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Synthesis of field observations and empirical studies**

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Transmission models for the fish pathogen *Ichthyophonus*: Synthesis of field observations and empirical studies

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Abstract: Empirical studies have demonstrated that *Ichthyophonus* is transmitted among piscivores via consumption of infected prey; however, this unidirectional mode of transmission from small to large fish cannot sustain *Ichthyophonus* within a population. To circumvent this problem, *Ichthyophonus* evolved an infective waterborne cell that has been shown to infect both fresh and saltwater fish. Successful transmission of *Ichthyophonus* via a waterborne stage is linked to the proximity of infected and susceptible individuals, a condition met when the two groups occupy the same habitat. It is posited that this occurs during annual inshore migrations when herring enter areas occupied by infected demersal predators. A plausible transmission scenario is that during inshore excursions planktivores are exposed to infective waterborne cells shed by demersal piscivores. Once planktivores are infected the parasite is recycled when consumed by predators. This model is supported by reports of ichthyophoniasis increasing in herring populations at the time of inshore migration. The possible role of an intermediate host for *Ichthyophonus*, as well as evidence for a free-living stage in its life cycle is presented.

Introduction

Ichthyophonus is one of several genera belonging to the Class Mesomycetozoea, an ancient group of protists that arose near the animal-fungal boundary >500 million years ago (Berbee and Taylor 2001; Heckman et al. 2001; Mendoza et al. 2002). The parasite has been identified and isolated from freshwater and saltwater fishes worldwide; it occurs over a wide range of habitats (McVicar

2011), and has been described as "...the most ecologically and economically significant pathogen..." of marine and anadromous fishes (Hershberger et al. 2016). Infections range from inapparent, i.e. subclinical, with no gross or microscopic signs of disease, to clinical disease that can present as external ulcers, extensive tissue and organ damage, and heavy mortality (Fish 1934; McVicar and McLay 1985; Rahimian and Thulin 1996; Kocan et al. 1999; Kocan et al. 2004; Hershberger et al. 2017).

Ichthyophonus was first described over a century ago (Hofer 1893) and was originally given the species name *I. hoferi*. Unfortunately the type specimen no longer exists and no morphologic features were described to distinguish this isolate from others. Currently there are no recognized criteria for species designation, so the organism is referred to generically throughout this paper as *Ichthyophonus*.

The parasite's complete life cycle, mechanism(s) of transmission, and development in the piscine host, have been both definitive and confusing. Transmission in piscivores is oral, via ingestion of infected prey, infected tissues, or ingestion of waterborne cells in the absence of carnivory. Conversely, in planktivores, such as herring, *Clupea* sp. and shad, *Alosa* sp., both of which frequently exhibit high infection prevalence and clinical disease, there is no confirmed mechanism for natural transmission. Early attempts to transmit the parasite to Atlantic herring, *C. harengus*, succeeded only after feeding massive doses of infected tissue on multiple occasions, something that is unlikely to occur naturally (Sindermann and Chenoweth 1993), while recent attempts to transmit the parasite by housing infected Pacific herring, *C. pallasii*, with uninfected sentinel herring have been unsuccessful (Gregg et al. 2012); however, the possibility of planktivore-to- planktivore transmission occurring in nature has not been ruled out. Because there is currently no unifying theory defining the mechanism(s) of transmission for both piscivores and planktivores, the life cycle of *Ichthyophonus* remains obscure.

A number of field studies have attempted to explain annual and seasonal transmission patterns as well as factors associated with periodic epizootics (Fish 1934; McVicar 1981; Kocan et al. 2004; Kramer-Schadt et al. 2010; Marty et al. 2010; Oskarsson & Palsson 2011; Hershberger et al. 2016) but no consensus has been reached.

Interpreting field studies is often hampered because the distinction between infection and disease is frequently blurred, sequential sampling of the same population is infrequent, and there is a lack of diagnostic uniformity among studies. Some authors report gross visible lesions, i.e. presumptive disease (Holst 1996; Kramer-Schadt 2010), the results of which can vary widely, and may or may not reflect infection prevalence (Kocan et al. 2011; Hershberger et al. 2017). Some authors identify the parasite from tissue squash preparations (Holst 1994; Rahimian and Thulin 1996), which does not distinguish between infection and disease. Histopathology is used to confirm the identity of the organism and determine the severity of disease, but is prone to miss infections in their early stages, as well as low-level infections. In vitro explant culture has been shown to identify infected individuals, even at low infection intensity (Kocan et al. 2004; Hershberger et al. 2017); however, this technique does not distinguish between infected and diseased individuals. None of these techniques can determine when a population or individual became infected, i.e. time of transmission. Recently, molecular techniques (PCR) have been proposed as a screening method (Whipps et al. 2006; Hamazaki et al. 2013). While some techniques are more sensitive than others for detecting infected individuals, it is generally recognized that the hierarchy of sensitivity is: in vitro culture > tissue squash > histology > gross lesions, with in vitro culture being described as the “gold standard” (Hershberger 2012). PCR is not included in this hierarchy because it does not detect the living organism; consequently the results of PCR screening should be backed up with an independent diagnostic test, e.g. explant culture or histopathology.

