Faecal indicators in scats from Black Swans (*Cygnus atratus*)

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1 SUMMARY

The aim of this project was to address whether Black Swans (*Cygnus atratus*) could be potential contributors to the high levels of *E. coli* and faecal coliforms sometimes identified in water and shellfish in New Zealand. A literature review identified a distinct lack of data. To better inform this work 46 Black Swan scats were collected from Golden Bay, weighed, and faecal coliforms, *E. coli* and enterococci enumerated. Each gram of Black Swan scat is estimated to have an average of 30,000 faecal coliforms, 26,000 *E. coli*, and 500 enterococci. When combined with previously published data on the mean daily output (wet weight) of individual Black Swans (418 grams/day), this suggests each Black Swan has a mean daily output of 11 million faecal coliforms, 8.6 million *E. coli*, and 270,000 enterococci.

We conclude that Black Swans carry potentially significant levels of the traditional microbial indicators (faecal coliforms, *E. coli* and enterococci) used to assess microbial water quality and the safety of shellfish. This study did not aim to assess the quantitative impact of Black Swan faecal material on the contamination of shellfish; however it has confirmed that Black Swans are a potential source of faecal contamination of water and shellfish.

1. INTRODUCTION

The Tasman District Council (TDC) sought advice on the impact of Black Swans on the microbial quality of water and shellfish, as measured by the faecal indicators *E. coli* and enterococci. The information will be used to advise the community and shellfish industry of the possible role of Black Swans in elevating microbial counts in water and shellfish. Identifying a potential link between high faecal indicator counts in shellfish and the presence of Black Swans in the Golden Bay area would be useful to TDC when addressing Public Health, community and shellfish industry concerns about faecal contamination of shellfish.

1.1 Background

The shellfish industries located within the marine environment of Golden Bay have experienced recurrent problems with harvested shellfish carrying higher bacterial loadings than the permitted quality standards of <300 faecal coliform and <230 *Escherichia coli* per 100 g of shellfish. The question being asked by the council and affected shellfish industries is: what is the origin of the high bacterial loadings? Possible sources include septic tanks leaking human waste into waterways; faecal runoff from agricultural land and the faecal deposition of wildfowl in the local region.

Modelling of the reduction in salinity in Golden Bay waters has been a useful indicator of increased freshwater inputs into the Bay from the three main river systems: Aorere, Takaka and Motueka. The assumption here is that high precipitation events increases the runoff of faecal pollution from agricultural lands and overflow of septic tank systems into river systems which drain into the Bay. Rain events increase the probability of high faecal microbial numbers suggestive of faecal contamination and concurrent health hazards. However in recent years the salinity based models have failed to predict with sufficient accuracy when it should be safe to harvest the shellfish. As a result shellfish have been harvested when they contain unacceptably high levels of faecal indicators. This suggests that sources of the faecal indicators are unrelated to freshwater inputs.

In the past, sources of faecal pollution in the area have included contamination from human sources such as faulty septic tanks, and direct inputs from farming sources such as dairy farms. These sources have been addressed through use of abatement notices, replacement of septic tanks, and improvements to farming practices. One potential source of faecal inputs is the population of Black Swans which frequents the area.

1.2 The Black Swan

There are seven species of swan worldwide, five of which are white. The two non-white species are the South American black-necked swan and the Australian Black Swan. In the 1860s, one hundred Australian Black Swans (*Cygnus atratus*) were introduced into New Zealand (http://www.nzbirds.com/birds/blackswan.html). It is also believed, that about the same time, the swans may have arrived naturally as their numbers grew rapidly to establish populations throughout the main islands. Their habitats are large lowland and coastal lakes, and estuaries. Studies suggest that Black Swans are moderately sedentary and divide into 10 regional populations with the exception of Farewell Spit which is the major moulting site in the country where 10,000 birds derived from all around the country congregate. The majority of Black Swans on Farewell Spit are believed to originate from breeding areas in Marlborough and the Wairarapa (Heather and Robertson, 2000).

