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Research Note

Ligula intestinalis infection as a potential source of bias in the bioindication of endocrine disruption in the European chub *Leuciscus cephalus*

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Abstract

European chub *Leuciscus cephalus* collected from five localities in the lowland and subalpine regions of Austria were analysed for oestrogenic effects of endocrine-disrupting chemicals and the presence of the plerocercoid of the tapeworm *Ligula intestinalis*. Of 1494 chub analysed, only seven (six males, one female) were found to be infected with single, but large plerocercoids up to 15 cm in length. *Ligula*-infected fish showed comparatively immature gonads, as demonstrated by the gonadosomatic index and gamete developmental stages. Plasma levels of the egg precursor protein vitellogenin also showed concentrations ranging below the detection limit. The present results indicate that chub infected with *L. intestinalis* and exposed to exogenous oestrogenic compounds can result in reduced gonadal maturation and produce false oestrogen-positive diagnoses in male fish. For plasma vitellogenin levels, *L. intestinalis* infections can result in false oestrogen-negative diagnoses in male and female fish.

A single and relatively small-sized plerocercoid of the tapeworm *Ligula intestinalis* is able to reduce the gonadal size of its fish host and can permanently inhibit gametogenesis (Arme, 2002). It is therefore not surprising that *L. intestinalis* can adversely affect the value of indication criteria such as the gonadosomatic index, stage of gonadal maturation and induction of vitellogenin production which are conventionally used in research on endocrine disrupting chemicals (EDCs) (Hecker, 2001). The European chub *Leuciscus cephalus*, with a wide autochthonous geographical spread and low status of endangerment in various types of water bodies (IUCN, 1996), is an upcoming indicator species in aquatic ecotoxicology (Andres *et al.*, 2000; Machala *et al.*, 2001;

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Flammarion *et al.*, 2002) and especially with reference to EDCs (Brion *et al.*, 2000; Flammarion *et al.*, 2000).

The present study contributes to the little data available on *Ligula* infections in *L. cephalus* and in particular to *Ligula*-mediated effects on male gonadal development and vitellogenin production.

During the course of co-operation on endocrine modulators in Austria *L. cephalus* was analysed for oestrogenic effects using the following two exposure scenarios:

1. Fish, maintained in 1000 l tanks, were exposed for 3 weeks in two on-site sewage treatment plants, with a dilution of effluent of 1:10, in the rivers Leitha and Wienfluss in the federal district of Lower Austria. Fish were caught by electrofishing in April 2002 and originated from feral populations in tributaries of the sub-alpine lake Mondsee and the river Enknach in the federal district of Upper Austria.
2. Field exposure in the lowland rivers Leitha, Schwechat, and Wienfluss in the federal district of Lower Austria, receiving effluents from municipal sewage treatment plants. Fish were collected from three locations by electrofishing over three sampling seasons from September 2001 until October 2002.

For an indication of oestrogenic effects, only fish in the size range 8.1–15.0 cm total length (mean value 11.6 cm) with a median age of 2 years (Grillitsch *et al.*, 2003) were analysed. This size range provided an optimum for accessable blood volume in the fish and the endogenous variability in the measurement parameters was minimal. Biometric variables included total body length (TL), condition factor (CF), gonadosomatic index (GSI), gamete stage of maturation (GST) after Blüm *et al.* (1988), and plasma vitellogenin concentration (VTG) analysed by anti-*L. cephalus*-vitellogenin enzyme-immunoassay (effective detection limit = 0.0019 mg ml^{-1}, Grillitsch *et al.*, 2003). Mean values of these parameters were compared in infected and uninfected chub from both on site sewage treatment plant experiments and field exposures.

Of 1494 *L. cephalus* examined, seven were infected with *L. intestinalis*. Each fish harboured a single and large plerocercoid up to 15 cm in length. All infected fish originated from populations in the subalpine localities (six chub from tributaries of the lake Mondsee and one from the nearby river Enknach) and were used in the on-site sewage treatment plant exposures. Infected fish were found in both exposure and control treatment groups in the sewage treatment plants under study (table 1). Infected exposed male fish from the on-site sewage treatment plant in the river Leitha showed lower values for GSI and GST compared with those in uninfected exposed males (table 1). Infected female chub demonstrated a lower GSI than uninfected females. Parasitized male fish in the control group showed similar values for GSI and GST as uninfected fish. In the sewage treatment plant exposures in the river Wienfluss, only one male chub in the control group harboured plerocercoids of

Table 1. The relationship between the total body length (TL in cm), condition factor (CF), gonadosomatic index (GSI), gamete stage (GST) and plasma vitellogenin concentration (VTG in mg ml^{-1}) of *Ligula*-infected and uninfected chub from on-site sewage treatment plants in the rivers Leitha and Wienfluss and from feral chub populations in the rivers Leitha, Schwechat and Wienfluss; the fish received effluents from municipal sewage treatment plants.

