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Abnormalities of the attachment clamps of representatives of the family Diplozoidae

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Abstract

A comparative study has been made of the haptoral morphology of four species of diplozoon (Monogenea: Diplozoidae) from the gills of fish exposed to different levels of water pollution in two river systems in eastern Europe. An examination of the haptors of *Paradiplozoon homoion* (Bychowsky & Nagibina 1959), *Paradiplozoon ergensi* (Pejčoch 1968) and *Paradiplozoon megan* (Bychowsky & Nagibina 1959) from chub caught in the River Morava, Czech Republic and of *Diplozoon paradoxum* (Nordmann 1832) from bream recovered from the River Volga, Russia has revealed abnormalities to the attachment clamps. Two abnormal conditions were found: structural alterations to the attachment clamps and changes in the number of attachment clamps; these occurred both singly and in combination. A higher frequency of abnormal attachment clamps was found in diplozoons from fish caught in the more polluted localities of both rivers. The abnormalities have been recorded and their morphology compared in the light of conditions of environmental stress.

Introduction

In a degraded aquatic environment, particularly where there are pollutants at chronic and sublethal concentrations, changes in the structure and function of aquatic organisms may take place more frequently than their mass mortality. One possible method of assessing the effects of pollutants on aquatic organisms is to examine susceptible organ systems for morphological changes. Of these, the gills of fish are perhaps the best example of a vulnerable target organ that readily responds to unfavourable environmental conditions (Evans, 1987; Müller & Lloyd, 1994). Mallatt (1985) extensively reviewed the structural alterations taking place in fish gills in the presence of environmental pollutants. Thus, the most common changes induced by a wide range of pollutants are the lifting of the epithelium covering the secondary gill lamellae, increased numbers of lymphatic spaces, changes in blood-flow patterns and the appearance of granulocytes in the epithelium. Hypertrophy and hyperplasia of the gill epithelial cells, including mucus and chloride cells, are also common pathological features.

The relationship between 'environment–fish host–parasite' is an exceedingly complex one involving a multiplicity of factors (see Kennedy, 1975; Esch *et al.*, 1990; Esch & Fernández, 1993; Rohde, 1993). Some of these factors may affect ontogenesis of fish parasites, including those ectoparasites that are in direct contact with both the fish host and its environment (e.g. Khan & Thulin, 1991; Williams & Jones, 1994; MacKenzie *et al.*, 1995; Paperna, 1997). It is reasonable, therefore, to expect that pollution and environmental stress most likely affect both host fish and its ectoparasites, notably those monogeneans inhabiting the gills of fish.

Abnormal development of the attachment clamps of diplozoons (Monogenea: Diplozoidae) can involve not only detrimental ecological factors, but also the health of the host fish or genetic factors of the parasites themselves. Bovet (1967) observed an increase in the number of abnormalities ('teratologies') of the attachment clamps of *Diplozoon paradoxum* from fish maintained in the laboratory, resulting from the effects of temperature. Kuperman (1992) reported finding specimens of *D. paradoxum* with structural anomalies, such as a reduction in the size of attachment organs or an unusual asymmetry in the arrangement and number of valves, and used these

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findings as evidence of the mutagenic effects of toxic substances of polluted waters on the morphogenesis of parasites. Oliver (1971) recorded several ‘haptoral teratologies’ to the attachment clamps of *Diplozoon gracile* from *Barbus meridionalis*, including the development of ‘supernumerary clamps’. It is possible that some of these abnormalities in parasite morphology could be related to the isolation of host-fish micropopulations, particularly in cases where they are exposed to non-optimal environmental living conditions. Harris (1998) also described extreme morphological variation between related individuals of *Gyrodactylus pungitii*.

Unfortunately, a common feature of most reports on morphological abnormalities in parasites is the absence of detailed descriptions of the cases recorded and the imprecise use of appropriate terminology. The present paper reports on morphological abnormalities in diplozoon parasites that have been recovered from the gills of fish in two river systems in eastern Europe and describes them with respect to conditions of environmental stress.

Materials and methods

Czech river system

As part of a comparative study of parasite communities of fish, 129 specimens of chub (*Leuciscus cephalus* L.) (length 5.2–33 cm) were caught by electrofishing (Penáz & Jurajda, 1993) during April–October 1994 from the River Morava, Czech Republic. Captured fish were placed immediately in a tank of aerated local river water and transported to the laboratory. They were then maintained in an aquarium for up to 3 days using a standard aquarium filter and charcoal. Fish were killed, measured, weighed, sexed and aged (using the scales of the fish) and examined for parasites by standard methods (Ergens & Gelnar, 1992).