Black Swans are attracted to the Golden Bay region and Farewell Spit by the abundance of marine eel grass which provides their primary diet. Monitoring of the population since 1977 has reported summer time numbers varying between 15,000 and the current 5,000 birds recorded in 2007 (personal communication, Neil Deans, Fish and Game). It is postulated that Black Swans may be a source of the faecal contamination in Golden Bay (as evidenced by high numbers of microbial indicators). The aim of this Envirolink project was to address whether Black Swans could be potential contributors to the high levels of *E. coli* and faecal coliforms identified in harvested shellfish.

2 LITERATURE SURVEY

2.1 Daily faecal loading of birds

The daily faecal output of various bird species has been measured by a number of researchers, and their findings presented in Table 1. The only New Zealand study was by a group at Otago University. They calculated the mean daily faecal output of an individual *Cygnus atratus* to be 418 grams fresh weight or 52 g dry weight of faeces (Mitchell and Wass, 1995). When compared with other bird species (Table 1), these results suggest that Black Swans have the highest average daily faecal loading of the wildfowl on which data has been collected. However to assess the potential impact of each bird species one must also factor in not only the number of each species present, but also the relative concentration of indicators and pathogens present within the faeces.

Table 1. Published faecal outputs of bird species

Bird species	Average weight of individual faecal samples (grams)	Estimated weight of faeces per day (grams)	Reference
Black Swan		418 (wet) 52 (dry)	Mitchell and Wass, 1995
Starlings		40 (wet)	Feare (2001) cited in Wither et al. (2005)
Seagulls		50 (wet)	Wood and Trust (1972)
Whistling swans		317 (wet)	Hussong et al. (1979)
Canada geese		250 (wet)	Hussong et al. (1979)
Canada geese	8.35 (wet)		Alderiso and DeLuca (1999)
Canada geese	1.1 (dry)		Feare et al. (1999)
Ring billed gulls	0.48 (wet)		Alderiso and DeLuca (1999)

2.2 Microbial indicator counts for birds

Researchers have published a number of studies which enumerated faecal coliforms, *E. coli* and/or enterococci in a range of bird species (Table 2). Literature searches did not reveal studies on the enumeration of *E. coli* or enterococci in the Black Swan (*Cygnus atratus*) which inhabits Australia and New Zealand. Kuntz et al. (2004) noted that counts of *Enterococcus faecalis* were highly variable in individual wild bird faeces – some with high numbers, others had none. This finding is supported by the range of counts of bacteria in Canada geese as identified by Middelton and Ambrose (2005) (Table 2). Geldreich (1966) estimated the faecal load from Black Swans to be 1.1×10^{10} faecal coliform (FC) bacteria per day.

Table 2. Colony forming units (CFU) of selected microbial faecal indicators per gram of bird faeces

Bird species	Faecal coliforms	*E. coli*	Enterococci	Reference
Gull	$1.4 \times 10^{6\#}$		$1.8 \times 10^{5\,\#}$	Wood and Trust (1972)
Starlings		3.4×10^{8}		Wither et al. (2005)
Whistling swans	2.5×10^{6}			Hussong et al. (1979)
Canada geese	3.6×10^{4}			Hussong et al. (1979)
Canada goose	1.5×10^{4}			Alderiso and DeLuca (1999)
Canada goose		$0\text{-}10^{7}$	$10^{2}\text{-}10^{7}$	Middleton and Ambrose (2005)
Gull		$<1 \times 10^{5} - 10^{9}$	$<1 \times 10^{6} - 10^{8}$	Fogarty et al. (2003)
Ring-billed gulls	$10^{6} - >10^{10}$			Levesque et al. (2000)
Ring-billed gulls	3.7×10^{8}			Alderiso and DeLuca (1999)

- per gram of intestinal contents, all other results in excreted scat.

2.3 Pathogen identification in the faeces of birds

There is little information on the pathogen load carried by the Black Swan (*Cygnus atratus*). One paper does report the identification of *Cryptosporidium* in the faeces of birds in a zoo which included the Black Swan (Rohela et al., 2005). A pilot study of the pathogens present in the faeces of mute swans (*Cygnus olor*) did not identify any of the target pathogenic bacteria, which were listed as follows: *Shigella*, *Salmonella*, *Campylobacter*, *E. coli* O157:H7 or *Yersinia enterolitica* (Watabe et al., 2004).

