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NOTE

Transmission of PKX, the causative agent of proliferative kidney disease (PKD), to rainbow trout *Oncorhynchus mykiss* following filtration of water and sediments

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ABSTRACT: PKX, the myxosporean causing proliferative kidney disease (PKD), was transmitted to rainbow trout *Oncorhynchus mykiss* following their exposure to filtered sediments and water from a recycled water system where PKD is enzootic. Suspended sediments (25 l) were passed sequentially through 500, 250, 100 and 50 µm screens. Infectivity was found with material trapped at 500 µm and in water passing the 50 µm screen. No infectivity was found with material trapped on the 250, 100 or 50 µm screens. In a second study, 180 l of water was passed sequentially through a series of filters identical to those used in the sediment trial. No infectivity was trapped on any size filter (500 to 50 µm) but 10 of 20 trout exposed to water passing all filters were infected with PKX. The association of PKX infectivity with material >500 µm in the sediments and with a stage smaller than 50 µm in the water is consistent with the hypothesis that a second host residing in the sediments releases a smaller infective stage found in the water, as shown for other myxosporean/actinosporean parasite life cycles. A small oligochaete (*Stylaria lacustris*) found on the 500 µm filter is under investigation as the possible second/alternate host of the PKX myxosporean.

Proliferative kidney disease (PKD) is a serious systemic myxosporean infection which is primarily found among rainbow trout *Oncorhynchus mykiss* in Europe and among rainbow trout and chinook *O. tshawytscha* and coho *O. kisutch* salmon in the western USA and Canada (Clifton-Hadley et al. 1984, Hedrick et al. 1986). PKX, the causative agent of PKD, possesses extrasporogonic and sporogonic phases similar to other members of the phylum Myxozoa (Kent & Hedrick 1985a, 1986, Feist & Bucke 1987, Hedrick et al. 1988, von Odening et al. 1988).

Infections with the PKX myxosporean are obtained by contact between the fish and an unknown infective stage present in the water (Ferguson & Needham 1978, D'Silva et al. 1984, Foott & Hedrick 1987). Although the source and nature of the infective stage are unknown, the seasonality of its infectivity has been suggested by epizootiological studies (Ferguson & Ball 1979) and confirmed experimentally (Foott & Hedrick 1987). Attempts to transmit the disease by feeding of infected kidney tissues or cohabitation with infected fish have failed (Ferguson & Ball 1979, D'Silva et al. 1984). Experimental infections can be induced by injecting healthy trout or salmon with cell suspensions obtained from the kidney, spleen or blood of infected fishes (Clifton-Hadley et al. 1983, Kent & Hedrick 1985b). Experimentally induced infections have been used to study the host range, sequential development, pathogenesis, effects of water temperature and response of the parasite to therapeutic regimes (Rafferty 1985, Clifton Hadley et al. 1986, Kent & Hedrick 1986, Clifton-Hadley & Feist 1989, MacConnell et al. 1989, Arkush & Hedrick 1990).

Chilmonczyk et al. (1989) were the first to demonstrate that contact between trout and sediments from a recycled water system sufficed to transmit PKX. Expanding on these observations we have performed filtration studies on both sediments and water from a recycled water system where PKD is enzootic to identify the size and potential source of infectivity of the PKX myxosporean.

Materials and methods. ***Experimental fish:*** Rainbow trout used in the study originated from the Gournay-sur-Aronde farm, a facility 90 km north of Paris, France, with no history of PKD. In the sediment study,

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groups of 50 (7 g) trout were placed in 6 replicate 14 l aquaria receiving flow-through dechlorinated tap water at 16 °C. The same conditions were used in the water filtration study except that the fish were larger (20 g). All fish were fed a dry trout ration once daily at 2 % of their body weight.

Sediment filtration: Sediments were removed by siphon from the bottom of the settling tank at the experimental fish installation (IPE), Institut National de Recherche Agronomique (INRA), Jouy-en-Josas, France. This recycled water installation is known to contain annually recurring infections with the PKX myxosporean (Chilmonczyk et al. 1989, authors' unpubl. obs.). Sediments were collected as a suspension in 25 l of water and were then passed sequentially through graded screens with mesh sizes of 500, 250, 100 and 50 µm. The 14 l of water passing the smallest screen was retained in a 20 l plastic tank, and this was used for the short exposure period (see below) of the fish prior to their transfer to a 14 l aquarium. Materials trapped on each of the screens were placed into 5 l of water in individual 14 l aquaria containing 50 rainbow trout. An additional 50 trout were placed directly into the 14 l of water (in the plastic tank) passing all of the screens. The fish were maintained in their respective aquaria or the plastic tank with aeration for 3 h. The water flow to all aquaria was then resumed and the fish in the plastic tank were transferred to a 14 l aquarium identical to those from other experimental groups. An unexposed control group of 50 trout was held in an additional 14 l aquarium. The fish were maintained in these aquaria throughout the study and examined daily. Fish dying during the study and all those surviving after 3 mo were examined for presence of PKX in kidney imprints stained with Giemsa (Clifton-Hadley et al. 1983).