Two sample sites (145 km apart) were chosen for study along the River Morava: a polluted site at Bolelouc, situated downstream of the industrial city of Olomouc and a relatively unpolluted site near the village of Brodské. To evaluate the level of environmental impact at the two sites, semi-quantitative kick-samples (10 min) of benthic macroinvertebrates were taken using a hand-net of mesh-size of 0.5 mm (Zahrádková *et al.*, 1995). Species composition was evaluated using the saprobic index $S_{csn} = s_i h_i I_i / h_i I_i$, where s_i is the saprobic value of individual taxon, h_i is the abundance of the individual taxon, and I_i is the indicator weight of the individual taxon. Data resulting from an investigation of macrozoobenthic invertebrates have been published in separate papers (Gelnar *et al.*, 1997; Dušek *et al.*, 1998). The evaluation of pollution was also based on chemical analysis (including biochemical oxygen demand, phosphate and nitrate content, conductivity) of water samples taken from the study localities during the investigation.

In total, 132 specimens of three species of *Paradiplozoon* (63 specimens from Brodské, 69 specimens from Bolelouc) were examined for abnormalities with the aid of a stereomicroscope system (Olympus BX 5O) using phase-contrast, differential interference contrast (DIC according to Nomarski) and microimage analysis. Whole haptors of all recorded specimens of the *Paradiplozoon* species were routinely fixed in ammonium picrate-glycerin (after Malmberg, 1956; Ergens & Gelnar, 1992) and the remaining part of the worms fixed for 4 h in 4% formalin, washed and then stained in iron acetocarmine (Georgiev *et al.*, 1986), dehydrated in an alcohol series, cleared and mounted in Canada balsam. Whole haptors of specimens were stained in Gomori’s trichrome stain (Kritsky *et al.* (1978) modified by B. Koubková *et al.*, personal communication) in order to help discern abnormalities of the attachment clamps. The morphology of the clamps was studied in detail and their dimensions measured and compared to those of haptors of worms exhibiting what was considered to be normal development (figs 1, 2).

Russian river system

In 1987, accidental effluent from metallurgical work at Cherepovetz, Russia caused severe pollution of the Rybinsk Reservoir in the Volga River System. Comparative analysis of the water quality in different zones of the reservoir revealed high concentrations of PCBs, PAHs, oil products and heavy metals such as Pb, Cd, Cu and Cr in Scheksna Bay, the closest bay to Cherepovetz (Flerov, 1990). All biotic components of the Scheksna Bay ecosystem, from algae to fish communities, were dramatically changed by this incident. In 1988–1991, 207 specimens of adult bream, *Abramis brama* L. (length 37–45 cm) from the Rybinsk Reservoir were caught by trawl at three bays: 77 samples from the Sheksna Bay (12 and 30 km from Cherepovetz); 66 samples from the Mologa Bay (110 km from Cherepovetz), and 64 samples from the Volga Bay (135 km from Cherepovetz). An examination of fish for parasites, with special emphasis on *Diplozoon paradoxum*, was undertaken immediately after catching in the ship’s laboratory facilities. The prevalence and intensity of infection by *D. paradoxum* were defined in fresh samples of fish. For scanning

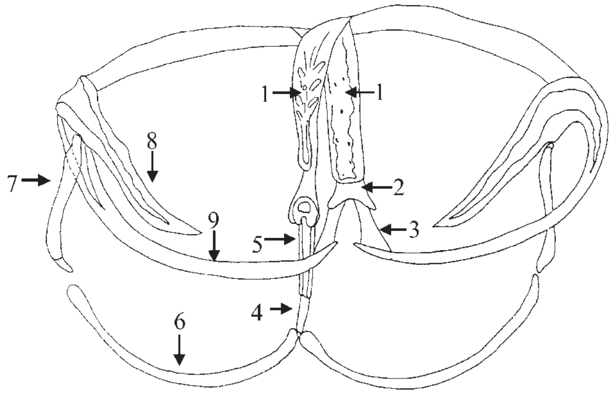
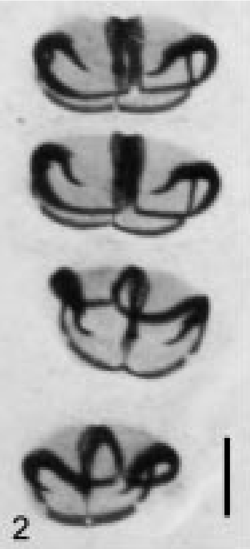
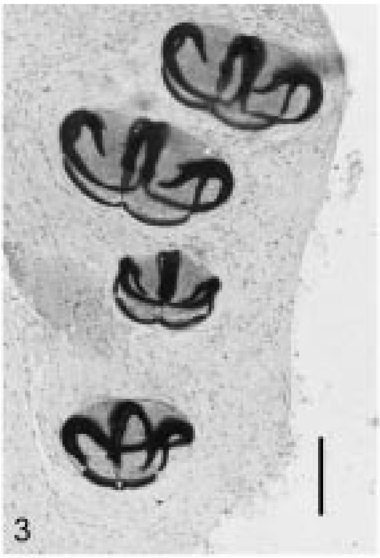


