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Ichthyophonus-infected walleye pollock *Theragra chalcogramma* (Pallas) in the eastern Bering Sea: a potential reservoir of infections in the North Pacific

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Abstract

In 2003, the Alaska walleye pollock industry reported product quality issues attributed to an unspecified parasite in fish muscle. Using molecular and histological methods, we identified the parasite in Bering Sea pollock as *Ichthyophonus*. Infected pollock were identified throughout the study area, and prevalence was greater in adults than in juveniles. This study not only provides the first documented report of *Ichthyophonus* in any fish species captured in the Bering Sea, but also reveals that the parasite has been present in this region for nearly 20 years and is not a recent introduction. Sequence analysis of 18S rDNA from *Ichthyophonus* in pollock revealed that consensus sequences were identical to published parasite sequences from Pacific herring and Yukon River Chinook salmon. Results from this study suggest potential for *Ichthyophonus* exposures from infected pollock via two trophic pathways; feeding on whole fish as prey and scavenging on industry-discharged offal. Considering the notable *Ichthyophonus* levels in pollock, the low host specificity of the parasite and the role of this host as a central prey item in the Bering Sea, pollock likely serve as a key *Ichthyophonus* reservoir for other susceptible hosts in the North Pacific.

Keywords: Alaska, Bering Sea, Chinook salmon, *Ichthyophonus*, offal, walleye pollock.

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Introduction

Many pathogens of wild fish species do not directly impact fishing industries; however, some are capable of affecting the product quality, mortality rates, recruitment and host stock fluctuations of commercially important species (Sindermann 1990). Members of the genus *Ichthyophonus* are cosmopolitan parasites of economic significance in both wild and cultured fisheries (McVicar 2011) and for some hosts can be transmitted trophically through feeding on infected prey (Jones & Dawe 2002; Prabhuji & Sinha 2009). *Ichthyophonus* spp. infections have been identified in over 100 marine and freshwater hosts with varying disease signs and severity (Rahimian & Thulin 1996; McVicar 2011). The majority of worldwide reports from this genus have been identified as *Ichthyophonus hoferi* (Plehn & Mulsow), the type species, originally isolated from rainbow trout in Europe (Plehn & Mulsow 1911; McVicar 2011). There are indications, however, that this species may represent a species assemblage of closely related organisms that has yet to be resolved (Spanggaard *et al.* 1996; Rasmussen *et al.* 2010); therefore, this protist will hereinafter be referred to by the genus name, *Ichthyophonus*.

In the North Pacific and adjacent rivers, *Ichthyophonus* has been identified in populations of numerous commercially important fish species including walleye pollock, *Theragra chalcogramma* (Pallas), Pacific herring *Clupea pallasii* Valenciennes and multiple rockfish and salmon species (Morado & Sparks 1990; Eaton, Kent & Meyers

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1991; Criscione *et al.* 2002; Kocan, Hershberger & Winton 2004; Tierney & Farrell 2004; White *et al.* 2013). Many of these susceptible hosts are distributed throughout the Bering Sea; however, in this northernmost region of the Pacific Ocean, no published reports document *Ichthyophonus* in any wild fish population. It has been suggested that the Aleutian Islands, bordering the Bering Sea to the south, may form a barrier to the spread of the pathogen (Kocan *et al.* 2004); however, this hypothesis needs further investigation.

Ichthyophonus has been reported in anadromous fish from rivers that discharge into the Bering Sea, such as the Yukon and Kuskokwim Rivers (Kocan *et al.* 2004). In the Yukon River, increased prespawning mortality and seafood product losses of Chinook salmon *Oncorhynchus tshawytscha* (Walbaum) have been attributed to this parasite (Kocan *et al.* 2004). A 12-year epizootiological study of *Ichthyophonus* infections in these fish revealed that parasite prevalence was synchronous with host population size, leading Zuray, Kocan & Hershberger (2012) to propose that a critical prey item could be contributing to infections. Chinook salmon smolts out-migrating from the Yukon River into the Bering Sea appear to be free of *Ichthyophonus*, suggesting that they acquire the parasite from infected prey in their ocean phase (Kocan *et al.* 2004). Pacific herring was initially suspected as an infection source because (i) it is a significant component of Chinook salmon diets (Davis *et al.* 2009b); (ii) it is a known host of *Ichthyophonus* in Alaskan waters (Marty *et al.* 1998); and (iii) in laboratory trials, feeding of infected Pacific herring tissue successfully transmitted the parasite to Chinook salmon (Jones & Dawe 2002). However, results from multiple surveys revealed an apparent absence of *Ichthyophonus* in numerous Pacific herring samples from the Bering Sea (Kocan *et al.* 2004). Currently, the source of infections in Yukon River Chinook salmon remains a mystery.

Walleye pollock, hereinafter referred to as pollock, is another major prey species of ocean-phase Chinook salmon (Davis *et al.* 2009b). Pollock also harbour *Ichthyophonus* infections (Morado & Sparks 1990); however, little is known about the parasite in this host. Low levels of *Ichthyophonus* were found in pollock south of the Aleutian Islands over 25 years ago (Morado & Sparks 1990; Eaton *et al.* 1991), and in a recent study, infections in Alaskan pollock were reported, but without distribution data (White *et al.* 2013). Nevertheless, the presence of this parasite in pollock has broad implications. Pollock is not only a key prey item linking many marine predators in Alaskan waters (Livingston 1993), but is also the target of one of the largest fisheries in the world (Shen *et al.* 2008). A portion of the pollock caught in Alaska is processed and inspected at sea (Wilen & Richardson 2008), and fish waste material, offal, is discharged into the ocean (Bluhm & Bechtel 2003). Offal can provide an alternate food source for various fish species including Chinook salmon (Lang, Livingston & Dodd 2005; Buser *et al.* 2009; Davis, Myers & Fournier 2009a); however, consumption of unpasteurized fish waste could also influence the spatial and temporal distribution of trophically transmitted parasites. The low host specificity of *Ichthyophonus* and its ability to infect marine, freshwater and anadromous hosts suggests potential for linkages between ocean and adjacent river systems.

