

Effects of Irgarol on the pond snail *Radix balthica* - indications for endocrine mediation

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Introduction

Irgarol is

- a triazine derivate with antifouling features,
- an inhibitor of the electron transfer in the photosystem II,
- substitute for organotin compounds (TBT),
- in paints and building materials since the mid 80s,
- increasing in surface waters levels,
- very toxic to aquatic macrophytes and algae,
- likely to induce endocrine mediation,
- likely to bioaccumulate in macrophytes,
- restricted in use or banned in 4 EU countries (S, DK, GB, NL)



Fig. 2. *Radix balthica*

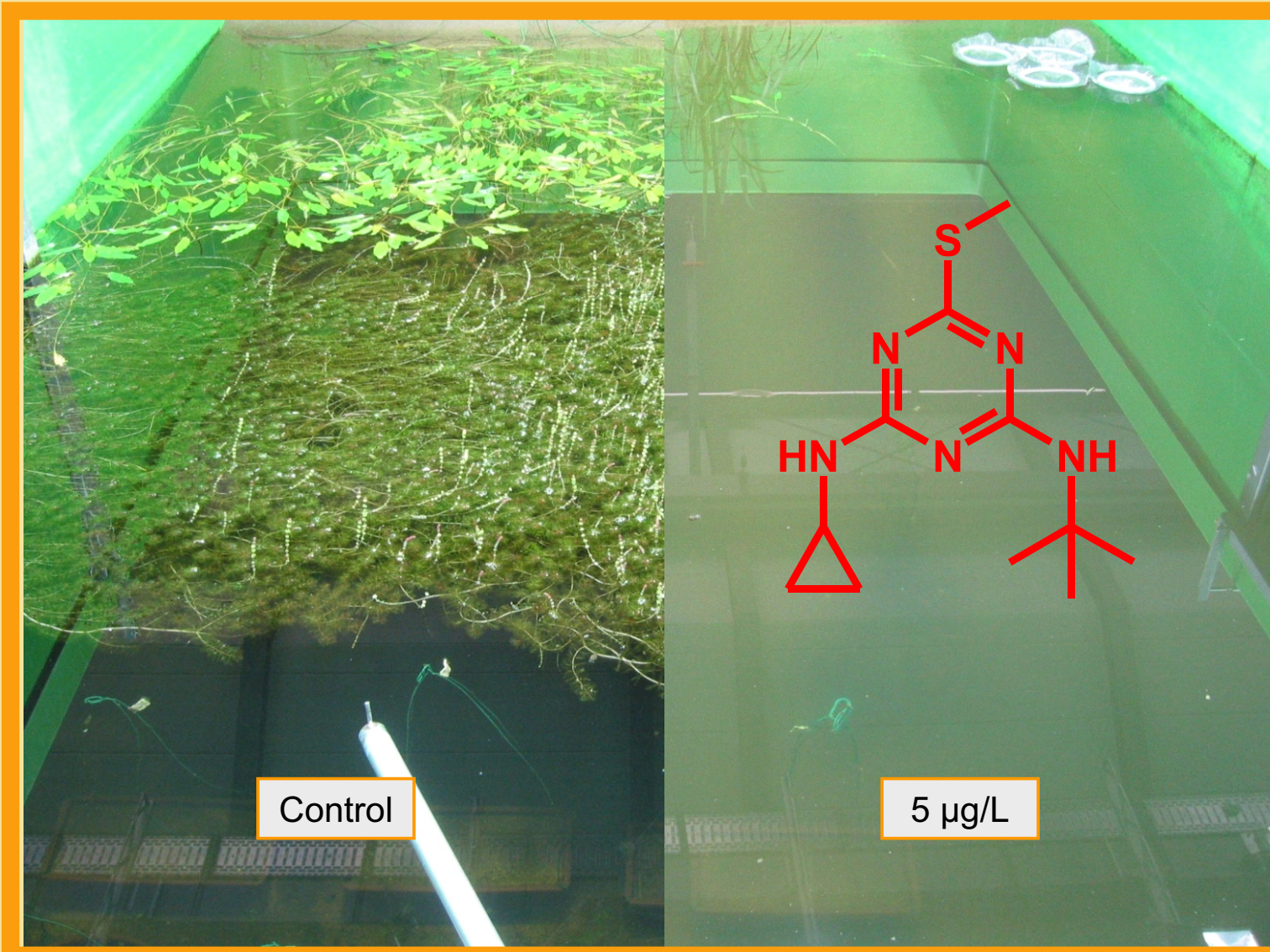


Fig. 1. Control pond and pond on Day 64 (14 June 2005) after single dosing of 5 µg/L Irgarol

As part of a fate and effect study in the indoor ponds of the artificial pond and stream mesocosm system (FSA) in Berlin (Fig. 1 and 3), several endpoints in the pond snail *Radix balthica* (Fig. 2) were analysed including endpoints under endocrine control.

Material and Methods

Ponds

Size: length 690 x width 325 x height 250 cm

Water volume: 15 m³

Artificial light: mean 13,000 lx

Nutrient regime: TP 0.045 mg/L; TN 1.5 mg/L

Biological establishing of the ponds

Ground: Sand, natural fine sediment, shore area (Fig. 3).

Macrophytes: *Myriophyllum verticillatum*, *Potamogeton nodosus*, *Chara* sp. had been planted in the littoral zone 15 months prior to dosing of Irgarol.

Stocking: Plankton and macroinvertebrates from nearby mesotrophic lakes and ponds.

Experimental design

Single application of Irgarol in 6 mesocosms, 4 different concentrations (1 x 0.04/ 2 x 0.2/ 1 x 1/ 2 x 5 µg/l nominal), 2 uncontaminated control ponds

