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The Pathogenic Copepod *Phrixocephalus cincinnatus* (Copepoda: Pennellidae) In the Eye of Arrowtooth Flounder, *Atherestes stomias*, and Rex Sole, *Glyptocephalus zachirus*, From British Columbia

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The pathogenic copepod *Phrixocephalus cincinnatus* (Copepoda: Pennellidae) in the eye of arrowtooth flounder, *Atherestes stomias*, and rex sole, *Glyptocephalus zachirus*, from British Columbia

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Abstract

We report *Phrixocephalus cincinnatus*, a pennellid copepod infecting the eyes of flatfishes, from a single specimen of rex sole, *Glyptocephalus zachirus*, for the first time. In the typical host, the arrowtooth flounder, *Atherestes stomias*, the parasite occurred commonly in sampled populations from the Broughton Archipelago in British Columbia, infected primarily the right eye of the flounder, and on only one occasion presented more than two parasites per eye. The copepod attached to the choroid layer and ramified throughout the posterior compartment of the eye, resulting in the disruption of the retina and probably impairing host vision. Inflammation and hyperplasia progressed to necrosis and proliferation of connective tissue, resulting in the total destruction of the eye.

Introduction

Phrixocephalus cincinnatus is a hematophagic pennellid copepod infecting the eyes of primarily flatfishes in the eastern Pacific Ocean. Originally described from the Pacific sanddab, *Citarichthys sordidus*, in California, it is now confirmed from at least 8 host species (*Atherestes stomias*, arrowtooth flounder; *C. sordidus*; *C. xanthostigma*, longfin sanddab; *Hippoglossus stenolepis*, Pacific halibut; *Lepidogobius lepidus*, bay goby; *Parophrys vetulus*, English sole; *Pleuronichthys decurrens*, curlfin sole; and *Zalembius rosaceus*, pink seaperch) ranging from southern California to British Columbia. In British Columbia, the parasite is well known from the arrowtooth flounder (Kabata, 1967).

Pennellids are unusual among copepods in that they utilize intermediate hosts in their development. Free-swimming nauplii develop into copepodids, which seek a fish or an invertebrate upon which to develop and mate. Mated females then search out the definitive host. Upon reaching the definitive host, the organisms undergo differential growth, producing a variety of structures that facilitate contact with the host's bodily fluids. Members of the genus *Phrixocephalus* develop a holdfast organ consisting of an elaborate, dendritic network of processes ("antlers" or "rami") that anchor the parasite deep within host tissue where it induces the formation of a hematoma from which it ingests the extravasated blood. In the case of *Phrixo-*

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cephalus cincinnatus, the newly-settled adult enters the eye, burrows into the choroid layer, ramifies throughout the globe of the eye, and elongates the trunk externally through the cornea where the egg sacs are extruded. Adults can be up to 30 mm long and as many as 5 individuals in one eye have been reported (Kabata, 1967; Kabata, 1969; Perkins and Gartman, 1997), though typically only single infections are observed. Severe physical damage to the eye can result in blindness, particularly when both eyes are infected (Kabata and Forrester, 1974). The prevalence can exceed 80% in some host populations (Kabata, 1969).

The host-parasite relationship has been investigated on two occasions. Kabata and Forrester (1974) studied the parasite in the arrowtooth flounder in British Columbia and identified a negative relationship between prevalence of the parasite and fish size and depth of capture. Perkins and Gartman (1997) studied the parasite's relationship with the Pacific sanddab in southern California and found differences in prevalence among localities and an increase in prevalence during the summer and in proximity to wastewater outfalls. Kabata (1969, 1970) gave a brief narrative on host pathology along with three general photomicrographs of sectioned material, but for the most part, the specifics of the pathological changes associated with the infection have not been well documented. In this paper, we use material collected near the northeastern coast of Vancouver Island to report a new host record for the parasite and document the pathological changes associated with the parasite and its development.

Materials and methods

Hundreds of individuals of various flatfish species (arrowtooth flounder; rex sole; slender sole, *Eopsetta exilis*; flathead sole, *Hippoglossoides elassodon*; butter sole, *Pleuronectes isolepis*; and English sole caught as by-catch in commercial shrimp fisheries within the Broughton Archipelago, Queen Charlotte Straight, northern Vancouver Island, British Columbia (50°43'N 126°36'W) during 2001-2002 were examined grossly for evidence of infection by *P. cincinnatus* and released. Representative specimens of both infected and uninfected hosts were collected and examined in detail.