The primary fishing grounds for pollock are located in the eastern Bering Sea, where approximately 1.2 million tonnes of pollock are caught annually (Ianelli *et al.* 2011). During industry quality inspections, an unspecified *Ichthyophonus*-like parasite was visualized in pollock flesh[1] and is believed to adversely affect seafood product quality by altering the flavour and texture of fillets (K. Burger, personal communication, 10 May 2006). The industry-based practice of screening fillets using backlit conveyor belts is called candling; this is a rapid method for determining parasite load, but it is not specific for identifying parasite taxa (Martin & Collette 1997; Shahidi, Jones & Kitts 1997). With the development of multiple methods for detecting members of the genus *Ichthyophonus* [i.e. *in vitro* culture, histology, conventional polymerase chain reaction (cPCR) and quantitative PCR (qPCR) (Kocan *et al.* 1999; Whipps *et al.* 2006; White *et al.* 2013)], visual observation, molecular identification and prevalence estimation of this parasite in Bering Sea pollock are possible.

Based on anecdotal information from the seafood industry, confirmed reports of pollock as a susceptible host (Morado & Sparks 1990; Eaton *et al.* 1991; White *et al.* 2013) and the central role of pollock in the Bering Sea food web (Dwyer, Bailey & Livingston 1987), we hypothesize that eastern Bering Sea pollock harbour *Ichthyophonus* infections and are a potential source of infections for other susceptible North Pacific fish hosts. The objectives of this study were to (i) identify the *Ichthyophonus*-like organism infecting

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pollock in the Bering Sea, (ii) estimate infection prevalence and distribution in eastern Bering Sea pollock, (iii) obtain an historical perspective on *Ichthyophonus* in age-0 pollock in Alaska and (iv) investigate the existence of *Ichthyophonus* in offal samples collected from the stomachs of ocean-stage Chinook salmon. Objective results were then used to reflect on the potential role of *Ichthyophonus* in pollock populations and speculate whether pollock could act as an *Ichthyophonus* reservoir.

Materials and methods

Sample collection

Between the months of June and July in 2006 and 2007, pollock were collected from 71 stations during NMFS annual eastern Bering Sea groundfish stock assessment surveys. Pollock were captured from a depth range of 21–174 m on the Bering Sea shelf using bottom trawl gear operated by the crew of chartered fishing vessels F/V *Northwest Explorer* and F/V *Arcturus*. Pollock collected in 2006 ($n = 83$) and 2007 ($n = 221$) ranged in size from 90 to 840 mm fork length.

Two to six individuals were collected daily, and the weight, length and sex for each were recorded. Five separate tissue samples were targeted from each fish sampled. A portion of heart and a portion of subdorsal skeletal muscle were excised aseptically from each fish. Each tissue was split into two samples and preserved separately: one in 100% ethanol for molecular analysis and the other in 10% sodium acetate-buffered formalin for histological processing. Otoliths were preserved in ethanol, and fish age was later determined by the AFSC's Age and Growth staff in Seattle, Washington.

Ichthyophonus identification by sequence analysis

We used histology (see Methods below) to visualize *Ichthyophonus*-like schizonts from the pollock samples collected. From the infected individuals, two pollock from the eastern Bering Sea (Fig. 1) were selected for parasite sequencing and DNA was extracted from corresponding ethanol-preserved skeletal muscle samples following the procedure mentioned in the study by White *et al.* (2013). Briefly, DNA was extracted using a single-column DNeasy®2 kit (Qiagen) following the manufacturer's protocol, except that DNA was eluted in 100 μL Buffer AE. Fragments of *Ichthyophonus* 18S rDNA (1530 bp) were generated from the extracted DNA by cPCR using primers PIchF1 and PIchR3 (White *et al.* 2013). For reference, Region B

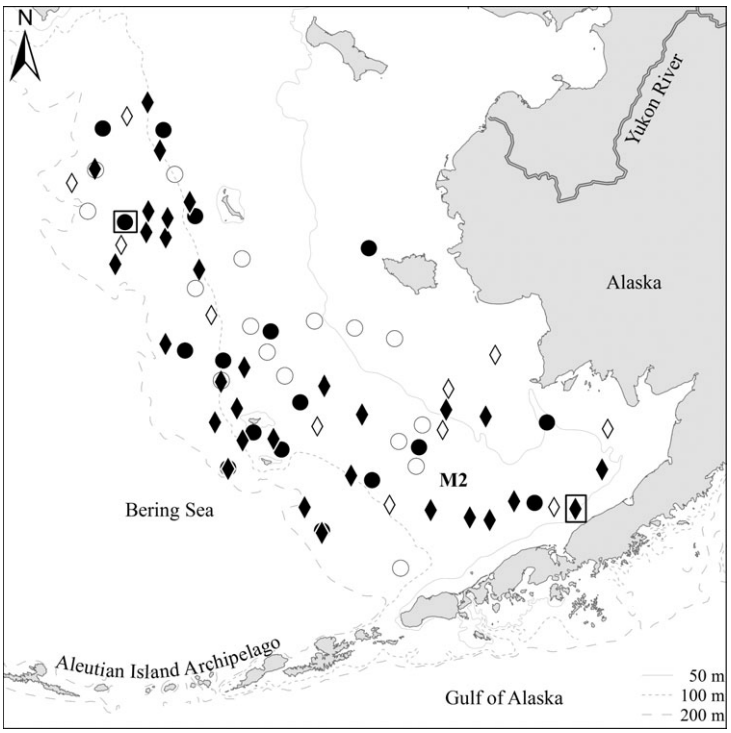

Figure 1 Walleye pollock (*Theragra chalcogramma*) bottom trawl capture stations in 2006 (circles) and 2007 (diamonds); solid markers indicate stations where *Ichthyophonus*-infected fish were identified by histology and/or PCR. Box-outlined markers indicate stations where *Ichthyophonus* 18S rDNA was sequenced. M2 identifies the site of Bering Sea oceanographic mooring 2 used for determining ice retreat index.