Fig. 1. Anatomical details of the clamp of a diplozoon: 1, median plate; 2, trapeze spur; 3, joining sclerites of the median plate; 4 and 5, sclerites of the distal tip of the median plate; 6, median sclerite of the posterior jaw; 7, lateral sclerite of the posterior jaw; 8, spiky spur; 9, anterior jaw.





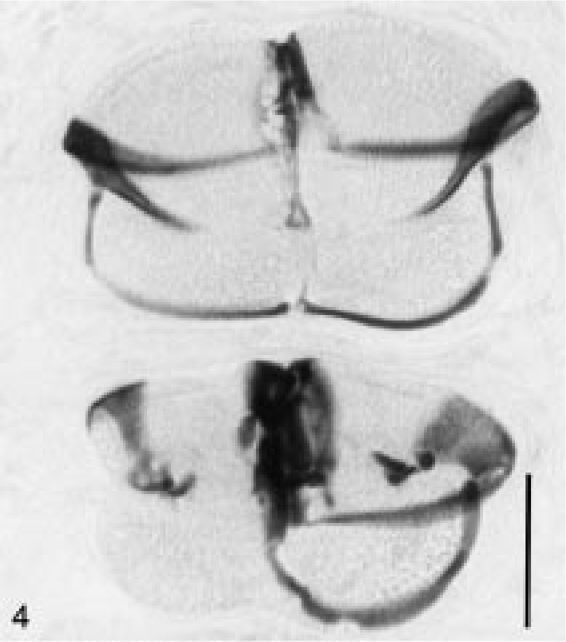


Fig. 2. *Paradiplozoon ergensi*. Left side of normal haptor. Gomori trichrome. Bar = 100 μm. Fig. 3. *Paradiplozoon ergensi*. Right side of haptor, abnormally small R3 clamp. Gomori. Bar = 100 μm. Fig. 4. *Paradiplozoon homoion*. R3, R4 clamps showing absence of some sclerites. Gomori. Bar = 50 μm. Fig. 5. *Paradiplozoon homoion*. R2, R3 clamps showing size differences. Gomori. Bar = 50 μm. Fig. 6. *Paradiplozoon homoion*. Left side haptor showing absence of four clamps (0:4). Phase contrast. Bar = 200 μm. Fig. 7. *Paradiplozoon megan*. R4 clamp showing deformed sclerites. Gomori. Bar = 50 μm.

electron microscopy (SEM), worms were fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for 4 h, postfixed in 1% osmium tetroxide for 1 h, dehydrated in a graded ethanol series, critical-point dried with liquid CO_2, sputter-coated with gold, and examined with a Hitachi JSM-25S scanning electron microscope operated at 15 kV.

Results

Czech river system

Of the chub caught at the polluted site of Bolelouc, 78.6% were infected with diplozoon species, with a mean intensity of 1.5 (range 1–6), compared to 53.4% prevalence, mean intensity 1.74 (range 1–10) of worms at the relatively unpolluted site at Brodske. Details of the prevalence and intensity of infection for the three individual species of *Paradiplozoon* recovered from both sites, together with dominance data, are given in table 1. In general, more specimens of each of the three species of diplozoon were found on fish in polluted water compared to fish from unpolluted water.

Abnormalities of the attachment clamps of the following monogenean gill parasites were observed: *Paradiplozoon homoion* (Bychowsky & Nagibina, 1959), *Paradiplozoon ergensi* (Pejčoch, 1968) and *Paradiplozoon megan* (Bychowsky & Nagibina, 1959). All recorded changes in morphology and dimensions of the attachment clamps were categorized into four basic types:

1. Changes in the size of clamps without accompanying alterations in their structure. During normal development of diplozoon parasites, the clamps grow and develop from the first to the fourth pair (fig. 2), and the presence of any significantly smaller clamps is considered to be abnormal (fig. 3).
2. Morphological alterations of the standard size of clamps, expressed as sclerite deformations or the absence of several sclerites from the clamp (figs 4, 5).
3. Abnormalities involving the absence of some clamps, so that the usual complement of 4 + 4 clamps in longitudinal rows (fig. 6) is lacking.
4. A combination of all three types of morphological abnormality listed above (figs 8–12).

