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Differences in Reproductive Characteristics Among Field Populations of *Polycelis tenuis* (Platyhelminthes) in a Metal Contaminated Stream

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Rhiophilous planarians (Platyhelminthes, Tricladida, Paludicola) are scavengers and/or predators inhabiting the interface layer between water and sediment (Reynoldson and Davies 1970). As a result, they may be hypothesized as being highly vulnerable to pollutants (Simkiss 1990). However, field studies assessing species diversity and density for a series of taxa suggest triclads to be relatively tolerant (Gower et al. 1994; Hurd et al. 1996). This study aims to give insight in whether and how triclads react on the population level towards metal pollution in the field.

Five populations of the planarian *Polycelis tenuis* (Ijima) were sampled up- and downstream of an ancient lead/zinc mine. In addition to the internal zinc concentrations measured for these field populations, their reproductive characteristics were estimated when culturing them under semi-natural conditions in the laboratory. The reproduction pattern was investigated in two seasons and explained in light of the internal zinc concentration in the study species and its life-cycle.

MATERIALS AND METHODS

Polycelis tenuis individuals were gathered from the heavy metal contaminated stream Geul (Swennen et al. 1994) flowing from Steinkaul (50°42'N, 6°7'E, province of Liege) in Belgium to Itteren (50°54'N, 5°43'E, province of Limburg) in The Netherlands, where it joins the Meuse river. In a downstream direction, we sampled at Moresnet center (1), Moresnet bridge (2), Plombieres (3), Belgian/Dutch border (4) and Epen (5) (Fig. 1). The ancient ore mine is located at Plombieres. Note that we will use the numbers in parentheses to indicate locations. The river substratum consists of a stony and calcareous-rich Carboniferous formation for populations 1, 2 and 3, and Tertiary sand for populations 4 and 5.

Flatworms were collected from populations 2, 3, 4, 5 in September 1995 (autumn season) and from populations 1, 3, 4, 5 in April 1996 (spring season). Replacement of population 2 by 1 in spring was due to low population density at site 2. Individuals were removed from the underside of stones using a soft paint-brush. Populations were kept separately in water from the sampling sites.

For the purpose of the measurement of internal zinc concentrations, around 50 specimens were collected randomly from the field at each site and size-selected in the laboratory on the following day. An image analysis system (*MicroScale TM/TC Image Analysis Systems*) connected to a Sony video camera (model AVC-D5CE)

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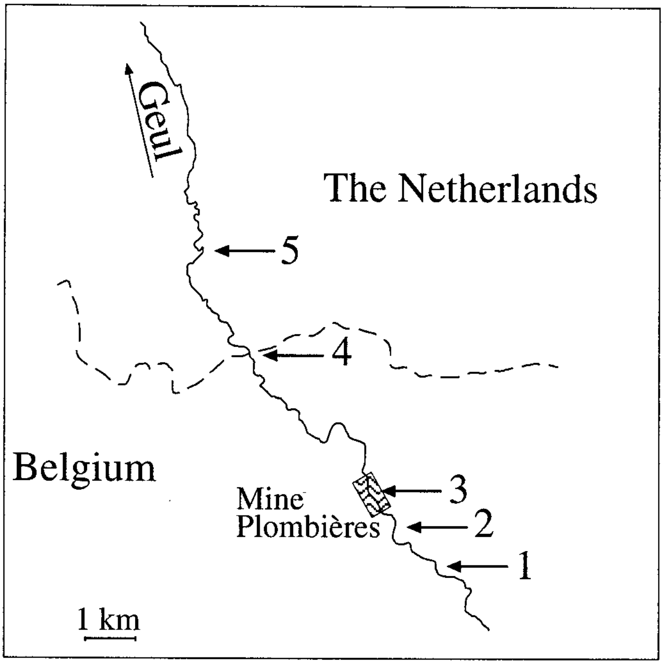