(Criscione *et al.* 2002) is completely contained within the fragment generated by primers PIchF1/PIchR3. Each 50-μL reaction mixture consisted of 0.05 U μL^{-1} MyTaq HS DNA polymerase (Bioline), 1× MyTaqHS PCR buffer (dNTPs and $MgCl_2$ included in buffer; Bioline) and 0.4 μM of each primer. The following were thermal cycling reaction conditions: *Taq* activation for 3 min at 95 °C, followed by 35 amplification cycles at 95 °C for 15 s, 62 °C for 15 s and 72 °C for 30 s. PCR products were purified (QIAquick PCR Purification Kit®; Qiagen), inserted into a pCR®2.1-TOPO® plasmid vector and cloned in TOP10 *Escherichia coli* (TOPO-TA Cloning® Kit; Invitrogen) according to the manufacturer's protocol. Clones were selected and amplified via cPCR using M13 forward and reverse primers following the manufacturer's protocol (TOPO-TA Cloning® Kit; Invitrogen). A total of 20 clones, 10 clones from each fish, were purified (QIAprep Spin Miniprep® Kit; Qiagen) and sequenced in both the forward and reverse directions using three sets of primers at the University of Washington High Throughput Genomics Unit. Sequence outputs and chromatograms were viewed and aligned using Geneious 5.3.6 (Drummond *et al.* 2010). Geneious and ClustalX 2.0 (Larkin *et al.* 2007) were used to perform multiple sequence alignments of 18S rDNA with other known isotypes of *Ichthyophonus* and other members of the class Ichthyosporea. This process was repeated using primers GO1 and Ich2R (Region A; Criscione *et al.* 2002) on *Ichthyophonus* from one of the two fish described previously, resulting in eight clones; the fragment generated from Region A is 661 bp and overlaps with the fragment generated from primers PIchF1 and PIchR3 by approximately 481 bp.

Ichthyophonus detection and prevalence estimation in the eastern Bering Sea

Three diagnostic tests (histology, cPCR and qPCR) were employed to detect *Ichthyophonus* in pollock heart and skeletal muscle samples. Combined results from these diagnostic tests were used to estimate *Ichthyophonus* prevalence in the eastern Bering Sea in 2006 and 2007.

For histological visualization of *Ichthyophonus*, formalin-fixed tissues of heart and skeletal muscle were processed following standard procedures (Sheehan & Hrapchak 1980). Deparaffinized tissue sections (4 μm) were stained with haematoxylin and eosin (Luna 1968). The entire tissue section of each tissue type was examined by light microscopy for the presence of *Ichthyophonus*.

To detect *Ichthyophonus* DNA by cPCR, extracted DNA from fish tissues was tested with the Whipps *et al.'s* (2006) *I. hoferi*-specific cPCR assay using primers Ich7f/Ich6r, following published methods. To confirm the presence of amplifiable DNA extracted from each fish sample, a cPCR was performed with 18S rDNA universal forward and reverse primers CS1/CAS2 (Le Roux *et al.* 1999). Reaction mixtures were prepared in 20-μl volumes and consisted of 1× GoTaq Flexi PCR buffer (Promega), 2.5 mM $MgCl_2$, 0.2 mM dNTP, 25 pmol each primer and 0.025 U μL^{-1} *Taq* DNA polymerase and 0.8 μL of template DNA. Reactions were carried out using an MJ Research DNA Engine PTC-200, with initial denaturation for 5 min at 94 °C, followed by 30 amplification cycles: 1 min at 94 °C, 1 min at 55 °C and 1 min at 72 °C, followed by a final adenylation at 72 °C for 10 min. PCR products were separated by electrophoresis on a 1–2% agarose gels, stained with ethidium bromide and visualized using UV illumination.

To detect *Ichthyophonus* DNA using qPCR, reaction mixtures were prepared with the extracted DNA samples (above) following the protocol mentioned in the study by White *et al.* (2013). Briefly, qPCRs were prepared in 25-μL volumes consisting of 2 μL extracted DNA template, *Ichthyophonus*-specific primers and probe (400 nM vc7F and vc5R primers, 300 nM 6-FAM-labelled probe ICH27), 15 μg bovine serum albumin, 12.5 μL 2× SensiMix II Probe mastermix (Bioline) and deionized H_20. Each 96-well reaction plate comprised 7 serially diluted standards in triplicate, unknown samples in duplicate and six no-template controls. The qPCR assay was conducted on a Mx3000P™ Real-Time PCR System (Agilent Technologies, Inc.) or a CFX96™ Real-Time PCR Detection System (Bio-Rad Laboratories, Inc.), using the manufacturers' software. On both instruments, *Taq* was activated for 10 min at 95 °C, followed by 40 amplification cycles at 95 °C for 30 s, 60 °C for 1 min and 72 °C for 1 min. Unknown samples were considered positive if the average quantity of replicates was >3 copies (White *et al.* 2013).

To estimate *Ichthyophonus* prevalence in eastern Bering Sea pollock in 2006 and 2007, individuals were scored as infected or uninfected based on combined test results from both tissue types. Pollock

with at least one positive diagnostic test result for *Ichthyophonus* were considered infected; fish with all negative test results were considered uninfected. For management purposes, Bering Sea pollock within the United States Exclusive Economic Zone are separated into three stocks: eastern Bering Sea, Aleutian Islands and Bogoslof (Ianelli *et al.* 2010). Pollock were collected for this study from only the eastern Bering Sea region; therefore, they were treated as a single binomial population consisting of infected and uninfected individuals, although it is recognized that there may be multiple subpopulations within this region (Ianelli *et al.* 2010). Within this population, groups were defined as male, female, adult, juvenile and categorized by fish fork length and fish weight. Pollock were considered to be juveniles if they were age 3 and under, while age 4 and older fish were categorized as adults (Duffy-Anderson *et al.* 2003). Prevalence differences between groups were determined using a 2×2 chi-square. For prevalence estimates, binomial proportion confidence intervals were calculated at a 95% confidence level. All data were analysed using SYSTAT 13 statistical software package (Systat Software, Inc.).

Historical *Ichthyophonus* prevalence in age-0 pollock

Archived unpublished pathology data records were obtained from National Marine Fisheries Service, Alaska Fisheries Science Center, Fisheries Resources Pathobiology Team. Data included juvenile pollock size (standard length), collection location and various anomaly identifications including *Ichthyophonus* infection status determined by histological visualization as described by Morado & Sparks (1990). Collections include age-0 pollock (age determined by standard length) captured by anchovy trawl in the eastern Bering Sea in 1994 and the Gulf of Alaska in 1986, 1987, 1988, 1990 and 2001.

