-of-tow location was 175° 05’ 23” E; −36° 40’ 38” S (Firth of Thames).

Chilled whole fish were delivered to Plant & Food Research, Mt Albert Research Centre, at 0727 h on 14 November 2022. On arrival, fish temperature was measured using a digital FoodPro infrared thermometer (Comark Ltd, England) and the fish were stored in a cool room (set at 0.7°C) until sampling commenced at 0830 h.

One staff member, experienced in quality assessment of fish, recorded observations on sensory quality indicators.

3.2. Microbiological Analysis

3.2.1. Aerobic Plate Count (APC) – Skin and Tissue

Microbiological analyses were performed on 10 cm^2 of skin, and separately on 10 g of flesh (excluding the skin) excised from the anterior dorsal region of the fish. Using aseptic technique, a sterile template with a 10 cm^2 circle cut out from the centre was placed on the skin surface. Using sterile scalpel and forceps, the skin was cut, held and then separated from the underlying flesh. 10 g of the underlying flesh was then aseptically excised. Samples were diluted in salt peptone water (0.1% Difco peptone, 1% NaCl in distilled water) and homogenised in stomacher bags for 2 min (Colworth Stomacher). Serial dilutions were prepared in salt peptone water, and using the drop plate technique, 10-µL drops were plated on Plate Count Agar (Difco) with 1% added NaCl (sPCA). Plates were incubated for 72 h at 20°C and the APC was determined by counting colony-forming units (cfu) of countable dilutions. Results were expressed as $\log_{10}$ cfu/cm^2 and cfu/g for skin and flesh, respectively.

3.2.2. Escherichia coli *analyses*

The method used was that of Feng et al. (2020) with modifications. Tissue (25 g flesh with skin attached) was excised aseptically from the dorsal region of the fish body. Each tissue sample was placed in a stomacher bag and diluted with 225 mL of Phosphate Butterfield buffer (PB) (KH_2PO_4, pH 7.2) (Ajax Finechem, Thermofisher Scientific, Albany, Auckland, New Zealand). Samples were then homogenised for 2 min using a stomacher. An aliquot of 10 mL from each sample was transferred into 10-mL double-strength Lactose Broth (LB) (Difco Laboratories, Becton, Dickinson and Company, Sparks, MD, USA) in glass tubes with an inverted Durham tube inside. Furthermore, another 1-mL aliquot of the homogenate was serially diluted in PB and distributed to 10-mL single strength LB in glass tubes with inverted Durham tubes. LB tubes were incubated at 35°C for 48 h. At the end of the 48-h incubation period, all samples that were deemed to be presumptive positive (displayed turbidity and gas bubbles inside the Durham tubes) were transferred (using a 10-µL loop) into *Escherichia coli* broth with 4-methylumbelliferyl-β-D- glucuronide (EC-MUG; Neogen Corporation, Lansing, MI, USA) and incubated for 48 h at 44.5°C. Positive samples (indicated by fluorescence under UV light) were recorded and the most probable number (MPN) was calculated using the Blodgett spreadsheet (Blodgett 2010). Results were expressed as MPN/g of tissue.

3.2.3. Vibrio *analyses*

Tissue (25 g flesh with skin attached) was excised aseptically from the dorsal region of the fish. Samples were diluted with 225 mL of phosphate buffered saline (PBS, pH 7.4), and then homogenised for 2 min by Stomacher. Aliquots were then serially diluted in PBS and 1-mL from each dilution was transferred into triplicate tubes of 10-mL Alkaline Peptone Water which were incubated for 24 h at 35°C for enrichment.

DNA from the dilution series tubes was extracted by boiling at 100°C for 10 min followed by cold shock on ice for 5 min. DNA extracts were analysed using two different PCR protocols for detection of different *Vibrio* genes. The first was the qPCR protocol described by Cruz et al. (2020, 2021) following the methods of Nordstrom et al. (2007) and Campbell & Wright (2003) for *Vibrio parahaemolyticus* and *Vibrio vulnificus*, respectively. Detection of *tlh* and *vvh* (*V. parahaemolyticus* and *V. vulnificus* specific genes, respectively) was performed and, if *tlh* was present, another qPCR was carried out to detect *tdh* and/or *trh* (*V. parahaemolyticus* pathogenicity markers). The second was a conventional PCR protocol of Whistler et al. (2015) designed for the detection of the *V. parahaemolyticus* pandemic strain sequence type 36 (ST-36). The ST-36 protocol detected the *tlh* gene and four pathogenicity genes; *flp*, *prp*, *tdh* or *trh*. Any positives in the dilution series from these PCR tests were subsequently calculated as MPN/g of fish tissue, Blodgett (2010).

4. RESULTS & DISCUSSION

The average fish temperature (measured at the skin surface) at the time of delivery was 4.0°C (s.d. 1.4°C).

4.1. Microbiological Results

The results from the microbiological tests for each fish are presented in Table 1. APC counts were low and in keeping with freshly harvested chilled fish. *E. coli* and *V. vulnificus* were not detected. *V. parahaemolyticus* were present in very low numbers. The initial q-PCR was negative for both *tlh* (*V. parahaemolyticus*) and *vvh* (*V. vulnificus*) detection genes in all samples; however, the conventional ST-36 PCR was positive for *tlh* (Table 1) but not for the presence of *flp*, *prp*, *tdh* or *trh* pathogenic genes. The detection of *tlh* in the ST-36 assay but not the qPCR assay confirms the greater sensitivity of the former assay that has been observed previously (Vidovic et al. 2020).

Table 1. Microbiology results for snapper samples

Sample#	APC Skin (log_{10} cfu/cm^2)	APC Flesh (log_{10} cfu/g)	*Escherichia coli* (MPN/g)	*Vibrio parahaemolyticus* (MPN/g)	*Vibrio vulnificus* (MPN/g)
SNA_1	2.55	2.88	<0.36	0.306	<0.086
SNA_2	3.07	2.78	<0.36	0.086	<0.086
SNA_3	3.09	2.95	<0.36	0.086	<0.086
SNA_4	2.87	2.88	<0.36	<0.086[#]	<0.086
SNA_5	2.86	2.95	<0.36	0.086	<0.086
Mean (S.D.)	2.89 (0.22)	2.89 (0.07)	<0.36	0.121 (0.105)	<0.086

The detection limits were 0.36 MPN/g for *E. coli* and 0.086 MPN/g for *Vibrio spp.*
Abbreviations: APC, Aerobic Plate Count; cfu, colony forming units; MPN, Most Probable Number; S.D., standard deviation.
[#]Count (<0.086) was treated as half the detection limit (0.043) for the purpose of calculating mean and S.D.

4.2. Post-harvest Sensory Observations

All fish were in a profoundly post-rigor state 17 h post capture (Figure 1).

Figure 1. Rigor assessment immediately before microbiological sampling.

Sensory attributes observed immediately prior to and during microbiological sampling are summarised in Table 2.

Table 2. Sensory attributes of "milky" fish

Tissue	Observations
Gills	Bright red or pink-red, no mucus, characteristic fresh odour
Eyes	Convex, pupil black-grey, iris clear
Body	Some scale loss associated with net marks, no slime, skin iridescent colour, characteristic fresh odour. Body condition (anterior dorsal) skinny
Flesh	Opaque – "milky"

5. DISCUSSION

5.1. Sensory Observations

In general, the snapper appeared to be in poor condition lacking muscle mass in the anterior dorsal region. They had passed through rigor very quickly post-capture, suggesting low energy reserves. Other quality indicators showed little indication of post-harvest quality deterioration, confirming that they had been freshly caught and had not been subjected to temperature abuse.

Changes in post-harvest tissue opacity have been documented from time to time over the past 60 years. Love (1962) first suggested that muscle tissue opacity could be used as an indicator of "condition" in North Sea cod. This author found that tissue opacity increased when spawning occurred, when food was scarce and when aquaculture reared fish were deliberately starved. The post-harvest appearance of

the muscle alters, being translucent in well-fed fish, but becoming whiter and opaquer as condition deteriorated. Tomlinson et al. (1965) documented something similar when describing halibut as having “chalky” tissue where the flesh was described as dull, white and opaque rather than translucent. This occurred post-mortem when muscle tissue pH fell to 6.0 or lower.

Factors contributing to the occurrence of “chalky” halibut include warmer bottom temperatures and/or starvation. Post-harvest chalkiness can be prevented if fish are allowed to rest for 1–2 days before being butchered (IPHC 2022).

Filleters from the Kai Ika Project, a project working to fillet fish for recreational fishers, said that “milky” flesh was not seen in fat, well-conditioned fish but was more common in “skinny” fish (Dewan 2023).

Figure 2. Chalky halibut fillet on right. Reprinted from International Pacific Halibut Commission (IPHC) website 2022.

5.2. Microbiology

Using similar isolation methods, the flesh APC for the snapper in this study (2.89 $\log_{10}$ cfu/g) was very similar to that recorded for New Zealand jack mackerel (Ryder et al. 1984) and hoki (Ryder et al. 1993) for the first 3 days of storage in ice. These methods usually find about one $\log_{10}$ more bacteria on the surface of the fish compared to the flesh. However, in this case, the APC of the flesh was the same as that of the skin and about 1 $\log_{10}$ lower than found on other freshly harvested fish (Ryder et al. 1984; Scott et al. 1986; Scott et al. 1989; Ryder et al. 1993). The exact reason for the difference between the skin surface and flesh count is not clear however, the lack of surface slime and the loss of scales may have contributed to this result. The APC results do not suggest any heightened food safety risk.

E. coli is a faecal microorganism, and its presence suggests contamination of faecal origin (Food Standards Australia New Zealand 2022) and the possible presence of enteric pathogens such as *Salmonella*. *E. coli* counts of over 100/g are considered unsatisfactory for ready-to-eat food (Food Standards Australia New Zealand 2022) which was the standard these fish were assessed against and some people eat raw fish flesh. *V. vulnificus* is a human pathogen that naturally occurs in seawater and can cause serious disease (septicaemia) when contaminated seafood is consumed by individuals with compromised health (e.g., liver disease). The absence of *E. coli* and *V. vulnificus* in the snapper flesh indicate no risk from these organisms.

V. parahaemolyticus occurs naturally in seawater and consumers (healthy or otherwise) eating seafood contaminated by this microorganism can contract gastroenteritis. In these samples, *V. parahaemolyticus* was present in very low numbers (maximum 0.3 MPN/g). The most stringent international regulatory criteria allow for up to 100 MPN/g (Canadian Food Inspection Agency 2019). Not all strains of *V. parahaemolyticus* cause illness and usually only those with known pathogenicity markers including thermostable direct haemolysin (TDH) and TDH-related haemolysin (TRH) cause outbreaks. Cases of *V. parahaemolyticus* illnesses in New Zealand have normally been attributed to strains carrying both *tdh* and *trh* genes. These haemolysin genes were not detected in any of the samples. There is therefore no indication of any heightened *V. parahaemolyticus* risk from "milky" fleshed snapper.

6. CONCLUSIONS

The microbiological results did not indicate that there would be any heightened risk of illness from consuming snapper with "milky" flesh. The "milky" or "chalky" flesh phenomenon has been documented elsewhere. It affects the post-mortem opacity of the flesh in fish that are harvested when they are in poor condition.

7. ACKNOWLEDGEMENTS

We are grateful to NIWA for supplying the "milky" fleshed snapper in good condition and in a timely manner.

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Ministry for Primary Industries
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January 2023

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New Zealand Government

Biosecurity New Zealand
Ministry for Primary Industries
Manatū Ahu Matua

Growing and Protecting New Zealand

Incursion Investigation Report

Snapper and Trevally with Milky-White Flesh Syndrome

Date: 24/02/2023

Case number: 121665
Accession number: N/A
Lead Investigator sending the report: 9(2)(a)
Advisor/Manager: 9(2)(a) Aquatic Health, Biosecurity Surveillance, and Incursion Investigation
MPI Group, receiving the report: Fisheries New Zealand
Version: 1.0
Status: Final
Permissions: In Confidence

Contents

Page

1. Situation report

1.1. Host organism

Australasian snapper (*Pagrus auratus*)

White trevally (*Pseudocaranx dentex*)

1.2. Risk organism

No risk organism identified

1.3. Legal status

N/A

1.4. Summary of findings

Biosecurity New Zealand did not find any evidence to indicate a biosecurity concern regarding exotic diseases or infectious agents.

Evidence of nutritional deficiencies was seen in affected fish.

A relatively heavy parasite load was observed in all fish analysed, including those submitted as healthy controls. There is no evidence to suggest any related biosecurity concerns regarding these parasites.

It is suspected that the Milky-White Flesh Syndrome seen in snapper is related to chronic malnutrition.

1.5. Background

On 01/09/2022 Biosecurity New Zealand was notified of a widespread issue occurring on the east coast of Northland and Auckland concerning Australasian snapper (*Pagrus auratus*) and white trevally (*Pseudocaranx dentex*). The notification came from NIWA who had been contacted by both recreational and commercial fishers. NIWA also reported that there was online discussion on fishing forums regarding the issue. The issue was reported to be white, soft, and mushy flesh occurring in between 10-50% of wild caught snapper, and a smaller number of trevally (Appendix 1). The condition was later named Milky-White Flesh Syndrome (the syndrome).

Discussion about the syndrome was reviewed within various fishing forums and Facebook pages and many recreational fishers mentioned its sporadic presence over the last 10 years, indicating it was not uncommon around spawning time, and that populations always seemed to recover. Many raised concerns around the current apparent prevalence and persistence of the issue. This was consistent with what recreational fishers that had spoken to NIWA had reported.

A community fish filleting programme had seen 50% of snapper (100-200) affected while operating at a pier in the Hauraki Gulf in the week prior to the notification. Commercial fishers had also reported that around 25% of their catch for both snapper and trevally were affected.

There are two prior cases logged in the SII investigation database referring to a similar issue:

- Investigation 36002 opened on 01/02/2012 - Flaccid fleshed snapper. This report concerned white flaccid fleshed snapper in the Auckland region. The notifier indicated they had caught a few of them since the beginning of spawning season but had not seen it before. A sample was collected during this investigation. Histopathology showed some abnormality in the skeletal muscle fibres that was thought to indicate acute exertional or nutritional degeneration. The changes were considered non-infectious.
- Investigation 37203 opened on 12/10/2012 - Snapper with soft flesh. A recreational angler caught two snapper in the Hauraki Gulf near Great Barrier Island that were emaciated and had soft muscle, unsuitable for eating. The samples had been frozen and were not suitable for analysis. The investigator advised the notifier to chill any future samples they collect and get back in contact. No further samples became available, and the investigation was stood down.