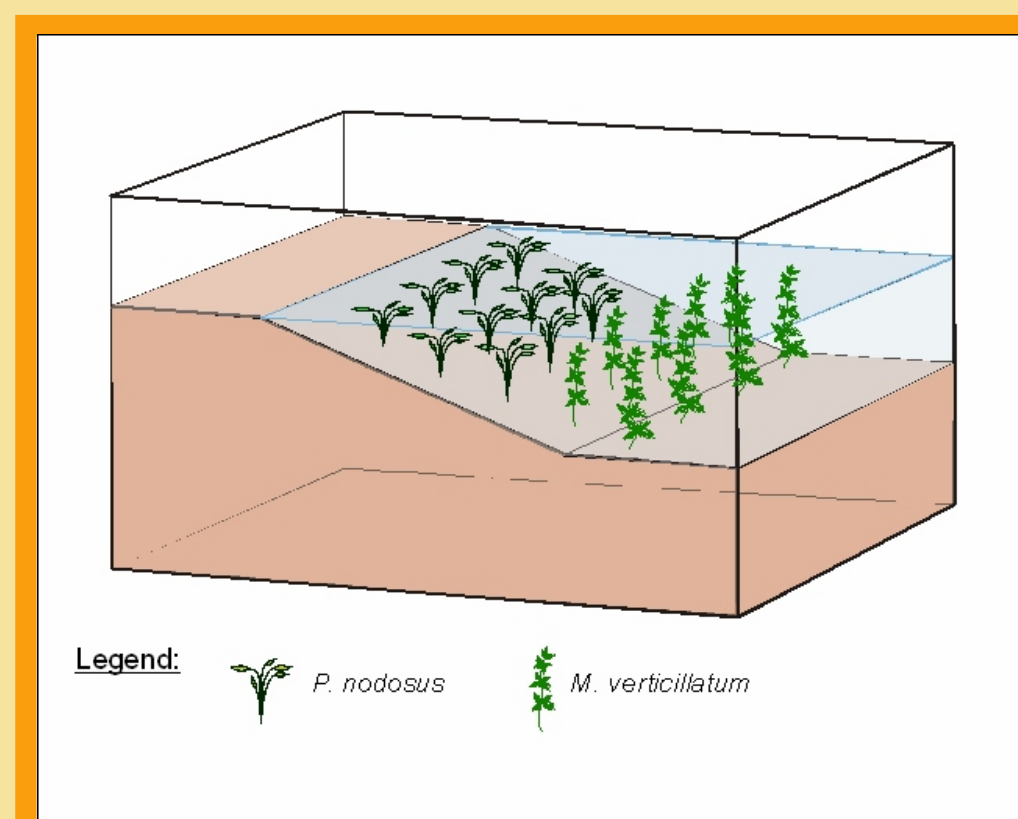


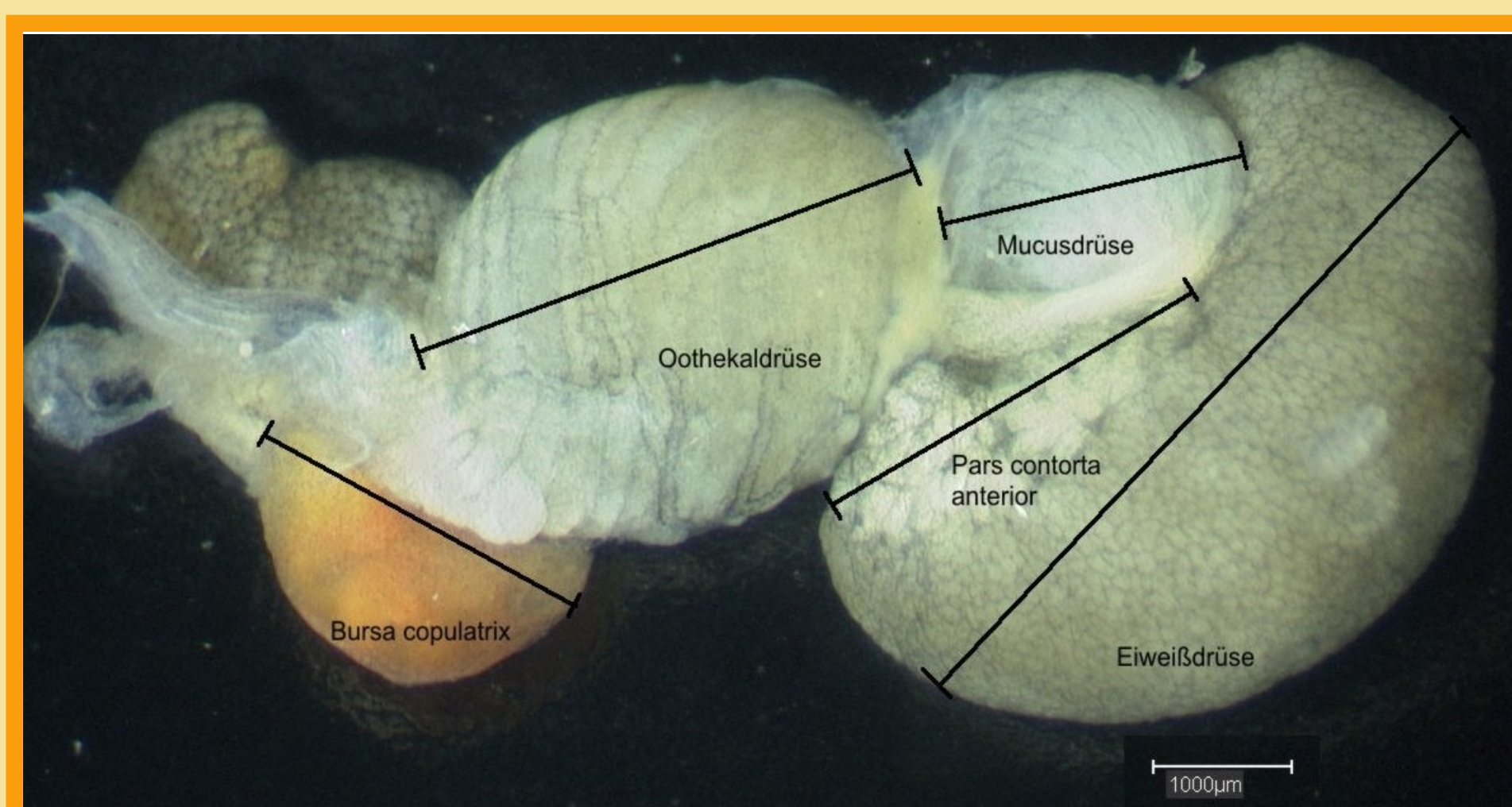
Fig. 3: Sketch of a pond

Duration of experiment: 150 days (application: 11-04-2005)

Table 1: Sampling scheme and number of specimens. C= control, * = no more snails to be found

Day	C 1	C 2	0.2 µg/L	1 µg/L	5 µg/L
15	20	20	20	20	20
30	20	20			
60	20	20	20	20	20
90	20	20			
150	20	20	*	*	20