Ichthyophonus detection in offal from Chinook salmon stomach contents

Buser *et al.* (2009) obtained stomach contents of Chinook salmon captured in the eastern Bering Sea from the winter 2007 pollock fishery. The stomach contents were identified as offal (e.g. fish body parts and pieces discarded after processing); Buser *et al.* (2009) extracted DNA from this offal and definitively identified most of the samples as pollock by sequencing. Archived samples of extracted DNA (20-9A, 48-13A, 50-7A, 52-3A, 52-4, 59-16, 60-19, 84-16) retained from the work of Buser *et al.* (2009) were kindly donated by Isadora Jimenez (University of Washington) and screened for the presence of *Ichthyophonus* DNA using the qPCR assay method described previously.

Results

Ichthyophonus identification by sequence analysis

Sequence analysis of the 18S rRNA gene of the *Ichthyophonus*-like organism found in eastern Bering Sea pollock revealed that consensus sequences from 2006 Region A fragments (GenBank KC500080 – KC500087) and PIchF1/PIchR3 fragments (GenBank JX509908 – JX509918) and 2007 PIchF1/PIchR3 fragments (GenBank KC500070 – KC500079) were identical to the *Ichthyophonus* sequences (Regions A and B) obtained from Pacific herring collected in British Columbia and Puget Sound, Washington; Chinook salmon from the Yukon River, Alaska; and cultured freshwater rainbow trout *Oncorhynchus mykiss* (Walbaum) from Buhl, Idaho (Criscione *et al.* 2002; Hershberger *et al.* 2008). [Correction added on 19 September 2013, after first online publication: The Genbank numbers for 2006 Region A fragments and 2007 PIchF1/PIchR3 fragments were amended.] Each of the 10 individual clones sequenced from each of the two eastern Bering Sea pollock was unique, with up to six single-point mutations per clone relative to the consensus sequence generated from each fish. Nearly all mutations were transitions; only one clone of 10 for each fish contained a single indel. Additionally, two separate preparations of one clone (1530 bp PIchF1/PIchR3 fragment) were sequenced side by side, resulting in identical sequences (GenBank JX509911 and JX509912). Although it appears as though the clonal mutations were not consistent and were uninformative (with consensus sequences conforming to the Pan-Pacific 18S type), this amount of variation in the 18S clones is unusual for such a highly conserved region.

Ichthyophonus prevalence in the eastern Bering Sea

Ichthyophonus-infected pollock were identified throughout the range of the sampling area in the eastern Bering Sea in both 2006 and 2007

(Fig. 1). *Ichthyophonus* prevalence in male pollock was 32% and 27% in 2006 and 2007, respectively; prevalence in females was 26% and 28% (Fig. 2). *Ichthyophonus* prevalence was not significantly different between sexes in 2006 ($\chi^2 = 0.28$, $n = 80$, $P = 0.59$) or 2007 ($\chi^2 = 0.08$, $n = 182$, $P = 0.78$). Similarly, *Ichthyophonus* prevalence was not significantly different between years 2006 and 2007 in males ($\chi^2 = 0.33$, $n = 132$, $P = 0.56$) or females ($\chi^2 = 0.07$, $n = 130$, $P = 0.79$).

Pollock ages 1 through 18 were infected with *Ichthyophonus*, and prevalence in adult pollock (age 4 +) was not significantly different in 2006 and 2007 ($\chi^2 = 0.021$, $n = 263$, $P = 0.88$). *Ichthyophonus* prevalence in adult pollock was 29% and 28% in 2006 and 2007, respectively (Fig. 2). *Ichthyophonus* prevalence in juvenile pollock (age 1–3) was 3% in 2007 (Fig. 2), which was significantly lower than that found in adult fish in the same year ($\chi^2 = 11.14$, $n = 221$, $P < 0.001$). A precise estimate of *Ichthyophonus* prevalence in juvenile pollock in 2006 was not determined as only three juveniles were collected that year and none of them were positive for *Ichthyophonus*.

In both 2006 and 2007, *Ichthyophonus* prevalence was 23% or greater in pollock with a fork length of 400 mm or greater (Fig. 3). *Ichthyophonus* prevalence could not be reliably estimated for pollock in the size range of 190–360 mm (approximate size range of age-2 and age-3 pollock) because of insufficient sample size, which is likely attributable to the gear selectivity of bottom trawls (Karp & Walters 1994). *Ichthyophonus* prevalence in age-1 pollock was 3% (Fig. 3).

Historical *Ichthyophonus* prevalence in age-0 pollock

Ichthyophonus schizonts were identified by histology in age-0 pollock collected near the Pribilof Islands in the eastern Bering Sea in 1994 and throughout the Gulf of Alaska sampling area over multiple years (Fig. 4). In the eastern Bering Sea, the smallest pollock identified with an *Ichthyophonus* infection was 45 mm, and overall prevalence in age-0 pollock was 2%, and prevalence appears to increase with size (Fig. 5). In the Gulf of Alaska, the smallest pollock identified with an *Ichthyophonus* infection was 61 mm (1988), and overall prevalence in age-0 pollock per year was 1, 0, 2.3, 2 and 2% in 1986, 1987, 1988, 1990 and 2001, respectively. Although not statistically significant, prevalence again appears to increase with host size in age-0 fish (Fig. 5). It should be noted that fine-scale fish age cannot be inferred from fish length, and 'age-0' is the term given to the subset of juveniles that hatched earlier in the same year and have not yet experienced a winter season. Age-0 fish hatch dates could vary as much as ~ 80 days, and growth rates may be dependent on environmental and geographic variability at the time of hatch (Yoklavich & Bailey 1990).