Figure 1. Studied field populations of the flatworm *Polycelis tenuis* at the river Geul and location of the ancient ore mine of Plombieres (see text)

with macro-lens was used to measure the planar dorsal surface of a flatworm while moving over the bottom of a Petri dish. The system allows to convert a measurement as a number of pixels in to a square millimeter measurement. The average of three separate measurements was used. Eventually, 20 individuals of similar size were selected out for each population, except for population 5 in autumn, from which only 16 individuals were set apart. Size selection was performed in order to avoid the interpretation of differences in internal concentrations between populations as a result of size differences between analyzed specimens. Individuals were then rinsed three times with distilled water and placed in groups of four in polyethylene sample cups in a way that average body size was comparable between the cups. After drying (60°C 24 hours),weighing (Cahn electrobalance model 4100, accuracy ±0.1 μg) and digestion (100 μl HNO_3) of the individuals a microwave-aided digestion was performed (Blust et al. 1985). Note that average dry weight of the samples, ranging from 2.81 mg (Plombières '95) to 3.75 mg (Moresnet bridge '95), did not differ between populations (ANOVA all effects: $F_{7,31}=0.6119$, $P>0.05$). Samples were analyzed by flame atomic absorption spectrometry (Perkin-Elmer 1100B) for their zinc content. Quality control for the analytical procedure was maintained by analysis of certified standards (bovine liver CRM185), which gave derivation within 5% of the certified values.

For culturing, a second randomly collected sample of 50 or 100 individuals was collected for each population for the autumn and spring experiment, respectively. During 14 days, each population was kept in water from the sampling site. Animals were kept at 16 ± 1°C at a constant day/night cycle of 12L: 12D (day starting at 07.00 am). Individuals were isolated in small (0.1 l) plastic vials that were placed in large (35 l), aerated containers. Water circulation was maintained with *Micra* water pumps. Each vial had a hole (diameter 25 mm) in each of two opposite sides, covered by a 0.125 mm mesh net to provide semi-natural, stream-like conditions. Animals were not fed during the experiment. In autumn and spring, individuals were measured during the first experimental week. The number and size (for method see below) of the cocoons produced, were recorded daily.

Prior to the experiments, we developed a method to measure the reproductive allocation of an individual, expressed as the volume of a cocoon as a percentage of the volume of the parent.

First, length and width of the ellipsoid cocoons were measured to the nearest 0.01 mm with a Wild measuring eyepiece mounted on a Wild M8 stereomicroscope (enlargement 50x). Next, linear measurements were converted to volume estimates with the formula: $\text{volume}=4/3.\pi.(\text{length}/2).(\text{width}/2)^{2}$.

Secondly, we developed a model (see two steps below) to estimate the volume of a living animal based on the measurement of its planar dorsal surface. In the first step, 93 individuals were measured and ranged in order of size in 31 groups, each group with three individuals of similar size. Groups were placed in polyethylene sample cups and dry weight was measured with a Cahn electrobalance. Next, we used a powercurve fit to describe the relationship between dry weight and dorsal planar surface: $\text{dry weight}=0.031 \times \text{dorsal surface}^{1.536}$ ($r^2=0.96$). The fit of the model to the data improves for larger individuals as can be inferred from the negative correlation between the relative residual value (=(|observed - predicted| / predicted) x 100) and the predicted dry weight ($N=31$, $r^2=0.214$, $p<0.01$). However, more than 99% of mature individuals measure at least 5 mm^2 and in 60% of these cases the 10% deviation from the model was not exceeded (maximum deviation = 25%).

In the second step, we determined the ratio fresh weight / dry weight. For this, six groups of six individuals were first weighed alive in weighing cups and then dry weight was determined after having kept the specimens for 24 hours at 60°C. The ratio fresh weight / dry weight was estimated at 5.860 (±0.174). Overall, the model assumes that an individuals' body volume in mm3 equals its fresh weight in mg assuming that the specific gravity is 1 mg/mm^3.

RESULTS AND DISCUSSION

In autumn, internal zinc concentrations differed significantly between populations (ANOVA after logarithmic transformation: $F_{3,15}=61.51$, $P<0.001$), with population averages ranging from 370-1658 µg/g. In detail, populations 4 and 5 contained significantly higher zinc concentrations than population 2 and 3 and the same was observed for population 3 compared to population 2 (Scheffé-test, $p<0.05$). In spring, differences in zinc concentration between populations were less pronounced (ANOVA after logarithmic transformation: $F_{3,16}=4.09$, $p=0.02$), with no significant differences between pairs of populations (Scheffé-test, $p>0.05$). Average internal zinc concentrations only varied between 753-993 µg/g (Fig. 2).