All these morphological changes fell outside the normal range of clamp variation that hitherto has been observed and recorded for the species examined. With the exception of attachment clamps, no other abnormal morphological changes were apparent in any of the specimens examined; moreover, the tissue surrounding the abnormal attachment clamps was not damaged in any way. It is therefore reasonable to assume that the absence of some sclerites or of whole clamps from the worms examined was not the result of incorrect fixation or adverse histological processing. Most of the morphological anomalies were recorded from specimens of *P. homoion* where 50%, i.e. 6 individuals, showed abnormalities of the attachment clamps. In most cases, clamps of the second and third pairs were damaged or deformed. Occasionally, attachment clamps with all sclerites fully reduced or deformed were found on specimens of *P. megan* from the polluted locality at Bolelouc (fig. 7). Tables 2 and 3 describe each recorded case of abnormal attachment clamps of diplozoons recovered from fish caught at Brodské and Bolelouc.

The two sample sites on the River Morava differed in the degree of organic pollution and in associated shifts in the community structure of benthic macroinvertebrates, a feature commonly indicative of environmental stress. According to the system of saprobity (Sládeček, 1981), β-mesosaprobity was found at the unpolluted site at Brodské ($S_{csn} = 2.16$), and α-mesosaprobity at the polluted site at Bolelouc ($S_{csn} = 3.15$). There was a higher frequency (8.69% specimens) of abnormalities of attachment clamps of diplozoons from fish caught in the more polluted locality, compared to the frequency (4.76% specimens) of recorded abnormalities of diplozoons from the unpolluted site.

Russian river system

The prevalence of *D. paradoxum* (Nordmann, 1832) on bream in the polluted Sheksna Bay was 61.0%, with a mean intensity of 4.3 (range 1–19). For bream caught in regions closest to Cherepovetz (12 km) these values increased to 79.2% and 5.8 (range 1–19). In contrast, fish caught in the relatively unpolluted waters of the Volga and Mologa had fewer diplozoons, with values significantly lower at 48.4%, 3.2 (1–12) and 13.6%, 3.4 (1–8), respectively. Overall, 12% of all diplozoons recovered from bream in the Scheksna Bay exhibited some form of clamp abnormality. Of these, 55% were found with

Table 1. Prevalence, mean intensity and dominance of *Paradiplozoon* species on chub from two localities* along the River Morava, Czech Republic.

	Prevalence (%)		Mean intensity (range)		Dominance	
Species	Brodské	Bolelouc	Brodské	Bolelouc	Brodské	Bolelouc
P. ergensi	12.3	12.5	1.3 (1–3)	2 (1–5)	0.79	2.18
P. homoin	13.7	21.4	2.1 (1–10)	2.2 (1–6)	2.06	4.04
P. megan	5.5	16.1	2 (1–4)	1.4 (1–3)	0.53	2.02
Total species	53.4	78.6	1.7 (1–10)	1.5 (1–6)		

* Brodské, unpolluted site; Bolelouc, polluted site.

Table 2. Abnormal cases of attachment clamps of *Paradiplozoon* species from fish recovered from the unpolluted site at Brodské, Czech Republic.

Species	Size of medial hooks (mm)	Position of clamp*	Description of abnormal cases
P. ergensi	0.026	2R	Clamp size smaller than 2L clamp
P. homoion	0.0242	2L	Absence of sclerites from the distal tip of the median plate
			Median sclerites of the posterior jaw grown together in the central part
			Spiky spur separated
			Distal tip of the median plate not fully developed
		3L	Clamp size smaller than 2L clamp
			Median plate deformed in proximal part of clamp
			Absence of joining sclerites of proximal tip of the median plate
			Sclerites of anterior jaw grown together
		3R	Clamp size smaller than 2R clamp and 4R clamp
			Spiky spur deformed in right half of clamp
			All sclerites deformed in left half of clamp
			All median plate deformed
		4L	Clamp size smaller than 2L clamp and 4R clamp
			All sclerites deformed
			Both posterior and anterior jaws grown together
			Absence of spiky spurs
			Median plate reduced
			Three separated ball-shaped sclerites, difficult to identify
		4R	All sclerites abnormally thin, clamp size normal
P. homoion	0.0192	4R	Clamp size smaller than 4L clamp

* L, left side of haptor; R, right side of haptor; numbers mark position of attachment clamp in longitudinal row.

clamps reduced from the normal number and arrangement of 4:4 (fig. 13) to 4:3, 3:3 and 4:1 (figs 14–16). Ten percent of the worms from fish from Sheksna Bay showed a complete reduction of the clamp from one side of the haptor (4:0) (fig. 17), and 35% had deformed clamps that were smaller than normal (figs 18, 19). None of the diplozoons examined from bream caught in the unpolluted River Volga and Mologa Bays showed structural abnormalities to the clamps.