Ichthyophonus detection in offal from Chinook salmon stomach contents

Of the eight archived offal samples tested for *Ichthyophonus* with the qPCR assay, sample 60–19 (Buser *et al.* 2009) yielded a positive result of 63 copies per reaction. Buser *et al.* (2009) were not

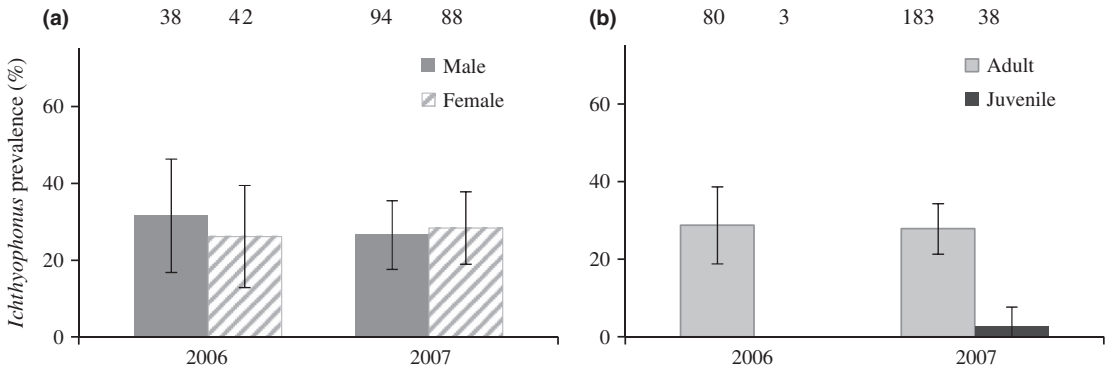

Figure 2 *Ichthyophonus* prevalence by (a) fish sex in adult walleye pollock and (b) fish maturity stage in walleye pollock (*Theragra chalcogramma*) captured in the eastern Bering Sea by bottom trawl during the summers of 2006 and 2007. Sample size (*n*) for each size group is at the top of each graph. Bars = 95% confidence intervals.

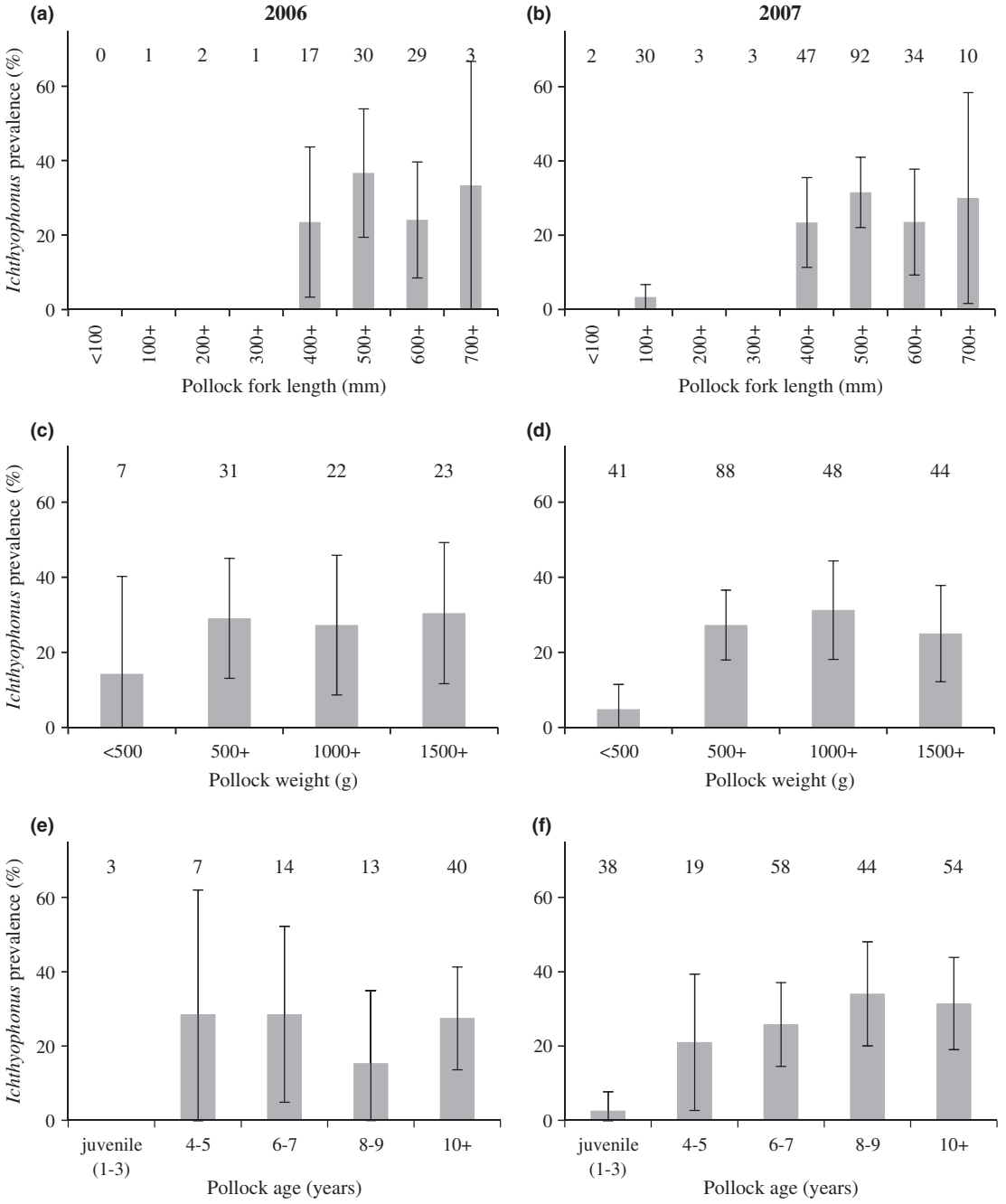

Figure 3 *Ichthyophonus* prevalence by fish size, weight and age in walleye pollock (*Theragra chalcogramma*) captured in the eastern Bering Sea by bottom trawl during the summers of 2006 (a, c, e*) and 2007 (b, d, f*). Sample size (*n*) for each size group is at the top of each graph. Bars = 95% confidence intervals. *Age data were not available for 6 fish (4 +, 2 −) in 2006 and 8 fish (3 +, 5 −) in 2007 – results not included in graphs e and f.

able to definitively identify the origin of all offal samples due to low and/or confounding peaks (including sample 60–19); however, all the offal samples that were verified by molecular methods were of pollock origin. Nevertheless, the positive qPCR result indicates the presence of *Ichthyophonus* DNA in what is presumed to be fishery waste that was consumed by a Chinook salmon in the eastern Bering Sea. The disease status of this individual Chinook salmon was not available.