The present investigation was initiated and NIWA was approached to assist with the collection of samples for analysis at the MPI Animal Health Laboratory (AHL).

Fisheries New Zealand (FNZ) and the Department of Conservation (DoC) was notified of the situation via heads-up communication in the week following the initial notification.

1.6. Initial Notification

BNZ was notified on 01/09/22 at 15:00 by 9(2)(a) NIWA. MPI were then directed to 9(2)(a) NIWA, for information.

The information 9(2)(a) provided had come from two recreational fishers 9(2)(a)

It was reported that the issue was prevalent within the Hauraki Gulf, but commercial fishers had seen the issue in their catch from "Area 1" which covers the east coast of the Auckland and Northland regions.

It was reported that 10-50% of fish were affected depending on the notifier.

1.7. Persons/Companies of interest

National Institute of Water and Atmospheric Research (NIWA)

- 9(2)(a) (Biosecurity coordinator, initial notifier)
  Phone: 9(2)(a)
  Email: 9(2)(a)
- 9(2)(a) (Marine ecologist, key contact for sampling and investigation)
  Phone: 9(2)(a)
  Email: 9(2)(a)
- 9(2)(a) (Key contact for sample collection)
  Email: 9(2)(a)
- 9(2)(a) (Key contact for sample collection)
  Email: 9(2)(a)

9(2)(a)

- 9(2)(a) (9(2)(a) lead, sample submitter)
  Phone: 9(2)(a)

Email: 9(2)(a)

Fisheries New Zealand

- 9(2)(a) Inshore Fisheries North)
  Phone: 9(2)(a)
  Email: 9(2)(a)
- 9(2)(a) Inshore Fisheries North)
  Phone: 9(2)(a)
  Email: 9(2)(a)
- 9(2)(a) Inshore Fisheries North)
  Phone: 9(2)(a)
  Email: 9(2)(a)

Food Safety New Zealand

- 9(2)(a) Food Risk Assessment)
  Phone: 9(2)(a)
  Email: 9(2)(a)

Biosecurity New Zealand

- 9(2)(a) Aquatic Health, lead investigator)
  Phone: 9(2)(a)
  Email: 9(2)(a)
- 9(2)(a) Aquatic Health, supporting investigator)
  Phone: 9(2)(a)
  Email: 9(2)(a)
- 9(2)(a) Immunology)
  Phone: 9(2)(a)
  Email: 9(2)(a)
- 9(2)(a) Aquatic Animal Diseases)
  Phone: 9(2)(a)
  Email: 9(2)(a)
- 9(2)(a) Science Products)
  Phone: 9(2)(a)
  Email: 9(2)(a)

Department of Conservation

- 9(2)(a) (Threats Manager Central)
  Email: 9(2)(a)
- 9(2)(a)
  Email: 9(2)(a)

1.8. Urgent measures

No urgent measures were performed.

2. Methods

Five submissions of fish were sent to AHL for analysis to investigate if any exotic disease, or another infectious cause was associated with the white-mushy flesh seen in snapper and trevally. NIWA were contracted to perform three rounds of sampling, consisting of four

submissions. 9(2)(a) also submitted snapper fillets from a commercial catch directly for analysis at AHL (submission five).

The first sampling round consisted of two submissions [W22_02933-168487 & W22_02965-167194] and included six whole affected fish (five snapper and one trevally) and ten preserved affected snapper flesh cubes (five preserved in formalin and five in ethanol). All samples were collected from boat ramps in the Hauraki Gulf area. This sampling commenced on 12/09/22 with samples received at AHL on 14/09/22.

Sampling for the third and fourth submission commenced on 07/11/22. Submission three [W22_03660-169679] consisted of six whole snapper collected from boat sheds in the Hauraki Gulf. Four of these were affected by the syndrome and the other two were collected as healthy control fish for comparison. Submission four [W22_03984-170652] consisted of four whole snapper collected from the Bay of Plenty. These were all apparently normal fish that were collected to provide a comparison from outside of the reported affected area.

For submissions one – four, AHL performed macroscopic assessment of the fish, including the calculation of condition factors (K= (weight (g) * 100) / (length (cm)^3)), and dissection to assess the internal condition of the fish. General aquatic bacteriology was performed using swabs from the kidneys of each fish. Cultured bacteria colonies were identified via sequencing performed by Ecogen (Auckland, New Zealand) with results analysed at AHL using BLAST, or biochemical characteristics. Histology was performed on H/E – stained glass slides, prepared using the formalin preserved flesh cubes and from samples taken from multiple areas of the whole fish: gills, skin, brain, kidneys, spleen, liver, heart, intestinal tract, spleen, gonad, and skeletal muscle.

Submission five consisted of snapper fillets submitted by 9(2)(a). Two affected snapper fillets and two healthy snapper fillets, taken from the Hauraki gulf, were received at AHL on 14/12/2022.

Only histology was performed on these samples as adequate bacteriology had already been performed during previous analysis.

Results from the first sampling round (submission one and two) were finalised on 30/09/22 (preserved cubes) and on 03/11/22 (whole fish samples). A summary report on these results was completed and shared with FNZ on 10/11/22. Results for the analysis of submission three were finalised on 15/12/22 and submissions four and five on 18/01/2023. Summaries of submission three - five were prepared and provided to FNZ on 31/01/23.

While samples of trevally were sought, only one was able to be collected during the first round of sampling. Further samples were logistically inviable for collection due to the populations position in deeper waters at the time. By the time samples were landed and transported to AHL they would be inviable for analysis. Trevally samples were placed on hold and a new investigation will be opened once they become available.

3. Results Summary

Overview

There was no evidence found in any of the analysis performed to indicate a biosecurity concern regarding exotic diseases or infectious agents. No clear evidence of a septic or infectious (viral, bacterial, fungal, or protozoal) cause for the muscle changes was diagnosed.

A relatively heavy internal parasite load was observed in these fish. However, there is no evidence to suggest that any specific parasite is linked to the syndrome, or that there are any related biosecurity concerns. No histologic or macroscopic evidence was found to suggest the presence of *Kudoa* parasites, which have been linked to mushy flesh in other fish species.

Affected fish observations

There were two key observations from affected fish samples:

Evidence of iron accumulation was seen in the livers of affected snapper and was also seen in the spleen of some affected snapper. These were observed as golden-brown pigments in the livers of the fish and confirmed by Perl's stain.

Nutritional deficiencies observed in the affected fish were indicated by:

- Poor condition factors ($\bar{x}$ = 1.69)
- Degenerative changes in skeletal muscle relating to muscle atrophy and a loss of polysaccharides within muscle tissue.
- Liver atrophy with an absence of polysaccharides.
- Abdominal cavities lacking healthy viscera.
- Presence of PAS positive, ceroid/lipofuscin macrophage aggregates within viscera, multiple organs, and the brain.

Healthy fish observations

Snapper submitted as "healthy" controls in submission three had good condition factors. One of them showed early changes suggesting a progression towards an affected state: iron accumulation and early abdominal visceral and liver atrophy. The other showed a very well-developed gonad, large plump liver, viscera filled abdominal cavity and no iron accumulation, suggesting normal liver activity.

All four snapper collected as healthy controls from the Bay of Plenty for submission four were found to have a good condition factors (μ = >1.95). They had no significant external abnormalities, appeared healthy, and histology showed healthy livers and no abnormalities of the other organs. No significant iron accumulation in the liver was seen in these fish.

Common observations

Variable but frequently abundant internal parasitism was present in all fish, including those submitted as healthy controls. Encysted and migrating nematode larvae and encysted helminths were present. All fish had at least one or more of the following signs of parasitism, evidence of parasite migration to the brain and further cysts within various body tissues, including the gonads, spleen, kidneys, heart, and brain was also present. This varied fish to fish. *Anisakis spp* was seen within the serosa of coelomic cavities in some fish. Inflammation in the intestinal tract (enteritis) associated with helminth parasites was commonly seen and intestinal intussusception was seen in three of the affected fish. No evidence of myxosporean parasites (e.g., *Kudoa* species) were detected in any fish.

4. Discussion

No biosecurity concerns were identified regarding exotic diseases or infectious agents. However, three observations of interest were noted during the laboratory analysis

- Evidence of nutritional deficiencies in affected fish.
- Iron accumulation within livers of affected fish.
- Internal helminth parasitism in both affected and healthy fish.

Nutritional deficiencies

Nutritional deficiencies in the affected fish are apparent. Externally these fish were in noticeably poor condition (skinny and sunken abdomens). Affected fish condition factors ranged from 1.45 – 1.92 ($\bar{x}$ = 1.69). Factors > 1.95 are considered heathy and were seen in the healthy "control" fish. Internally, the soft white and watery muscle tissue was obvious. Degenerative changes in skeletal muscle relating to muscle atrophy, as well as liver atrophy and a lack of healthy viscera was seen. The muscle changes are associated with tissue breakdown following a prolonged period of starvation, hypoproteinaemia (lower-than-normal levels of protein) and loss of muscle glycogen. Liver mass was significantly reduced and evidenced by glycogen depletion and hepatocyte atrophy. There were aggregates of PAS positive, ceroid/lipofuscin macrophage in multiple organs. These aggregates are associated with the breakdown of fat and connective tissue cells, allowing the intake of polysaccharides stored within them.

Iron accumulation

Iron accumulation has been previously reported in aquarium and wild fish. Dietary issues, metabolic defects, high iron levels in the environment, or exposure to heavy metals and PCB may cause the condition, but impacts are uncertain (Terio et al. 2018). Increased iron accumulation in circulation may also be driven by tissue and blood breakdown, secondary to chronic malnutrition, and an inability to excrete and manage iron due to poor nutritional intake and reduced liver and bile function. It is suggested that the iron accumulation seen in the affected snapper is attributed to chronic starvation, tissue breakdown (releasing iron from cells) and poor haemostasis of iron in the body and tissue.

Parasitism

Internal helminth parasitism was seen in all fish analysed and could contribute to or exacerbate poor body condition in affected fish. This would also explain the marked increase in eosinophilic granulocytes that were seen in kidneys and tissues- eosinophilic granulocytes are inflammatory cells frequently associated with parasitic infestations.

Significant inflammation of the intestines (enteritis) was seen and is associated with helminth parasites. This would be expected to affect digestion and nutrient absorption as well as result in a loss of body proteins and blood through the parasite activity and mucosal damage. Intestinal intussusception, a condition where part of the intestine slides into an adjacent part, was seen in three of the affected fish. This would have further prevented feeding in the affected fish. This condition is rarely reported in fish but has been described in aquaculture fish with enteritis (Liu et al. 2009, Cao et al. 2016, Hoitsy et al. 2021). In this case it is likely associated with heavy intestinal parasitism and inflammation, and a reflux effect in the intestine. Parasitism has been described as a cause of intestinal intussusception in terrestrial animals (Hoitsy et al. 2021).

It is noted that the types of helminth parasites observed are transmitted to the fish via predation of other species, and do not infect the fish directly.

Summary

It is suspected that the Milky-White Flesh Syndrome seen in snapper is related to chronic malnutrition. However, the driving factors behind the manifestation of this syndrome are likely multifactorial.

Potential contributing factors that could be explored further include:

- Poor body condition post-winter.
- Migration to spawning areas.

- Decreased food availability, due to increased competition, higher fish densities, ecosystem changes, seabed changes etc.
- Increases in parasite burdens in prey species.
- Increases in water temperature.
- Anthropogenic factors preventing feeding inshore, such as increased boat presence, diving, or water sports.
- Possible role of toxins, chemicals, and pollutants in the environment.

More research is needed to understand the drivers behind the nutritional deficiencies seen in the analysis and their relation to the syndrome present in these fish. However, these are not considered biosecurity concerns.

5. Risk assessment

5.1. Risk identification - Risk organism

No risk organism was identified during the analysis of samples submitted during this investigation. AHL did not find any evidence of exotic diseases or infectious agents. BNZ does not believe there is any biosecurity risk present that is affecting snapper populations. More sampling will be completed to confirm these results for trevally when samples become obtainable. However, findings in the one trevally that was assessed so far corroborate what was seen in snapper.

6. Recommendations

No biosecurity concerns have been identified and BNZ has now closed this investigation. BNZ will pass findings on to FNZ who are investigating potential environmental drivers behind the syndrome.

It is recommended that trevally are collected once samples are available to confirm that the condition presented within their population is of the same nature and not of biosecurity concern. This will be opened as a new investigation.

It is recommended that any sightings of affected snapper from outside of the affected area, or sightings of newly affected species are investigated to confirm they are of the same nature and not a biosecurity concern.

7. References

Cao, H., He, S., Li, Y., Yang, Y., & Ai, X. (2016). Hafnia alvei: A pathogen causing Infectious Intussusception Syndrome (IIS) in farmed channel catfish Ictalurus punctatus. The Israeli Journal of Aquaculture, 68, 1305–1311.