Water filtration: Water taken directly from the supply to the raceways in the IPE was passed sequentially through an identical set of screens to those used in the sediment trial and the final filtrate was retained in a 200 l plastic tank. A total of 180 l of water was filtered. Material trapped on each screen was then placed into aquaria containing 20 rainbow trout (20 g) as described for the sediment trial. An additional 20 fish were placed into the 180 l of water (with aeration) that passed all of the filters. After 3 h, the fish in the plastic tank were netted and placed into an aquarium identical to that used for other experimental groups. The water flow was resumed to all aquaria including an unexposed control group. As in the sediment trial, dead fish and all fish surviving at the end of the study (3 mo post initiation) were examined for evidence of the PKX myxosporean.

Results. ***Sediment filtration:*** The infectious stage of the PKX myxosporean was associated only with material trapped on the 500 µm screen and to a lesser extent in the water passing all filters tested. More than 50 % of the fish (24 of 41) were infected with PKX at 3 mo post exposure to material trapped on the 500 µm screen, and 4 of 9 fish that died were infected (Table 1). An examination revealed that the predominant forms present in the material were two species of oligochaetes (*Lumbriculus variegatus* and *Stylaria lacustris*). In addition, snails, crustaceans and insect larvae were abundant. In earlier studies we have washed, removed and isolated the larger oligochaetes (*L. variegatus*), snails, crustaceans and insect larvae (unpubl. data). We did not observe actinosporeans nor could we infect fish following exposure of trout to these isolated invertebrates (unpubl. data).

Other than the material trapped on the 500 µm screen the only infectivity found was in the final filtrate, where 2 of 43 fish were positive for PKX (Table 1). PKX was not detected in any dead fish from any other treatment groups.

Water filtration: In contrast to sediment filtration studies, infectivity was not detected on material trapped on any filter size but resided solely in the water passing all filters tested (Table 1). PKX was detected in 6 of 13 dead fish from the final filtrate group but not among dead fish from any other group. An outbreak of ichthyophthiriosis reduced the number of fish surviving to 3 mo but of the 7 surviving fish from the final filtrate, 4 were infected with PKX. PKX was not detected among dead fish in any other groups. In contrast to studies with the sediment, there was no evidence of oligochaetes, snails, insect larvae or crustaceans trapped on the 500 µm screen following filtration of 180 l of water.

Discussion. The results of filtration studies indicated that infectivity is associated both with larger material (>500 µm) and with a smaller body (<50 µm). This pattern of infectivity is consistent with previous findings for other myxosporeans in which a second host (oligochaete) releases a smaller infective stage (actino-

Table 1. PKX infectivity (no. fish infected/no. fish) among rainbow trout 3 mo after exposure to filtered sediments and water from a recycled water system enzootic for proliferative kidney disease

Origin	Filter size (µm)				Filtrate[a]	Control
	500	250	100	50		
Sediments	24/41[b]	0/41	0/43	0/46	2/43	0/48
Water	0/20	0/18	0/19	0/17	4/7[c]	0/22

[a] Water and particulates that passed all filters
[b] PKX was detected in 4 of 9 fish that died during the experiment
[c] PKX was detected in 6 of 13 fish that died during the experiment

sporean). Markiw & Wolf (1983) and Wolf & Markiw (1984) in their pioneering studies with *Myxobolus cerebralis*, the causative agent of whirling disease in salmonid fish, were the first to demonstrate that the intermediate host (second host), an oligochaete (*Tubifex tubifex*), trapped on the largest filter size (500 µm) was the source of an infective stage (an actinosporean). Although this hypothesis with *M. cerebralis* was challenged (Hamilton & Canning 1987), it has now been proven by others (El-Matbouli & Hoffmann 1989, Hedrick et al. 1989). Additional myxosporean/actinosporean life cycles have now been established for several other myxosporeans including *Hoferellus cyprini*, *Myxobolus cotti* and *M. pavloskii* (El-Matbouli & Hoffman 1989, El-Matbouli et al. 1992, Großheider & Körting 1992, Hoffmann pers. comm.). In addition, strong evidence exists for similar alternating cycles for *M. arcticus* (Kent et al. 1990) and a *Myxobolus* sp. from goldfish *Carassius auratus* by Yokoyama et al. (1991). Burtle et al. (1991) have also described an actinosporean found as the probable infective stage of proliferative gill disease in channel catfish *Ictalurus punctatus* presumably caused by *Sphaerospora ictaluri* (Hedrick et al. 1990). Similarly, the second host and actinosporean stages for *Ceratomyxa shasta* have presumptively been identified (Bartholomew et al. 1992).