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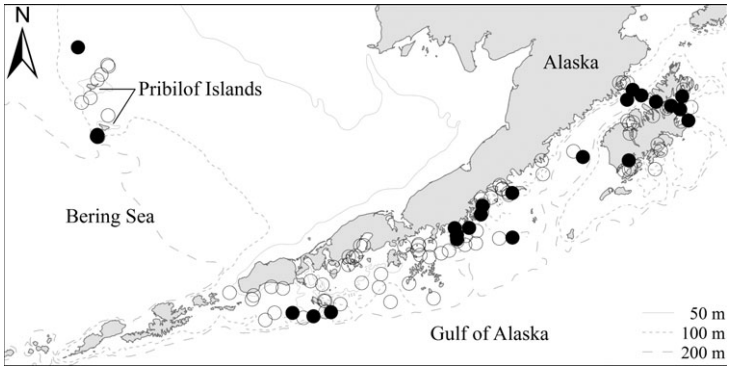

Figure 4 *Ichthyophonus*-infected age-0 walleye pollock (*Theragra chalcogramma*) captured by Anchovy trawl in the eastern Bering Sea in 1994 and in the Gulf of Alaska 1986, 1987, 1988, 1990 and 2001. All circles indicate age-0 walleye pollock collection stations, and solid circles indicate stations with *Ichthyophonus*-infected age-0 walleye pollock determined by histology.

Discussion

Ichthyophonus in pollock

To our knowledge, this is the first documented record of *Ichthyophonus* from the Bering Sea, in pollock or any other fish species; however, our historical data indicate that *Ichthyophonus* is not a recent introduction to this region. Age-0 pollock from the eastern Bering Sea were infected in 1994, while *Ichthyophonus* infections have occurred regularly at low levels in this host in the Gulf of Alaska since 1986 (Fig. 5). More recently, infections identified in adult pollock indicate that the distribution of this parasite is considerably more widespread in the North Pacific than previously reported (Fig. 1). Moreover, ongoing studies and preliminary reports (Dykstra *et al.* 2013; Nichols *et al.* 2012) suggest this parasite is likely present in multiple hosts in the Bering Sea.

Ichthyophonus prevalence in juvenile pollock was significantly lower than in adult fish (Fig. 2). This observation is consistent with studies of *Ichthyophonus* in Pacific herring that show an increase in prevalence with age (Hershberger *et al.* 2002; Marty *et al.* 2003). Similarly, *Ichthyophonus* prevalence appears to increase with size in age-0 pollock in both the eastern Bering Sea and Gulf of Alaska (Fig. 5). However, in adult pollock, *Ichthyophonus* prevalence appeared to level off in fish greater than 40 cm (Fig. 3), about age 4 and older. Although multiple factors may contribute to the observed patterns and prevalence of *Ichthyophonus* in pollock, these factors likely relate back to two assumptions: (i) *Ichthyophonus* is frequently maintained in a host as a chronic infection which is marked by tissue encapsulation of the parasite (Rahimian 1998), and (ii) after early onset of infections, cumulative exposures over time contribute to increasing prevalence with age (Hershberger *et al.* 2002). Exposure to *Ichthyophonus* and ultimately transmission of the parasite likely occurs through feeding over the lifespan of the fish, but on a finer scale, diet composition at-age, cannibalism and climate conditions could play a role in *Ichthyophonus* prevalence levels in pollock.

Ichthyophonus transmission mechanisms are thought to differ between piscivorous (i.e. Chinook salmon) and planktivorous fishes (i.e. Pacific herring) (Kocan, Gregg & Hershberger 2010). Researchers have demonstrated *Ichthyophonus* transmission to piscivorous fish through feeding on infected fish tissue (Jones & Dawe 2002), but a route of transmission for planktivorous fish has yet to be defined (Gregg *et al.* 2012). Presumably, planktivorous hosts consume *Ichthyophonus*-infective stages via a paratenic or transport host such as zooplankton (Hershberger *et al.* 2002; Gregg *et al.* 2012), but no such carrier species has been confirmed. Because pollock are primarily planktivorous at age-0 and increase piscivory and cannibalism as they grow (Bailey & Dunn 1979; Lang *et al.* 2005), diet composition at-age may be related to the prevalence trends with fish size.

Examining pollock size at first exposure may provide direction for future research to identify a specific source of infection for age-0 fish and confirm a planktonic reservoir species for *Ichthyophonus*. Age-0 pollock feed primarily on small and large copepods, pteropods and euphausids among other zooplankters (Brodeur *et al.* 2002). With increasing body size, diet shifts from mostly small copepods to a variety of larger prey items (Schabetsberger *et al.* 2003). Because *Ichthyophonus* infections were not detected in the smallest size categories of age-0 fish examined, it is possible that the infection source may be associated with a prey item that is restricted by fish gape size or