Morphological endpoints (Fig. 4)



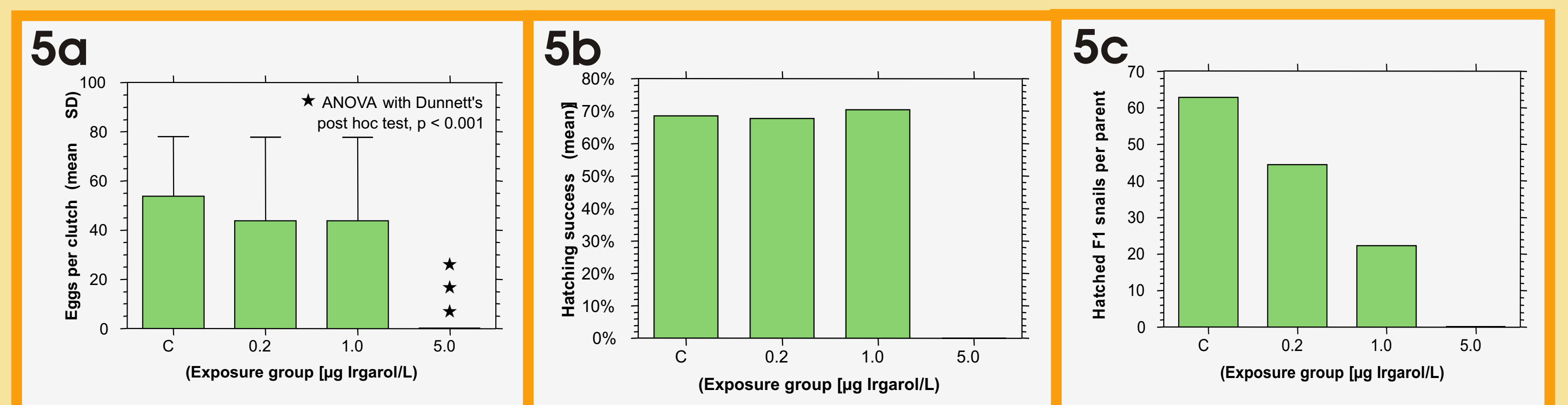
Histological endpoints

- Ovotestis including check of spermatogenesis and oogenesis in the acini, presence of the gamete states as well as follicle and Sertoli cells
- Accessoric sexual glands in female and male organs, emphasis on oothecal, mucus, and albumen gland
- Ductus hermaphroditicus, Pars contorta anterior and posterior, Prostata and Bursa copulatrix
- Copulation apparatus with penis sheath, praeputium, penis and praeputial gland

Results and Discussion

1. Reproduction after 15 days

There was no reproduction at the highest concentration already 15 days after application (Fig. 5 a, b). The number of hatched F1 per parent decreased with increasing Irgarol concentration (Fig. 5c).