Hoitsy, M, Hoitsy, G, Jakab, C, Molnár, T, Gál, J, Baska, F. (2021). Intussusception caused by intestinal neoplasia in mature rainbow trout (Oncorhynchus mykiss, Walbaum 1792). Journal of Fish Diseases. 44: 893– 898. https://doi.org/10.1111/jfd.13347

Liu, J.-Y., Yang, W.-M., Li, A.-H., & He, G.-W. (2009). Preliminary study on the etiology of channel catfish intussusception disease. Acta Hydrobiologica Sinica, 32(6), 824–831. https://doi.org/10.3724/SP.J.1035.2008.00824

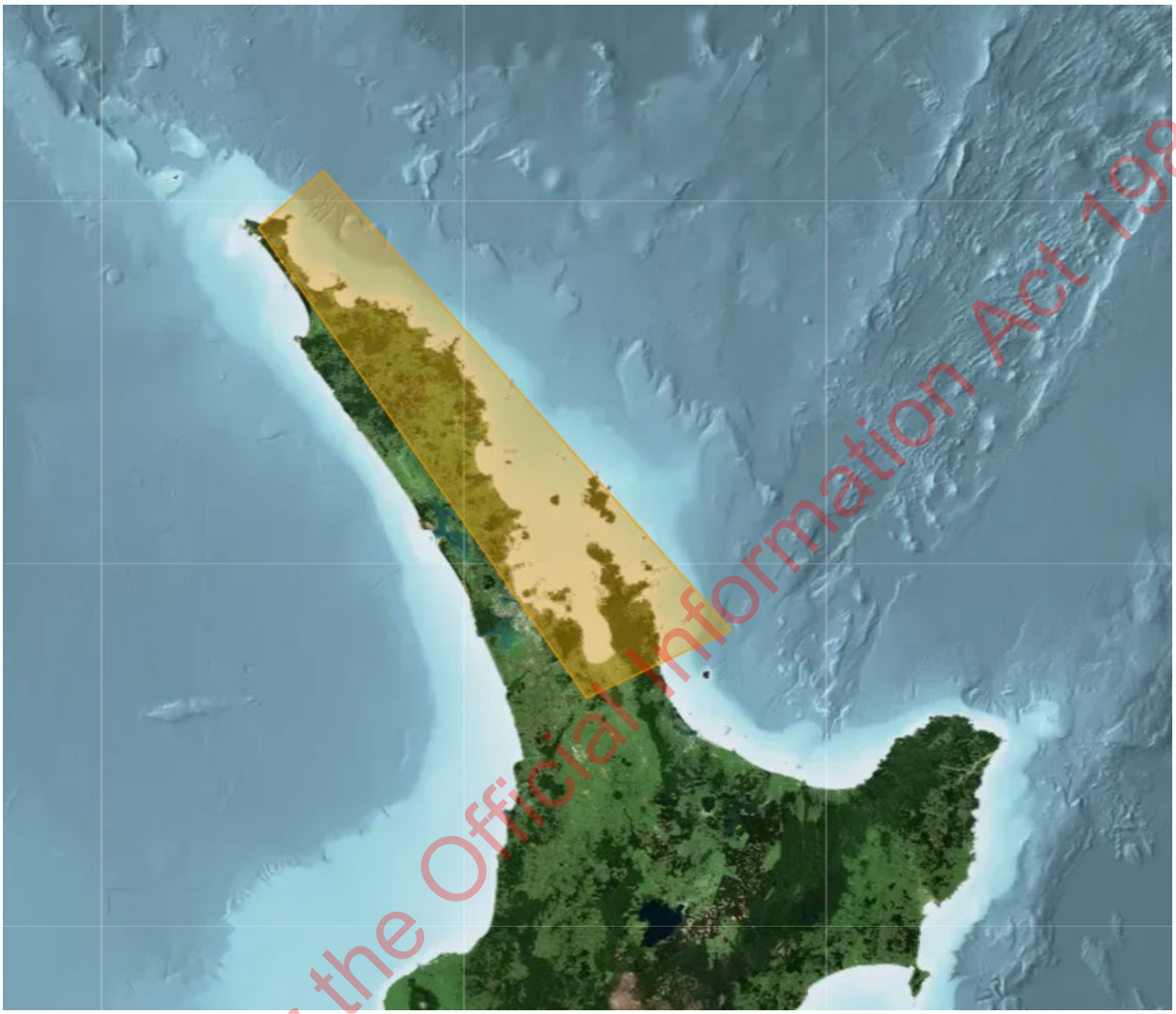
Terio K, McAloose D, St Leger J (eds)(2018). Chapter 39, Osteichthyes, Non-infectious diseases, Iron overload, p947-948. Academic Press, Elsevier.

8. Appendix 1

Image 1. showing the white flesh found in snapper.

Image 2. Image showing a comparison of affected snapper fillets (left) and healthy snapper filets (Right).

Image 3. Image showing the reported affected area.



Ministry for Primary Industries
Manatū Ahu Matua

Biosecurity New Zealand
Tiakitanga Pūtaiao Aotearoa

Note to Incursion Investigator:
Please put your address, phone and email details into this box before sending the work request to the supplier

Supplier, please quote the following numbers on your invoice and treatment certificates for MPI's reference:	
Investigation Number*	121665
Purchase Order Number*	To be advised.

Work Request

Section A – to be completed by MPI

Service to be undertaken by:

Approved operator	National Institute of Water and Atmosphere
City/Town:	Auckland
Phone:	9(2)(a)
Email:	9(2)(a)

Location of task(s) to be completed:

Contact:	9(2)(a)
Address:	9(2)(a)
Phone:	9(2)(a)
City/Town:	Auckland

Background:

Snapper tissue and fish samples to be collected to investigate reports of milky white and soft / mushy tissue within snapper caught in the Auckland to Northland region.
NIWA contracted to perform collection of samples for MPI.

Task(s) to be completed:		☑
1	Section B (Health and Safety) emailed to Incursion Investigator, prior to commencing work.	
2	5 affected tissue samples to be collected and stored in 10% formalin (5 different fish if possible, 1cm cubed per sample) – storage volume ratio of 1:10 – sample:fixative	
3	5 affected tissue samples to be collected and stored in 100% ethanol (5 different fish if possible, <1cm cubed per sample) – storage volume ratio of 1:10 – sample:fixative	
4	Formalin and ethanol samples to be couriered to MPI AHL via dangerous goods courier.	
5	3-5 Whole fish or fillets and remains (depending on condition) to be collected fresh and chilled but not frozen.	
6	Fresh fish samples to be couriered overnight to be received within 24 hours of collection at MPI AHL.	
7	Invoice emailed to MPI accounts payable.	

Please report any delay in actioning any of the above tasks as soon as possible

Please email invoice to **accountspayable@mpi.govt.nz** Include the Investigation number and purchase order number as a reference on your invoice. Include the name of your MPI contact (Incursion Investigator).

MPI Standard Terms and Conditions @ www.mpi.govt.nz/about-mpi/information-for-suppliers

Terms of Reference:

Treatment Specifications	**Item Description** (Consignment/Container/BL/AWB):
	Short Code: (Treatment type as per requirements from the Import Health Standard: Approved Biosecurity Treatment; BNZ-STD-ABTRT)

Authority:

Authorised by:	9(2)(a)	9(2)(a) Biosecurity New Zealand, Surveillance & Incursion Investigation Group
Signed:	9(2)(a)	**Email:** 9(2)(a)
Date: 09/09/2022		**Contact phone:** 9(2)(a)

Section B (Health & Safety) – Must be completed by the Supplier and emailed to the II, before undertaking the requested work

What are the health and safety risks associated with the task(s)? *Tick all that apply:*

Y Aggression	Y Biological hazards/ unknown organisms	Y Driving	Y Hazardous Substances	☐ Remote / Isolated locations	☐ Working with animals	Y Working on or around water	Y Working around moving vehicles & equipment

What is in place to manage each of the H&S risks ticked above?

Risk:	Controls in place to manage that risk:
Aggression: Encountering aggressive/drunk/intimidating members of the public Indirect intimidating, aggressive or nuisance behavior, Physical harassment etc.	If you feel at all threatened or intimidated by an individual, group or someone nearby, politely and quietly leave the ramp to avoid any potential escalation of the situation. Do not talk back to anyone who is harassing you, no matter what they say. If a situation does escalate, call 111. Stop the job.
Biological hazards/ unknown organisms:	In between each sample of fish, equipment will be sterilised and rinsed with ethanol 100% to prevent contamination and gloves replaced. As this is a suspected incursion, all gear will be thoroughly cleaned and disinfected with Ster-Bac before returning to the lab.
Driving:	• Driver training • Vehicle selection and maintenance • Check road and weather conditions before departing • Plan the journey with appropriate rest stops. • Share the driving or stay overnight when travelling long distances.
Hazardous Substances: Spills in workspace. Unprotected chemical contact personnel.	Bring portable spill kit in vehicle and to sampling site. Only personnel who are correctly equipped and trained in vicinity of sampling. First aid kit to contain barrier cream, eye wash and other necessary chemical first aid supplies.
Working around moving vehicles & equipment: Reversing vehicles and trailers	Always wear a hi-vis vest. Never walk or stand behind a boat as it may begin reversing or boat may not be properly secured to trailer. Avoid going down to the water's edge.
Working on or around water: Slippery ramp surfaces	Always wear appropriate closed toe footwear. Familiarize yourself with the worksite and identify slippery areas of ramp. Avoid going down to the water's edge.

Requirements to keep workers and others safe while carrying out the task(s):	☑
Tick the box to the right to confirm all workers carrying out the task(s) have been advised of the H&S risks, and the controls in place to manage them:	Y
Tick the box to the right to confirm that the workers carrying out the task(s) have received training to enable them to carry out the task(s) safely.	Y
Tick the box to the right to confirm that the workers know how to respond to foreseeable emergencies (e.g. earthquake, medical emergency) while they are carrying out the task(s).	Y

Who has overall responsibility in the business for ensuring the task(s) are carried out safely?

9(2)(a)

What safety checks (hazard assessments) will you carry out prior to starting the task(s)?

See Section 2 in NIWA Fieldwork Plan Snapper sampling SCJ231GOV for safety procedures prior to sampling.

Confirm the Personal Protective Equipment that will be used to safely carry out the task(s):			
Item:	**☑**	**Item:**	**☑**
Hi-Viz vest	Y	Hearing protection	
Steel-capped boots	Y	Hard hat	
Overalls/coveralls	Y	Face mask	
Safety gloves	Y	Other (specify): safety goggles	Y

Requirement to report health and safety incidents:

- **Important: You must report all H&S incidents including near misses that happen while carrying out the task(s) to MPI via email to the Incursion Investigator**
- Report a **Notifiable Event** to WorkSafe NZ via free-phone 0800 030 040 immediately (Notifiable event includes a death, a notifiable illness or injury, or a notifiable incident). For more information visit WorkSafe's website: https://worksafe.govt.nz/notifications/notifiable-event/what-is-a-notifiable-event/

Declaration:

By signing this form, I confirm that the information provided in this Section is true and correct.

Name:	9(2)(a)
Signature:	9(2)(a)
Date:	**09/09/2022**

Supplier – please send this completed Section to the Incursion Investigator prior to proceeding with this request.

Milky flesh snapper notifications that were stood down

Notify #: 306716 ; Suspect Organism: Milky Flesh Snapper, Bell Block Reef, New Plymouth
14/01/2024 Bell Block Reef, New Plymouth

9(2)(a) calling as they have Milky white flesh inside snapper which he caught in the New Plymouth area at the Bell Block Reef. 9(2)(a) has advised that they caught 2 snappers yesterday and only one of them had the milky white flesh. 9(2)(a) has photos of the fish but has the sample of the flesh which is frozen.

9(2)(a) did not reply or send the photos through to me. As no samples were available, an investigation was not opened.

Stood down as no fresh whole samples available. Emailed notifier to thank for report and advise we need fresh whole samples for any testing.

Notify #: 306663 ; Suspect Organism: Milky flesh snapper Coromandel
9/01/2024 Opito Bay, Coromandel 9(2)(a)

Caller has found a snapper which looks to have a milky flesh well fishing in the Coromandel region Opito bay area between that and great mercury they had caught 5 4 were okay as well as some other fish which were okay , they had also found a few milky flesh on a diff trip that had a milky flesh as well plus possibly a couple on a diff trip in opito which they had given a friend that were also milky

Advised on what we require for disease testing fresh, whole fish and thanked for report.

Notifier did not reply.

Notify #: 306432 ; Suspect Organism: Milky flesh snapper, Awhitu Peninsula (West Coast Auckland)
21/12/2023 Awhitu Peninsula 9(2)(a)

Milky flesh snapper caught off Awhitu

Stand down - carcass disposed of, no samples for testing

Notify #: 306332 ; Suspect Organism: Milky Flesh Snapper - Tauranga Harbour, Tauranga
11/12/2023 Tauranga Harbour

Milky flesh snapper caught in land at the Tauranga harbour last night (10/12/23) at 5pm. It was iki'd straight away, bagged and put into our motorhome fridge. Would have been no more than 2 minutes from water to fridge.

Spoke to notifier, unfortunately it has now been frozen and is not viable for analysis. I have shared with the info on catching and preserving these fish for analysis in future and asked him to share this

around his friends and family.

Notify #: 285904 ; Suspect Organism: Milky flesh snapper (Pagrus auratus) - Karioitahi Waiuku Auckland

18/09/2023 Location: Karioitahi Centroid 9(2)(a)

Notifier reports catching a Kowhai - very thin but flesh was ok. certainly not in good condition.

Main concern was the snapper - milky flesh not able to be used and meat was mushy. Caught on the west coast.

No samples available - sent educational material on how to sample if they run into the issue again, asked them to spread the word in their community.

Notify #: 266515 ; Suspect Organism: Milky flesh snappper (Pagrus auratus) - East of Whangarei harbour, Whangarei

18/09/2023 Location: due East of Whangarei harbour, in 54 metres;

Notifier has been fishing in the Whangarei area - due East of Whangarei harbour, in 54 metres; and has been catching some milky flesh snapper

9(2)(a) is part of a couple of fishing clubs in their area and would be keen for info he can pass onto those folks;

Has read info on the website;

Stood down - no samples available, passed on educational material on sampling for next time.

Notify #: 257398 ; Suspect Organism: Milky flesh snapper and trevally Whitianga
8/08/2023 Location: Whitianga

Caller 9(2)(a) advises the fish he has been catching around mercury bay have been very skinny white looking. Thinks the fish might have some sort of. 9(2)(a) caught a Trevally today and barley any flesh on it. Thinks this has been noticed by him and his friends for the last three weeks. Seems to be affecting trevally and snapper

Stand down as no samples available, advised on current info available and to provide samples in future if possible.

Notify #: 257372 ; Suspect Organism: Milky flesh syndrome - Snapper - Waitemata Harbour, Auckland
2/08/2023 Location: Waitemata Harbour

Has caught some snapper with friends on a fishing trip recently and one of the 2 he caught had the mikly flesh has not yet heard from any of his friends to see if they had any

fished at the sergeant channel and across to zeno rock is unsure which spot they came from only noticed when fileting but suspects was zeno rock as thats where majority of catch were from

has a photo of one of the milky fillets

Gave the notifier more information on the syndrome and asked him to call back when he has samples

Notify #: 257327 ; Suspect Organism: Milky Snapper - Snells Beach, Opotiki, Bay of Plenty
24/07/2023 Location: Opotiki District

Hello there here a a few picks of a poor conditioned skinny snapper that was caught white surfcasting from the beach by Opotiki,

off Snells Beach, Snell Road, Opotiki.