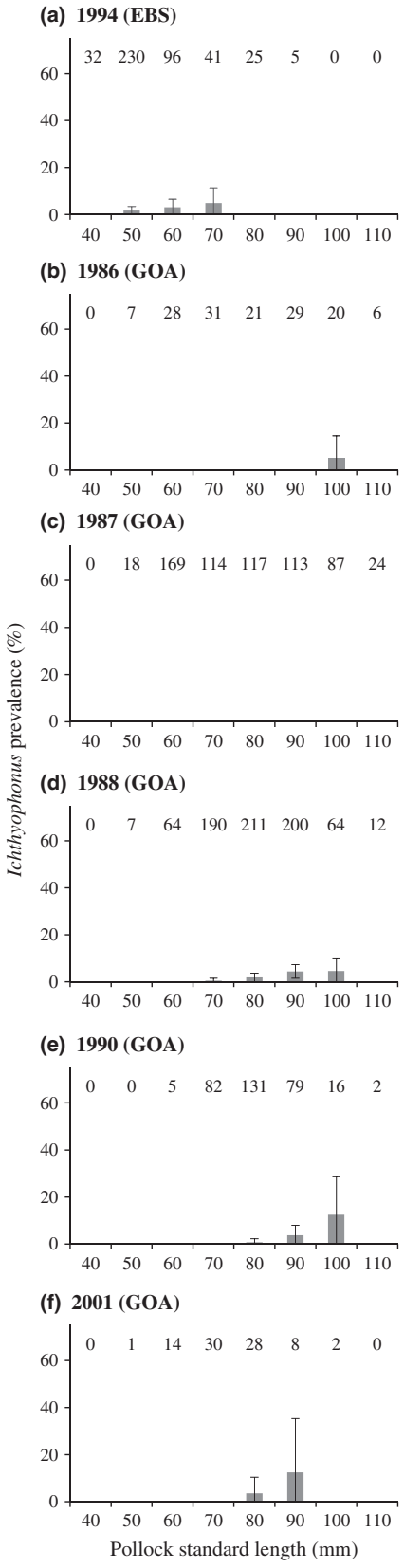

Figure 5 *Ichthyophonus* prevalence by fish size (mm) in age-0 walleye pollock (*Theragra chalcogramma*) captured in the eastern Bering Sea (EBS) in September 1994 (a), and the Gulf of Alaska (GOA) in September of 1986 (b), 1987 (c), 1988 (d), 1990 (e) and 2001 (f). Sample size (*n*) for each size group is listed at the top of each graph; size labels indicate the upper limit of the size range. Bars = 95% confidence intervals.

seasonal availability of specific prey. Results of limited sampling suggest that initial infections appear in smaller fish in the eastern Bering Sea than in the Gulf of Alaska (Fig. 5). This discrepancy mirrors the difference in the mean size of age-0 fish between the regions; eastern Bering Sea age-0 pollock tend to be 20–30 mm smaller than the Gulf of Alaska fish, which generally grow at a faster rate (Brodeur & Wilson 1996; Brodeur, Wilson & Ciannelli 2000). Additional research is needed to determine a source of infections in juvenile pollock and other planktivorous fish.

As pollock continue to grow, their prey selection includes age-0 pollock (Bailey & Dunn 1979; Dwyer *et al.* 1987; Lang *et al.* 2005), so infections could be acquired through cannibalism. Cannibalism in this species occurs year-round but is highest on age-0 pollock in autumn (Dwyer *et al.* 1987). Estimates suggest that larger pollock consume approximately 400 billion age-0 pollock annually in the eastern Bering Sea (Dwyer *et al.* 1987). Even though *Ichthyophonus* prevalence is low in age-0 pollock (Fig. 5), transmission events could occur from the sheer number of small fish consumed. Because intensity of cannibalism on pollock in the Bering Sea appears to be related to ocean conditions, *Ichthyophonus* exposure could also vary with climate conditions. In anomalously warm years, more cannibalism on young pollock occurs. In cool years, when large copepod and euphausiid prey are more abundant, cannibalism appears to be less severe (Moss *et al.* 2009; Hunt *et al.* 2011). Exposure to *Ichthyophonus* likely continues throughout the lifespan of the fish, although it is uncertain what forces stabilize prevalence in larger fish (Fig. 3).

It is not possible to assess the effect that *Ichthyophonus* could have on pollock populations or recruitment to the fishery with our limited data. However, results of captive studies on juvenile Pacific herring and *Ichthyophonus* (Gregg *et al.* 2011; Vollenweider *et al.* 2011) could provide insight into potential effects of *Ichthyophonus* on age-0 pollock recruitment. For example, experimental results showed that juvenile Pacific herring

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incurred significant energetic costs from *Ichthyophonus* infections (Vollenweider *et al.* 2011). In age-0 pollock, reduced energy reserves were associated with weak recruitment, but this observation also coincided with a climate-related prey shift (Hunt *et al.* 2011). In years with less favourable climate conditions (i.e. early ice retreat) (Hunt *et al.* 2011), increased energetic costs due to *Ichthyophonus* infections (Vollenweider *et al.* 2011) could exacerbate already weak recruitment conditions for age-0 pollock.

From a commercial fisheries standpoint, pathogens can have multiple economic effects on targeted resources (Sindermann 1990). Disease-related mortalities are the most obvious. Even though it appears that more than a quarter of adult pollock harbour *Ichthyophonus* infections (Fig. 2), the eastern Bering Sea pollock fishery has not reported significant pollock morbidity or mortalities attributed to this parasite. There are other economic costs of marine fish diseases, however, which can include reduced product quality or rejection by processors, as well as spread of disease from one species of fish to other species of commercial importance (Sindermann 1990). From the perspective of disease spread, the presence of *Ichthyophonus* in a host such as pollock is of both economic and ecological concern. Pollock is widely distributed, the single most abundant fish species in the Bering Sea and is the dominant fish prey species in the eastern Bering Sea for a variety of predators (Wespestad *et al.* 2000). These host characteristics and presumptive evidence that pollock can survive with chronic *Ichthyophonus* infections suggests a potential for parasite spread. A survey of other eastern Bering Sea fish species for *Ichthyophonus* could provide insight into both parasite dynamics and species interactions. Considering that *Ichthyophonus* can be transmitted via predation and has low host specificity (Prabhuji & Sinha 2009; McVicar 2011), pollock likely serve as a key reservoir providing opportunities for transmission to susceptible piscivorous fish species in the Bering Sea, Gulf of Alaska and adjacent watersheds.

Pollock as a reservoir of *Ichthyophonus* in the North Pacific

In the Yukon River, Alaska, *Ichthyophonus* infections were reported in the declining Chinook salmon stock, but a source for these infections could not be found (Kocan *et al.* 2004; Zuray *et al.* 2012). Recently, Zuray *et al.* (2012) identified an epizootiological pattern suggesting that a prey item critical to survival may be a source of infections. We propose that pollock is the previously unidentified prey-based infection source for Yukon River Chinook salmon. There are a number of findings from this study as well as host life history strategies that support this hypothesis. Food habit studies conducted in the Bering Sea identified pollock as an important prey item (≥10% of prey composition by weight) of Chinook salmon (Davis *et al.* 2009b), and the range of their ocean stage overlaps with the geographic distribution of pollock infected with *Ichthyophonus* (Fig. 1). Because *Ichthyophonus* infections are present in juvenile pollock as well as adults, the opportunity for transmission to occur through feeding would not be restricted by Chinook salmon gape size. Moreover, consensus sequences of *Ichthyophonus* 18S rDNA from pollock and Yukon River Chinook salmon are identical. It should be noted, however, that little SSU variability has been found in this genus in the North Pacific as well as worldwide; sequencing of ITS1 and ITS2 regions of *Ichthyophonus* from both hosts may provide additional information (Criscione *et al.* 2002; Rasmussen *et al.* 2010).

