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Abnormalities in two *Bosmina longirostris* S.L. (OF Müller, 1785) populations (Crustacea: Branchiopoda: Bosminidae)

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Abstract
Abnormal trunk morphology is observed in two populations of *Bosmina longirostris*. One population is from a trout cultivation pond, the other from a pond with a very high angling pressure. The length and shape of the first antenna (trunk) of part of the population are abnormal. No abnormalities were observed at three locations, with low or no angling pressure. The observations are considered in the context of the described variation in the first antenna of this species. Possible causes of the abnormalities are discussed.

Keywords: Morphology, Xenobiotics, fishpond, angling

1. Introduction
The description of *Lynceus longirostris* is very brief and there is wide morphological variation in *Bosmina longirostris* (Müller, 1785) [5]. Several European species are described: *B. cornuta* (Jurine, 1820) [20]; *B. brevicornis* Hellich, 1877; *B. brevirostris* Fischer, 1854; *B. pelagica* Stingelin, 1895; *B. pellucida* Stingelin, 1895; and *B. similis* (Sars, 1890). The primary differences between the species are the shape and length of the trunk (antenna I and rostrum grown together) and shell spine. Today, these species are considered synonyms of one very variable species: *B. longirostris* s.l. [1, 2]. The varieties are distinguished by the length and shape of trunk and shell spine [2-4], which vary due to environmental factors. The environmental factors known to influence the morphology of the trunk are temperature [5-9] and predation [10-13]. Predation influences morphology through chemical cues, Kairomones [14], and physical stimulation [13].
Morphological abnormalities in Cladocera have recently been described for the genera *Ilyocryptus* [15, 16], *Daphnia* [17, 18], *Coronatella* [19], *Ceriodaphnia*, *Chydorus*, and *Bosmina* [18], and *Pleuroxus* [20]. This article describes morphological abnormalities in *B. longirostris* s.l. populations and discusses the possible causes.

2. Materials and Methods
Samples were taken at four locations in the Netherlands: Assen, Groningen, Lelystad, and Weert. The samples were as follows: (1) Assen trout cultivation and fishing pond Forellenplas (52°59′25″ N; 6°30′49″ E); (2) Assen Baggelhuizerplas (52°59′15″ N; 6°30′40″ E); (3) Weert holiday resort Roompot (51°15′03″ N; 5°38′44″ E); (4) Weert Vosseven (51°11′29″ N; 5°39′29″ E); (5) Groningen swimming pond Karelingerplas (53°14′20″ N; 6°36′06″ E); and (6) Lelystad Bultpark (52°30′50″ N; 5°27′43″ E). The samples were taken on 26 March 2019 around Assen, on 3 January 2020 around Weert, on 9 March 2020 in Groningen, and on 6 May 2020 in Lelystad. The locations differ in angling pressure. Assen Forellenplas and Weert Roompot have very high angling pressure, while Assen Baggelhuizerplas, Weert Vosseven, and Lelystad Bultpark have low angling pressure and Groningen Karelingerplas has none, as it is used only for swimming.
One hundred animals were examined for abnormalities in a cuvet (Hydrobios 5 ml), using an inverted microscope (Olympus IM70) at 100x magnification. Animals were selected under a binocular microscope (Olympus SZX12) to take photographs, then mounted in a drop of glycerin on a slide and covered with a cover glass. Photographs were taken using Olympus CellSense on an Olympus BX51 microscope.

3. Results

Abnormalities were observed in Assen Forellenplas, Weert Roompot and in one animal in Assen Baggelhuizerplas. The normal morphs for all six locations are given in Figure 1.

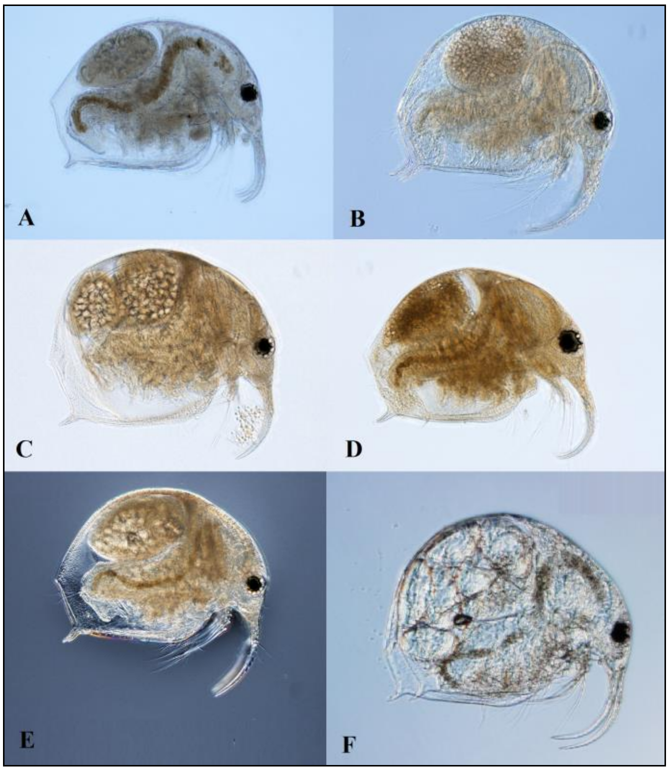

Fig 1: Normal animals A-Forellenplas; B-Roompot; C-Baggelhuizen; D-Vosseven; E-Bultpark; F-Groningen

