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NOTE

Ichthyophonus in sport-caught groundfishes from southcentral Alaska

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ABSTRACT: This report of *Ichthyophonus* in common sport-caught fishes throughout the marine waters of southcentral Alaska represents the first documentation of natural *Ichthyophonus* infections in lingcod *Ophiodon elongates* and yelloweye rockfish *Sebastes ruberrimus*. In addition, the known geographic range of *Ichthyophonus* in black rockfish *S. melanops* has been expanded northward to include southcentral Alaska. Among all species surveyed, the infection prevalence was highest (35%, n = 334) in Pacific halibut *Hippoglossus stenolepis*. There were no gross indications of high-level infections or clinically diseased individuals. These results support the hypothesis that under typical conditions *Ichthyophonus* can occur at high infection prevalence accompanied with low-level infection among a variety of fishes throughout the eastern North Pacific Ocean, including southcentral Alaska.

KEY WORDS: *Ichthyophonus* · Pacific halibut · Lingcod · Rockfish · Pacific cod · Alaska

INTRODUCTION

Ichthyophonus spp. are mesomycetozoean parasites that have been reported in more than 145 fish hosts throughout the world (Gregg et al. 2016); however, some of these reports likely involve misidentifications, as other parasite(s) have been incorrectly reported as *Ichthyophonus* in several species of amphibians (Conway et al. 2015). High-severity infections have been associated with recurring epizootics in several commercially and recreationally important marine and freshwater fishes, including Atlantic and Pacific herring *Clupea harengus* and *C. pallasii*, respectively, mackerel *Scomber scombrus*, yellowtail flounder *Pleuronectes ferruginea*, Chinook salmon *Oncorhynchus tshawytscha*, American shad *Alosa sapidissima*, and several species of rockfish (Kent et al. 2001, McVicar 2011, Burge et al. 2014). Gross signs of the disease vary among host species and may include white nodular lesions on the heart and other internal organs, ulcers in skeletal muscle, and 'sandpaper' skin — often accompanied by black ulcers on the epidermis. Sublethal effects may include reduced growth, reduced swimming stamina, and compromised overall fish health (McVicar 2011). The parasite genus contains several morphologically indistinguishable genotypes (Gregg et al. 2016), which may separate into distinct species. Neither distinguishing characteristics between species nor consensus on species nomenclature have been fully resolved; therefore, the parasite will hereafter be referred to generically as *Ichthyophonus*.

Although *Ichthyophonus* is known to infect many marine fishes, its known host distribution in the

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marine waters of Alaska is limited to Pacific herring in the Gulf of Alaska, walleye pollock *Gadus chalcogrammus* in the Gulf of Alaska and Bering Sea, Chinook salmon in the Yukon and Kuskokwim rivers, and Pacific halibut *Hippoglossus stenolepis* in Prince William Sound, Cook Inlet, and the eastern Bering Sea (Eaton et al. 1991, Marty et al. 1998, 2010 Kocan et al. 2004, White et al. 2014, Gregg et al. 2016, Hershberger et al. 2018). The presence of *Ichthyophonus* in these fishes, all of which are important prey species at some point in their life histories, suggests that many other predatory fishes in the region are likely exposed to the parasite through trophic interactions. Further, *Ichthyophonus* infections are common in several rockfishes *Sebastes* spp. off the coasts of Oregon and Washington (Kent et al. 2001); however, its occurrence in these species in Alaskan waters has not yet been reported. The objective of this study was to determine the prevalence of *Ichthyophonus* in common sport-caught groundfishes in southcentral Alaska.