Adding to the uncertainty of using different diagnostic methods are the significant differences in infection and disease prevalences observed when different capture gear is used for surveying wild populations (Holst 1996). Field surveys are beneficial in identifying changes in seasonal and annual infection and disease prevalence, as well as differences among populations. Although difficult, if field diagnostic and capture methods were to be standardized, horizontal comparison of results among studies would be more meaningful.

Most investigators would agree that the schizont is the most recognizable stage of *Ichthyophonus* and probably represents the ‘primary stage’ of the

94 parasite. Several investigators have proposed plausible life cycles, with 'life
95 cycle' being defined as:

96 *The series of stages in form and functional activity through which*
97 *an organism passes between successive recurrences of a specified*
98 *primary stage* (Merriam-Webster 2006).

99 Although predator-prey transfer is a recognized route of transmission, it
100 cannot explain sustained transmission of *Ichthyophonus*. Daniel (1933)
101 and Dorier and Degrange (1960) proposed life cycles that included all of
102 the recognized stages of *Ichthyophonus* in both the living and dead host,
103 but offered no convincing explanation as to how the parasite was
104 transferred between hosts in the absence of carnivory. Likewise,
105 Sindermann and Scattergood (1954) hypothesized a life cycle that
106 involved ingestion of "spores" (i.e. schizonts) that were released into
107 seawater via pustules (i.e. epidermal ulcers), or from infected corpses.
108 They also described a possible alternate route of transmission that
109 utilizes a "...copepod or possibly other planktonic forms..." Although
110 plausible, neither of these routes of transmission has been
111 experimentally confirmed.

112 The missing element in the life cycle of *Ichthyophonus* is how the parasite
113 navigates the environment between hosts and infects both planktivores and
114 piscivores in the absence of carnivory. This paucity in our knowledge raises the
115 question, "Does *Ichthyophonus* have a 'direct' (simple) or 'indirect' (complex) life
116 cycle"; i.e., does it utilize a single host, multiple hosts, or both. Equally relevant
117 is what constitutes a 'host' for this parasite?

118 The schizont and merozoites contained within it are presently the only
119 stages of *Ichthyophonus* known to occur in the living host, and have been
120 conclusively identified as infective cells (Okamoto et al. 1987; Kocan and
121 LaPatra 2016). Additionally, an as yet unidentified waterborne cell has been
122 shown to be infective via ingestion (LaPatra and Kocan 2016). Significantly,
123 once an infected host dies, tissue schizonts metamorphose into several different
124 cell types in the carcass, and similar cells occur postprandial in the intestinal

tract of fish fed infected tissue (Kocan et al. 2014; Kocan and LaPatra 2018).

The role of these cells in the parasite's life cycle is currently unknown.

Objectives

This perspective summarizes the current data on natural and experimental transmission of *Ichthyophonus* and synthesizes it into plausible models that could explain observations reported in the scientific literature as well as explain natural transmission in wild populations.

Direct life cycle

Unidirectional transmission (confirmed)

The most recognizable mode of transmission for *Ichthyophonus* is via consumption of infected prey, a phenomenon that has been confirmed by both field and empirical studies. In wild fish, *Ichthyophonus* was found in the digestive tract of Atlantic cod, *Gadus morhua*, after feeding on Infected herring (Sindermann & Chenoweth 1993; Kramer-Schadt et al. 2010), and cultured fish are known to develop ichthyophoniasis following ingestion of infected fish or fish tissue (Athanasopoulou 1992; Franco-Sierra et al 1997; Jones and Dawe 2002; Kocan et al. 2013).

Although predator-prey transfer is an effective mode of transmission it cannot sustain *Ichthyophonus* within a population because parasite transfer is unidirectional from small prey to larger predators (Fig. 1). This ultimately results in a host population consisting of large infected individuals with no mechanism for transmitting the parasite back to smaller prey. Therefore, an alternative method of transmission must exist to ensure cyclical transmission from large piscivores to small prey fish.