The abnormalities in Assen Forellenplas are shown in Figure 2. The deformations are of three primary types (Fig. 2–3): blunt, club, or balloon-shaped (Fig. 2A, F and 3F), elongated (Fig. 2B, E), and deformed (Fig. 2C and 3C). Different types can be present in one animal (Fig. 2A–B and 3A–B).

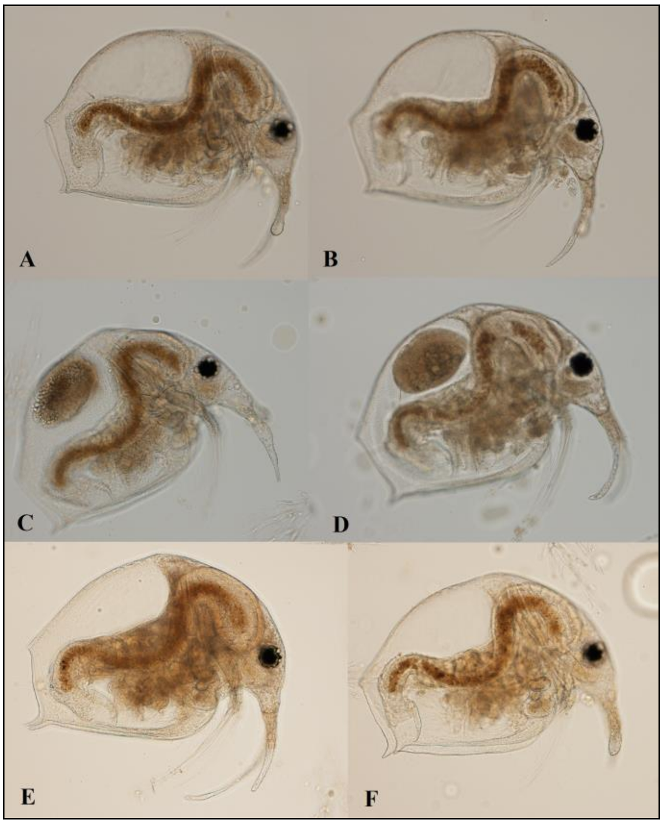

Fig 2: Abnormalities in Assen forellenplas. A - Balloon shaped; B - Elongated, same animal as A; C - Deformed; D - Elongated and Slightly deformed; E - Elongated; F - Club-shaped

The abnormalities in Weert Roompot are shown in Figure 3.