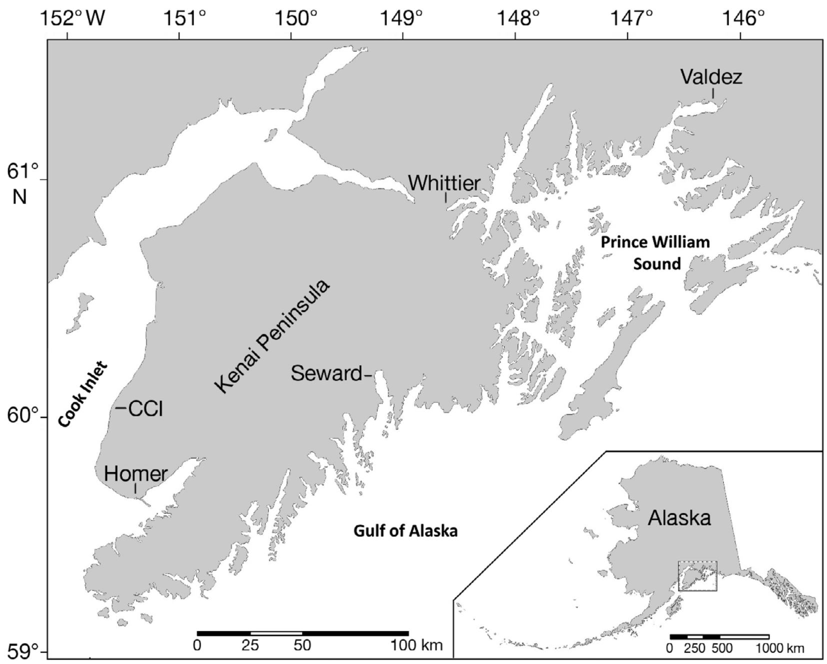

Fig. 1. Fishes were sampled at the ports of Homer, Central Cook Inlet (CCI), Seward, Whittier, and Valdez. Inset shows the location of the sampling areas in southcentral Alaska

MATERIALS AND METHODS

The prevalence of *Ichthyophonus* infection in fishes from the marine regions of southcentral Alaska was assessed by sampling sport-caught specimens at municipal fish processing stations in the ports of Homer, Seward, Valdez, Whittier, and from Central Cook Inlet (CCI) near Ninilchik and Deep Creek (Fig. 1). Samples were collected after anglers were finished cleaning fish, but before the carcasses were discarded. Data from each fish included species identification, fork length (±1 cm), sex, and *Ichthyophonus* infection status (described below). All sampling occurred at these ports simultaneously between 24 and 28 August 2011.

Ichthyophonus infection prevalence was determined by tissue explant culture. Heart tissue (approx. 0.5 g) was collected from fish carcasses for *in vitro Ichthyophonus* culture. Only fish with intact pericardial cavities were sampled, and sampling tools were sterilized between fish to avoid cross-contamination (LaPatra et al. 2008). The tissue was placed into 5 ml of Eagle's minimum essential medium supplemented with fetal bovine serum (5% v/v), penicillin (100 IU ml^{-1}), streptomycin (100 µg ml^{-1}), gentamycin (100 µg ml^{-1}), and buffered to a pH of 7.8 with 1 M Tris (Hershberger et al. 2002). Samples were refrigerated for 12–72 h before being incubated for 4 wk at 16–18°C. Cultures were examined microscopically (×40–100 magnification) after 7, 14, and 28 d of incubation for the presence of *Ichthyophonus* life stages. A total of 5 reads were performed on each tissue sample, and samples were considered positive if *Ichthyophonus* stages including schizonts and germinating bodies were detected in at least 2 reads[1]. Infection prevalence (the percentage of samples testing positive) and the corresponding binomial proportion 95% confidence interval was calculated for each port and species; in addition, the prevalence in Pacific halibut — the only species sampled in all 5 ports — was compared between ports. Statistical comparisons were performed using a χ^2 test where sample sizes were ≥30 individuals per species. Prior to initiating the tissue explant cultures, hearts were

[1] This protocol (multiple reads with >1 = positive infection) was employed because the readers were university students with *Ichthyophonus* microscopy experience limited to training by US Geological Survey staff for the purposes of this study.

examined macroscopically for gross signs of ichthyophoniasis, including white nodules on the surface; infections were scored as high severity when gross signs were observed, and low severity when explant cultures were positive but gross signs were not observed.

RESULTS

Of the 668 fishes sampled from the 5 ports, 12% (n = 83) of the *Ichthyophonus* cultures were unreadable because of overgrowth of mold and/or yeast contaminants; results presented here are based on observations from the uncontaminated cultures (n = 585). *Ichthyophonus* was detected in fishes from all 5 ports and in 5 of the 10 species examined, including yelloweye rockfish *Sebastes ruberrimus* (5%, n = 20), lingcod *Ophiodon elongates* (16%, n = 45), black rockfish *S. melanops* (9%, n = 56), Pacific cod *Gadus macrocephalus* (9%, n = 58), and Pacific halibut (35%, n = 334). The parasite was not detected in dusky rockfish *S. ciliates* (n = 46), canary rockfish *S. pinniger* (n = 2), copper rockfish *S. caurinus* (n = 2), silvergray rockfish *S. brevispinis* (n = 4), or quillback rockfish *S. malinger* (n = 18; Table 1). The combined infection prevalence in all species varied by port (17–44%) and was strongly influenced by the proportion of Pacific halibut in each sample (Table 1). Samples from CCI had the highest *Ichthyophonus* prevalence (44 ± 10.1%). In Pacific halibut — the only species examined in all 5 ports — infection prevalence varied (26–45%) but was not significantly different among ports ($\chi^2 = 6.89$, df = 4, $p = 0.14$). Among infected fishes with n ≥ 30 samples (Pacific halibut, Pacific cod, black rockfish, and lingcod), *Ichthyophonus* prevalence did not vary by sex ($p > 0.8$; Table 2). The minimum, mean and maximum fork lengths of the fishes sampled in this study are provided by port and species in Table 3 to support comparisons with other studies.