Size distributions (Fig. 3), for all populations and both sampling occasions, demonstrated the presence of large individuals in the populations sampled in spring. At least 25% of the individuals had a planar dorsal surface of minimum 10 mm^2.

During autumn, this counts only for the outermost upstream population whereas, at the most, 6% of the individuals reached a 10mm^{2}size for populations 3, 4 and 5.

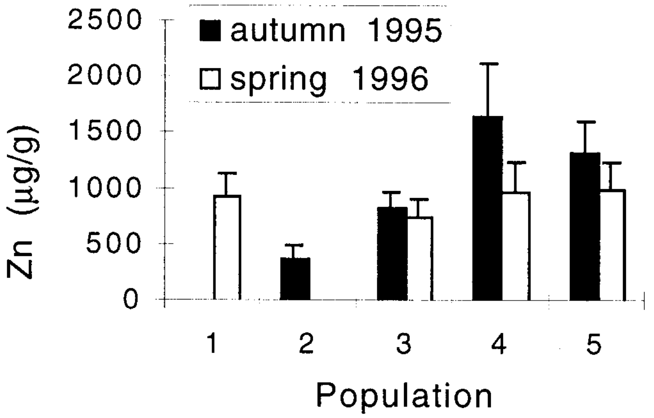

Figure 2. Average internal zinc concentrations with standard deviation for the studied field populations in two seasons.

Some reproductive characteristics of the different populations are summarized in Table 1. In autumn, individuals tended to reproduce at a smaller size in the downstream direction (2→5). Analysis of variance verified this observation (ANOVA: $F_{3,38}$=12.90, P<0.0001), with cocoon producing individuals of population 4 and 5 being significantly smaller than those of population 2 (Scheffé-test, P<0.005). In spring, size at which cocoons were produced did not differ between populations (ANOVA: $F_{3,123}$=0.41, P>0.05). Note that the populations upstream the ancient ore mine showed similar patterns in both sampling seasons.

Table 1. Population parameters indicating size at which cocoons are produced in two seasons. Missing values indicate uninvestigated populations.

	Autumn experiment (1995)		Spring experiment (1996)	
Population	Number of cocoons per individual	Number, size range and average size of cocoon producing individuals (mm2) (±SD)	Number of cocoons per individual	Number, size range and average size of cocoon producing individuals (mm2) (±SD)
1			0.25	N=25, [7.56, 16.60], 12.42 ±2.60
2	0.26	N=13, [7.80,19.46], 11.77 ±3.43		
3	0.04	N=2, [6.80, 7.67], 7.23 ±0.62	0.44	N=44, [8.35, 26.01], 12.52 ±3.71
4	0.40	N=20, [5.88, 12.33], 8.12 ±1.58	0.32	N=32, [8.18, 16.41], 12.38 ±2.20
5	0.16	N=8, [3.70,7.61], 5.82 ±1.15	0.29	N=29, [7.02, 16.28], 11.81 ±0.25