Its the first and only fish that I have caught here with this problem, any enquires il can be contacted by this email or the below supplied cell phone number. thanks.

Notifier has caught 1 Snapper. Filleted it and discarded the rest of the fish making it unfit for testing for Milky Flesh Syndrome. I have sent him below email containing information on the syndrome as well as sample preservation for better quality specimens.

Notify #: 257318 ; Suspect Organism: Milky fleshed snapper (Pagrus auratus) - Ruawai, fishing off the stop banks south of Dargaville in Kaipara Harbour.
24/07/2023 Location: Ruawai 9(2)(a)

A few days ago, 9(2)(a) caught a few snapper that had bloodshot eyes but no milky flesh. However today he has caught and filleted one snapper that had severely bloodshot eyes and very milky flesh, unsure if safe to consume. Only one in his catch today with the issue, has kept separate if team is wanting him to drop this off to an office to check further. Was caught in Ruawai, fishing off the stop banks south of Dargaville in Kaipara Harbour.

Notifier did not have a whole fish to submit for testing. Discussed ways to keep a look out for the syndrome and how to check pre filleting. Notifier will keep a look out and will keep this fish whole, chilled and get in touch if he finds another.

Notify #: 257239 ; Suspect Organism: Milky Fish Syndrome - Snapper - Kapiti Coast
10/07/2023 Location: Kapiti Coast

9(2)(a) has caught some snapper with milky white flesh on the kapiti coast recently.not sure if this is still reportable.

Notifier's number not in service. Have emailed them. No response :(

Notify #: 257221 ; Suspect Organism: Milky flesh snapper Bay of Islands

6/07/2023 Location: Bay of Islands 9(2)(a)

Reporting milky fleshed snapper caught by her husband 9(2)(a) ;

Caught fishing Papa Aroha; Motuarohia Island

2 our of 4 caught had milky white flesh

Stand down as not collecting samples from Northland or Auckland east coast areas.

Biosecurity New Zealand remain interested in further reports of:

- affected snapper from outside the east of Auckland and Northland areas
- in finfish other than snapper.

Sent an informative email to the notifier.

Notify #: 254788 ; Suspect Organism: Milky white flesh syndrome - Snapper - Otaki Beach
20/03/2023 Location: Otaki Beach

9(2)(a) called to report that he caught 2 Snappers 1Km off Otaki Beach, near Kapiti Island on 19/03/2023 at around 4pm. One of the Snapper had milky white flesh. He filleted the snapper and fed it to his dog.

Investigation opened - potential disease - ongoing research

Sampling not possible as the notifier fed the sample to his dog. Investigation closed.

Notify #: 253900 ; Suspect Organism: Snapper, trevally, parore and kahawai - suspected Milky white flesh syndrome - Herekino Harbour, Northland
12/01/2023 Location: Herekino Harbour

9(2)(a) wants to report issues with fish around the Herekino Harbour area. Snappers are described as Milky, fish are skinny, flesh is white and fish are Lethargic.9(2)(a) has noticed this back in 2016 and a lot of other people on facebook are talking about this also affecting other fish types also. Wants to inform MPI of this.

Potential Investigation to look into Snapper Milky white flesh syndrome within a newly emerging area. Test to ensure this is the same issue as what is seen on the East coast and also get samples of the other affected fish into AHL for diagnostics.

Notifier has received sampling kits, but no specimens have become available to date.

This notification is stood down and a new one will be opened if samples become available.

Notification #256602 : Milky Flesh Syndrome in snapper (Pagrus auratus) - Paparoa, Northland

19/06/2023 15:15

9(2)(a) caught a snapper that had mushy milky white flesh. poke to 9(2)(a), who had already gutted the fish and disposed of the guts. Advised of common external traits to watch out for that indicates MFS. Advised if he sees those traits to cut a small incision into the side of the fish to check if the flesh is milky. If so keep the whole fish, with guts intact chilled and give us a call.

9(2)(a) noted the fish had a blue dye like liquid that came out of its guts when he gutted it. This has not been reported on other MFS cases.

This was the first report of the issue in the Wairoa area.

No viable samples are available so this case is stood down.

Status: Stood down

No images available

Notification #256521 : Milky Flesh Snapper Syndrome Manukau West Auckland

06/06/2023 14:57

Location: 9(2)(a)

Used to getting milky snapper out east coast, got my limit off whenuapai a few weeks ago and all 7 were milky and inedible. But ive been fishing manukau recently and usually really fat snapper but today caught a milky flesh not skinny mid 30s snap. Wondering if this is any use to you guys to know cheers. Caught in im water off jenkins bay titirangi near high tide.

Sent an initial email asking for a few more details and whether samples are available - standing down unless I hear back.

Status: Stood down

Photos of filleted fish:

Notification #256419 : Milky Flesh Snapper - Mount Maunganui

02/06/2023 14:30

Location: 9(2)(a)

9(2)(a) ; Firth of Thames on Wednesday 31/05. About 4 out of 14 fish they caught were affected. Has questions that need answering so they can advise their club. Are people eating the affected fish? Has photos and a sample of the affected fish.

All fish caught with mushy flesh are snapper. Likely the usual mushy flesh syndrome seen many times. Known to be in BoP. Asked for pictures to check it is presenting typically. Stood down as no need for further investigation.

Status: Stood down

Photos of fish:

Notification #256399 : Milky Snapper Syndrome - Houhora Harbour, Northland

31/05/2025 18:30

Location: Houhora Harbour, Northland

Customer has been fishing recently and caught a few milky flesh snapper in Houhora Harbour a he had one himself and a friend fishing with him at same time found 3 snapper in same situation , He didn't think to take any pictures only through about it after talking to friend and realising they had both found same thing . they do have a few more from fishing the next day which have yet to be filleted will take photos if any are affected.

Stood down. Notifier does not have any photos. We have already had several reports of Milky flesh Snapper from Houhora harbour and AHL have run tests on them as well. I have asked him to report it through the 0800 number if he finds any more milky fish from other regions.

Status: Stood down

No images available

Notification #256062 : Milky flesh syndrome Snapper Trevally Tarakihi Northland

03/05/2023 16:05

Location: Kaitaia

Fisheries Officer reporting on behalf of commercial fishers in the far north who are concerned about skinny fish and milky flesh in snapper, trevally and maybe tarahiki. They believe the issue is getting worse.

Stood down as FO was reporting complaints and did not have any samples for testing. Advised to let commercial fishers know that Fisheries New Zealand is still investigating and that we are still interested in receiving samples of species other than snapper. The FO was advised to call the hotline to organise for affected fish to be sent whole and chilled to the Animal Health Laboratory if he is able to collect samples at any point.

Status: Stood down

No images available

Notification #256056 : Milky flesh snapper south Waihi beach

02/05/2023 09:45

Location: Waihi Beach

A member of the public found a snapper with milky flesh on the 13th of April. They have since buried the fish so no samples available.

The notifier has been informed that if they will find other snappers with milky flesh to call us again and we can look into organizing a fresh adn entire sample to be tested at AHL

Status: Stood down

No images available

Notification #255072: Snapper Milky Flesh Opua Bay, Bay of Islands

11/04/2023 14:30

Location: 9(2)(a)

9(2)(a) is fishing out on a boat in opua bay of islands and has caught a snapper which has a milky flesh has not taken any photos and no longer has the fish had seen an article and realised he should have reported it found it a couple of days ago

Spoke to caller and identified the issue is likely the known Milky Flesh syndrome seen in snapper that is present in the east Northland and Auckland area (see investigation 121665).

Advised notifier that the issue has been thoroughly looked into by BNZ and no biosecurity issues had been identified. Advised that FNZ has an ongoing investigation into drivers behind the issue.

Advised notifier that we are currently interested in collecting samples from outside of the east Northland and Auckland areas.

Status: Stood down

No images available

Notification #255047: Snapper Milky Flesh Syndrome - Takou Bay Northland

05/04/2023 11:45

Location: 9(2)(a)

Caller is wanting to report finding milky type residue coming out of snappers they commercially catch, has never seen it before but snappers come out ill as well.

Spoke to caller and identified the issue is likely the known Milky Flesh syndrome seen in snapper that is present in the east Northland and Auckalnd area (see investigation 121665). Have requested pictures to ensure it does not appear to be a separate issue to the one known to be present.

Advised notifier that the issue has been thoroughly looked into by BNZ and no biosecurity issues had been identified. Advised that FNZ has an ongoing investigation into drivers behind the issue.

Provided following links for further info:

https://www.mpi.govt.nz/fishing-aquaculture/recreational-fishing/information-on-popular-fish-in-nz/snapper-status-and-information/milky-white-flesh-in-snapper-and-some-other-finfish/

https://www.rnz.co.nz/news/national/486867/snapper-in-hauraki-gulf-found-with-milky-white-flesh-syndrome-scientists-investigating

Status: Stood down.

No images available

Notify #: 37203 ; Suspect Organism: snapper with soft flesh
12/10/2012

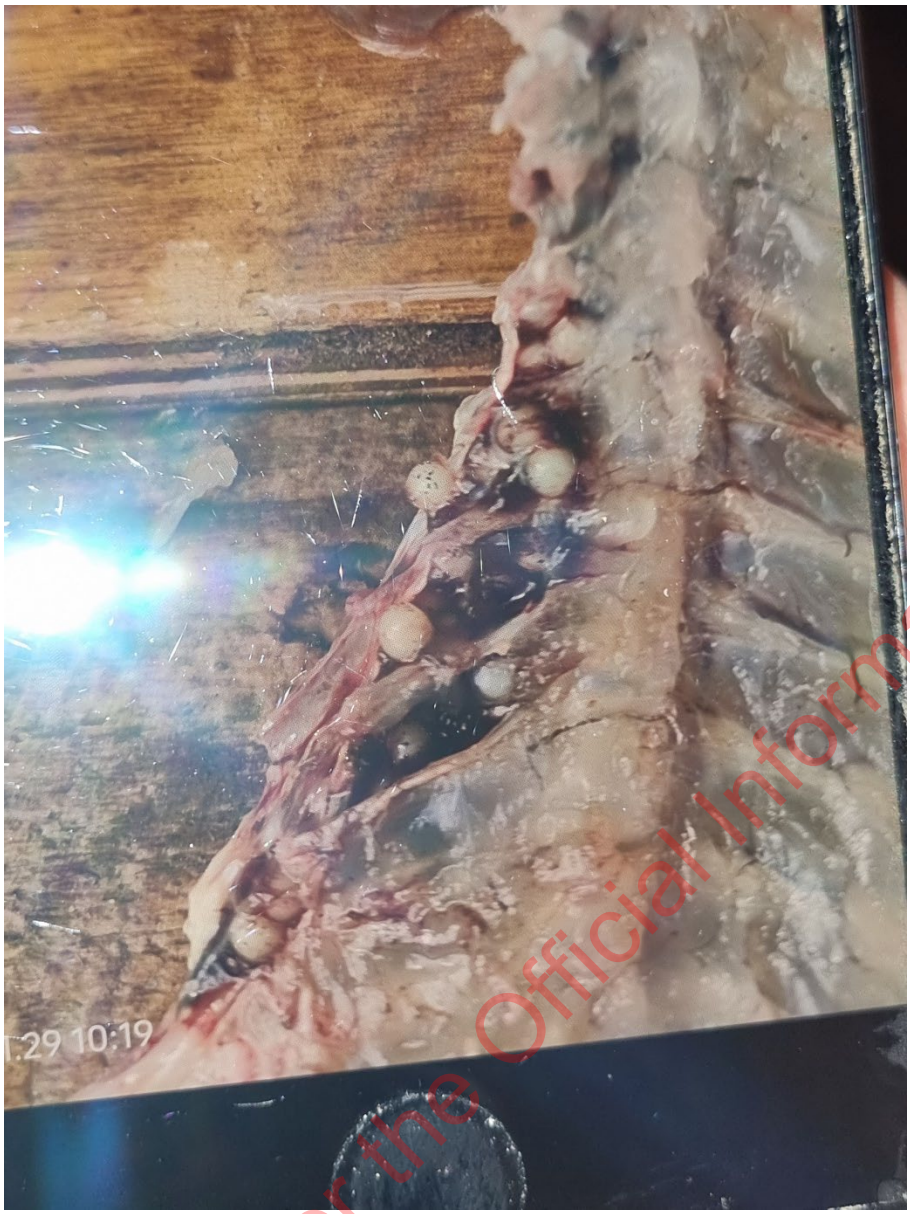
Reason for call: Recreational angler caught two snapper inHauraki Gulf near Great Barrier Island over past 3 weeks that wereemaciated and had soft muscle, unsuitable for eating. Fish were 350-450mmlong.

Stand down / investigation justification Notifier had frozen one whole fish,unsuitable for testing. Requested that in the future he refrigerate (notfreeze) the fish if he caught another displaying same signs, and to call MPI again.

All investigated reports of milky / white flesh snapper

Notify #: 307141 ; Suspect Organism: Snapper with white cysts and milky flesh - Bland Bay (Northland)
Notification Date: 01/02/24
Location: Bland Bay
Reason for call: A member of the public found a snapper with white cysts and milky flesh. Pest Management colleagues emailed us following talking with [redacted], a member of the [redacted] 9(2)(a) , who came along to their hui there and the one at Rawhiti about caulerpa. Her friend caught this snapper in the area and it had very milky flesh and cyst like growths throughout the fish
Stand down / investigation justification: Pictures to be sent to AHL to see whether a sample is needed.
Investigation summary: A member of the public contacted MPI after finding lumps in a snapper caught in Northland. They described the snapper as having very milky flesh and cyst like growths throughout the fish. Reports like this have been received several times in the past. However, to ensure they were not of biosecurity concern an investigation was opened and images were sent to the MPI Animal Health Laboratory in Wallaceville (AHL). Based on photographs the team at AHL were able to identify the suspicious lumps as Metacercarial trematode and cestode (tapeworm and fluke) cysts. These are common parasites of fish and do not represent a biosecurity risk. Similarly, white flesh in snapper does not represent a biosecurity concern. This phenomenon, named “milky flesh syndrome” has been previously investigated thoroughly by BNZ. During this previous investigation, numerous samples were assessed at AHL and there were no findings that suggested a biosecurity concern was present. Fisheries NZ has continued to monitor and work on the issue, but a specific cause is yet to be determined. As no biosecurity issues were identified, this investigation was closed.