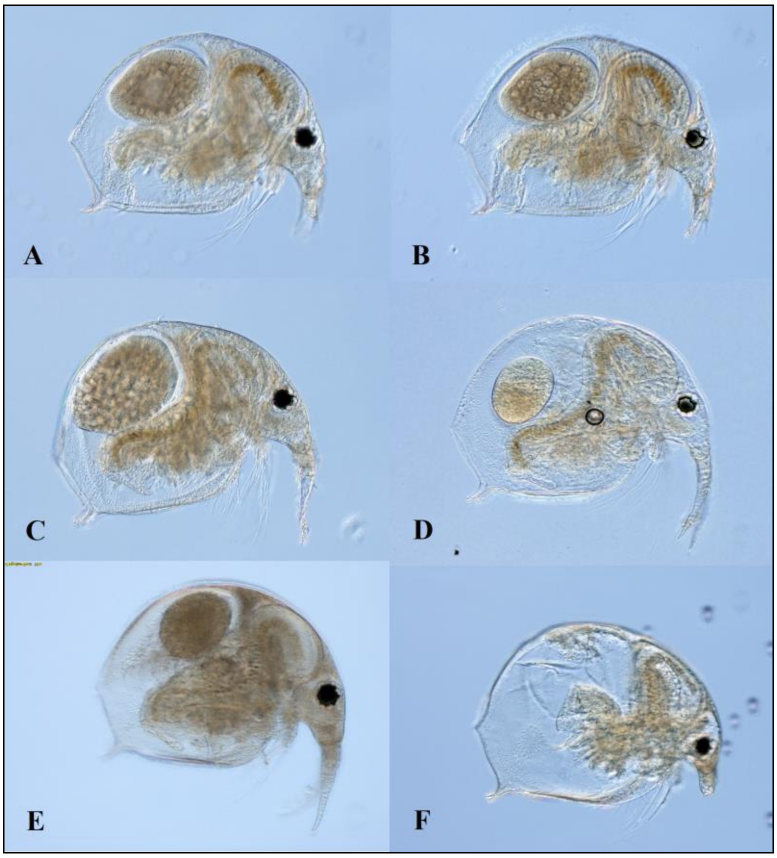

Fig 3: Abnormalities in weert. A - Blunt; B - Deformed, same animal as a; C - Deformed; D - Deformed; E - Deformed; F - Blunt.

The distribution of abnormalities in the populations is shown in Figure 4. Half (50%) of the abnormalities are found in Assen Forellenplas. Adult parthenogenetic females show 72% of the abnormalities, juvenile females just 14%. The population Weert Roompot has 80% of the abnormalities, solely in adult females. One juvenile female with an elongated trunk was found in Assen Baggelhuizerplas. Juveniles with abnormalities are rare. Elongated trunks were only found in Assen Forellenplas.

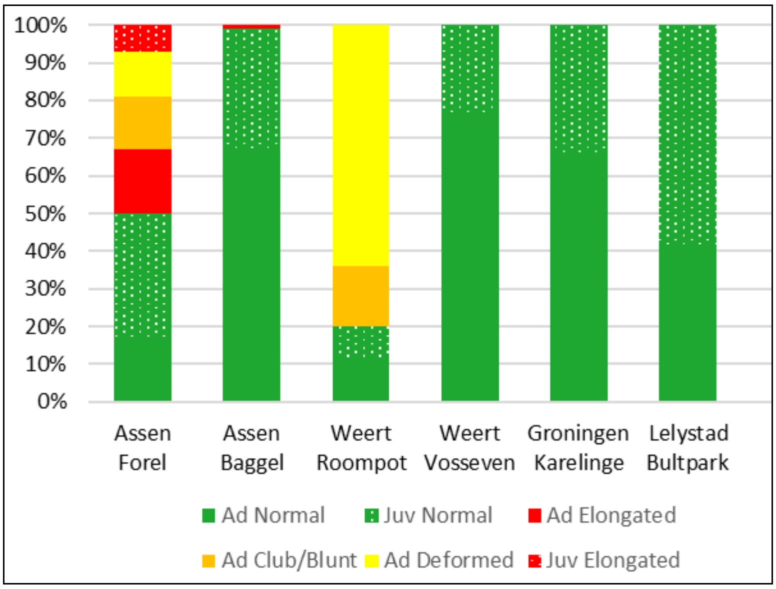

Fig 4: Trunk shape in adult (Ad) and juvenile (Juv) females (%)

4. Discussion

Two ponds are characterized by a very high percentage of abnormalities. There are several causes of abnormalities in cladocerans suggested in the literature. These include toxic substances [11, 14, 17, 19], eutrophication [18], and extreme environments [15, 20]. De Melo *et al.* (2017) [18] observed abnormalities in *Bosmina* intestines, which were probably due to the eutrophication of the reservoir.

Deformations in *Bosmina longirostris* s.l. populations are described by Timm (1904) [21], who mentions maimed antennae in *Bosmina cornuta* (Fig. 5). These are recovered injuries that are sometimes observed in low numbers and characterised by shorter regrown ends. However, the shape of the abnormalities in Assen clearly indicate that these are not recovered injuries.

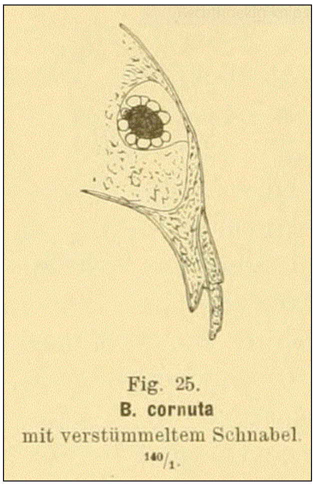

Fig 5: *Bosmina cornuta* with maimed trunk (Timm, 1904) [21]

Some of the abnormalities in Weert (3A and B) are of similar appearance to the maimed trunk depicted by Timm (1904) [21]. The deformed trunks are shorter than the normal trunks (1B). The number of deformations is, in my experience, extraordinary high.

