Water sports could contribute to the translocation of ranaviruses

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BACKGROUND Ranaviruses have been identified as the cause of explosive disease outbreaks in amphibians worldwide and can be transmitted between hosts both via direct and indirect contact, in which humans might contribute to the translocation of contaminated material. Pathogen pollution of ranavirus by humans through adhesion of virions to boat or other recreational gear surfaces has been suggested, however, no studies have demonstrated this so far. Gray et al. (2018) provided evidence that ranavirus could adhere to examination gloves and hence contribute to the transmission of the virus among amphibians that are processed as part of a pathogen surveillance study if gloves are not changed between animals.

AIM The aim of this study was to evaluate the possible role of water sports in the human translocation of ranavirus and *Batrachochytrium* spp. using molecular biology techniques. For that purpose, boats were sampled during the spring Spanish Canoe Championship which took place in Pontillón de Castro (northwestern Spain), a reservoir with a history of ranavirosis.

METHODS A total of 234 boats were sampled during the Championship in Pontillón de Castro (500 participants), in May 2017. Boats were tested for the presence of ranavirus and *Batrachochytrium* spp. DNA, using quantitative real-time polymerase chain reaction techniques (qPCR) (Picco et al., 2007; Blooi et al., 2013). Samples were collected both when boats (either kayaks or canoes) arrived at the Nautical Complex and Race Course and were still dry (i.e., before entering the complex and prior to a disinfection treatment), and after each race when the boats were still wet. Thirty two kayaks were tested at arrival and after the race. Moreover, water samples were collected and processed from the northern end, middle and southern end of the reservoir.

RESULTS A total of 22 swabs (22/234, 9.40%) yielded positive qPCR-results for *Ranavirus* DNA while *Bd* or *Bsal* were not detected in any of the tested samples (Figures 1 and 2). Interestingly, there were four positive kayaks at arrival that became negative after race and one kayak negative at arrival that became positive after race. The three water samples analyzed yielded positive results.

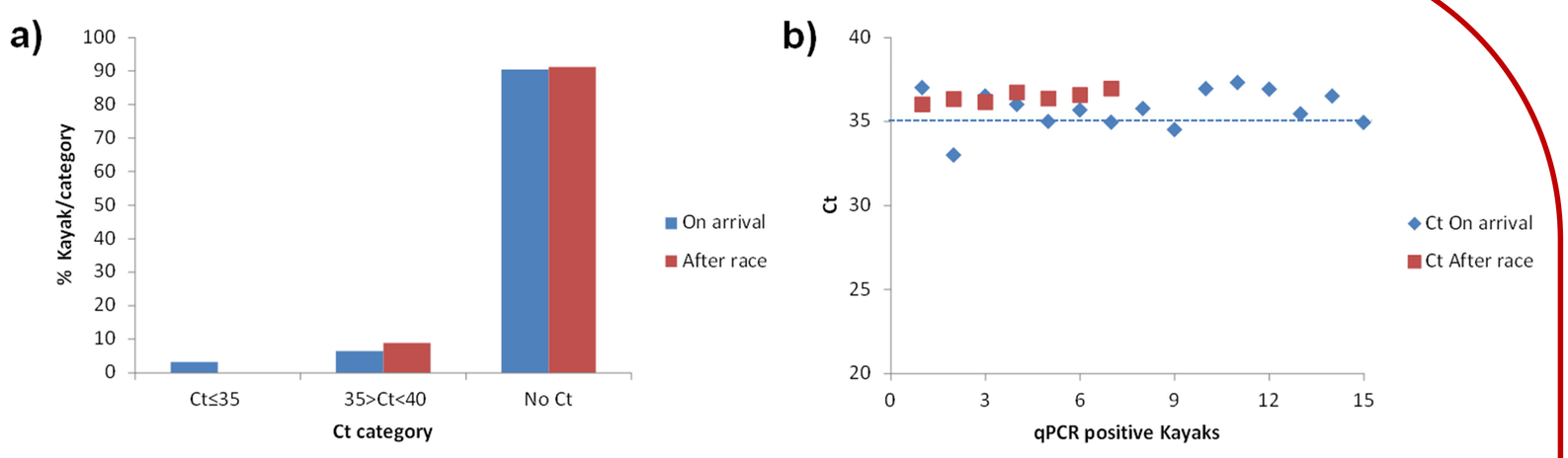

Figure 1. Graphs representing the percentage of kayaks within each ranavirus Ct category (a), before and after the Spring Spanish Canoe Championship held in the Pontillón de Castro reservoir (Northwestern Spain) in 2017, and the Cts obtained in quantitative real-time polymerase chain reaction (qPCR) positive kayaks (b). Samples with a cycle threshold (Ct) ≤35 were considered qPCR positives. Samples with a Ct between 35 and 40 were considered weak qPCR positives. Samples with no Ct or no typical amplification curves were regarded as qPCR negative.