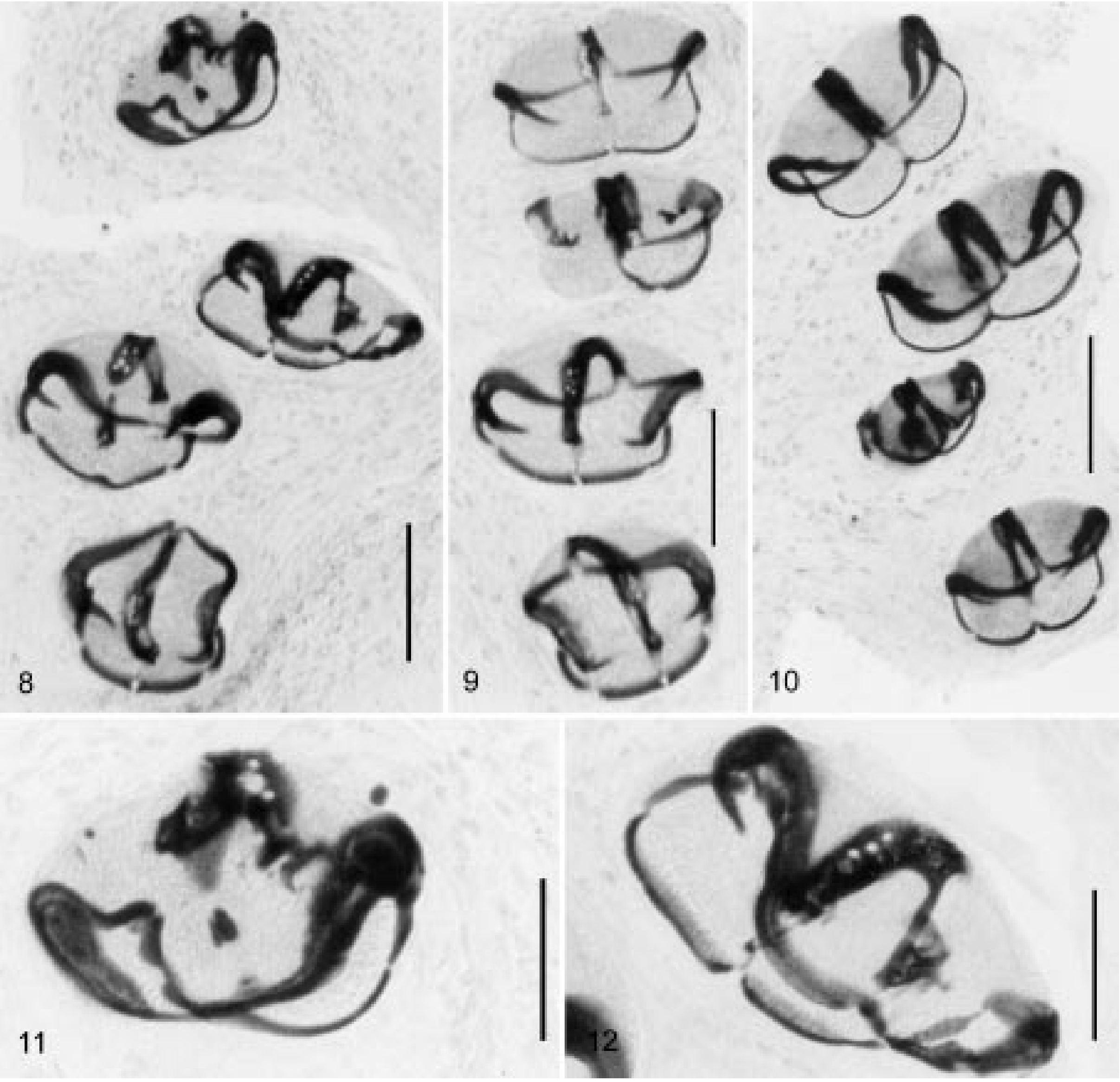
Discussion

Parasites are an integral component of animal communities and seem to be sensitive to a wide array of ecological factors, including environmental stress (e.g. Koskivaara, 1992; Kuperman, 1992; Poulin, 1992; Zharikova, 1993; Landsberg *et al.*, 1998). Numerous published accounts describe the effects of pollution on fish parasites (see reviews by MacKenzie, 1987; Möller,

Table 3. Abnormal cases of attachment clamps of *Paradiplozoon* species from fish recovered from the polluted site at Bolelouc, Czech Republic.