(No lab report as no testing was done. Only assessment of images)



Notify #: 257308 ; Suspect Organism: Milky Flesh Syndrome - Snapper - Firth of Thames, Coromandel
Notification Date: 20/07/23
Location: 9(2)(a)
Reason for call: Caller is wanting to report they went fishing out on the muscle farm in the Coromandel and out of 10 fish they caught 4 had the milky white stuff on it. Was advised to call it through.
Stand down / investigation justification: Stood down as the notifer forgot to send the whole fish instaed of just the fillets.
Notifier has said he will go fishing again - another sampling kit will be sent to him in case more milky fish are caught.
Investigation summary: A keen angler caught 10 Snapper, 2 Kahawai and 20 Jack Mackerel from the Firth of Thames, Coromandel, Waikato Region. From this catch 4 Snapper had milky, and mushy flesh. The Snapper were about 30cm in length, all skinny. He also made an interesting observation that only the smallest of the fish he had caught so far had milky flesh. These observations were reported to MPI through the exotic pest and disease hotline 0800 80 99 66. An investigator got in touch with the angler and requested images of the fish which were forwarded to the Animal Health Laboratory (AHL). After examining the photos, AHL requested for the samples to be sent to them for testing. It is key to note that in order to understand the how the Milky Flesh Syndrome impacts the fish, the whole intact fish needs to be examined. While this angler had filleted the fish, he had kept the carcass and fillets refrigerated. A sampling kit was sent over to the notifier and the samples picked up to be sent to AHL. Upon arrival of samples, AHL noted that only the fillets were sent and not the whole fish. Due to this, a holistic examination was not possible. The notifier had been made aware of this and acknowledged that he had mistakenly sent only the fillets. Due to this no testing could be performed and the investigation was closed.

(No lab report as no testing was done.)

Notify #: 256030 ; Suspect Organism: Milky flesh Snapper - Tauranga harbour
Notification Date: 30/04/23
Location: 9(2)(a)
Reason for call: Caught snapper on Friday in Tauranga Harbour, most fine but x2 in the catch had quite milky texture and discoloration. No photos but has a sample vacuum-sealed if needed, only has photos of fillets and has frozen the frames.
Stand down / investigation justification: Samples not collected for testing due to sub-optimal conditions of the samples. The notifier has been informed of the ideal conditions the sample needs to be in for testing - whole chilled fish.
Investigation closed.
Investigation summary: Biosecurity New Zealand (BNZ) was contacted by a member of the public on 30/04/2023 regarding a couple milky flesh Snapper they had caught on 28/04/2023. The notifier had the frozen carcass and a few refrigerated fillets. After consulting pathologists at the Animal Health Laboratory, it was determined that the fish were not in ideal condition for testing. For milky flesh fish cases, the diagnosis requires seeing the whole organism, its overall condition and visceral content in-situ. Thus, whole fresh chilled fish are the ideal conditions for a reliable diagnosis. The MPI (Fisheries NZ) website has been updated to reflect the correct sample conditions and the notifier has been informed of the same. The investigation was closed.

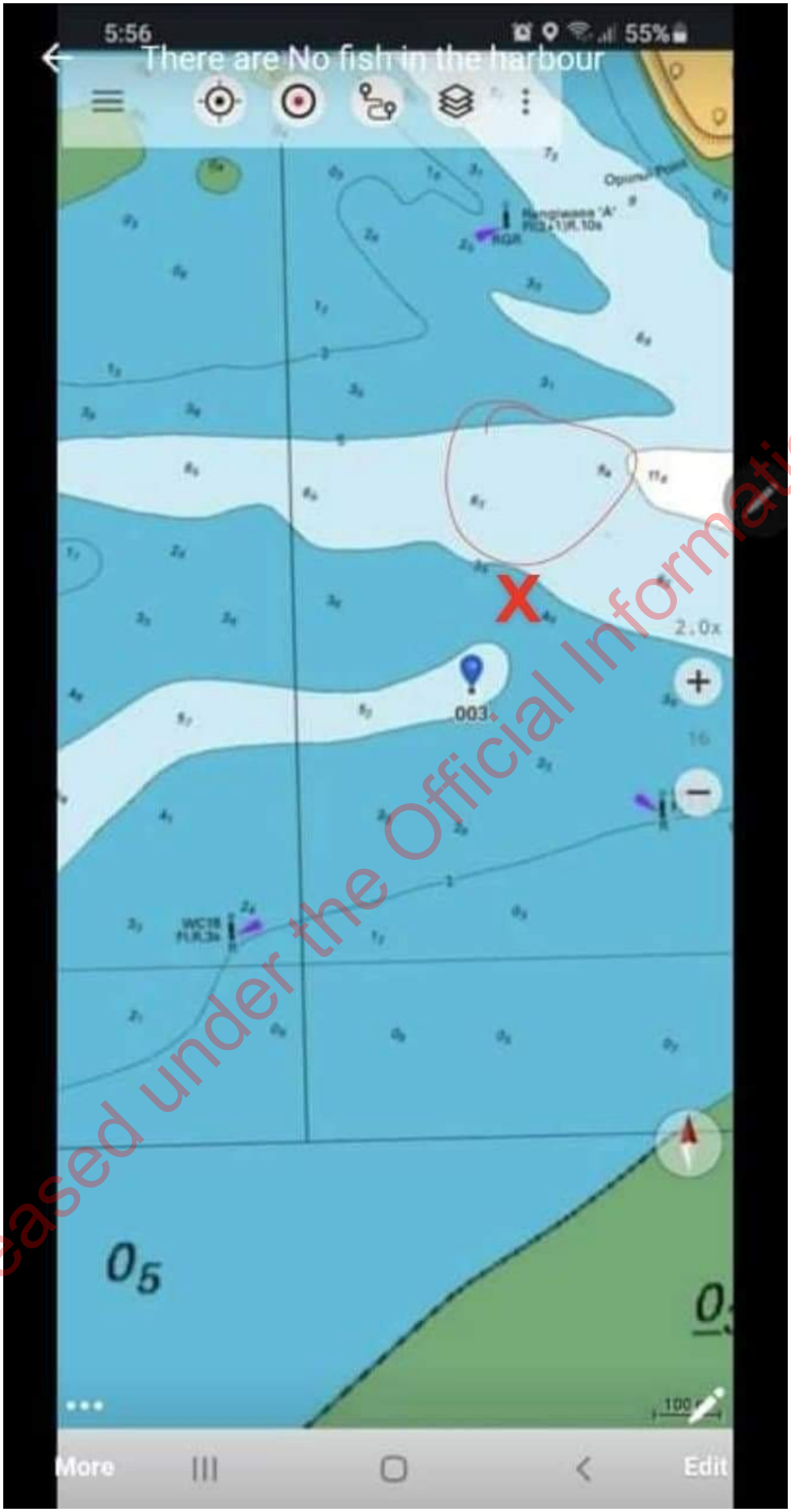
(No lab report as no testing was performed)

8:31 30%

www.google.com

Maps | Bay of Plenty Regional...

boprc.govt.nz



Notify #: 255056 ; Suspect Organism: Milky Snappers in Foxton Beach
Notification Date: 09/04/23
Location: 9(2)(a)
Reason for call: 30m Deep off Foxton Beach they have caught 3 milky snappers.
Stand down / investigation justification: It would seem that this is another case of milky white flesh syndrome which has been affecting snappers in the Hauraki Gulf and East Northland areas. As this is a different area the fish will be sent to AHL for testing.

https://www.mpi.govt.nz/fishing-aquaculture/recreational-fishing/information-on-popular-fish-in-nz/snapper-status-and-information/milky-white-flesh-in-snapper-and-some-other-finfish/
Investigation Summary A member of the public notified MPI after finding three milky snappers 30m Deep off Foxton Beach. One fish fillets were left and they were sent to the Animal Health Laboratory for testing. Scientists noticed that the fillets had areas of normal muscle tissue with large sections and separate pieces of tissue having a macerated, "mushy" appearance, consistent with the milky white flesh syndrome. The fish pathology testing confirmed muscle necrosis, which was not associated with any visible parasites, bacteria fungi, or inflammation. A cause for the changes seen was uncertain and muscle necrosis, as seen in this case, may be associated with nutritional or exertional factors. In order to have a deeper understanding of this condition, the whole fish for overall body condition are required. As the finding did not represent a biosecurity issue, the case was closed.

Please note there is a publicly available write up of the following investigation in:
Surveillance Magazine, Vol 50, No 2 – June 2023 (mpi.govt.nz) page 34

Notify #: 121665 ; Suspect Organism: Multiple Snapper with Milky White Mushy Flesh / Poor Condition - East coast Auckland and Northland
Notification Date: 01/09/22
Location: Multiple, East coast of Northland and Auckland
Reason for call: Original Notification 01/09/22
9(2)(a)
Phone: 9(2)(a)
Email: 9(2)(a)

9(2)(a) called on behalf of NIWA. Noted that: 9(2)(a) is the person who has received most reports (9(2)(a), 9(2)(a)
Multiple reports from rec /commercial fishers.
Location – Hauraki Gulf up to Northland region.
Issue has not been seen before recent reports
He was given a suggestion that it could be Kudoa parasite – noted that it was not known to NZ. Later followed up to note Kudoa sp. has been recorded from the musculature of a red cod some time ago. Record in page 10 of the attached 1982 pub.
Mentioned NIWA would look at collecting tissue sample in microscope to look for cysts.
The only other suggestion was environmental issues.
Only Snapper, no other fish effected

9(2)(a)
Phone: 9(2)(a)
Email: 9(2)(a)

Couple of enquiries from rec fishers through NIWA media enquiries line:
Mon / Tue - One rec fisher reports snapper with milky white flesh when filleting – soft/mushy, some slimy. Also reported poor condition – skinny. Noted that it had been noticed before but was infrequent.
Tue / Wed – One rec fisher reports from Great Barrier Island of the same thing, reporter said they had noticed similar over the last few months.
9(2)(a) – reported around 50% of snapper coming through over the weekend had this issue. They called during the week but reported on last weekends finds. Multiple areas, they travel around and fillet fish.
9(2)(a) reached out to a local fisheries factory (9(2)(a) who hadn't noticed anything at the time but has since reported the issue and sent in multiple pictures. – Area 1 – Snapper 1 – North cape to East cape.
9(2)(a) has contacted a friend who is a pathologist at 9(2)(a) who mentioned the Kudoa parasite and has suggested taking flesh samples, dry pressed to look at under a microscope. Mentioned potential detection from cysts in the flesh then ID from DNA analysis if cysts present. 9(2)(a) has said he was looking at doing that this weekend and will also store the samples in 100% ethanol for DNA analysis after looking at them under a microscope.
He is looking at doing this in house at NIWA I believe.
9(2)(a) also noted that it was mentioned to him that this has been widely discussed in the fishing community, checking fish.net or nzfishingcommunity Facebook page could be helpful.

9(2)(a) followed up with full details of the reports he has received:

From 9(2)(a)
"All of ours have been out on the mud between Tiri and Flat Rock in 35-45 meters during the first three weeks of August. Having said that I am seeing other reports come in from Coromandel to Mangawhai.
The fish that we have taken when we have been fishing the shallows and the reefs have been fine and very healthy."

From 9(2)(a)
"We live on the north east coast of Great barrier Island. Most of the fishing I do is on the east coast.
I have noticed this condition for some time now. I can't recall when I first saw it, as I did not see it is of any concern until now. I would only see the odd snapper.
The last trip I made, we had to throw away 3 good size fish out of 10.
We started fishing in the shallow foul area, no more than 10 m deep. All those 4 fish were fine.
We then moved to a foul deep spot of about 30 m. 3 of the 6th fish where affected and one questionable.
Two weeks before that we went out to 80m hapuku fish. Caught some snapper and half were affected.
2 weeks before that, I was fishing at 30 m on the sand and also had a couple of fish like this.
I find that the fish are of bad condition. The fish seem to have a sunken look just passed the rib cage.
A note to also consider is that the fishing has been incredible, especially on the sand deep. And the 40 years fishing out here I have never seen it so good and abundant."

From 9(2)(a)
"As discussed, we are receiving an increasing number of inquiries about snapper in the Hauraki Gulf that have white, milky tissue. The most alarming account came from the 9(2)(a) where a reported 50% of fish (100 of 200 fish) were in this condition.

We have never experienced anything like this and from the increased social media activity, nor has the general public. We do tend to see a small number of fish in this condition usually toward the end of the year but not in the quantities as they are now."

From 9(2)(a) earlier this week:
"we also noticed the similar quality issue in the factory today.
I have attached few photos – the good quality fillets vs the whitish fillets.
Also, the whole fish was not very "firm".
All these fish were from Area 1."

I suspect there will be a wealth of information on Fishing.net and NZFishing community face book page as well.
As discussed, please keep the photos and any mention of 9(2)(a) confidential until they have been approached for permission.

It has now been noted that Trevally may also be affected.

Further correspondence from 9(2)(a) has indicated 25% of commercial snapper and trevally are now affected, with growing concern within the industry. He has outlined some potential issues regarding fisheries management in light of the growing concern. I have attached his email to the documents section of the investigation.

10/02/23 9(2)(a) notified of 2 fish in northland east cost had the issue. Adding this notification to this investigation as it is the same area and issue.

Stand down / Investigation justification: Il requested pictures from both 9(2)(a) - Photos provided showing the issue.

Risk of potential aquatic disease affecting these fish - Investigation opened.

NIWA Sampling round 1 - Affected fish (5 Snapper 1 Trevally) - East coast of Auckland - Hauraki Gulf - No Exotic disease or infectious agent found.
NIWA Sampling round 2 - Affected and non-affected snapper (4:2) - East coast of Auckland - Hauraki Gulf - No Exotic disease or infectious agent found.
NIWA Sampling round 3 - Non affected snapper from a non-affected area (Bay of Plenty).
9(2)(a) sampling - Affected Fillets sent in.

All sampling and results have now been finalised.
No biosecurity risk is present. The issue is thought to be related to chronic starvation in these fish.
Only one trevally was sampled and if more become available a separate investigation will be opened.
Investigation has been stood down.
A close out report has been completed and provided to FNZ.