The majority of work on wild fish has concentrated on commercial species (Rand 1994; Sindermann & Chenoweth 1993; Kent et al. 2001; Kocan et al. 2004; Huntsberger and Smolowitz 2014; Hershberger et al. 2017), but many non-commercial wild species are also infected via ingestion of infected prey, or are themselves ingested; these non-commercial species may play a critical role in the overall life history of the parasite.

Waterborne transmission (confirmed)

To circumvent the conundrum of unidirectional transfer, *Ichthyophonus* evolved a second direct fish-to-fish mode of transmission, that of an infective waterborne cell. This route of transmission has been demonstrated in freshwater trout (Gustafson and Rucker 1956; Yakota et al. 2008; LaPatra and Kocan 2016) as well as in saltwater between sculpins (*Leptocottus armatus*) and flatfish (*Lepidopsetta bilineata*) (Kocan, R. unpublished).

The infective cell, which has not been described, is consumed by predator species (confirmed), and possibly planktivores (Fig 2). Waterborne transmission can sustain the parasite within a population of piscivores regardless of size, thus ensuring a ready supply of infected individuals that could serve as both a small prey item, and when mature, a source of waterborne infective cells. It has not been determined if this mechanism of transmission functions among planktivores.

Reservoir infections (posited)

A 'reservoir' is defined by Parasitologists as:
...one or more epidemiologically connected populations or environments in which the pathogen can be permanently maintained and from which infection is transmitted to the defined target population (Haydon 2002).

A key element for the successful transmission of a pathogen is the proximity of the reservoir and target host, and these conditions are met when individuals of a single or multiple populations occupy the same habitat. Proximity of the reservoir and target species can be continuous or punctuated, as occurs during the annual inshore migration of herring when they enter inshore zones occupied by demersal predators (Cass et al. 1990; Smale 1992; Johnson et al. 2003).

Between inshore migrations a waterborne infective stage cycling among demersal predators would maintain an infected population as well as confer a survival advantage to the parasite in the absence of infected prey. Because demersal piscivores have limited latitudinal migrations, are generally long-lived (Duijn 1956), and once infected do not readily clear their infection (Hershberger

et al. 2012), they are ideal reservoirs of infection for susceptible planktivores when they repopulate a common geographic area. During inshore migration, planktivores and demersal species come into close proximity where planktivores are more likely to be exposed to waterborne cells shed by demersal species. This in turn produces a population of infected prey, which then serves to recycle the parasite via direct consumption by piscivores (Fig. 3).

Supporting this model are multiple reports of a significant increase in ichthyophoniasis in different herring species and populations at widely separated geographic locations at the time of inshore migration (Fish 1934; Scattergood 1948; Holst 1996; Rahimian & Thulin 1996; Holst et al. 1997; Hershberger et al. 2016). Sindermann and Scattergood (1954) reported that in Atlantic herring (*C. harengus*), “Negative findings at the time of the first inshore migration indicate that infection “...occurs in inshore waters and that the disease spreads rapidly”. Likewise, Sindermann & Chenoweth (1993) proposed that “...the disease persists in other fish species in periods between outbreaks in herring, and reinvades when changes occur in the (herring) population . . .” They also posited that demersal taxa (e.g., halibut, flounder, plaice, lingcod, rockfish, sculpins) could serve as infected reservoir hosts until a susceptible host reappeared. Recently, *Ichthyophonus* prevalences were reported to increase in Pacific herring overwintering inshore, compared with those overwintering offshore (Hershberger et al. 2016).

The evidence for transmission from demersal to pelagic species following inshore migration is more compelling than that for transmission within or among pelagic species. If direct transmission occurs within pelagic populations (e.g. herring) while in open water, then infection prevalence should increase over time. In nature however, infection prevalence spikes at the time of inshore migration, supporting the hypothesis that transmission occurs at this time. Planktivores that become infected following inshore migration would also be a source of infection for demersal predators via direct consumption, thus completing the cycle of transmission depicted in Figure 3. Differential rates of transmission among populations during inshore migration could explain the

observed seasonal and annual variation in infection prevalence. Likewise, repeated exposure to the parasite during annual inshore migrations would explain the increase in infection and disease prevalence reported in older individuals.

The three models described above can explain *Ichthyophonus* transmission among all fish species, however they do not preclude the possibility of an additional complex life cycle.