Normal and infected eyes, including copepods, were removed, fixed either in 70% ethanol or 10% formalin, and shipped to the Gulf Coast Research Laboratory (GCRL). Of those arrowtooth flounders from which eyes were taken and fish size was known, the average size was 20.3 cm total length (10.5-31.8 cm). Limited size data are available for the other species, but all fish were comparable in size due to extruders in the trawl nets. At GCRL, representative material was selected and processed for histological examination. All material processed for histological examination came from the arrowtooth flounder. Eyes were washed in tap water, dehydrated through an ethanol series, embedded in paraffin, sectioned at 4 μm, mounted, stained with hematoxylin and eosin, and examined using a compound microscope. In total, 14 eyes representing the ontogeny of the parasite and normal, uninfected eyes were sectioned, stained, and examined.

Figure 1. Arrowtooth flounder, *Atherestes stomias*, with two *Phrixocephalus cincinnatus* protruding from the eye. Scale bar = approximately 15 mm.

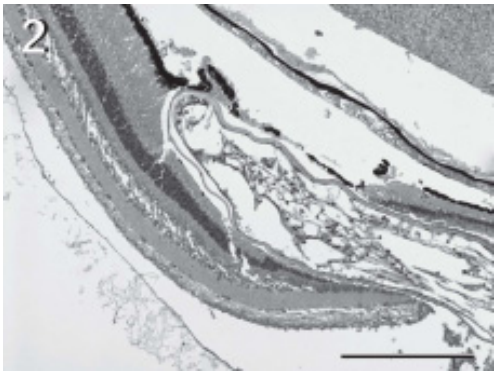

Figure 2. Ramus of *Phrixocephalus cincinnatus* in the retina of *Atherestes stomias*, H & E preparation, showing largely normal tissue with no appreciable immune response. Scale bar = 230 μm.

Results

The finding in a single rex sole constitutes a new host record for this parasite and brings the total of known host species to 9. No species other than the arrowtooth flounder and the rex sole was infected. The findings in this study, when taken together with those of Kabata (1967, 1969) and Kabata and Forrester (1974), indicate that the arrowtooth flounder is the typical host for this parasite in British Columbia. As many as 6 parasites per eye were observed, but 1 or 2 per eye was the most common finding (Figure 1). The right (lower) eye was more commonly infected than the left (upper) eye. The prevalence in arrowtooth flounder per trawl often approached 100%.

All lesions from the arrowtooth flounder were similar in type but variable in severity depending on the stage of development. Lesions began with localized inflammation and hyperplasia surrounded by largely normal tissue (Figure 2) and progressed to involve the sclera, choroid layer, and retina. Rootlets penetrated deeply into the choroid, often abutting the junction with the sclera (Figure 3). The capillary beds of the choroid rete were congested, particularly at the site of insertion; however, blood vessels throughout the uvea, including the iris, were distended and convoluted (Figure 4). Blood exuded from the vessels surrounding rootlets. The choroid, including the junction with the sclera was basophilic, inflamed, hyperplastic, disorganized, and either necrotic or fibrotic in the vicinity of the attachment site (Figure 5). Macrophages and lymphocytes infiltrated the area around the insertion (Figures 3, 5 and 6)

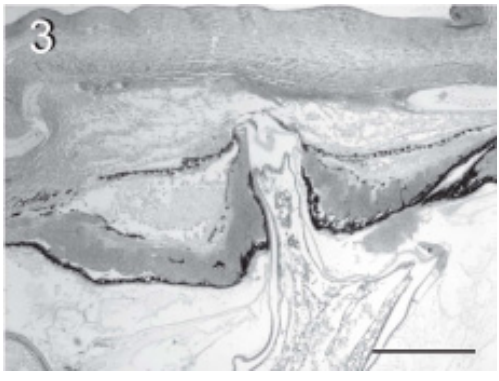

Figure 3. Insertion of *Phrixocephalus cincinnatus* into choroid of retina in *Atherestes stomias*, H & E preparation. Scale bar = 400 μm.