Investigation summary On 01/09/2022 Biosecurity New Zealand (BNZ) was notified of a widespread issue occurring on the east coast of Northland and Auckland concerning Australasian snapper (*Pagrus auratus*) and white trevally (*Pseudocaranx dentex*). The notification came from NIWA who had been contacted by both recreational and commercial fishers. The issue was reported to be white, soft, and mushy flesh occurring in between 10-50% of wild caught snapper, and a smaller number of trevally. The condition was later named Milky-White Flesh Syndrome

NIWA also reported discussions occurring online on fishing forums and Facebook pages. Many recreational fishers mentioned its sporadic presence over the last 10 years, indicating it was not uncommon around spawning time, and that populations always seemed to recover. Many raised concerns around the current apparent prevalence and persistence of the issue.

Samples were sent to the Animal Health Laboratory (AHL) for analysis to investigate if any exotic disease, or another infectious cause was associated with the syndrome. Samples consisted of both 'healthy' and 'affected' snapper from within the Hauraki gulf, and healthy fish from the Bay of Plenty, an apparently unaffected area. While samples of affected trevally were also sought, only one was able to be collected due to the population being in deeper waters at the time.

AHL performed macroscopic assessment, general aquatic bacteriology, and histology on the samples.

There was no evidence found to indicate a biosecurity concern regarding exotic diseases or infectious agents. Neither was there clear evidence of a septic or infectious (viral, bacterial, fungal, or protozoal) cause for the changes in muscle condition.

However, nutritional deficiencies were consistently observed in the affected fish. This was indicated by:

- Poor condition factors calculated for affected fish.
- Degenerative changes in skeletal muscle relating to muscle atrophy and a loss of polysaccharides within muscle tissue, associated with tissue breakdown following a prolonged period of starvation.
- Liver atrophy with an absence of polysaccharides.
- Abdominal cavities lacking healthy viscera.
- Evidence of the breakdown of fat and connective tissue cells.

Evidence of iron accumulation was also observed in the affected snapper. Iron accumulation can be associated with chronic starvation, tissue breakdown (releasing iron from cells) and poor haemostasis of iron in the body and tissue.

A relatively heavy internal parasite load was observed in all the sampled fish. However, there is no evidence to suggest that any specific parasite is linked to the syndrome, or that there are any related biosecurity concerns. No histological or macroscopic evidence was found to suggest the presence of myxosporean parasites such as *Kudoa spp.*, which have been linked to mushy flesh in other fish species.

Based on these observations, it is suspected that the Milky-White Flesh Syndrome seen in snapper is related to chronic malnutrition.

Information of these results have been passed on to Fisheries New Zealand who are further investigating the drivers behind the syndrome.

Notify #: 36002 ; Suspect Organism: Flaccid fleshed snapper
Notification Date: 01/02/12
Location: Auckland
Reason for call: snapper with strange flesh - caught few ofthem since beginning of spawning season....around Novemberflesh is white(not like snapper - looks painted)condition factor bad - flesh flaccidsimilarto post spawning troutnever seen anything like this before - now
Investigation / Stand down justification:

Investigation summary A fisherman contacted the MAF exotic pest and disease hotline to report that he had been catching snapper (Chrysophrys auratus) in the Auckland region that had a very white flesh. He estimated that two animals out of every ten that he had caught were affected. The flesh looked like the fish had already been cooked. The fish looked unaffected from the outside and it was only on dissection that the change in flesh color was evident. . A sample was sent to the Animal Health laboratory for histology. The sample submitted was suitable for histology only. Histopathology showed some abnormality in the skeletal muscle fibres that was thought to indicate acute exertional or nutritional

degeneration. The changes were considered non-infectious. Due to the non infectious etiology and the lack of additional samples for testing, the investigation was closed.

(No lab report as no testing was performed)

Animal Health Laboratory

Te Whare Rerenga Koiora o Aotearoa

Final Test Report

Ministry for Primary Industries
Manatū Ahu Matua

Accession Number: W22_02933
AHL Report Number: 168487

Telephone 9(2)(a)
Email: 9(2)(a)
Physical Address: 66 Ward St, Upper Hutt, New Zealand

Investigation Number: JW121665
Investigator Name: 9(2)(a)

Customer Supplied Information:

Submitter Name: AHL

Submitter Address: Po Box 40742
Upper Hutt
Wellington
New Zealand

Samples Collected: 12-Sep-2022
Samples Received: 14-Sep-2022
Sender Ph: 9(2)(a)

Bacteriology

General Aquatic Bacteriology

Sample #	Animal ID (name)	Species	Breed	Age	Microchip	Sample Type	Result
0001	Snapper 1	Fish	Not Supplied	Not Suppl.	Not Supplied	Whole Fish	Moderate mixed growth of Photobacterium phosphoreum, Vibrio cortegadensis, Aliivibrio wodanis, Shewanella woodyi
0002	Snapper 2	Fish	Not Supplied	Not Suppl.	Not Supplied	Whole Fish	Moderate mixed growth of Photobacterium phosphoreum, Vibrio cortegadensis, Aliivibrio sifiae, Aliivibrio wodanis, Photobacterium sp.
0003	Snapper 3	Fish	Not Supplied	Not Suppl.	Not Supplied	Whole Fish	Heavy mixed growth of Photobacterium phosphoreum, Vibrio cortegadensis, Aliivibrio sifiae, Aliivibrio wodanis, Photobacterium toruni.
0004	Snapper 4	Fish	Not Supplied	Not Suppl.	Not Supplied	Whole Fish	Light mixed growth of Photobacterium phosphoreum, Vibrio cortegadensis.
0005	Snapper 5	Fish	Not Supplied	Not Suppl.	Not Supplied	Whole Fish	Heavy mixed growth of Photobacterium phosphoreum, Vibrio cortegadensis, Aliivibrio wodanis.
0006	Trevalley	Fish	Not Supplied	Not Suppl.	Not Supplied	Whole Fish	Minimal growth of Tenacibaculum dicentrarchi.

Test Method Description: General Aquatic Bacteriology *AHL IANZ Accredited: No*

Bacteriology Lab Comment:

The condition factor for each Snapper was calculated (K= (weight (g) x 100)/(length (cm)^3). Results noted below. Normal condition factor for snapper averages 1.95 or higher (personal communication, Plant & Food Research)

1. K= 1.83
2. K= 1.86
3. K= 1.46
4. K= 1.92
5. K =1.81

Swab samples aseptically taken from the kidney of each fish were plated onto blood agar plus 3% salt and TCBS then monitored for bacterial growth.

From the Snapper there were 7 different bacteria types identified. Analysis of the sequencing data using BLAST showed highest similiarities to *Photobacterium phosphoreum* (99.88%), *Vibrio cortegadensis* (93.29%), *Aliivibrio wodanis* (97.17%), *Aliivibrio sifiae* (99.63%), *Shewanella woodyi* (99.4%), *Photobacterium* sp.(99.44%), and *Photobacterium toruni* (99.86%).

From the Trevalley there were 2 colonies. Analysis of the sequencing data using BLAST showed highest similiarities to*Tenacibaculum discentrarchi* (97.10%).

Given the histological results, the presence of these bacteria are mostly due to being part of the normal bacterial flora of the fish.

Note: sequencing was performed by Ecogene (Auckland, New Zealand) and results were analysed by AHL.

Signed

9(2)(a)

9(2)(a) t - Bacteriology & Aquatic Animals)
Ph. 9(2)(a)

Fish Pathology

History

Wild snapper catches presented with white milky and 'mushy' flesh and poor body condition. This affected a large proportion of fish caught. A trevally was found with similar presentation in the skeletal muscle.

Samples received

Six chilled whole fish. Five snapper (fish 1 to 5) (*Chrysophrys auratus*) and one trevally (fish 6) (*Pseudocaranx dentex*).

Macroscopic examination

In all fish incisions had been made into the skeletal muscle along the dorsal edges of the spine. Penetrating lesions were present on the side of the head, suggestive of pithing lesions. Blood clots were present around the brain and heart.
Fish 1: 720g, 34cm long. Had pale skeletal muscle on sectioning and a full stomach.
Fish 2: 870g, 36cm length. Very pale skeletal muscle, food in stomach and intestine, calciferous material in distal intestine. Multiple round spherical dense structures in the kidney and viscera.
Fish 3: 710g, 36.5cm length. Pale skeletal muscle. Multiple round spherical dense structures in the caudal kidney and coiled nematodes in viscera (suggestive of *Anasakis* spp).
Fish 4: 490g, 33cm length. Pale yellow and small liver. Crab leg in the distal intestine.
Fish 5: 650g, 33cm length. Liver greenish and pale skeletal muscle.
Fish 6: 1380g, 46.5cm length. Watery appearing skeletal muscle. Petechiation on the serosa and visceral connective tissue overlying the pyloric caeca. Intestines had watery content. Coiled nematodes (suggestive of *Anasakis* spp) on the serosa surfaces of the liver and intestine.
Samples were taken from the visceral tissues, skeletal muscle, gills and brain for histological examination.

Histological examination

Twenty four, H/E stained, glass slides were examined.
In all fish there was autolysis of the gills and visceral tissues. Autolysis was marked for the gills and intestinal tract and variable for the liver, spleen and kidney. Skeletal muscle was least affected.
Similar changes were present in all the snapper, fish 1 to 5.
Cardiac myocyte bundles appeared narrowed.
The kidneys, spleen and liver had moderate to abundant melanomacrophage nodular aggregates.
Hepatocellular cords of the liver appear narrow and shrunken with a low cytoplasm to nucleus volume and a fine scattered to moderate fine to coarse intra-cytoplasmic pale golden-brown pigment. Pancreatic tissues appeared within normal limits.
PAS stains of the liver showed aggregates of PAS positive material (stain for polysaccharides ie: glycogen) associated with macrophages forming clustered groups in the

hepatic parenchyma. However, hepatocytes have no evidence of polysaccharide material.
Perl's stain of the liver for iron showed moderate to marked diffuse hepatocellular iron accumulation, corresponding to the golden brown pigment seen on H/E sections.

Fish 1 and 5 had multiple well circumscribed, nematode structures within the outer neuropil of the brain.
Fish 1 and 4 had multifocal nodular aggregates of light golden pigmented macrophage aggregates within the neuropil and meningeal connective tissues.
Fish 1,2, 4, had some degenerative changes in the skeletal muscle. Scattered myofiber bundles showing swollen peripheral nuclei, scattered centralised nuclei, areas of loss of cross striation and scant to mild basophilic tissue fluid between muscle bundles and associated with nerve bundles. Occasional muscle bundles show vague aggregates of globular pale material in the sarcoplasm. PAS stains of muscle show no PAS positive staining.
Scattered muscle bundle necrosis was present in skeletal muscle of the eye of fish 4 and seen in the red muscle tissue of the subcutaneous tissue near the lateral line.

Fish 2, 3, 5: Multifocally within the intestinal sections are intramuscular to deep mucosal cystic and degenerate lesions lined by epithelioid macrophages, occasionally containing degenerate material or rare parasitic structure.

Fish 6, trevally:
Liver: hepatocellular cords were narrowed with decreased cytoplasm to nucleus ratio and scattered fine to moderate golden-brown pigment in the cytoplasm. Multifocal nodular aggregates of pale golden brown pigment laden macrophages and scattered nodular melanomacrophage aggregates. PAS stains of the liver showed aggregates of PAS positive material (stain for polysaccharides ie: glycogen) associated with macrophages forming clustered groups in the hepatic parenchyma. However, hepatocytes have no evidence of polysaccharide material.

Kidney: Abundant nodular melanomacrophage aggregates. Karyolysis of haemopoietic and lymphoid cells was present.
Stomach, intestine: Multifocal encysted nematodes within the serosa and parasitic cystic structures in the deep mucosa of the stomach.
Brain: Multifocal pigment laden macrophage nodular aggregates in the neuropil and meningeal connective tissue.
Heart: Focal encysted nematode. Cardiomyocytes were thinned and had fine vague globules visible within the cardiomyofibres.
Skeletal muscle: Scattered muscle bundles with centralised nuclei, pale swollen and vacuolated appearing bundles with fine basophilic tissue fluid within the interstitial tissue of muscle bundles and nerve bundles. In areas muscle fibres have a frayed appearance and in red muscle bundles globular aggregates were present within myofibres.

Diagnosis
Liver, fish 1 to 6: Suspected, moderate to marked, diffuse hepatocellular atrophy.
Liver, fish 1 to 6: Moderate to marked, diffuse haemosiderosis.
Skeletal muscle, fish 1, 2, 4, 6: Multifocal, mild, degenerative myopathy.
Brain, fish 1, 5: Multifocal, verminous encephalopathy, nematode.
Fish 2, 3, 5, 6: Multifocal, moderate, intra-mural, submucosal and serosal, verminous serositis and enteritis, nematode.

Comment

Thank you for submitting these fish for examination.
Unfortunately, there was autolysis which may have affected some of the changes seen on histology. However, there are some features that appear consistent between the fish.

The most significant change appears to be within the liver. Hepatocytes appear smaller, hepatocellular cords narrower and there is a fine to coarse golden-brown pigment accumulating in the hepatocytes.
The hepatocytes show no accumulation of polysacchardide material, based on PAS stain, suggesting complete los of glycogen stores. The brown pigment is identified as iron by Perl's stain, confirming significant haemosiderosis in these fish.
Haemosiderosis/iron overload has been reported to occur in a wide variety of aquarium and wild fish (fresh and marine). It is uncertain whether the accumulation in the liver is due to environmental or dietary factors or due to metabolic defects.
Iron accumulation in the liver of fish has been associated with high environmental iron levels as well as exposure to heavy metals and PCB contaminated sites/waters.
The impact of high iron accumulation on the fish is uncertain but may serve as an indicator for contaminant exposure. It is unsure if the iron in these fish is part of the manifestation of the pale muscle and examination of a 'healthy control' is needed.

There are scattered degenerative changes in the skeletal muscle, especially the red muscle and finer motor muscles ie: the eye. Changes are subtle and significant inflammation or cell infiltrates are not seen. It is difficult to correlate the changes seen histologically to the pale flesh seen macroscopically. The colour and consistency change may be associated with changes in glycogen storage and tissue fluid in the muscle.
Nodular aggregates of pale golden pigmented macrophages were scattered in various visceral tissues and the brain. These nodules of macrophages are through to be associated with tissue breakdown of fat and connective tissues cells and intake of polysaccharides.
There is evidence of parasitism in most of the fish, either encysted nematodes in the brain, intestine or heart and *Anasakis* spp within the serosa of the coelomic cavity.