Indirect life cycle

Intermediate host (proposed)

Several authors have proposed that *Ichthyophonus* utilizes an intermediate host, but the case for an indirect life cycle is not currently supported by field or laboratory data. Sindermann and Scattergood (1954) proposed a direct life cycle as well as an indirect life cycle that utilized a copepod, but presented no experimental evidence in support of the latter. The persistent belief that an intermediate host exists likely stems from there being no evidence for direct transmission among planktivores. The most frequently touted candidate for an intermediate host is a calanoid copepod, but to date there is no evidence to support this mechanism (Jepps 1937; Sindermann and Scattergood 1954; Hirche and Kwasniewski 1997; Torgersen et al. 2002; Gregg et al. 2012), however, the possible role of other arthropods, annelids, and mollusks has not been investigated.

A conundrum of definitions

The following definition would define a true intermediate host for *Ichthyophonus*:

“A host which is used by a parasite in the course of its life cycle and in which it may multiply asexually but not sexually” (Roberts and Janovy 2005; Merriam-Webster 2006).

This is in contrast to a ‘definitive’ host, which is one in which the parasite replicates by sexual reproduction.

In the current *Ichthyophonus* literature ‘intermediate host’ implies a non-piscine invertebrate, which if correct poses a conundrum. If *Ichthyophonus* has a stage that

undergoes asexual reproduction in a non-piscine intermediate host, then by definition the definitive host would be a 'fish' in which sexual reproduction occurs, a phenomenon that has yet to be demonstrated. Consequently, until an intermediate host is identified, or sexual reproduction is observed in the fish host, the concept of definitive (sexual reproduction) and intermediate (asexual reproduction) hosts for *Ichthyophonus* does not apply to this host-parasite relationship. Alternatively, there may be a 'paratenic' or transport host, in which the parasite remains infective but does not undergo any further development. At this time no evidence exists for either an intermediate or paratenic host for *Ichthyophonus*.

The definition of 'host' poses another dilemma. Are all fish species considered a single host, or do different fish species constitute different hosts for *Ichthyophonus*? Protozoan parasites in the Family Sarcocystidae are known to alternate between different mammalian host species, e.g. *Toxoplasma gondii* alternates between feline (definitive) hosts and human (intermediate) hosts, while *Sarcocystis cruzi* alternates between canines (definitive host) and bovines (intermediate host) (Roberts and Janovy 2005). Consequently, if *Ichthyophonus* has a complex life cycle it could alternate between different fish species similar to that which occurs in mammals infected with Sarcocystidae, with each fish species playing a different role in the parasite's life cycle. Alternatively, if all fish are considered a single host for *Ichthyophonus*, and sexual reproduction does not occur, then the parasite has a direct or simple life cycle.

These issues of definition cannot be answered until the parasite's life cycle is clearly defined for both piscivores and planktivores, and investigators concur on what constitutes a 'host' species for this parasite.

Free-living stage (presumptive evidence)

A parasite reservoir can be defined as:

"Living, or rarely nonliving, means of maintaining an infectious agent in nature that can serve as a source of infection..." (Roberts and Janovy 2005).

If a reservoir were a nonliving component of the environment (e.g. sediment), *Ichthyophonus* could exist as a free-living autonomous organism while waiting to encounter a susceptible host. There is presently no evidence for transmission of *Ichthyophonus* via this mechanism, but there is presumptive evidence that supports the plausibility of such a reservoir.

Ichthyophonus utilizes several different exit routes from the infected host, but the fate of parasite cells once released has yet to be determined (Fig 4).

Following the death of infected herring, tissue schizonts rapidly morph into several distinct cell types that persist in the corpse for several weeks (Kocan et al. 2014); these same cells have also been observed in post-mortem trout tissue (Dorier and Degrange 1960). Low tissue pH produced by elevated lactate following cessation of blood flow (Donaldson and Lamont 2013) likely stimulates schizont growth. Similar morphologic changes to the schizonts are also produced in vitro under low pH conditions, supporting the effect of low pH on the parasite (Spanggaard et al. 1994).

In order for these newly formed cells to play a role in the life history of *Ichthyophonus* they must somehow exit the dead host and ultimately infect a new host. Ingestion of post-mortem tissue may be one mechanism. When infected herring carcasses were fed to naïve sculpins (*L. armatus*), transmission was observed for up to 5 days post-mortem (Kocan et al. 2014).