DISCUSSION

This report represents the first documentation of natural *Ichthyophonus* infections occurring in lingcod and yelloweye rockfish; in addition, the known geographic range of *Ichthyophonus* in black rockfish has been expanded northward to include southcentral Alaska. The results further expand on earlier documentation of *Ichthyophonus* in Pacific halibut in the region, which indicated 20–30% infection prevalence in lower Cook Inlet and Kachemak Bay, AK (Grenier 2014).

Table 2. *Ichthyophonus* infection prevalence in 4 species of sport-caught fishes from southcentral Alaska with total sample sizes ≥30. Total fishes sampled (n), *Ichthyophonus* prevalence (prev, %), the 95% confidence interval (±) by species and sex

Species	Male			Female			χ^2	df	p
	n	Prev	±	n	Prev	±			
Lingcod	13	8	14.5	39	15	11.3	0.048	1	0.83
Black rockfish	27	7	9.9	35	11	10.5	0.009	1	0.93
Pacific cod	29	7	9.2	34	9	9.5	0.032	1	0.86
Pacific halibut	113	35	8.8	217	35	6.4	0.0002	1	0.99

Table 1. *Ichthyophonus* infection prevalence in 10 species of sport-caught fishes from southcentral Alaska. Total fishes sampled (n), *Ichthyophonus* prevalence (prev, %) and the 95% confidence interval (±) by species and port. CCI: Central Cook Inlet

Species	CCI			Homer			Seward			Valdez			Whittier			All ports		
	n	Prev	±	n	Prev	±	n	Prev	±	n	Prev	±	n	Prev	±	n	Prev	±
Canary rockfish	0	–	–	0	–	–	0	–	–	0	–	–	2	0	0.0	2	0	0.0
Copper rockfish	0	–	–	0	–	–	0	–	–	1	0	0.0	1	0	0.0	2	0	0.0
Silvergray rockfish	0	–	–	0	–	–	0	–	–	0	–	–	4	0	0.0	4	0	0.0
Quillback rockfish	0	–	–	0	–	–	1	0	0.0	0	–	–	17	0	0.0	18	0	0.0
Yelloweye rockfish	0	–	–	0	–	–	2	0	0.0	3	33	53.3	15	0	0.0	20	5	9.6
Dusky rockfish	0	–	–	39	0	0.0	7	0	0.0	0	–	–	0	–	–	46	0	0.0
Lingcod	0	–	–	2	0	0.0	17	6	11.2	25	24	16.7	1	0	0.0	45	16	10.6
Black rockfish	0	–	–	3	0	0.0	49	12	9.2	2	0	0.0	2	0	0.0	56	9	7.5
Pacific cod	0	–	–	5	0	0.0	45	11	9.2	0	–	–	8	0	0.0	58	9	7.2
Pacific halibut	93	44	10.1	126	26	7.7	41	39	14.9	36	27	14.3	38	45	15.8	334	35	5.1
All species	93	44	10.1	175	19	5.8	162	17	5.8	67	25	10.3	88	19	8.2	585	23	3.4

Table 3. The minimum, mean and maximum fork lengths (cm), by species and port, of 10 species of southcentral Alaska sport-caught fishes sampled for *Ichthyophonus*. CCI: Central Cook Inlet