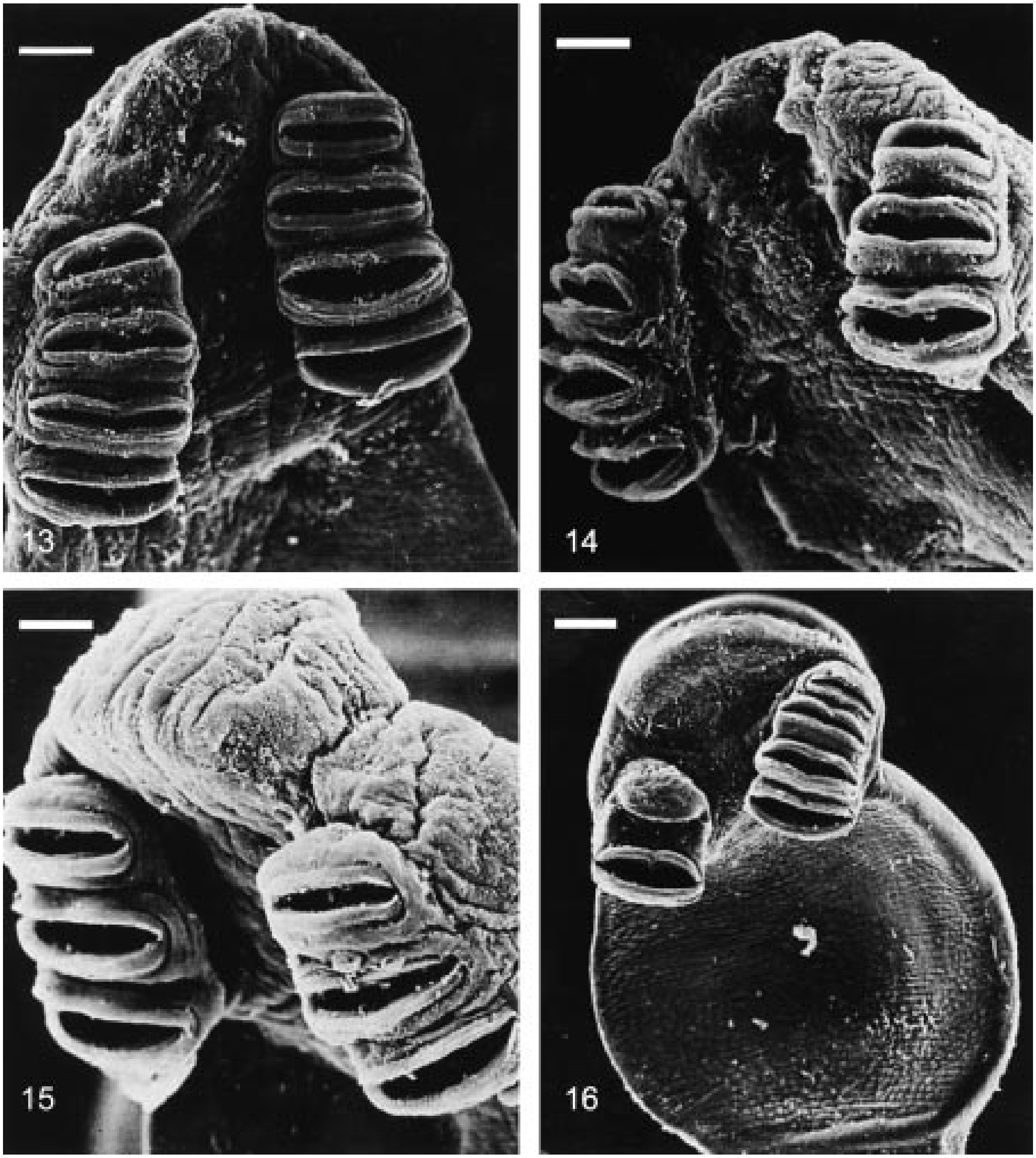
Species	Size of medial hooks (mm)	Position of attachment clamp*	Description of abnormal cases
P. ergensi	0.0256	3L	Clamp size smaller than 2L clamp, normal development
P. homoin	0.0198	3R	Clamp in longitudinal row is not observed
P. homoion	0.0211	2L	Median plate is fully deformed
			All sclerites abnormally thin
		3L	Clamp size smaller, normal development
P. homoion	0.0211	2L	Superfluous, unspecific sclerite in right half of clamp
			Small ball-shaped sclerite between median and lateral sclerite of posterior jaw
		3R	Clamp size smaller than 2R clamp and 3L clamp
			Absence of both median and lateral sclerites from the left posterior jaw
			A malformation of all median plate including those sclerites of both distal and proximal tips of the median plate
P. homoion	0.0214	3R	Clamp size smaller than 2R clamp and 4R clamp
			All sclerites deformed
			Absence of spiky spurs
P. megan	0.0267	2R	Clamp size smaller, normal development
		3R	Clamp size smaller, normal development
		4R	All sclerites reduced totally, determination of individual sclerites is difficult