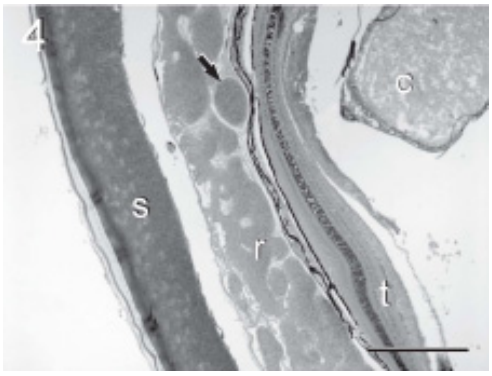

Figure 4. Capillary bed in choroid rete (r) of *Atherestes stomias* infected with *Phrixocephalus cincinnatus* showing distended, convoluted, and engorged blood vessels (arrow), sclera (s), retina (t), and ramus of *P. cincinnatus* (c), H & E preparation. Scale bar = 450 μm.

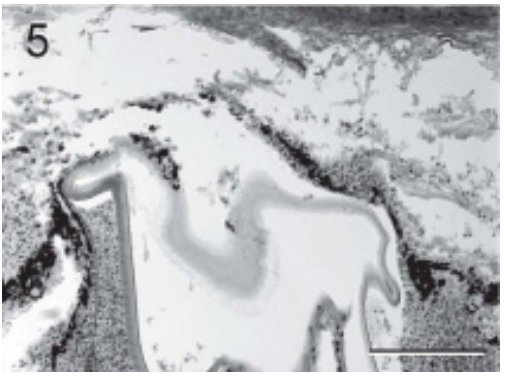

Figure 5. Insertion of *Phrixocephalus cincinnatus* into choroid of retina in *Atherestes stomias* showing necrosis, H & E preparation. Scale bar = 100 μm.

and were present to some degree throughout both the anterior and posterior compartments of infected eyes. In other areas within the posterior compartment, particularly in early infections, rami also compressed the retina against the choroid and infiltrated the retina without breaching it (Figures 2 and 4). Retinal detachment (typically between the photoreceptors and the choroid layer) was common, but difficult to distinguish from histological artifact. In developing infections, there was no evidence of immune cells or hyperplasia in the retina itself except at the site of penetration into the choroid. In mature infections, some rootlets were completely surrounded by basophilic, hyperplastic, fibrous connective tissue (Figure 7), and a substantial portion of the retina was replaced by proliferated, wispy strands of fibrous connective tissue (Figure 8). In all cases, regardless of the stage of the infection, the retina was functionally compromised.

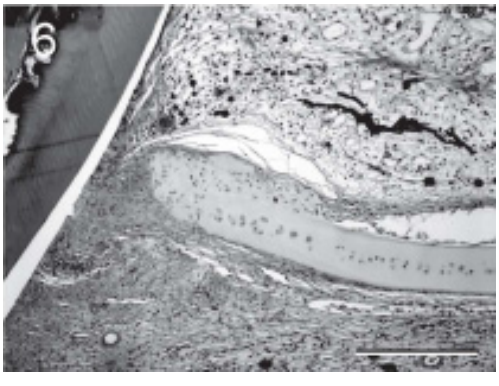

Figure 6. *Phrixocephalus cincinnatus* in eye of *Atherestes stomias* showing hyperplasia and macrophage/lymphocyte infiltration, H & E preparation. Scale bar = 270 μm.

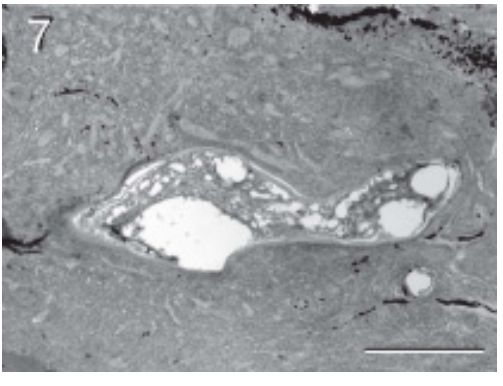

Figure 7. Rootlet of *Phrixocephalus cincinnatus* in eye of *Atherestes stomias* surrounded by basophilic, fibrous connective tissue, late infection, H & E preparation. Scale bar = 500 μm.

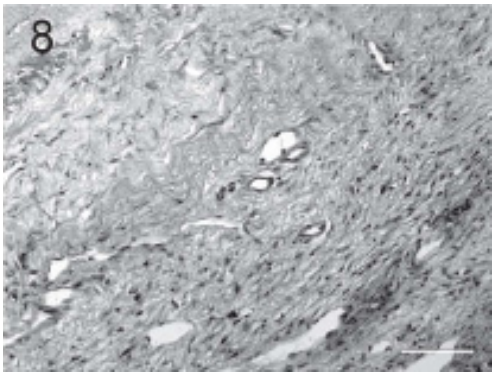

Figure 8. Wispy strands of proliferated fibrous connective tissue in retina of *Atherestes stomias* in late stages of infection with *Phrixocephalus cincinnatus*, H & E preparation. Scale bar = 70 μm.