Viewed in terms of a myxosporean/actinosporean alternating life cycle, our results suggest that the second host for PKX resides in the sediments and is trapped upon filtration at 500 µm. Fish exposed to suspensions containing this host are readily infected either by direct contact with released infective stages in the water or by ingestion of the second host. As shown by studies with the actinosporean causing whirling disease, the most probable route of entry of the infectious stage is the skin or gills (Markiw 1989). The lack of infectivity at 500 µm in our water filtration study is consistent with the absence of this second host in the water column. Observations of material trapped on the 500 µm screen in the water study confirmed the absence of the rich aquatic invertebrate life found in the sediment trial. Infectivity in the final filtrates of both the sediment and water studies indicates that the infectious or waterborne stage of PKX is less than 50 µm in size. Attempts to visualize this stage have so far failed but the difficulty encountered in observing actinosporeans may in part explain this difficulty (authors' unpubl. obs.). The source of the waterborne infective stage remains unknown but examinations of material trapped on the 500 µm screen in sediment trial have shown that a small oligochaete (*Stylaria lacustris*) unknowingly removed by rinsing procedures is present in the material with infectivity for PKX. This oligochate is now under suspicion and investigation as the potential second host for the PKX myxosporean.

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LITERATURE CITED

Arkush, K. D., Hedrick, R. P. (1990). Experimental transmission of PKX, the causative agent of proliferative kidney disease, to three species of Pacific salmon. J. appl. Ichthyol. 6: 237–243

Bartholomew, J. L., Fryer, J. L., Rohovec, J. S. (1992). *Ceratomyxa shasta* infections of salmonid fish. In: Kimura, T (ed.) Salmonid diseases. Proceedings of the OJI International Symposium on Salmonid Diseases, Sapporo, Japan, October 22–25, 1991 Hokkaido Univ. Press, Sapporo, p. 267–275

Burtle, G. J., Harrison, L. R., Styer, E. L. (1991). Detection of triactinomyxid myxozoan in an oligochaete from ponds with proliferative gill disease of channel catfish. J. aquat. Anim. Health 3: 281–287

Chilmonczyk, S., Thomas, P., de Kinkelin, P. (1989). Occurrence of proliferative kidney disease in an indoor recirculation unit and indirect experimental contamination of the rainbow trout. Bull. Eur. Ass. Fish Pathol. 9: 38–41

Clifton-Hadley, R. S., Bucke, D., Richards, R. H. (1984). Proliferative kidney disease of salmonid fish: a review. J. Fish Dis. 7: 363–377

Clifton-Hadley, R. S., Feist, S. W. (1989). Proliferative kidney disease in brown trout *Salmo trutta*: further evidence of a myxosporean aetiology. Dis. aquat. Org. 6: 99–103

Clifton-Hadley, R. S., Richards, R. H., Bucke, D. (1983). Method for the rapid diagnosis of proliferative kidney disease in salmonids. Vet. Rec. 112: 609

Clifton-Hadley, R. S., Richards, R. H., Bucke, D. (1986). Proliferative kidney disease (PKD) in rainbow trout *Salmo gairdneri*: further observations on the effects of temperature. Aquaculture 55: 165–171

D'Silva, J., Mulcahy, M. F., De Kinkelin, P. (1984). Experimental transmission of proliferative kidney disease in rainbow trout, *Salmo gairdneri* Richardson. J. Fish Dis. 7: 235–239

El-Matbouli, M., Fischer-Scherl, Th., Hoffmann, R. (1992). Transmission of *Hoferellus carassii* Achmerov, 1960 to goldfish *Carassius auratus* via an aquatic oligochaete. Bull. Eur. Ass. Fish Pathol. 12(2): 1–2

El-Matbouli, M., Hoffmann, R. (1989). Experimental transmission of two *Myxobolus* spp. developing bisporogeny via tubificid worms. Parasitol. Res. 75: 461–464

Feist, S. W., Bucke, D. (1987). Ultrastructural aspects of PKX, the causative agent of proliferative kidney disease in rainbow trout, *Salmo gairdneri* Richardson. J. Fish Dis. 10: 323–327

Ferguson, H. W., Ball, H. J. (1979). Epidemiological aspects of proliferative kidney disease amongst rainbow trout *Salmo gairdneri* Richardson in Northern Ireland. J. Fish Dis. 2: 219–225