When carcasses of infected herring were placed on the surface of sterilized natural marine sediment, plasmodia and hyphenated schizonts with cells in the apical tips of the hyphae were observed for up to 14 days in sediment underlying the area where the carcasses decomposed (Fig 5) (Kocan, R. unpublished). Since these cells do not occur in the living host, they may represent a non-piscine, or free-living phase of *Ichthyophonus* that develops post-mortem and functions as a non-piscine stage the parasite. Further research in this area is clearly warranted.

Additional evidence suggesting a possible non-piscine, or free-living stage of *Ichthyophonus*, is the morphological transformations that occur as schizonts transit the gastrointestinal tract of a host following ingestion of an

infected meal (Kocan and LaPatra 2018). When schizonts were monitored hourly as they transited the host's digestive tract, morphological forms indistinguishable from those seen in post-mortem host tissue were observed, demonstrating that not all schizonts participate in the initial infection process and remain capable of further development. These cells were ultimately encased in fecal casts, which when voided would be dispersed in the aquatic environment and deposited in sediments where they could become an extra-piscine reservoir. This hypothesis has yet to be tested. Dorier and Degrange (1960) also observed that some "Cysts (i.e. schizonts) passed through the gastrointestinal tract of trout unchanged while others developed into plasmodia and hyphenated cells containing spherical masses located at the end of hyphae" (author's translation).

The longer *Ichthyophonus* can survive outside the living host, the better are its chances of encountering a new host. Although not extensively studied, *Ichthyophonus*' survival in synthetic and natural seawater as well as 0.9% physiologic saline has been reported to be up to 1 year, making long-term survival outside the host plausible (Sindermann and Scattergood 1954; Spanggaard and Huss 1996).

Although viable *Ichthyophonus* cells predictably appear in post-mortem host tissue, their role in the life history of *Ichthyophonus* has not been empirically determined. A plausible hypothesis is that they are a part of the parasite's life cycle and confer a survival advantage to the parasite in the absence of susceptible hosts.

Epidermal ulcers

One of the most perplexing features associated with ichthyophoniasis are the epidermal papules and ulcers that appear on planktivores and piscivores in both fresh and saltwater habitats (McVicar 2011). Ulcers have been reported to appear within 9-10 days post-exposure, show no correlation with mortality, and to persist for > 1 year in experimentally infected herring (Hart et al. 2015). Several authors have proposed that ulcers are a route of exit for schizonts,

which when released are ultimately consumed by new hosts (Daniel 1933; Sindermann and Scattergood 1954; Kocan et al. 2010); there is currently no evidence to support this transmission strategy.

Lesions first appear as small papules on the skin and progress to open ulcers in which schizonts of various sizes can be seen (Kocan et al. 2010; Hart et al. 2015). Scraping or vigorous shaking of the host in seawater dislodges some of the cells, which under laboratory conditions are infective when injected into the coelom of herring; however, infections did not result when cells were administered by gavage. The cells grew only at low pH in vitro and were negatively buoyant in seawater (Kocan et al. 2010). Attempts to directly infect naïve herring by housing them with ulcer-bearing cohorts for >100 days were unsuccessful (Gregg et al. 2012), suggesting that herring with epidermal ulcers are not the source of infective cells for other herring. Based on these findings it might be concluded that schizonts associated with epidermal ulcers may not play the role in transmission that was initially proposed, but may serve another biological function in the *Ichthyophonus* life cycle.

Experimental exposures frequently involve feeding chopped or minced pieces of *Ichthyophonus* positive tissue, which greatly increases the surface area of infected tissue at the time of consumption. Although this is an efficient method of experimentally initiating infections, it does not replicate the natural mode of exposure where infected prey are consumed whole. When prey is ingested whole, schizonts on the skin surface immediately contact the stomach wall, where they are exposed to digestive fluids, i.e. HCl and pepsin. Because the majority of schizonts in living prey are distributed throughout the host's tissue, many remain embedded in the digesting bolus and do not directly contact the stomach epithelium before being passed into the intestines. The rapid and intimate contact of ulcers/schizonts with the stomach epithelium could explain the rapid penetration of the stomach epithelium by schizont-derived merozoites observed in controlled studies (Kocan et al. 2013; Kocan and LaPatra 2018).

In order to test this alternative hypothesis, the chemical environment encountered by *Ichthyophonus* in the host's stomach was simulated in vitro

(Battle 1935; Kitamikado and Tachino 1960; Grabner and Hofer 1989; Horn 1997). Infected herring tissue was placed into 0.5% 1N hydrochloric acid (HCl) and 0.5% pepsin in un-buffered saline and then incubated at 15°C. Within hours ameboid plasmodia were observed in the digestion fluid and were indistinguishable from those observed in the digesting bolus of fish previously fed an infected meal (Kocan et al. 2013).