The parasite was observed in the anterior chamber of some eyes, but regardless of the position of the parasite in the eye, the cornea of eyes infected with pre-emergent parasites was normal. Further, there was no indication of past injury to the cornea. The lens of all infected eyes appeared largely normal, although there were occasional spots of apparent degeneration not necessarily associated with the parasite. Some lens displacement was observed, but we could not differentiate either the displacement or the degeneration from that which normally occurs when sectioning eyes. The iris was normal in eyes infected with pre-emergent parasites, though blood vessels in the iris were engorged with blood in some hosts.

Discussion

Although *P. cincinnatus* is now confirmed from 9 hosts, only 2 (Pacific sanddab and arrowtooth flounder) are considered primary hosts. Even so, the levels reported in the Pacific sanddab are lower than those reported from the arrowtooth flounder. We have limited information regarding the actual prevalence in the population(s) from which our samples came; however, the parasite was common in the sampled populations. Kabata (1969) reported a prevalence of 83% in his sample of 53 fish. The biological importance, if any, of the difference in prevalence between the Pacific sanddab and the arrowtooth flounder is unknown. The studies on the sanddab examined considerably more fish from more localities than the studies on arrowtooth flounder; thus, the difference may be attributable to differences in sample size. Also, the zoogeographic distribution of the parasite and its intermediate host may more closely match that of the arrowtooth flounder than the Pacific sanddab.

Perkins and Gartman (1997) found substantial differences in prevalence related to season and locality as well as variation among years. Neither Kabata's data nor ours can address temporal or geographic patterns, but Perkins and Gartman's conclusion is consistent with a system in which a vagile host is variably exposed to infectious stages that are themselves patchily distributed. Thus, when the parasite is present, it can be quite common. All hosts except the arrowtooth flounder and Pacific sanddab, including those listed as unconfirmed (Gulf sanddab, *C. fragilis*; California tonguefish, *Symphurus atricauda*; and California skate, *Raja inornata*) by Perkins and Gartman (1997), are regarded as accidental hosts whose infections can be explained by incidental ecological or behavioral overlap with the primary hosts.

The size of the infected fish examined in this study is comparable to that of those examined in Kabata's studies. Kabata and Forrester (1974) noted that the prevalence of the parasite

decreased with the size of the fish, but the study did not attempt to elucidate the cause for the relationship. The authors did suggest a few possible mechanisms that could produce the pattern. Infection could 1) impede the growth of the host by affecting the ability of the host to catch prey, 2) kill the host outright, 3) be related to the fact that larger fish by virtue of being in a different niche are not exposed to the parasite, or 4) be related to larger fish having larger, tougher eyes that are more difficult to penetrate and navigate through. Perkins and Gartman (1997) found that the parasite did not decrease the condition of the Pacific sanddab in comparison with uninfected fish. Both Kabata and Perkins and Gartman agreed that fish infected in both eyes are lost from the population. However, with respect to monocular infections, the situation is less clear. Perkins and Gartman (1997) postulated that infected eyes, by virtue of fish retinas responding mainly to movement, were still functional, but that even if they were not, fish could thrive with one eye. Nonetheless, they saw few examples of one-eyed fish in their samples, which suggests selective removal. Kabata and Forrester's (1974) data noting a decrease in prevalence with age and length of the host also suggests selective removal. We can not summarily eliminate any possibility; however, the damage we observed strongly suggests visual impairment which could put a predatory fish like the arrowtooth flounder at a disadvantage both for obtaining food and avoiding predators. Borucinska et al. (1998) noted (citing Berland (1961)) that in the lore of Norwegian fisherman the copepod *Ommatokoita elongata* was believed to serve as a lure which would attract food to the vicinity of the impaired host's mouth. If that were true, it could mitigate some of the difficulty in finding food for a blind host. Nevertheless, we find it difficult to believe that a fish infected with *P. cincinnatus* could thrive, particularly when the indirect effects of infection are considered. Kabata (1970) lists pathological conditions ranging from anemia to reduced fat content to castration associated with infections by pennellid copepods. Thus, whether directly or indirectly, monocular infections with this parasite almost certainly contribute to impairment and, perhaps, host death.