Decrease in number of eggs per clutch

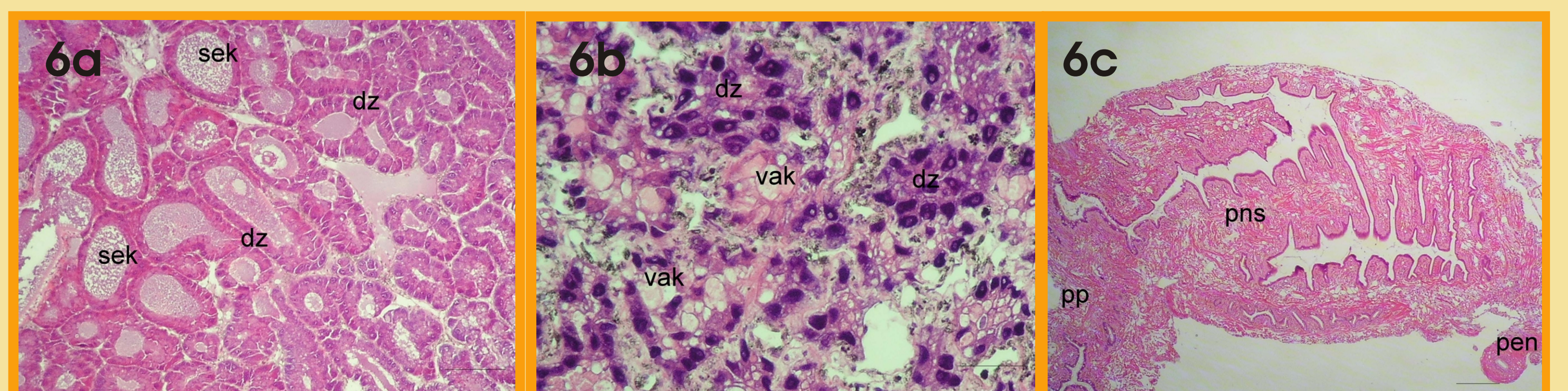
Decrease in hatching success

Reduced number of F1 snails per parent

2. Morphological effects

The morphological effects (Table 2) were confirmed by the histological analysis (Fig. 6 a-c).

3. Histological effects (Day 15)



Atrophic prostate gland, 1 µg/L, dz = glandular cells, sek = secretion, bar = 50 µm

Hypertrophic albumen gland 5 µg/L, dz = de-differentiated glandular cells, vak = intracellular vacuoles, bar = 50 µm

Penis degeneration 5 µg/L, pen = penis, pns = penis sheath, pp = praeputium, bar = 0.1 mm

Table 2: Summary of endocrine effects in *Radix balthica*. EC10 = calculated effect concentration at the 10% level. C.I.= confidence interval

	EC10 [ng/L]	95% C.I.
Day 15		
Reduction in reproductive effort (hatching success)	59	15 - 225
Reduced spermatogenesis	66	15 - 351
Hypertrophy of albumen glands	96	37 - 251
Day 60		
Reduced spermatogenesis	32	6 - 184
Changes at female sexual organs	57	4 - 794
Day 150		
Apparent gametogenesis with dominating spermatogenesis - in contrast to the controls, which had already passed the active phase of the reproduction cycle		

Conclusions

- Toxic effects of Irgarol on the reproduction of *Radix balthica* are in the range of Irgarol levels in surface waters (not shown here).
- Specific effects on reproductive organs and spermatogenesis were induced at low effect concentrations.
- Consequently, Irgarol is likely to have induced endocrine action in *R. balthica*, which can be referred to as „estrogenic“ or „anti-androgenic“.
- For the first-time adverse effects of Irgarol on faunal organisms in the ng/L concentration range have been shown.

References

Mohr S, Feibicke M, Ottenströer T, Meinecke S, Berghahn R, Schmidt R (2005): Enhanced experimental flexibility and control in ecotoxicological mesocosm experiments. A new outdoor and indoor pond and stream systems. Environ Sci & Pollut Res 12 (1), 5-7.