* L, left side of haptor; R, right side of haptor; numbers mark position of attachment clamp in longitudinal row.



Figs 8–12. *Paradiplozoon homoion* haptors showing a combination of clamp abnormalities. Gomori trichrome. Fig. 8. Left side of haptor showing deformed clamps. Bar = 100 μm. Fig. 9. Right side of haptor showing absence of sclerites and deformation of clamps. Bar = 100 μm. Fig. 10. Right side of haptor showing different sized clamps. Bar = 100 μm. Fig. 11. Detail of clamp L1 from fig. 8. Bar = 100 μm. Fig. 12. Detail of clamp L2 from fig. 8. Bar = 100 μm.

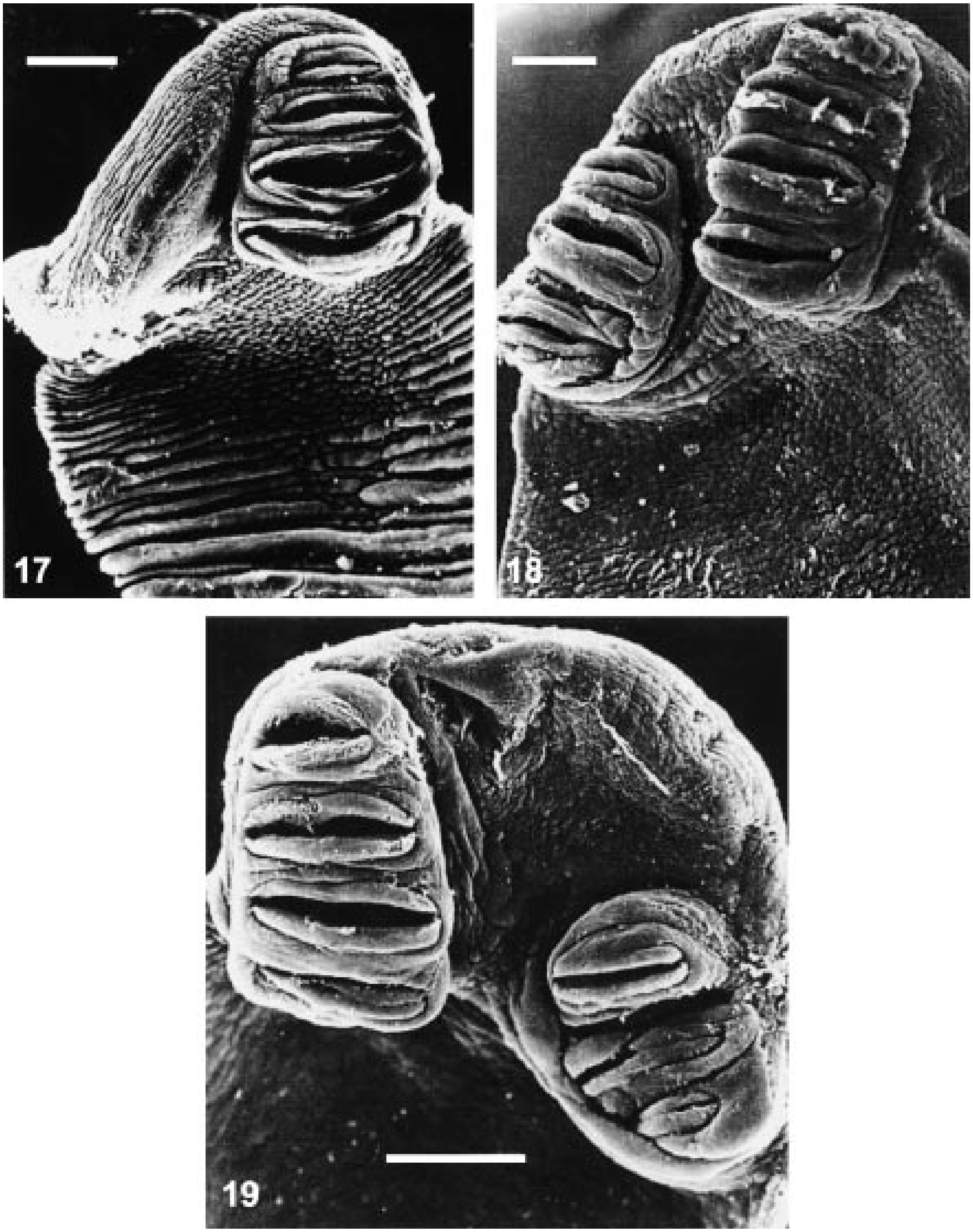
1987; Khan, 1991; Khan & Thulin, 1991; Poulin, 1992; Bucke, 1993; MacKenzie *et al.*, 1995; Lafferty, 1997; Sures *et al.*, 1999), such that they represent a very appropriate group of organisms for environmental risk assessment. Ectoparasites that live in immediate contact with aquatic pollutants and have a direct life cycle with a single host are ideally situated to reflect changes in the health status of the ecosystem. In this respect, it would seem reasonable to expect that fish-gill monogeneans could serve as sensitive bioindicators of environmental quality (see Koskivaara *et al.*, 1991a,b; Koskivaara, 1992; Koskivaara & Valtonen, 1992; Margoliese & Cone, 1996; Gelnar *et al.*, 1997; Dušek *et al.*, 1998). The idea is well supported by reports of diplozoons having structural anomalies that can be linked with non-optimal living conditions or with mutagenic effects of toxic substances from polluted waters (Bovet, 1967; Oliver, 1971; Oliver & Reichenbach-Klinke, 1973; Kritscher, 1991; Kuperman, 1992; Zharikova, 1993). It is clear from the present results that pollutants have a direct impact on diplozoon morphology of which the most vulnerable external structures are the attachment clamps of the haptor. The normal arrangement of clamps of all known diplozoons is 4:4, these being arranged longitudinally and symmetrically on both sides