Summary:

Based on the finding of apparent hepatic atrophy and nodular accumulation of foamy golden macrophages and early scattered muscle degenerative changes, it is suspected these fish are suffering from long term poor nutritional intake with muscular atrophy.
There is marked liver iron accumulation, and this may indicate exposure to heavy metals, PCB contamination in the environment and high iron levels in the environment.
The nematode infestation would have contributed to poor body condition.
Ideally examination of two fresh control fish with good body condition and 'normal' appearing skeletal muscle will allow for a better comparative study of the liver and muscle.
An infectious or parasitic process affecting the skeletal muscle was not visible.

References

Terio K, McAloose D, St. Leger J (eds)(2018). *Chapter 39, Osteichthyes, Non-infectious diseases, Iron overload, p947-948.* Academic Press, Elsevier.

Test Method Description:Histology on Fish samples

AHL IANZ Accredited: No

Signed

9(2)(a)

9(2)(a) (Pathologist)
9(2)(a)

Dates of Lab Activities: 14/09/2022 - 03/11/2022

Animal Health Laboratory

Te Whare Rerenga Koiora o Aotearoa

Final Test Report

Ministry for Primary Industries
Manatū Ahu Matua

Accession Number: W22_02965
AHL Report Number: 167194

Telephone 9(2)(a)
Email: 9(2)(a)
Physical Address: 66 Ward St, Upper Hutt, New Zealand

Investigation Number: JW121665
Investigator Name: 9(2)(a)

Customer Supplied Information:

Submitter Name: AHL

Submitter Address: Po Box 40742
Upper Hutt
Wellington
New Zealand

Samples Collected: 12-Sep-2022
Samples Received: 15-Sep-2022
Sender Ph: 9(2)(a)

Bacteriology

Samples on Hold - Bacteriology

Sample #	Animal ID (name)	Species	Breed	Age	Microchip	Sample Type	Result	Comment
0001	817 - 5 Ethanol fixed tissues	Fish	Not Supplied	Not Suppl.	Not Supplied	Tissue - Fixed	Cancelled	

Test Method Description: Samples On Hold - Bacteriology *AHL IANZ Accredited: No*

Signed

9(2)(a)

9(2)(a) (Pathologist)

Ph.

Fish Pathology

History
Multiple wild caught snapper (*Chrysophrys auratus*) presented with milky white 'mushy' flesh and were in poor condition.

Samples received
Five pottles of formalin each containing one small section of skeletal muscle.

Histological examination
Two H/E-stained glass slides with representative sections of each section of tissue examined.
The sections were similar and scattered muscle bundles contained fibrillar appearing muscle fibres and some areas of loss of cross striation and scattered fibres with centralised and prominent peripheral nuclei. In cross sections muscle bundles appear angular and shrunken from the sarcolemma. There was a scant to mild interstitial accumulation of tissue fluid, seen as pale basophilic staining. PAS stains of muscle show no PAS positive staining.

Diagnosis
Scattered, mild, degenerative myopathy with mild interstitial oedema.

Comment
Thank you for submitting these sections of muscle tissue for examination.
The sections from the five fish all reveal similar and subtle changes. Significant inflammation or tissue necrosis is not seen.
There is mild tissue oedema between the muscle bundles and suggestion of early and subtle muscle degeneration (fibrillar appearance, loss of cross striation and centralised nuclei). However, a cause for this is not apparent and it is uncertain how these changes relate to the markedly pale flesh seen macroscopically. PAS stains do not show any evidence of polysaccharide staining in the muscle bundles and it may suggest depletion of polysaccharides in the muscle tissue.
The changes in the skeletal muscle are similar as those seen in the fresh whole fish submitted (our accession W22_02933) and appear to relate to early degenerative changes, suggestive muscle atrophy.
An infectious or parasitic process affecting the skeletal muscle was not visible.

Test Method Description:Histology on Fish samples *AHL IANZ Accredited: No*

Signed
9(2)(a)
9(2)(a) (Pathologist)
Ph.

Dates of Lab Activities: 15/09/2022 - 30/09/2022

Animal Health Laboratory

Te Whare Rerenga Koiora o Aotearoa

Final Test Report

Ministry for Primary Industries
Manatū Ahu Matua

Accession Number: W22_03660
AHL Report Number: 169679

Telephone 9(2)(a)
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Physical Address: 66 Ward St, Upper Hutt, New Zealand

Investigation Number: JW121665
Investigator Name: 9(2)(a)

Customer Supplied Information:

Submitter Name: AHL

Submitter Address: Po Box 40742
Upper Hutt
Wellington
New Zealand

Samples Collected: 13-Nov-2022
Samples Received: 14-Nov-2022
Sender Ph: 9(2)(a)

Bacteriology

General Bacteriology Culture

Sample #	Animal ID (name)	Species	Breed	Age	Microchip	Sample Type	Result
0001	1 Affected	Fish	Not Supplied	Not Suppl.	Not Supplied	Whole Fish	No growth.
0002	2 Affected	Fish	Not Supplied	Not Suppl.	Not Supplied	Whole Fish	No growth.
0003	3 Affected	Fish	Not Supplied	Not Suppl.	Not Supplied	Whole Fish	Light mixed growth of Enterovibrio spp., Shewanella woodyi, and Photobacterium sp.
0004	4 Affected	Fish	Not Supplied	Not Suppl.	Not Supplied	Whole Fish	No growth.
0005	5 Unaffected	Fish	Not Supplied	Not Suppl.	Not Supplied	Whole Fish	No growth.
0006	6 Unaffected	Fish	Not Supplied	Not Suppl.	Not Supplied	Whole Fish	No growth.

Test Method Description: General Bacteriology Culture *AHL IANZ Accredited: Yes*

Bacteriology Lab Comment:

Swab samples aseptically taken from the head kidney of each Snapper were plated onto Blood agar, Blood agar plus 3% salt and TCBS then monitored for bacterial growth.

From Snapper sample # 3 there were 3 different bacteria types identified. The *Shewanella woodyi* isolates were identified by MALDI-ToF. The *Enterovibrio* spp. and *Photobacterium* sp. were identified by 16s rRNA sequencing with highest similarities of 98.89% and 93.00 % respectively.

Note: sequencing was performed by Ecogene (Auckland, New Zealand) and results were analysed by AHL.

Signed

9(2)(a)

9(2)(a)

Ph. 9(2)(a)

Fish Pathology

History

A further 6 fresh caught snapper (*Chrysophrys auratus*) from the East coast of Auckland and Northland were submitted as part of an ongoing investigation into the increased occurrence of pale, milky and soft flesh.

Samples received

Six entire chilled snapper. Four affected and two unaffected.

Macroscopic examination

Affected group

Snapper 1: 455.9g, 30cm length. Very thin body condition with concave dorsal musculature. Reddening of the skin around the pectoral, pelvic and behind the anal fins. Very pale watery muscle tissue. Internal examination showed no body reserves, very empty coelomic cavity with atrophied liver and intestinal tract. Empty stomach. Pyloro-gastric intussusception was present.

Snapper 2: 507.1g, 31.5cm length. As for Snapper 1 with an abrasion on the upper right dorsal area. Very pale watery muscle tissue. Internal examination showed no body reserves, very empty coelomic cavity with atrophied liver and intestinal tract. Empty stomach with pyloro-gastric intussusception.

Snapper 3: 522.5g, 33cm length. Very thin and similar to Snapper 1. Very pale watery muscle tissue. Internal examination showed no body reserves, very empty coelomic cavity with atrophied liver and intestinal tract. Empty stomach.

Snapper 4: 481.9g, 31.5cm length. As for Snapper 1 with some haemorrhage in the iris. Very pale watery muscle tissue. Internal examination showed no body reserves, very empty coelomic cavity with atrophied liver and intestinal tract. Empty stomach. Pyloro-gastric intussusception was present.

Unaffected

Snapper 5: 808.2g, 34.5cm length. Good body condition with rounded dorsal musculature. Firm, normal appearing muscle tissue. Internal examination showed a moderately empty coelomic cavity, with small to moderate mesenteric fat reserves and smaller liver.

Snapper 6: 1057.1g, 37cm length. Good body condition with rounded dorsal musculature. Firm, normal appearing muscle tissue. Internal examination showed abundant mesenteric body reserves, plump, large rounded and pale liver with very prominent enlarged gonads and filling of entire body cavity by internal organs.

In all snapper large 3-5mm, firm, spherical masses were scattered throughout the kidney parenchyma.

Histological examination

Sixteen, H/E stained, glass slides were examined.

Common findings in the Snapper:

In all fish there was autolysis of the gills and visceral tissues. Autolysis was moderate to marked for the gills and intestinal tract and variable for the liver, spleen and kidney. Skeletal muscle was least affected.

The kidneys, spleen and liver had moderate to abundant nodular aggregates of melanomacrophages and ceroid/lipofuscin PAS positive (mostly in the liver) containing macrophages. The liver of snapper 6 had scattered small aggregates of ceroid/lipofuscin macrophages.

Heart:

Multifocal, small to large, aggregates and infiltrates of ceroid/lipofuscin PAS positive macrophages were present within especially the cardiac atrium and scattered in the ventricles.

Liver:

Snapper 1 to 4: Hepatocellular cords of the liver were narrow and shrunken with a low cytoplasm to nucleus volume and a fine scattered to moderate fine to coarse intra-cytoplasmic pale golden-brown pigment. Pancreatic tissues appeared within normal limits.

Perl's stain of the liver for iron showed moderate to marked diffuse hepatocellular iron accumulation, corresponding to the golden-brown pigment seen on H/E sections. Perl's stain also revealed aggregates of iron containing macrophages within the melanomacrophage centres of the spleen, but not the kidneys.

The liver of Snapper 5 (unaffected) had well defined trabeculae and hepatocellular architecture and hepatocytes had moderate cytoplasm. Hepatocytes showed mild to moderate iron accumulation in the hepatocytes. Iron accumulation was not seen in melanomacrophage centres within the spleen.

Skeletal muscle:

Snapper 1 to 4 had similar changes composed of shrunken angular muscle bundles in cross section, fibrillar and wavy myofibers and bundles in cross and longitudinal section, multifocal, dense eosinophilic and necrotic myofibers and rare scattered small aggregates of lymphocytes and macrophages.

Snapper 5 and 6: Muscle bundles were plump rounded and appeared within normal limits.

Kidney:

Snapper 1 to 6, all showed variable mild to abundant aggregates of eosinophilic granulocytes throughout the stroma of the kidney. Large round well circumscribed mass of fibrosed tissue with dense infiltrates of eosinophilic granulocytes (fibrosed parasitic cyst).

Spleen:

Multifocal scattered to moderate numbers of encysted, degenerate parasitic structures (suggestive of helminth eggs) were present within the stroma frequently associated with melanomacrophage centres.

Stomach and intestine:

Stomach and intestine: Mild to moderate fibrosis of the lamina propria of the stomach with scattered encysted parasitic structures and mild to moderate infiltrates of lymphocytes and eosinophilic granulocytes. Within the intestinal lamina propria were moderate to marked infiltrates of lymphocytes, eosinophilic granulocytes, moderate parasitic cysts, moderate fibrosis and larval nematodes within foci of granulomatous inflammation.

Occasional helminths organisms were present within the lumen of the intestine.

The inflammation of the stomach and intestine was milder in snapper 6, as compared to other snapper.

Individual findings

Snapper 1:

- Multifocally within the liver were large helminths containing multiple calcerous corpuscles suggestive of cestodes. Extensive hepatic necrosis was present surrounding the organisms.

- Brain: Scattered stromal infiltrates of ceroid/lipofuscin containing macrophages.
- Male gonad, moderate development with multifocal moderate aggregates of ceroid/lipofuscin containing macrophages and scattered encysted degenerate parasitic structures.

Snapper 2:
- Male gonad markedly depleted with moderate stromal ceroid/lipofuscin macrophage aggregates.

Snapper 3:
- Brain: Multiple nodules of calcified material and moderate multifocal aggregates of ceroid/lipofuscin containing macrophages were present within the white matter adjacent to the ventricle surface.
- Small poorly developed female gonad with moderate aggregates of ceroid/lipofuscin macrophages.

Snapper 4:
- Brain, brainstem and proximal spinal cord: mild to moderate multifocal infiltrates of lymphocytes and macrophages with ceroid/lipofuscin containing macrophages within the grey matter.

Snapper 6:
- Liver: Hepatocytes had abundant cytoplasm and scant cloudy vacuolation. No pigment was present, and no iron was seen on Perl's stain.
- Female well developed mature and maturing gonad.
- Kidney: Less melanin present in melanomacrophages as compared to the other snapper.
- Brain: Multifocal moderate, lymphocytic and macrophage aggregates in the brain and in occasional peri vascular areas. Frequently associated with dense round mineralised nodular masses and round bodies composed of a well-defined cytoplasmic border surrounding a cluster of dense round bodies (parasitic).

Diagnosis

Macroscopic diagnosis:

Snapper 1- 4: Marked, muscle, hepatic and gastrointestinal atrophy, suggestive of chronic malnutrition.
Snapper 1, 2, 4: Gastro-pyloric intussusception.

Histologic diagnosis:

Muscle, Snapper 1- 4: Moderate to marked diffuse, skeletal muscle degeneration and multifocal necrosis, suggestive of nutritional myopathy.
Liver, Snapper 1- 4: Moderate to marked, diffuse, hepatic atrophy with moderate hepatocellular iron accumulation.
Liver, Snapper 5: Mild, diffuse, hepatic atrophy and hepatocellular iron accumulation.
Liver, Snapper 1: Multifocal, intra hepatic, helminths suggestive of cestodes.

Kidney, Snapper 1 - 6: Mild to marked, multifocal to diffuse, eosinophilic granulocytic, stromal aggregates.
Stomach and intestine, Snapper 1- 6: Mild to marked, diffuse, chronic active, lympho-histiocytic and eosinophilic, gastro enteritis with scattered to abundant intra mural encysted parasites and helminth larvae (suggestive of nematodes).
Brain, Snapper 3, 4, 6: Mild to moderate, multifocal, chronic, lympho-histiocytic, encephalitis with parasitic cysts and mineralised bodies (suspected degenerate and calcified parasitic cysts).