Other species of wildfowl have been studied and are recognised to be potential sources of pathogenic microbes. Murphy et al. (2005) identified potentially pathogenic bacteria in the faeces of New Zealand ducks, including *Campylobacter jejuni*, *Bacillus cereus* and *Clostridium perfringens*, - all of which have all been implicated in cases of food poisoning. *Salmonella, Vibrio, Listeria* and *Campylobacter* including *C. jejuni* and *C. coli,* which are implicated in the majority of human cases of campylobacteriosis, have all been isolated from the faeces of gulls (*Laridae*) (Hatch, 1996). Canada geese can carry *Salmonella* and *Aeromonas* species potentially pathogenic to humans (Feare et al., 1999). Garczyk et al. (1998) identified *Giardia* and the human pathogenic species of *Cryptosporidium parvum* in the faeces of Canada geese. These examples of pathogenic microbes carried by wildfowl strongly suggest the role of birds as vectors of infectious disease. Furthermore, these findings support the need for an investigation of the potential of Black Swans to disseminate pathogenic microbes in the environment and subsequently contaminate shellfish beds.

3 ENUMERATION OF *E. COLI* AND ENTEROCOCCI IN BLACK SWAN FAECES

The initial hypothesis of this research was that the high levels of the microbial indicators *E. coli* detected in shellfish may be due to inputs from Black Swans that inhabit the environs of Golden Bay. Due to the scarcity of data available on levels of microbial indicators in the faeces of Black Swans (Table 2), we quantified levels of faecal coliforms (FC), *E. coli* and enterococci in Black Swan scats collected from the Nelson District. Enumeration was performed using Colilert (Idexx Laboratories Inc., Westbrook, ME, US) assays incubated at 44.5°C for FC and *E. coli*, and Enterolert (Idexx Laboratories Inc., Westbrook, ME, US) for counts of enterococci. Arithmetic means were calculated and 95% confidence intervals (CIs) determined using a bootstrapping approach.

The recently defecated scats were weighed, with an average mass of 44.9 grams with a 95% confidence interval range from 37.1-53.5g. Of the 46 fresh faecal scats analysed, more than 91% contained detectable levels of enterococci, with a mean of 474 enterococci per gram of faecal material (wet weight). Faecal coliforms and *E. coli* were detected in more than 89% of the scats with a mean level of 30,000 faecal coliforms per gram of faeces, and 26,000 *E. coli* per gram of faeces. These results are summarized in Table 3, with full results for individual scats presented in Appendix 1.

Table 3. Data collected on faecal scats of Black Swans during this study

	Number of Black Swan scats tested		
Count	46		
	Wet weight of faeces		
Mean	44.9 g		
95% CI*	37.1 – 53.5 g		
Range	14-141 g		
	Bacterial count (CFU per gram faeces)		
	Enterococci	Faecal coliforms	*E. coli*
Mean	4.7×10^2	3.0×10^4	2.6×10^4
95% CI*	1.5×10^2 - 9.7×10^2	5.6×10^3 - 7.0×10^4	1.8×10^3 - 6.8×10^4
Range#	5.0×10^0 - 8.7×10^4	5.0×10^0 - 8.0×10^5	5.0×10^0 - 8.0×10^5
	Bacterial count (CFU per scat)		
	Enterococci per scat	Faecal coliforms per scat	*E. coli* per scat
Mean	2.9×10^4	1.2×10^6	9.2×10^5
95% CI*	5.5×10^3 - 6.4×10^4	2.1×10^5 - 2.7×10^6	7.8×10^4 - 2.6×10^6

* CI = Confidence interval. # Range excludes values below the detection limit of the assays

This study was carried out in the winter month of July and therefore, seasonal variation may affect the counts obtained. Alderisio and DeLuca (1999) studied the seasonal variability of faecal coliform counts in the ring-billed gull and the Canada goose. Their study showed that faecal coliform concentrations for the ring-billed gull were stable (within the same averaged order of magnitude, 10^8) between seasons but that the Canada goose displayed greater variability including averages ranging from 10^3 to 10^7 between the two winter seasons during which data was collected. It may be important to enumerate microbial indicators from Black Swans throughout the seasons to identify if their microbial levels follow the same trend as the Canada geese.

