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Spring Carp Mortality Syndrome (SCMS) Transmission Study

Science Report – SC020054/SR1

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Our work includes tackling flooding and pollution incidents, reducing industry's impacts on the environment, cleaning up rivers, coastal waters and contaminated land, and improving wildlife habitats.

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Science at the Environment Agency

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The work of the Environment Agency's Science Group is a key ingredient in the partnership between research, policy and operations that enables the Environment Agency to protect and restore our environment.

The science programme focuses on five main areas of activity:

- **Setting the agenda**, by identifying where strategic science can inform our evidence-based policies, advisory and regulatory roles;
- **Funding science**, by supporting programmes, projects and people in response to long-term strategic needs, medium-term policy priorities and shorter-term operational requirements;
- **Managing science**, by ensuring that our programmes and projects are fit for purpose and executed according to international scientific standards;
- **Carrying out science**, by undertaking research – either by contracting it out to research organisations and consultancies or by doing it ourselves;
- **Delivering information, advice, tools and techniques**, by making appropriate products available to our policy and operations staff.

Steve Killeen

Head of Science

1 Introduction

The carp, *Cyprinus carpio*, is the most popular sporting fish in England and Wales. As a result, both match and specimen fisheries dominated by this species are common throughout the country.

During the late 1980s and 1990s concern grew over widespread and large-scale carp mortalities in fisheries throughout England and Wales. In some cases over 90% of the carp population died, however there was no obvious cause of mortality. The mortalities occurred during spring or early summer and did not appear to be related to fish size, age or sex. Mortalities of this nature have been termed Spring Carp Mortality Syndrome (SCMS).

The cost of SCMS to a fishery can be substantial. The annual cost of SCMS to fisheries throughout England and Wales has been estimated to be at least £200,000. Though the number of suspected cases of SCMS has declined in recent years, the cause of the mortalities remains unclear. As part of the programme of research into SCMS investigations, a collaborative study was undertaken by the Environment Agency and Centre for the Environment, Fisheries and Aquaculture Science (Cefas) to investigate the transmission of SCMS and to determine whether an infectious agent was involved in the mortalities.

2 Methods

Five large carp were sampled from a suspected SCMS mortality at Riverside Lake, Doncaster in May 2003. The carp were transported to the Cefas Laboratory, Weymouth. Samples of naïve carp (<1yr, 4-6cm) were sourced from two suppliers. Mirror carp and ghost carp were obtained from Bow Lake, Hampshire Carp Hatcheries and mirror carp from Priory Fisheries. An additional sample of larger carp(12-16cm) was obtained from Fishers Pond, Hampshire.

Effluent water from the tank containing two Riverside carp was passed through a coarse in-line filter into two small tanks; one containing 20 Bow Lake carp and the other containing 20 Priory Fisheries carp. The initial water temperature was 11 ºC, which was increased to 16 ºC over a five-day period. A control group of Bow Lake and Priory Fisheries carp was maintained in a separate tank at 15 ºC for the duration of the study.

After 24 days of exposure to Riverside carp effluent water, 13 Bow Lake carp and 15 Priory Fisheries carp were transferred to two separate flow-through tanks. A group of 5 naïve Bow Lake carp and 5 naïve Priory Fisheries carp were introduced to each tank, (10 naïve carp in each). The water temperature was increased from 16 ºC to 21 ºC over a 5-day period.

Gill and visceral tissue was dissected from one Riverside carp. Tissue from six Priory Fisheries carp, exposed to Riverside effluent water, was also sampled and pooled with the tissue from the Riverside carp. Separate extracts of gill and liver/spleen/kidney were prepared and filtered through a 45 µm membrane. A volume of 10 ml of filtered extract was added to 10 ml of tank water and added to each of three new experimental tanks holding 10 naïve Bow Lake carp, 10 naïve Priory Fisheries carp and 5 Fishers Pond carp respectively. A control tank was also set up containing a similar group of fish. The water temperature in the tanks was increased from 16 ºC to 21 ºC over a period of four days.

A selection of dead and moribund carp was sampled from each of these tanks at varying intervals throughout experiment and tissue was collected for virological and histopathological examination. Virological examination was conducted using cell culture isolation on Koi Fin (KF) and *Epithelioma papulosum cyprini* (EPC) cell lines. Molecular virology included Koi Herpesvirus (KHV) PCR assay and nested PCR assay.

3 Results

The two Riverside carp died on day 13 and 18 of the experiment. The first mortalities in the Bow Lake and Priory Fisheries group, exposed to the effluent water, occurred on day 19. An additional four carp from the Bow Lake group died within 15 days and 12 carp died from the Priory Fisheries group within 34 days . No mortalities or clinical signs of disease were observed in the control group of carp.

Following the transfer of exposed Bow Lake and Priory Fisheries carp to separate tanks containing naïve carp, five mortalities occurred in the Bow Lake group and 10 in the Priory Fisheries group. No mortalities occurred in the naïve group of fish.