These findings suggest that epidermal ulcers represent a mechanism that ensures rapid and efficient invasion of the host when *Ichthyophonus*-infected prey is eaten, rather than a mechanism for releasing infective cells into the aquatic environment. Schizonts that do not contact the gastric epithelium are subsequently passed into the intestines where they encounter elevated pH levels (Kocan and LaPatra 2014). These cells are then reintroduced into the environment where they may continue to play a role in transmission of the parasite.

Summary

A direct transmission life cycle for *Ichthyophonus*, dependant on the ingestion of infected tissue as well as water-borne infective cells, has been empirically demonstrated and verified in piscivores. A natural route of transmission for planktivores has not been empirically demonstrated, but the existence of a waterborne infectious cell makes it plausible that this mode of transmission can also infect particle and filter feeders.

Although a complex life cycle utilizing an intermediate or paratenic host has been proposed for *Ichthyophonus*, none has yet been confirmed. If such a transmission mechanism were identified, then *Ichthyophonus* would have both a direct and complex life cycle.

The germination of schizonts and development of multiple forms of the parasite outside the living host suggests the existence of an extra-piscine phase of the parasite's life cycle. Although there is currently no evidence to support this hypothesis, it is reasonable to conclude that the numerous morphologic forms of

the parasite that develop following the death of the host must play some role in the parasite's survival strategy.

Numerous fish species have been identified as being infected with *Ichthyophonus*, but little experimental work has been done on species other than herring and trout, and the majority of field studies have focused on commercially important species. Because the natural history of commercial and cultured fish species is artificially modified as a result of population manipulation, the true life history of *Ichthyophonus* may not be known until extensive studies on non-commercial wild species are conducted. In the meantime, *Ichthyophonus* continues to be a significant pathogen of both wild and cultured fish.

Draft

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416 Figure 1. Predator-prey model demonstrating direct transmission of
417 *Ichthyophonus* via unidirectional transfer of schizonts.

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420 Figure 2. Multidirectional waterborne transmission model for *Ichthyophonus* within a
421 population of susceptible hosts. * Infective cell

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423

424 Figure 3. Model demonstrating cyclical transmission of *Ichthyophonus* between
425 piscivores and planktivores as well as among demersal piscivores.

426 (+) infected prey; (*) waterborne infective cell.

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428

429 Figure 4. Routes of entry and exit of *Ichthyophonus* cells from a fish host. Solid
430 arrows - experimentally confirmed routes of entry and exit; the ultimate fate of
431 cells exiting the host is unknown. Empty arrow – exit route unknown but cells
432 have been confirmed to be infective. (Kocan et al. 2014; Kocan and LaPatra
433 2018)

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435

436 Figure 5. *Ichthyophonus* cells observed in marine sediment 14 days following
437 death of infected herring. A. Schizont (s) with intact capsule (arrow); B.
438 Plasmodial cell emerging from schizont (arrow); C. Bifurcated plasmodium
439 exiting schizont capsule (c) through germ tube; D. Multiple germ tubes
440 containing plasmodia derived from the original schizont capsule (c).

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FIGURE LEDGENDS

Figure 1. . Predator-prey model demonstrating direct transmission of *Ichthyophonus* via unidirectional transfer of schizonts.

Figure 2. Multidirectional waterborne transmission model for *Ichthyophonus* within a population of susceptible hosts. (*) Infective cell

Figure 3. Model demonstrating cyclical transmission of *Ichthyophonus* between piscivores and planktivores as well as among demersal piscivores.

(+) infected prey; (*) waterborne infective cell.

Figure 4. Routes of entry and exit of *Ichthyophonus* cells from a fish host. Solid arrows - experimentally confirmed routes of entry and exit; the ultimate fate of cells exiting the host is unknown. Empty arrow – exit route unknown but cells have been confirmed to be infective. (Kocan et al. 2014; Kocan and LaPatra 2018)

Figure 5. . *Ichthyophonus* cells observed in marine sediment 14 days following death of infected herring. A. Schizont (s) with intact capsule (arrow); B. Plasmodial cell emerging from schizont (arrow); C. Bifurcated plasmodium exiting schizont capsule (c) through germ tube; D. Multiple germ tubes containing plasmodia derived from the original schizont capsule (c).

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