3.1 Survival characteristics of the faecal indicators

The decay rate of faecal coliforms and enterococci is varied, and is dependent on the temperature and the environment in which they are present. In freshwater and autoclaved river water, the decay rate of faecal coliforms was found to be significantly lower than that of enterooccci (Medema et al., 1997; Anderson et al., 2005). While in sewage in an estuarine environment, the natural population of enterococci survived longer than the *E. coli* population (Lessard and Sieburth, 1983).

4 CONCLUSIONS

- The mean daily faecal output of individual *Cygnus atratus* has been estimated to be 418 grams fresh weight of faeces (Mitchell and Wass, 1995).
- Our data indicates a mean of 44.9 g per Black Swan scat. This would suggest an individual bird produces approximately 9 scats per day.
- Each gram of Black Swan scat is estimated to have an average of 30,000 faecal coliforms, 26,000 *E. coli,* and 474 enterococci.
- This equates to a mean daily output of 11 million faecal coliforms, 8.6 million *E. coli*, and 270,000 enterococci.

We conclude that Black Swans carry potentially significant levels of the traditional microbial indicators (FC, *E. coli* and enterococci) used to assess microbial quality in either water or shellfish samples. This study did not aim to assess the quantitative impact of Black Swan faecal material on the contamination of shellfish; however it has determined that Black Swans have the potential to be a source of faecal contamination of shellfish.

To more fully inform management decisions on the impact of Black Swans on microbial contamination of water and shellfish, future research could:

- Identify and enumerate pathogens in Black Swan scats – what is the health risk posed by Black Swan faeces?
- Determine the survival of *E. coli*, other indicators, and relevant pathogens in Black Swan faeces deposited directly on land, and in saline environments
- Enumerate microbial indicators from Black Swans in additional seasons to more accurately estimate variability in the presence and levels of these indicators.
- Identify source specific markers able to identify faecal contamination from Black Swans. This would aid the discrimination between microbial indicators derived from Black Swans and those from other faecal sources.

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6 APPENDIX 1 - MICROBIAL INDICATORS IN INDIVIDUAL BLACK SWAN FAECES

Collection Date	Weight (g)	Faecal coliforms	*E. coli*	Enterococci
09/07/07	24.49	3320	3320	410
	25.27	3890	740	20.5
	14.22	<5	<5	91.5
	35.72	12230	610	42.5
	19.16	121	109	126.5
	47.88	<5	<5	90.5
	25.32	110	110	50
	16.11	155307	155307	69
	62.37	160	160	5
	23.94	5630	1180	40
	53.36	199	199	30
	22.1	402	382	50
	16.33	10500	8360	61
	22.37	<5	<5	177
	36.76	3880	3680	5
16/07/07	50.49	20	20	40.5
	52.25	10462	748	80
	28.91	173287	173287	1191
	23.5	3968	645	40
	39.78	19863	1624	125
	88.2	77010	9600	365
	83.41	5	5	<5
	93.44	1162	906	25
	147.48	594	426	4902
	73.17	31	31	5
	82.38	5172	2382	<5
	42.2	54750	12340	450.5
	30.77	5794	675	8664.5
	31.74	4884	845	102
	96.14	3130	359	195
	56.32	530	31	<5
23/07/07	51.18	1650	50	10
	33.11	3255	3255	486
	26.07	213	20	5
	43.86	<5	<5	45
	56.66	1956	1223	91
	31.41	10	10	60.5
	39.58	749	677	25
	32.73	<5	<5	25
	32.17	437	70	<5
	33.41	41	31	50
	39.64	798500	798500	2333
	39.26	1722	1722	10
	43.35	9208	8664	594
	51.64	1046	1046	50
	43.12	4160	4160	564

All microbial results Most Probable Number (MPN) per gram of faeces.