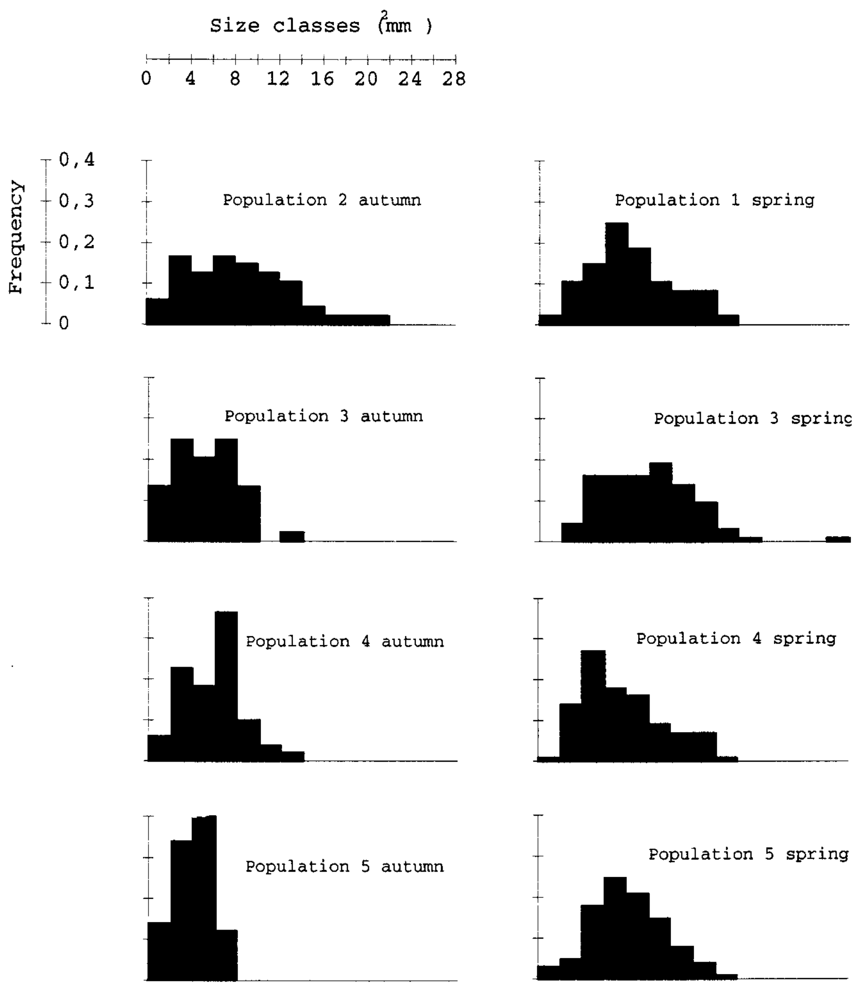

Figure 3. Size distribution of populations collected in autumn 1995 (left) and spring 1996 (right).

In autumn, reproductive allocation increased significantly in the downstream direction (ANOVA all effects: $F_{3,38}$=3.48, P=0.03). A gradual change eventually lead in to a 64% increase as observed for the outermost downstream population 5 (Fig. 4). Post-hoc testing indicated a significant difference between the outermost populations of the gradient (Scheffé-test: P=0.03). However, after correction for parent size (ANCOVA covariable: $F_{1,37}$=3.85: P=0.06), these differences disappeared (ANCOVA population effect: $F_{3,37}$=0.55, P>0.05). In spring, reproductive allocation also differed between populations (ANOVA all effects: $F_{3,123}$=3.31, P=0.02). However, the maximum difference in allocation (17%) was smaller than in autumn (Fig. 4). More specifically, reproductive allocation in spring only differed between populations 3 and 4 (Scheffé-test: P=0.04). Size of the parent also had an effect (ANCOVA covariable: $F_{1,122}$=5.73, P= 0.02), but could not explain the relatively small population differences (ANCOVA population effect: $F_{3,122}=3.66$, $P=0.01$).

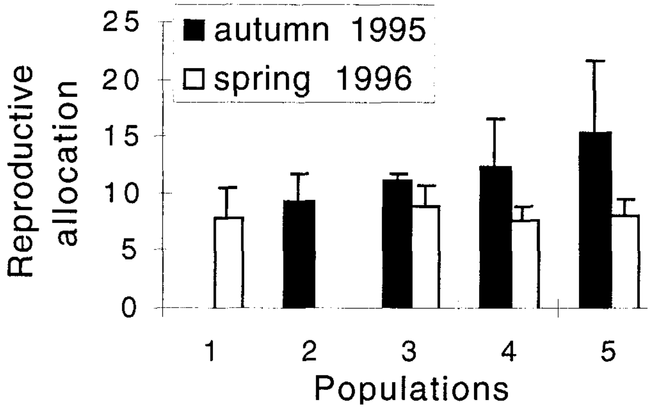

Figure 4. Average reproductive allocation (cocoon volume as a percentage of the volume of the parent) for all the populations and for both sampling occasions. Standard deviations are indicated.