Comment

Thank you for submitting the Snapper for examination, as part of the ongoing investigation into the occurrence of pale milky flesh.
These snapper are showing more advanced and significant changes as compared to the previous submission, W22_02965.
There is no clear evidence of a septic or infectious (viral, bacterial, fungal, protozoal) cause for the muscle changes seen.
The marked muscle degeneration seen macroscopically and on histology is not inflammatory in nature and is a degenerative process, strongly thought to be associated with chronic nutritional deficiencies.

The accumulation of ceroid/lipofuscin/PAS positive macrophage aggregates in multiple body organs and brain supports the process of tissue breakdown.

In all snapper there is evidence of inflammation of the intestinal tract with variable but frequently abundant parasitism. There is also evidence of aberrant parasite migration to the brain and further parasitic cysts (possible cestode eggs) within various body tissues including the gonads, spleen, kidney and brain.
These snapper have a significant parasite burden which could contribute or exacerbate a poor body condition. The high parasite burden also accounts for the marked increase in eosinophilic granulocytes in the kidney and tissues. Eosinophilic granulocytes are often associated with parasitic infestations.
Intestinal intussusception is very rarely reported in fish. It has been described in aquaculture fish with enteritis (Liu *et al.* 2009, Cao *et al.* 2016, Hoitsy *et al.* 2021).
In this case the finding of intussusception of the intestine into the stomach is very interesting and is likely associated with the heavy intestinal parasitism and inflammation seen and a reflux effect in the intestine (parasitism has been described as a cause of intestinal intussusception in terrestrial animals, Hoitsy et al. 2021). This would have prevented feeding in the affected fish.

Snapper 5, collected as 'unaffected' did show early changes that suggest a progression to the same body state as for the affected group. There was iron accumulation in the liver, early abdominal visceral and liver atrophy and parasitism.

Snapper 6, unaffected, had a very well-developed gonad, suggesting she was nearing spawning and the liver was large and plump and the viscera filled the abdominal cavity. There was no iron accumulation, suggesting that in a normal active liver, iron accumulation should not be as evident as seen in the affected snapper.

The exact cause for the iron accumulation is uncertain, but factors that may contribute to the iron accumulation includes:

- Increased iron accumulation in circulation due to tissue and blood breakdown, secondary to chronic malnutrition,

- An inability to adequately excrete and manage iron due to poor nutritional intake and reduced liver and bile function,
- High iron loads in the environment and food chain,
- Possible effect of toxins or chemicals.

Summary

- There is no clear evidence of an underlying viral, bacterial, fungal, protozoal or protist cause for the muscle degeneration,
- The pale milky flesh and very poor body condition is suspected to be associated with chronic malnutrition,
- There is a parasite burden causing secondary intestinal inflammation and damage which could cause or worsen the poor body condition.

Factors to consider that may be predisposing to poor body condition include:

- Snapper coming out of winter in poor body condition and moving to spawning areas but not getting adequate feed and there is higher competition for food, due to increased fish population density associated with spawning and migrations.
- Ecosystem collapse and decreased food availability and changes in the seabed and feeding areas of adult snapper.
- Density of the snapper in the area, larger fish numbers would increase competition.
- Higher parasite burdens in prey species due to increase water temperatures and proliferation of parasites.
- Anthropogenic factors that prevent fish feeding inshore ie: increased numbers of boats, diving, water sports.
- Possible role of toxins, chemical and pollutants in the environment.
- Environmental factors ie: increased water temperatures with lower dissolved oxygen, possibly reducing feeding activity of the fish.

References

Liu, J.-Y., Yang, W.-M., Li, A.-H., & He, G.-W. (2009). Preliminary study on the etiology of channel catfish intussusception disease. *Acta Hydrobiologica Sinica*, 32(6), 824-831. <https://doi.org/10.3724/SP.J.1035.2008.00824>

Cao, H., He, S., Li, Y., Yang, Y., & Ai, X. (2016). Hafnia alvei: A pathogen causing Infectious Intussusception Syndrome (IIS) in farmed channel catfish Ictalurus punctatus. *The Israeli Journal of Aquaculture*, 68, 1305-1311.

Hoitsy, M, Hoitsy, G, Jakab, C, Molnár, T, Gál, J, Baska, F. (2021). Intussusception caused by intestinal neoplasia in mature rainbow trout (Oncorhynchus mykiss, Walbaum 1792). *Journal of Fish Diseases*. 44: 893- 898. <https://doi.org/10.1111/jfd.13347>

Test Method Description:Histology on Fish samples *AHL IANZ Accredited: No*

Signed

9(2)(a)

9(2)(a) (Pathologist)

Ph. 9(2)(a)

Dates of Lab Activities: 14/11/2022 - 15/12/2022

Animal Health Laboratory

Te Whare Rerenga Koiora o Aotearoa

Final Test Report

Ministry for Primary Industries
Manatū Ahu Matua

Accession Number: W22_03984
AHL Report Number: 170652

Telephone 9(2)(a)
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Physical Address: 66 Ward St, Upper Hutt, New Zealand

Investigation Number: JW121665
Investigator Name: 9(2)(a)

Customer Supplied Information:

Submitter Name: AHL

Submitter Address: Po Box 40742
Upper Hutt
Wellington
New Zealand

Samples Collected: 12-Dec-2022
Samples Received: 13-Dec-2022
Sender Ph: 9(2)(a)

Comments: Species: Pagrus auratus - Australasian snapper

Bacteriology

General Bacteriology Culture

Sample #	Animal ID (name)	Species	Breed	Age	Microchip	Sample Type	Result
0001	846 1	Fish	Not Supplied	Not Suppl.	Not Supplied	Whole Fish	No growth.
0002	846 2	Fish	Not Supplied	Not Suppl.	Not Supplied	Whole Fish	No growth.
0003	846 3	Fish	Not Supplied	Not Suppl.	Not Supplied	Whole Fish	No growth.
0004	846 4	Fish	Not Supplied	Not Suppl.	Not Supplied	Whole Fish	No growth.

Test Method Description: General Bacteriology Culture *AHL IANZ Accredited: Yes*

Bacteriology Lab Comment:

The condition factor for each Snapper was calculated (K=(weight (g) x 100)/length (cm) ^3). Results are noted below.

Snapper #1 - K=1.97
Snapper #2 - K=1.95
Snapper #3 - K=1.92
Snapper #4 - K=1.96

Swab samples aseptically taken from the kidney of each fish were plated onto blood agar, blood agar plus 3% salt and TCBS then monitored for bacterial growth.

Signed

9(2)(a)

9(2)(a) Bacteriology & Aquatic Animals)
Ph. 9(2)(a)

Fish Pathology

History
Entire fresh caught Australasian Snapper (*Chrysophrys auratus*) submitted as 'healthy' controls in an ongoing investigation into pale, milky and soft flesh affecting snapper.

Samples received
Four entire fresh chilled snapper.

Macroscopic examination
No significant abnormalities.

Representative sections of tissue from each of the four snapper, comprising: skeletal muscle, skin, gills, heart, brain, intestinal tract, liver, anterior and posterior kidney, spleen and gonad (some fish) were taken, fixed in formalin and processed to H/E-stained glass slides.

Histological examination
Twelve H/E-stained glass slides.
In all four snapper there was autolysis affecting the gills and mucosa of the intestinal tract.
All four-snapper had similar findings on histology.
Spleen, liver, pancreas, brain, muscle, skin, heart: No significant abnormalities detected.
The liver hepatocytes were rounded, plump and formed well defined broad trabeculae with no significant abnormalities recognised.

Fish 1 to 4
Gastrointestinal tract: There was moderate to marked fibrosis in the submucosa of the lamina propria with a moderate to marked infiltration of eosinophilic granulocytes, lymphocytes and plasma cells through the submucosa with scattered diffuse to multifocal nodular aggregates of macrophages. Multifocally encysted and migrating nematode larvae and encysted helminths were present.
Anterior kidney: Mild to marked infiltrates of eosinophilic granulocytes.
Posterior kidney: Fibrosed parasitic cysts with scant to moderate eosinophilic granulocytes.

Fish 4
Spleen: Multifocal, helminth cysts (suggestive of metacercarial cysts)

Perl's iron stain of the liver, spleen and kidney for all four fish showed scant to occasionally moderate accumulation of iron in macrophages within melanomacrophage nodules of the liver and spleen. No iron was seen in the kidney melanomacrophage centres.

Occasional scattered iron pigment was present in hepatocytes.

Diagnosis

Fish 1 to 4

Gastrointestinal tract: Moderate to marked, diffuse, chronic active eosinophilic, lympho-plasmacytic and histiocytic, enteritis with helminths.

Kidney: Mild to marked, eosinophilic infiltration with multifocal, fibrosed encysted parasitic remnants.

Fish 4

Spleen: Encysted metacercarial cysts

Comment

Thank you for submitting these four snapper for comparative examination in the ongoing examination for pale, milky and soft flesh in Snapper.

Overall, these snapper appear healthy with no clear evidence of significant degenerative changes in the muscle or body tissues, especially the liver.

Significant accumulation of iron in liver hepatocytes are not a feature. This suggest that the iron accumulation seen in affected snapper may be attributed to poor diet and poor haemostasis of iron in the body and tissue.

Of interest is the significant inflammation in the intestinal tract associated with helminth parasites. The inflammation and parasitism would be expected to affect digestion and nutrient absorption as well as result in loss of body proteins and blood through the parasite activity and mucosal damage.

This parasite burden was seen in affected snapper as well. Snapper may carry a large parasite burden, but it is possible that significant parasitism together with other factors (ie: poor nutrient availability, systemic illness, heavy metals, toxins/chemical, stressors) may be resulting in the progressive debility of the snapper.

Test Method Description:Histology on Fish samples

AHL IANZ Accredited: No

Signed

9(2)(a)

9(2)(a) (Pathologist)

Ph. 9(2)(a)

Dates of Lab Activities: 13/12/2022 - 18/01/2023

Animal Health Laboratory

Te Whare Rerenga Koiora o Aotearoa

Final Test Report

Ministry for Primary Industries
Manatū Ahu Matua

Accession Number: W22_03990
AHL Report Number: 170651

Telephone 9(2)(a)
Email: 9(2)(a)
Physical Address: 66 Ward St, Upper Hutt, New Zealand

Investigation Number: JW121665
Investigator Name: 9(2)(a)

Customer Supplied Information:

Submitter Name: AHL

Submitter Address: Po Box 40742
Upper Hutt
Wellington
New Zealand

Samples Collected: 27-Oct-2022
Samples Received: 14-Dec-2022
Sender Ph: 9(2)(a)

Fish Pathology

History
Ongoing sample submission from Snapper presenting with pale milky soft flesh.

Samples received
Four samples of skeletal muscle tissue. Two affected and two unaffected.

Histological examination
Four H/E-stained glass slides containing a representative section of skeletal muscle per slide.
Slides 1 and 2: Overall muscle bundles are plump, rounded and had good cross striation. Rare muscle bundles show some swelling and fibrillar change and degeneration.
Slides 3 and 4: Scattered interstitial small lymphoid infiltrates with rare macrophages. Scant stromal oedema. Multifocal scattered muscle bundles show swelling, pallor, occasional necrosis, loss of cross striations and fibrillar appearance.

Diagnosis
Slides 1 and 2: No significant abnormalities
Slides 3 and 4: Multifocal, mild to moderate, degenerative myopathy.

Comment
Thank you for submitting these sections of muscle tissue.
Slides 1 and 2 appear mainly to be from healthy muscle tissue and would correspond to samples taken from 'unaffected' snapper.
Slides 3 and 4 show degenerative muscle changes and would correspond to samples taken from 'affected' snapper. The changes are degenerative and significant inflammation, infectious or parasitic agents are not recognised.
This suggest muscle degeneration may be associated with nutritional, exertional or other non-inflammatory/non-infectious causes of muscle damage.

Test Method Description:Histology on Fish samples *AHL IANZ Accredited: No*

Signed
9(2)(a)
9(2)(a) (Pathologist)
Ph. 9(2)(a)

Dates of Lab Activities: 14/12/2022 - 18/01/2023

Animal Health Laboratory

Te Whare Rerenga Koiora o Aotearoa

Final Test Report

Ministry for Primary Industries
Manatū Ahu Matua

Accession Number: W23_01062
AHL Report Number: 174941

Telephone 9(2)(a)
Email: 9(2)(a)
Physical Address: 66 Ward St, Upper Hutt, New Zealand

Investigation Number: GR255056
Investigator Name: 9(2)(a)

Customer Supplied Information:

Submitter Name: AHL

Submitter Address: Po Box 40742
Upper Hutt
Wellington
New Zealand

Samples Collected: 09-Apr-2023
Samples Received: 11-Apr-2023
Sender Ph: 9(2)(a)

Fish Pathology

History
A fresh caught Australasian Snapper (*Chrysophrys auratus*) was noticed to have abnormal changes in the muscle tissue during filleting.

Samples received
Fillets of muscle taken from a wild caught snapper.

Macroscopic examination
Muscle fillets had areas of normal muscle tissue with large sections and separate pieces of tissue having a macerated, 'mushy' appearance.

Histological examination
Two H/E-stained glass slides comprising multiple sections of muscle tissue.
There were similar findings across the tissue sections. Normal muscle bundles were present, interspersed with individual to extensive areas showing necrosis of muscle bundles. In areas muscle bundles had a fibrillar fragmented appearance with individualised muscle fibres being prominent within muscle bundles.
Gram and PAS stains do not show any clear bacteria, fungal or parasitic structures.

Diagnosis
Multifocal to extensive, monophasic, muscle necrosis.

Comment
Thank you for submitting these sections of muscle tissue from the affected snapper. The sections of muscle tissue examined confirm the presence of muscle necrosis.
The necrosis is not associated with any visible parasites, bacteria, fungi, or inflammation.
A cause for the changes seen is uncertain and muscle necrosis, as seen here, may be associated with nutritional or exertional factors.
Other factors to consider may be the presence of bacteria causing post-mortem autolysis and possible chemical or toxins that can cause muscle degeneration.

It is not possible to determine on examination of the muscle tissue alone if the degenerative changes seen in the muscle is associated with long term nutritional deficiency in this fish. Ideally examination of the entire fish for overall body condition is required.

Test Method Description:Histology on Fish samples *AHL IANZ Accredited: No*

Signed
9(2)(a)

9(2)(a) (Pathologist)
Ph. 9(2)(a)

Dates of Lab Activities: 11/04/2023 - 19/04/2023