Qualitative data provided by fishermen confirm the preference of the parasite for the right (lower) eye. Kabata (1969) found that about 66% of infected flounders harbored copepods in only the right eye. He reasoned that the right eye by virtue of being elevated in the center of the head was more exposed to the infectious stage of *P. cincinnatus* than the left eye which sits close to the sediment. Kabata's logic appears to be supported in Perkins and Gartman's work on the Pacific sanddab, whose eyes are nearly level on the head. Perkins and Gartman (1997) found only a slight preference for the left (anterior) eye.

The host response to the copepod follows a fairly typical pattern including inflammation, hyperplasia, granulation, and proliferation of fibrous connective tissue. The cornea is avascular and relatively devoid of mast cells, thus it is limited in its ability to mount an immune response to injury (Niederkorn, 1994). Proper function of the cornea is dependent on a relatively thin layer of delicate cells, damage to which can quickly result in opacity and blindness (Wilcock and Dukes, 1989; Niederkorn, 1994). Apart from

tearing that may have resulted from the extraction of the eye or sectioning, there, however, was little evidence of host response in the cornea, which is particularly remarkable given that the mature parasites had penetrated the cornea twice. In mammalian corneas and presumably in fish corneas, response to injury involves neuronal signals that stimulate organ-wide degranulation of mast cells which results in vasodilation, vasopermeability, edema, and inflammation characteristic of the typical host response to a parasite (Niederkorn, 1994). Thus, any response in the cornea may be a by-product of processes occurring at the organ level. Corneas of elasmobranchs did demonstrate some hyperplasia, dysplasia, necrosis, and inflammation in response to infection by *O elongata* (Lernaeopodidae) (Borucinska et al., 1998; Benz et al., 2002), and Figure 94 in a book by Kabata (1970) shows corneal inflammation and necrosis associated with *P. cincinnatus* in arrowtooth founder. Neither paper discusses the mechanisms of the response.

The retina, while avascular, is capable of hypertrophy, hyperplasia, fibrous metaplasia, necrosis, and phagocytic activity (Wilcock and Dukes, 1989). Our data indicate inflammation, hyperplasia, fibrosis, and necrosis in the vicinity of the retina, but we can not necessarily conclude that those pathological changes are of retinal origin. In fact, we observed the parasite surrounded by retinal tissue with no corresponding response (Figure 2). Wilcock and Dukes (1989) warned that the diagnosis of retinal pathology in teleosts must be made cautiously because of the variability among teleost species and the lack of good comparative data. The choroid layer, being highly vascular, can marshal the body's entire immune repertoire. Thus, the non-specific responses inside the globe of the eye combined with the direct insult of choroid penetration provide ample opportunity for the host to produce pathological changes that would at the very least impair visual acuity. At the very worst, the reaction could completely destroy the eye. Wilcock and Dukes (1989) indicated that the mechanism for destruction of infected eyes is death of the parasite followed by devastating inflammation. We did observe severe inflammation in some of the infected eyes; therefore, it is likely that we observed the end result of infection by *P. cincinnatus*. However, because our observations were limited to previously fixed material, we do not know if any of the parasites were dead before fixation.

The eye is a somewhat unusual habitat. Copepods have exploited this habitat in a couple of different ways. *Ommatokoita elongata* only superficially infects the eyes of Greenland sharks and Pacific sleeper sharks and grazes on the epithelial cells of the cornea and conjunctiva, thus limiting its exposure to the host's immune response and only partially affecting the host's vision. Because of the limited immune response in the cornea, Benz et al. (2002) considered the shark eye a relatively less hostile environment than other parts of the body and thus an advantageous habitat for *O. elongata*. Indeed, several generations of *O. elongata* can use the same eye (Borucinska et al., 1998; Benz et al., 2002). In the case of *P. cincinnatus*, the cornea also is relatively unaffected, but the deep infection results in vasodilation, increased vasoperme-

ability, and edema that provides access to the blood and other fluids that nourish the parasite. Further, Kabata (1969) proposed that *P. cincinnatus* expels digestive enzymes through the anus to digest the eye to facilitate extrusion of egg strings. This could contribute to the organ-wide, internal destruction we observed. Kabata (1967) indicated that blindness and total destruction of the eye was the inevitable result of infection by *P. cincinnatus*. Death of the parasite, whether through failure to develop or senescence, results in total destruction of the eye (Kabata and Forrester, 1974).

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