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Disease threat to European fish

The deliberate introduction of new species can have unexpected negative consequences[1,2] and we show here how a recently introduced fish, the invasive Asian cyprinid *Pseudorasbora parva*, is causing increased mortality and totally inhibiting spawning in an already endangered native fish, the European cyprinid *Leucaspius delineatus*. This threat is caused by an infectious pathogen, a rosette-like intracellular eukaryotic parasite that is a deadly, non-specific agent. It is probably carried by healthy Asian fish, and could decrease fish biodiversity in Europe, as well as having implications for commercial aquaculture.

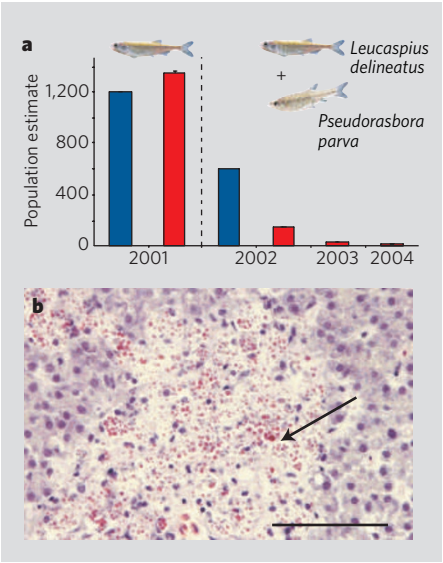

Figure 1 | Decline of *Leucaspius delineatus* population in a large natural pond after the introduction of *Pseudorasbora parva* and its associated pathogen, *Sphaerothecum destruens*. **a,** The exact maximum likelihood estimates (red bars, showing s.e.m.) for *L. delineatus* populations in the autumn are shown for four years. Starting populations in April of 2001 and of 2002 are shown as blue bars: these represent 1,200 *L. delineatus* in 2001, and 600 *L. delineatus* present in a mixed population with 600 *P. parva* introduced in 2002. **b,** High-power light micrograph of a section of a *L. delineatus* liver. There is a focus of phagocytic cells (arrow) containing conspicuous eosinophilic rosette-like agents (pink). (Slide stained with haematoxylin and eosin; scale bar, 50 micrometres.)

The sunbleak, *L. delineatus,* is the only representative of its genus and the only nest-guarding fish among European cyprinids. Once widespread in Europe, in the past 40 years it has inexplicably declined[3] and is now on the European list of threatened freshwater fishes[3]. By contrast, since its introduction in 1960 into Romanian ponds near the River Danube, the Asian topmouth gudgeon, *P. parva,* has spread rapidly throughout Europe[4] and has locally coincided with *L. delineatus* extinction[5,6].

In laboratory experiments (for methods, see supplementary information), we found that the holding water of *P. parva* acted as an absolute inhibitor of spawning for *L. delineatus* (no eggs produced in *P. parva* water compared with $1,596 \pm 840$ in control, clean water), and caused a large increase in fish mortality ($69 \pm 3\%$ deaths in the treatment group, compared with $16 \pm 2\%$; $P < 0.05$, Mann-Whitney *U*-test; 4 experiments). These results were confirmed in a large natural outdoor pond, where *L. delineatus* populations declined by 96% over three spawning seasons (2002–04; Fig. 1a) after being mixed with *P. parva*, despite an increase of 13% in the year before *P. parva* arrived (2001; Fig. 1a). Spawning was totally inhibited in *L. delineatus* after *P. parva* was introduced.

We found that the decline in *L. delineatus* (caused by total inhibition of spawning, loss of body condition, and death) that resulted from sharing water with *P. parva* was caused by an infectious organism. Histological findings from moribund *L. delineatus* indicated extensive infection of visceral organs, including the reproductive tissues, with an obligate intracellular eukaryotic pathogen (Fig. 1b; see also supplementary information) similar to the lethal rosette agent *Sphaerothecum destruens*[7] that infects Chinook salmon, *Oncorhynchus tshawytscha,* and Atlantic salmon, *Salmo salar*.

The presence of this pathogen in *L. delineatus* that had been exposed to holding water from *P. parva*, and its absence in the source population and in the control group (n=20), was confirmed by polymerase chain reaction amplification of its DNA using primers specific to a small segment of the ribosomal DNA of *S. destruens*. The prevalence of the rosette-like agent in moribund or dead *L. delineatus* was 67% (n=12). The parasite was also detected in subclinical fish in the treatment group, but at a lower prevalence (28%; n=32). This level of infection is consistent with that reported in tissues of salmon exposed to *S. destruens*[8].

Preliminary examination indicates that other cyprinids, such as the fathead minnow *Pimephales promelas*, are also susceptible to this pathogen, which causes effects identical to those in *L. delineatus* (prevalence, 20%; n=5). All *P. parva* specimens (n=10) tested for the rosette-like agent were negative: however, this is to be expected, given that pathogen concentrations in healthy carrier fish are very low and difficult to detect using conventional diagnostic tests[9]. Cohabitation studies are a recognized method for detecting carrier states for different fish pathogens[9,10] and, as our results illustrate, they are currently the most reliable way to detect a healthy carrier.

Our results have three important implications. First, the most invasive fish species in Europe[4] is a healthy host for a deadly, non-specific pathogen that could threaten aquaculture trade, including that of salmonids. Second, it is difficult to identify fish populations that are carriers of pathogens. Third, this pathogen could pose a threat to the conservation of European fish diversity.

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