Species	CCI			Homer			Seward			Valdez			Whittier			All ports		
	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.	Min.	Mean	Max.
Canary rockfish	–	–	–	–	–	–	–	–	–	–	–	–	67	70.5	74	67	70.5	74
Copper rockfish	–	–	–	–	–	–	–	–	–	34	34	34	36	36	36	34	35.0	36
Silvergray rockfish	–	–	–	–	–	–	–	–	–	–	–	–	31	39.0	46	31	39.0	46
Quillback rockfish	–	–	–	–	–	–	40	40	40	–	–	–	33	38.8	45	33	38.8	45
Yelloweye rockfish	–	–	–	–	–	–	43	53.5	64	32	49.7	60	40	50.1	59	32	50.4	64
Dusky rockfish	–	–	–	29	37.3	44	34	37.7	43	–	–	–	–	–	–	80	105.6	129
Lingcod	–	–	–	93	93.0	93	80	109.3	123	89	104.5	129	93	93	93	29	37.3	44
Black rockfish	–	–	–	45	46.7	48	38	47.0	59	59	61.5	64	31	33.5	36	31	47.0	64
Pacific cod	–	–	–	62	69.6	75	54	65.3	81	–	–	–	63	71.6	83	54	66.6	83
Pacific halibut	65	85.1	152	62	80.5	119	59	78.5	120	63	84.9	147	58	82.5	105	58	82.2	152

The relatively high prevalence of *Ichthyophonus* in Pacific halibut (35%, n = 334) and lingcod (16%, n = 45) throughout the study region may be a reflection of the piscine generalist feeding strategies of these species. *Ichthyophonus* is easily transmitted to susceptible hosts through the consumption of infected fish tissues (Kocan et al. 2010). Throughout the eastern North Pacific Ocean, Pacific herring (Hershberger et al. 2016) and other forage species often demonstrate high infection prevalence and therefore represent a likely source of infection for piscivorous fishes, including Pacific halibut (Roseneau & Byrd 2000, Orlov & Moukhametov 2007) and lingcod (Cass et al. 1990, Tinus 2008). In contrast, the relatively low prevalence of *Ichthyophonus* in Pacific cod (9%, n = 58) may reflect reduced exposure to the parasite via the dominance of invertebrates in their diets (Urban 2012).

Lack of any apparent association between *Ichthyophonus* infection prevalence and Pacific halibut sex (Table 2) contrasts with a previously published report of the parasite in this host, where the infection prevalence was significantly greater in females (Hershberger et al. 2018). Reasons for this discrepancy remain unresolved, but may involve differences in geographical scale between the 2 studies; Hershberger et al. (2018) reported patterns over a much broader study area, including the eastern North Pacific Ocean and Bering Sea. Analogous differences in infection prevalence occur between male and female Atlantic herring (Kramer-Schadt et al. 2010) but not Pacific herring (Hershberger et al. 2016).

Complex and poorly understood epizootiological relationships between rockfishes and *Ichthyophonus* are indicated by dramatic differences in host infection prevalence and parasite genotypes throughout the eastern North Pacific Ocean. For example, high infection prevalence occurs in some species throughout Oregon, Washington, and British Columbia including Pacific Ocean perch *Sebastes alutus* (48%) and yellowtail rockfish (51%; Kent et al. 2001); however, low infection prevalence occurs among sympatric species including canary rockfish (5%), yellowmouth rockfish (10%; Kent et al. 2001), and Puget Sound rockfish *S. emphaeus* (11%; Halos et al. 2005). An analogous low prevalence of infection occurred among all rockfishes surveyed throughout southcentral Alaska (Table 1), though inferences about *Ichthyophonus* prevalence in rockfishes based on these data are limited by small sample sizes. Interspecies differences in infection prevalence possibly result from differences in host species susceptibility, life history characteristics, and/or diet differences. In addition, differences in parasite tissue tropisms may also account for the apparent low prevalence in some rockfishes, as the parasite sometimes occurs in rockfish livers more often than in hearts, which were evaluated in this study (Kent et al. 2001, Halos et al. 2005).

Evaluation of the impacts to the host was beyond the scope of this study; however, there was no indication of overt disease in any sampled fishes. Although the infection prevalence in Pacific halibut (35%) was relatively high, the documented infection severity in this host is typically very low (Grenier 2014, Hershberger et al. 2018). In addition, neither internal nor external gross signs, indicative of heavy infections, were detected in any of the fishes examined in this study. These observations support the hypothesis that *Ichthyophonus* often occurs as a chronic infection in Pacific halibut (Hershberger et al. 2018) and other marine hosts, causing little or no apparent harm under typical conditions. However, in response to poorly understood environmental conditions that likely involve periods of punctuated exposure and high population densities, overt ichthyophoniasis epizootics can occur in association with massive fish kills (reviewed in McVicar (2011) and Burge et al. (2014)), emphasizing the need to continue to monitor *Ichthyophonus* prevalence and infection severity throughout its known host and geographic range.

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