Ferguson, H. W., Needham, E. A. (1978). Proliferative kidney disease in rainbow trout, *Salmo gairdneri* Richardson. J. Fish Dis. 1: 91–108

Foott, J. S., Hedrick, R. P. (1987). Seasonal occurrence of the infectious stage of proliferative kidney disease (PKD) and resistance of rainbow trout, *Salmo gairdneri* Richardson, to reinfection. J. Fish Biol. 30: 477–483

Großheider, G., Körting, W. (1992). First evidence that *Hoferellus cyprini* (Doflein, 1898) is transmitted by *Nais* sp. Bull. Eur. Ass. Fish Pathol. 12(2): 17–20

Hamilton, A. J., Canning, E. U. (1987). Studies on the proposed role of *Tubifex tubifex* (Muller) as an intermediate host in the life cycle of *Myxoxosma cerebralis* (Hofer, 1903). J. Fish Dis. 10: 145–151

Hedrick, R. P., Kent, M. L., Smith, C. E. (1986). Proliferative kidney disease of salmonid fishes. Fish Dis. Leaflet 74, U.S. Dept. of Interior, Fish and Wildlife Service, Washington, D.C.

Hedrick, R. P., Kent, M. L., Toth, R. J., Morrison, J. K. (1988). Fish infected with *Sphaerospora* spp. Thélohan (Myxosporea) from waters enzootic for proliferative kidney disease of salmonids. J. Protozool. 35: 13–18

Hedrick, R. P., McDowell, T., Groff, J. M. (1990). *Sphaerospora ictaluri* N. Sp. (Myxosporea: Sphaerosporidae) observed in the kidney of channel catfish, *Ictalurus punctatus* Rafinesque. J. Protozool. 37: 107–112

Hedrick, R. P., Wishkovsky, A., Groff, J. M., McDowell, T (1989). Transmission trials with three mxyosporeans of salmonid fish. Abstract. In: Diseases of fish and shellfish. IV EAFP International Conference, Santiago de Compostela, Spain, September 24–28, 1989, p. 38

Kent, M. L., Hedrick, R. P. (1985a). PKX, the causative agent of proliferative kidney disease (PKD) in Pacific salmonid fishes and its affinities with the Myxozoa. J. Protozool. 32: 254–260

Kent, M. L., Hedrick, R. P. (1985b). Transmission of the causative agent of proliferative kidney disease (PKD) with the blood and spleen of infected fish; further evidence that the PKX parasite belongs to the phylum Myxozoa. Bull. Eur. Ass. Fish Pathol. 5: 39–42

Kent, M. L., Hedrick, R. P. (1986). Development of the PKX myxosporean in rainbow trout *Salmo gairdneri*. Dis. aquat. Org. 1: 169–182

Kent, M. L., Whitaker, D. J., Margolis, L. (1990). Experimental transmission of the myxosporean *Myxobolus arcticus* to sockeye salmon using an aquatic oligochaete, *Eclipidrilus* sp. (Lumbriculidae). Am. Fish. Soc. Newslett. (Fish Health Sect.) 18(4): 4–5

MacConnell, E., Smith, C. E., Hedrick, R. P., Speer, C. A. (1989). Cellular inflammatory response of rainbow trout to the protozoan parasite causing proliferative kidney disease. J. aquat. Anim. Health 1: 108–118

Markiw, M. E. (1989). Portals of entry for salmonid whirling disease in rainbow trout. Dis. aquat. Org. 6: 7–10

Markiw, M. E., Wolf, K. (1983). *Myxosoma cerebralis* (Myxozoa: Myxosporea) etiological agent of salmonid whirling disease requires tubificid worm (Annelida: Oligochaeta) in its life cycle. J. Protozool. 30: 561–564

Rafferty, M. D. (1985). The influence of temperature on experimentally-induced proliferative kidney disease. Ph.D. thesis, National University of Ireland, Cork

von Odening, K., Walter, G., Bockhardt, I. (1988). Koinzidentes Auftreten von PKX und *Sphaerospora* sp. (Myxosporidia) in Beständen von *Salmo gairdneri* (Osteichthyes). Angew. Parasitol. 29: 137–148

Wolf, K., Markiw, M. (1984). Biology contravenes taxonomy in the myxozoa: new discoveries show alternation of invertebrate and vertebrate hosts. Science 225: 1449–1452

Yokoyama, H., Ogawa, K., Wakabayashi, H. (1991). A new collection method of actinosporeans – a probable infective stage of myxosporeans to fishes – from tubificids and experimental infection of goldfish with the actinosporean, *Raabeia* sp. Fish Pathol. 26: 131–138

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