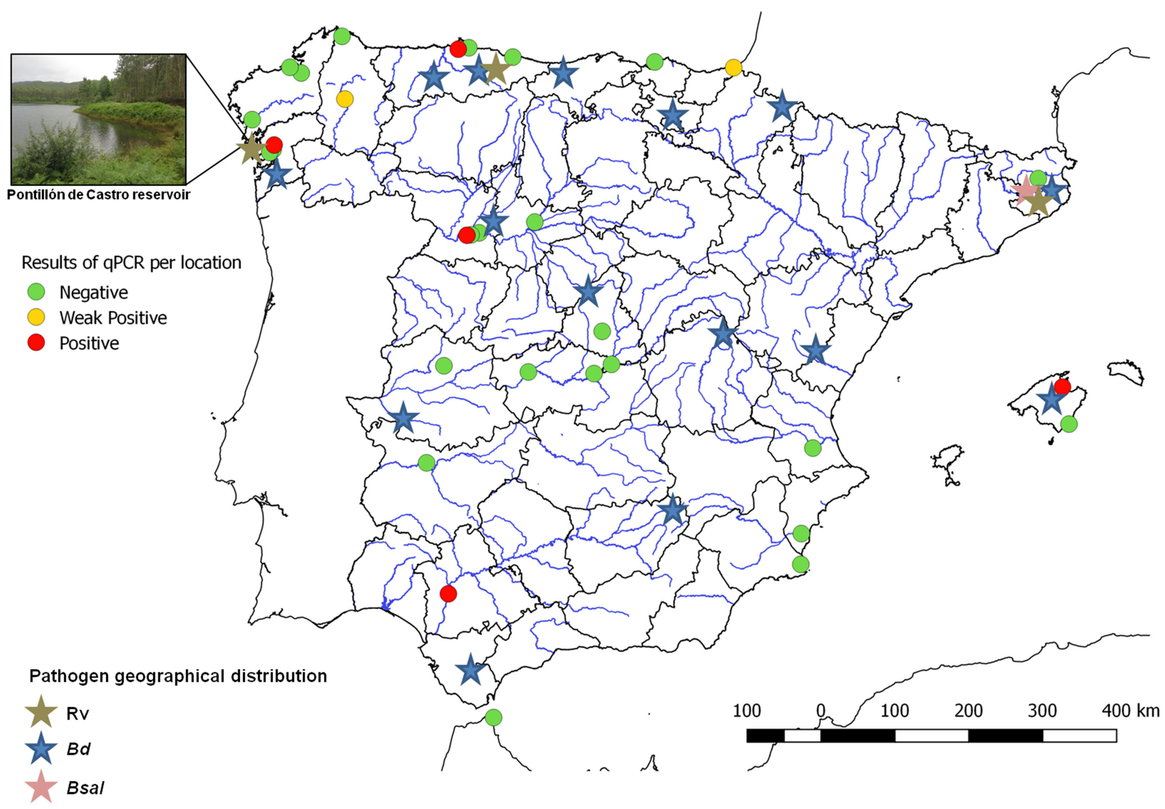

Figure 2. Origin (usual training place) of the participant kayaks in the Spring Spanish Canoe Championship held in the Pontillón de Castro reservoir in 2017, studied for the presence of ranavirus using quantitative real-time polymerase chain reaction (qPCR). Red circles represent training places with at least one positive kayak (threshold point (Ct) ≤35) by qPCR. Yellow circles represent training places with at least one weak positive kayak (Ct between 35 and 40). Green circles represent training places of negative kayaks. Brown, blue and pink stars represent the geographical distribution of Ranavirus (Rv), *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*) in Spain, respectively.

DISCUSSION AND CONCLUSIONS This research demonstrates for the first time how water-related sports such as kayaking can be a source of pathogen pollution for amphibians. We do not know if the virions on the boat were viable, because qPCR only detects DNA presence, or if there were sufficient quantities of live virus for it to be a source of infection; however, there is the possibility that some virions might remain viable, and present a translocation risk. In this study, neither *Bd* nor *Bsal* were detected in any of the kayak samples, which suggests that these pathogens have not been introduced into the reservoir or that their survival in or on watercraft is more limited when compared to ranavirus. Other human activities such as fishing and swimming could be additional sources pathogen pollution through contaminated equipment, this possibility needs to be investigated. Our results provide justification for public disinfecting stations in key areas where human traffic from water sports is high.

Key references Blooi, M. *et al*. Duplex real-Time PCR for rapid simultaneous detection of *Batrachochytrium dendrobatidis* and *Batrachochytrium salamandrivorans* in amphibian samples. *J. Clin. Microbiol.* 51, 4173-4177 (2013); Picco, A. M., Brunner, J. L. & Collins, J. P. Susceptibility of the endangered California tiger salamander, *Ambystosoma californiense*, to ranavirus infection. *J. Wild. Dis*. 43, 286-290 (2007); Gray, M. J. *et al*. Poor biosecurity could lead to disease outbreaks in animal populations. *PLoS One*. 13, e0193243 (2018).

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