Variation in trunk length is due to temperature and predation. The research on temperature or predation mentions no resulting abnormalities. However, the literature indicates coupling between length of antenna and shell spine when affected by temperature and/or predation [9, 12, 22, 23]. Only trunks are deformed here, which suggests that temperature or predation were not the causes. The possible causes of the abnormalities can be found in the differences between the locations. These differences include the following:

- High angling pressure in Assen Forellenplas and Weert Roompot
- Assen Forellenplas is used for trout cultivation
- Weert Roompot is situated in a former severely polluted area (cadmium and zinc), where sanitation finished in 2015

Assen Baggelhuizen is located directly besides Assen Forellenplas, connected by a culvert. The measured acidity and electric conductivity are almost equal (pH 8.2 and 7.8 and EC 84 and 63). The surroundings and water quality are the same and the only difference between the ponds is the land use, with fish stocking with trout and angling in Assen Forellenplas and no stocking and much less angling in Assen Baggelhuizen.

Weert Vosseven and Weert Roompot are located in an area previously polluted by a zinc factory. In four other samples taken in this area at the same time, *Bosmina longirostris* s.l. was present (number of observations in brackets): De Hoort (12), Grote IJzeren Man (74), Ringselven (39), and Tungelroyse beek (27). No abnormalities were found in these four samples. Abnormalities would be expected in these samples if pollution were still the cause of the abnormalities. No significant differences between the temperatures of the samples were identified.

In his article on combat between copepod predators and *Bosmina*, Kerfoot (1978) [24] describes an attack as follows: "Often the copepod may grab a mucro or antennule, constricting or severing the appendage; such an injury is temporary, and the part will regenerate in subsequent moults". Both shell spine (mucro) and trunk (antennule) are injured by attacks. No regenerated or abnormal shell spines were observed in the samples, which implies that predation was not the cause of the abnormalities. This raises the question of why trunks were the only feature affected. The answer could be a vulnerable period during molting (Fig. 6). The trunk is soft during molting [21] and permeability is thus higher for all kinds of substances. Xenobiotics have an impact on somatic growth [14] and molting [25], and they can induce deformed antennae [25, 26].

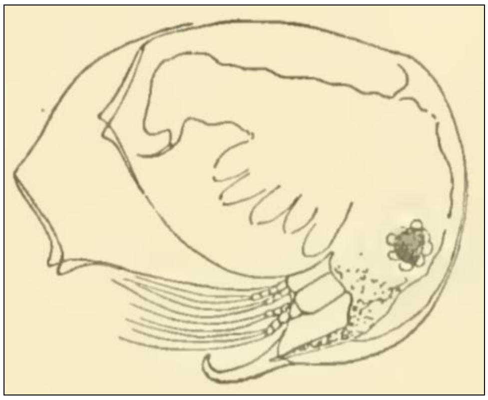

Fig 6: *Bosmina cornuta* during molting (Timm, 1904) [21]

The utilization of the ponds for fisheries can introduce xenobiotic substances by bait and fish gear. Eutrophication is suggested as a cause of abnormalities [18]. The use of bait may cause eutrophication, but research has shown that eutrophication caused by bait is neglectable [27, 28].

Lead is a known xenobiotic, accumulating in cladocerans [25, 29]. Each year, a weight of 2 – 11 tonnes of lead is lost in fresh waters by anglers in the Netherlands [30]. Lead can damage nerve cells and ganglia and alter cell structure and enzyme function [31]. The effects of lead on organisms are most pronounced at elevated water temperatures and reduced pH, in soft waters, and with long exposure time [30].

Baiting is a source of xenobiotics, as all kinds of additives are used such as dyes, flavourings, fragrances, glitter, luminous substances, and solvents. These substances are either natural or synthetic and are often used in concentrated form. Baiting works on chemoreception by fish [32, 33], formation of defence structures (for example longer antennae) in cladocerans is also induced by chemoreception [34], which might interfere [35]. Several organic and inorganic xenobiotics are known to cause deformities of carapace or antennae (trunks) [26]. In cladocerans, the modes of chemical reception, subsequent neurosystem information-processing, and growth or inhibition of structures remains elusive [34]. The formation of the defence structures induced by kairomones involves the stimulation of the endocrine system in the chitinase pathways associated with moulting [36-38]. This system may be involved in the induction of abnormalities. Pesticides and heavy metals are known to disrupt chemical information systems of cladocerans [39]. Disruption of the growth of the trunk after moulting by a xenobiotic could be the cause of the abnormalities. However, it is not possible to give an unambiguous answer to this question of the cause of the abnormalities observed, and more observations from locations with high angling pressure are needed.

5. Conclusions

Trunk deformations in *B. longirostris* are a new abnormality found in cladocerans. It is suggested that abnormalities of the trunk originate from exposure to some xenobiotics during sheeting. The locations in which these observations were made have in common a high angling pressure, which suggests the involvement of xenobiotics. However, the cause of the abnormalities cannot be unambiguously identified. More research is required to identify whether angling has an impact on development of abnormalities in cladocerans.

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