Following bath exposure of tissue extracts from Riverside and Priory Fisheries carp, no mortalities were recorded in the naïve carp six weeks post-challenge.

Histopathological examination was conducted on one Riverside carp, three Bow Lake carp and three Priory Fisheries carp following exposure to effluent water. A number of different pathologies were recorded including the presence of bacteria within the gill tissue. Cellular necrosis and lamellae fusion of the gill tissue was recorded extensively.

Virological examination was conducted on one Riverside carp that died before the start of the experiment. KHV nested PCR assay returned negative for the presence of KHV. A cytopathic effect (CPE) was recorded on KF cells isolated with gill tissue after 12 days, however no effect was noted on the EPC cells after 21 days, incubated at 20^{o}C. The Riverside carp that died on day 13 showed no CPE on either the KF or EPC cells, however the KHV nested PCR assay produced a PCR amplification similar to the KHV positive control. Reverse hybridisation with DNA probes and southern blotting confirmed a low-level KHV DNA positive result. The 2 Bow Lake carp and 2 Priory Fisheries carp from the exposure study showed no CPE on the KF or EPC cells after 28 and 21 days respectively. The 6 Priory Fisheries carp used in the bath exposure study also showed no CPE on the KF or EPC cells after 22 and 21 days respectively and were negative in the KHV nested PCR assay.

4 Conclusion

This study has provided some evidence for the presence of an infectious agent in fish from Riverside Lake. Virological investigation recorded a slow growing agent causing a CPE on KF cells from a Riverside carp. Further investigation showed that the CPE could be passaged onto EPC cells to produce a productive infection. Investigation by electron microscopy also shows evidence of the presence of herpes-like virus particles. However, this CPE was not consistent with KHV, as KHV produces a transient CPE in EPC cells with no productive infection. KHV DNA was however detected in another Riverside carp, though this was a low-level positive result. During summer and autumn 2003, the Environment Agency and Cefas investigated several other carp mortality events. A small number of these sites tested positive for KHV DNA by single-round or nested PCR. At one of these sites, KHV was isolated from gill tissue on KF cells. The source of these outbreaks remains unknown, however they could have occurred through linked stockings of fish from an infected site. Ornamental carp species were also present at some of the sites, providing a potential route of infection. Clinical signs of KHV occur between 17 and 27^{o}C (experimentally between 15 and 28^{o}C). It is possible that water temperatures at Riverside Lake could have been as high as 17^{o}C during May and therefore conducive to KHV, however evidence from virological examination indicates that despite KHV being present in the sample, another pathogen was present that could have been implicated in the mortality.

Further evidence of the presence of an infectious agent is provided by mortalities of naïve carp (Bow Lake and Priory Fisheries) exposed to effluent water from Riverside carp, even after transfer to new tanks. Failure of the exposed Bow Lake and Priory Fisheries carp, and bath exposure, to infect other naïve carp suggests that the conditions were unsuitable for replication of the infectious agent. This could be attributed to an unsuitable temperature range whereby the initial temperature for the Riverside and exposed carp was 11-16 ºC, which was increased to 21 ºC for the later studies.

Histopathological investigation identified a number of pathologies, most of which are considered non-specific. The histology tissue sections showed no evidence of an infectious agent, although bacterial pathogens were identified in the gill tissue of the Riverside carp. However as this fish was not fresh when sampled it is likely that these were secondary bacteria that invaded after the fish had died.

No specific bacterial investigation was conducted on the examined fish. These investigations would normally involve obtaining swabs of the kidney in order to grow bacterial cultures, if present, from which the bacterial species could be identified. This method determines whether a systemic bacterial infection is present within an individual fish. Therefore in the absence of these tests, the significance of bacterial pathogens within the sample could not be confirmed.

Gill necrosis and extensive lamellar fusion were evident in the most of the samples. These pathologies are similar to those recorded during bacterial exotoxin events. In order to differentiate between exotoxins and other causes, the loss of micro-ridging on the gills is required. In the absence of this information, it is not possible to determine whether bacterial exotoxins were involved in the mortalities. It has been suggest by the Cefas team overseeing the work that the bacteria recorded within the sample could have originated from the in-line filter that was used to reduce parasite load from the Riverside carp. The filter was washed through at regular intervals allowing a slow, continuous flow of water. A yellow-brown scum was also noted in the tanks that had to be removed at regular intervals. This scum could have occurred through the use of pellet food to feed the carp, however it was not investigated.

Evidence suggests that the presence of an infectious agent within the Riverside carp is the likely cause of the mortalities in the naïve carp. Though KHV was detected in one Riverside carp, it seems unlikely that this was the primary cause of the mortality. In the absence of additional investigations, there is also little evidence to suggest the presence of bacterial toxins within the sample. Following this experiment, the causative agent of SCMS remains undetermined. Collaborative work between the Environment Agency, Cefas and the English Carp Heritage Organisation (ECHO) will continue to further the understanding of SCMS and identify the causative agent.

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