Traits	TL*	CF*	GSI*	GST**	VTG***
Leitha					
Fish exposed in tanks					
Male					
Uninfected (37)	11.54 ± 1.79	0.81 ± 0.24	0.37 ± 0.22	1.5 [1; 2]	0.022
Infected (4)	10.9	0.84	0.19 ↓	1 ↓	< DL
	11.2	0.80	0.16 ↓	1 ↓	< DL
	13.5	0.55	0.14 ↓	1 ↓	< DL
	14.2	0.50	0.24 ↓	1 ↓	< DL
Female					
Uninfected (58)	11.83 ± 1.47	0.75 ± 0.19	0.69 ± 0.23	1 [1]	0.003
Infected (1)	9.5	1.11	0.11 ↓	n. a.	< DL
Control fish in tanks					
Male					
Uninfected (22)	11.33 ± 1.51	0.82 ± 0.20	0.27 ± 0.11	1 [1; 1.4]	0.030
Infected (1)	9.5	1.11	0.27	1	n. a.
Wienfluss					
Control fish in tanks					
Male					
Uninfected (18)	11.20 ± 1.28	0.83 ± 0.18	0.92 ± 1.15	2 [1; 2]	0.002
Infected (1)	11.6	0.74	0.32 ↓	1.5 ↓	< DL
Feral fish					
Male (613)	11.56 ± 1.67	0.80 ± 0.23	1.46 ± 1.84	2 [1; 3]	0.229
Female (593)	11.32 ± 1.69	0.83 ± 0.24	0.83 ± 0.29	1 [1]	0.965

The number in brackets () indicates the number of fish examined. Arithmetic mean ± standard deviation*; median [25%; 75% quantil]**; maximum, all mean values below detection limit ***; < DL below detection limit; n.a. not analysed; ↓ lower than the mean value of uninfected fish in the same exposure scenario.

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L. intestinalis and showed lower GSI and GST levels compared with other males. Among feral chub from three lowland rivers, no *Ligula*-infected fish were found. Gonads of parasitized male chub were mainly at the maturation stage 1 which comprised cystic cells, only a small number of primary spermatogonia, and no lumina in the tubuli of the testes with a maximum GSI of 0.32. The mean level of gonadal maturation in uninfected male feral fish was at stage 2 when spermatocyte A was present and the tubuli were larger and a mean GSI value of 1.46. In all *Ligula*-infected chub, mean values of VTG concentrations were below the detection limits as in the case of uninfected chub, although maximum VTG concentrations of uninfected fish exceeded those of infected ones. This trend became particularly evident in exposed fish from the Leitha exposures (table 1).

The present study has shown a low prevalence of infection of *L. intestinalis* in *L. cephalus* in Austrian rivers and creeks. The low *Ligula* infection rate may be due to the high water velocity of the waterbodies which limits the abundance of zooplankton, especially copepods, which act as the first intermediate host and this would explain the presence of *Ligula* infections in chub examined from lakes and reservoirs (Rydlo, 1994).

Despite the low number of infected chub found in the present study, the effects of ligulosis on the gonads of male and female fish should be emphasized in the context of bioindication for xenohormones. To date, the potential impact of *L. intestinalis* on bioindication criteria conventionally used in research on EDCs has rarely been considered. Hecker (2001) described endocrine disruption in the bream, *Abramis brama* at ten sampling sites in the river Elbe, Germany, with a significant negative influence of *L. intestinalis* on the gonadosomatic index, plasma vitellogenin level, $P450_{arom}$-activity, and blood-plasma concentrations of the sex steroid hormones 17β estradiol, 11-keto-testosterone and testosterone.

Minier *et al.* (2000) investigated the incidence of intersex roach due to endocrine disruption in one English and three French rivers. A significantly lower gonadosomatic index was evident in male and female roach (*Rutilus rutilus*) in the river Seine and this was likely to be due to the higher prevalence of *L. intestinalis* at this site, i.e. 46% compared with less than 5% in other sampling sites.

Ligula-infected fish have therefore to be excluded from bioindication for xenohormones and consequently further attention is needed to detect this tapeworm in fish population surveys. In fish communities with high prevalences of *Ligula*, an additional effort is needed to catch a sufficient number of uninfected fish as parasitized fish have a higher catchability in gillnets due to an increase in girth caused by the plerocercoid in the body cavity (Museth, 2001). Furthermore, heavily infected fish, which show an alteration in behaviour by spending more time close to the water surface and in the littoral zone (Loot *et al.*, 2001), are thus more likely to be caught by electrofishing in these shallow areas.

In summary, the present results indicate that all tapeworm-infected fish demonstrate comparatively immature gonads, as shown by the gonadosomatic index and developmental stage of the gametes, along with the plasma levels of the egg precursor protein vitellogenin all of which range below the detection limit. The effects of *L. intestinalis* infection on chub are similar to those described by Minier *et al.* (2000) and Hecker (2001) and confirm that both infection with *L. intestinalis* and exposure to exogenous oestrogenic compounds can result in reduced gonadal maturation and thus, false oestrogen-positive diagnoses in male fish. In contrast, for plasma vitellogenin levels, *L. intestinalis* can result in false oestrogen-negative diagnoses in male and female fish, although larger samples are needed to confirm the significance of these results.

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