A more detailed examination of Chinook salmon feeding behaviour related to seasonality reveals the potential for two pathways of *Ichthyophonus* transmission from pollock: juvenile pollock and fishery offal. In the summer months, pollock prey (primarily age-1) made up a substantial portion of Chinook salmon diets but, in the winter months, small pollock were considerably less important (Davis *et al.* 2009a). Fish offal identified as pollock (Buser *et al.* 2009) was present in the diets of all age groups of ocean-stage Chinook salmon in the eastern Bering Sea, but only in the winter; it was suggested that offal may provide an alternate food resource when other natural prey items are scarce (Davis *et al.* 2009a). This feeding behaviour could result in increased *Ichthyophonus* infection pressures, as schizonts can survive in sea water for extended periods of time (Hershberger *et al.* 2008). From our qPCR analysis of eight fishery offal samples obtained from Bering Sea Chinook salmon stomachs in 2007, one contained *Ichthyophonus* DNA. Although the presence of parasite DNA does not necessarily indicate the presence of a live parasite or viability (Kocan, Dolan & Hershberger 2011), this finding

supports the possibility that fishery offal could contribute to *Ichthyophonus* infections. From an epizootiological standpoint, the release of unpasteurized fishery waste at sea should be evaluated as timing of releases are unnatural and may occur in different areas than the fish were caught. Additionally, at-sea processing of pollock could augment natural rates of pathogen dispersal considering processors target adult fish, which possess the highest infection rate (Fig. 2). Regulation requires that tissue must be macerated prior to release to less than a half-inch in any dimension (Environmental Protection Agency 40 C.F.R. § 408.202), but these small pieces of fish tissue are then available to predators that would not normally be able to eat a whole adult pollock. Because several fish species, including pollock (Lang *et al.* 2005), are known to consume waste from fish processors (Brodeur & Livingston 1988; Lang *et al.* 2005) and host specificity of the parasite is low, the *Ichthyophonus* host list in the eastern Bering Sea could continue to grow.

Although at-sea processors release significant amounts of offal in the eastern Bering Sea (Aydin *et al.* 2007), many measures have been implemented by the industry and managers to reduce waste over the last decade. With the creation of the American Fisheries Act and Pollock Conservation Cooperative in 1998, rationalization of the pollock fishery allowed factories to take the time needed to process fish more efficiently (Wilen & Richardson 2008). The number of operating catcher-processors was reduced by about half, allocating a greater portion of harvest to shoreside plants (Wilen & Richardson 2008; NPFMC 2012). Moreover, by-product advances have increased the types of products that can be made from seafood processing waste (Crapo & Bechtel 2003), thereby reducing at-sea discharge of offal. This improvement is reflected by an increasing trend in the portion of catch resulting in products (by weight); in 1998, 27% of the catch resulted in products in comparison with 48% by 2009[3]. These types of improvements are beneficial for long-term stock management and industry revenue (Witherell, Pautzke & Fluharty 2000; Wilen & Richardson 2008) while also reducing spread of pathogens and disease. However, pasteurization of fish waste would likely eliminate the spread of most pathogens through offal. It is important for future regulation discussions to consider how management decisions and commercial fishing practices could affect pathogen dispersal to target stocks as well as other North Pacific species.

Concerns about climate change and the future of commercial fish stocks have prompted much research concerning climate impacts on eastern Bering Sea food webs (Coyle *et al.* 2011). This body of work focuses on linking oceanographic conditions (e.g. water temperature, ice cover) with zooplankton production, prey shifts, energy reserves and ultimately pollock recruitment (Moss *et al.* 2009; Coyle *et al.* 2011; Hunt *et al.* 2011). Connecting disease prevalence with the aforementioned factors could better explain this dynamic ecosystem and possibly aid in identifying predictors of disease. For example, the timing of spring ice retreat in the eastern Bering Sea (measured from oceanographic mooring M2; Fig. 1) may be a factor influencing *Ichthyophonus* prevalence in returning Yukon River Chinook salmon. Over a 12-year period, *Ichthyophonus* prevalence was significantly higher in returning females 1 year after an early ice retreat (before March 15th) than prevalence in returning females 1 year after a late ice retreat (after March 15) in the Bering Sea (ANOVA, $P = 0.001$, based on data presented by Zuray *et al.* (2012) and NOAA (2012)). The underlying mechanisms of this pattern remain unclear and additional contributing factors need to be defined, but predictive disease factors such as oceanographic conditions should be considered in future research.

Summary

A review of available temporal and spatial data suggests that *Ichthyophonus* is widely distributed in the eastern Bering Sea and has been present in pollock in this region for nearly two decades. The extensive range and prevalence of this trophically transmitted parasite in all pollock year classes is an important finding because this host is a key prey species in the eastern Bering Sea (Livingston 1993) and the target of one of the world's largest commercial fisheries (Shen *et al.* 2008). Although the limited data presented here only allow us to speculate on the dynamics of this parasite in this system, it does offer a foundation for future research. *Ichthyophonus* may spread in this region through live pollock as prey, and offal from at-sea fish processors may augment pathogen dispersal, although recent advances in waste repurposing may reduce potential dispersal in the future. Because pollock harbour chronic *Ichthyophonus*

infections, it is likely that this host aids in the persistence of this parasite in the North Pacific. Considering the low host specificity of *Ichthyophonus* and the interconnectivity of the eastern Bering Sea, coastal ecosystems and adjacent watersheds, it is challenging to predict the effects this parasite might have on the future of multiple fisheries. Yet, the presence of this parasite in several commercially important species, its ability to spread through food webs and suspected connection with climate conditions exemplifies how fish disease studies have an important role in an ecosystem-based approach to fisheries management.

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Note

1. Communications with industry initiated in 2003.
2. Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.
3. $\text{Calculation} = \frac{\text{weight of all pollock products}}{\text{weight of retained pollock catch}}$;

data acquired from NOAA Fisheries Catch and Product Reports available at http://alaskafisheries.noaa.gov/sustainablefisheries/catchstats.htm

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