In this study, inter-season and inter-population differences concerning size distribution, size at which cocoons are produced and reproductive allocation were demonstrated for the planarian *P. tenuis* . Inter-season differences may be explained as a result of an elevated competition for food during the end of the breeding season. Reynoldson and Young (1965) demonstrated that the increase in population density in autumn caused shrinkage of mature individuals in *P. tenuis* . The authors assumed that within a population, the threshold size for cocoon production is constant over the year. This is not supported by the present results. Summarizing, shrunken adults still producing cocoons at a smaller size, may partly explain the small size of reproducing individuals in autumn. Furthermore, we demonstrated that a large reproductive allocation was mainly a consequence of the temporary small size of the *P. tenuis* individuals in autumn. A size-dependent reproductive effort has also been shown for other triclad species (Vreys and Michiels 1995, Taylor and Reynoldson 1962). Several studies apply the reproductive allocation of a population as a bioindicator for metal pollution (Donker et al. 1993; Maltby 1991). This study underlines that the size-dependence of the reproductive allocation should be taken into consideration when tracing population differences, especially in field studies.

Shrinkage of individuals in autumn does not seem to be equally important among populations as size and size at reproduction decreased gradually going in a downstream direction along the river reach. Again, a difference in food availability among populations can serve as an explanation. Prey species and abundance can be affected by river specifications such as: local size and sediment characteristics of the river. These are clearly different for the upstream (1, 2 and 3) and downstream (4 and 5) locations as mentioned in M&M. Reynoldson and Davies (1970) describe isopods, amphipods, oligochaetes and molluscs as being the most important food sources for *P. tenuis.* (*From the latter prey items, the isopod Asellus aquaticus and the amphipod Gammarus pulex are regularly encountered in the Geul river. The smaller Asellus prey is at least 9 times more frequent at locations 4 and 5, whereas*

Gammarus comprises at least 60% of both preys at locations 1, 2 and 3.- unpublished results)

As a supplementary explanation for these inter-population differences, the pollution level may be considered. In autumn, exposure is largest downstream the ancient ore mine, based on the internal zinc concentrations in *P. tenuis* . (*In addition, another study on eight Polycelis tenuis populations in the river Geul, demonstrated that internal concentrations of Zn tend to be correlated positively with those of lead* ($N=8$, $r^2=0.51$, $P<0.1$), *copper* ($N=8$, $r^2=0.40$, $P<O.1$) *and cadmium* ($N=8$, $r^2=0.62$, $P<0.05$). *High internal zinc concentrations are therefore likely to be accompanied by high internal lead, copper and cadmium concentrations.- unpublished results.*)

In addition, it has been stated that organisms may incur direct energy costs to resist contaminants and that these energy expenditures may reduce the energy left to invest in survival, reproduction, storage of energy reserves and growth (Calow 1989). For *P. tenuis* , size reduction as a result of environmental pollution may be particularly relevant at the end of the breeding season when adverse pollution effects on the energy budget cannot be compensated for by feeding because of food scarcity. However, in this study linear relationships between the internal Zn concentration on the one hand and population characteristics (average size of al individuals, average size of reproducing individuals, reproductive allocation,...) on the other hand were not significant ($p>0.05$).

The observed fluctuations in internal metal concentrations among seasons may result from regular fluctuations in the metal exposure level related with variations in the height of the water. Leenaers (1980) demonstrated that more than 80 percent of the heavy metal transport in the river Geul occurs during flood events. Remarkably, internal Zn concentrations are relatively low at the height of the ancient Plombieres mine when compared with the downstream populations. (*For P. tenuis in the river Geul, support is found for this appointment because also peaks of internal Cu, Pb and Cd concentrations were localized 5.2 till 7.9 km downstream the Plombitres mine. - unpublished results*). Leenaers (1989) recognized that, due to constant reworking of the Geul sediment, readily soluble metal fractions became more important downstream the ancient ore mine of Plombieres. It appears that this phenomenon is (temporarily) reflected in internal metal concentrations in *P. tenuis.*

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