Figs 13–16. *Diplozoon paradoxum* haptors examined by scanning electron microscopy. Bar in all cases = 100 μm. Fig. 13. Frontal view of normal haptor (4:4 clamps). Fig. 14. Shows a reduction in the number of clamps: (4:3). Fig. 15. Shows a reduction in the number of clamps: (3:3). Fig. 16. Shows a reduction in the number of clamps: (1:4).

of the haptor. Abnormalities were observed in the clamps of all four species of diplozoon examined and included a reduction in the number and size of clamps, asymmetry in their arrangement, and considerable deformities.

However, in order to investigate associations between parasites and environmental pollution it is worth remembering that environmental factors differ in their effects and that parasites differ in their responses. For example, eutrophication can increase levels of parasitization, while heavy metals and an unspecified human



Figs 17–19. *Diplozoon paradoxum* haptors examined by scanning electron microscopy. Bar in all cases = 100 μm. Fig. 17. Shows a reduction in the number of clamps: (0:4). Fig. 18. Structural abnormalities and a reduction in the number of clamps: (3:4). Fig. 19. Structural abnormalities and a reduction in the number of clamps: (4:3).

'disturbance' invariably reduce parasitism (Lafferty, 1997). According to Lafferty's analysis, monogeneans represent a group of parasites that respond positively to the impact of eutrophication, thermal effluent, crude oil and industrial effluent, but negatively to the effects of pulp-mill effluent, acid precipitation and general disturbances. It seems that they can also exhibit a high degree of resistance to environmental pollution. Thus, in the present study, the level of infection of bream by *D. paradoxum* was markedly higher in the polluted areas of Sheksna Bay (61%), particularly in the vicinity of metal/oil effluent (79.2%), than in the relatively clean waters of the Volga and Mologa Bays (48.4% and 13.6%, respectively). A similar trend was apparent for diplozoon species in the Czech river system. Dušek *et al.* (1998) found that monogeneans also differed in their responses with respect to their systematics (genera *Dactylogyrus*, *Gyrodactylus*, *Paradiplozoon*), microhabitat distribution (location on gills or fins versus body surface of host fish) and evolutionary strategy (specialists versus generalists). There is no doubt that different pollutants can affect different species of parasites in different ways, and a more precise approach is needed to clarify associations between parasites and different environmental impacts.

In general, structural abnormalities such as those of haptoral clamps can be traced to deviations from normal development, often in early embryonic stages (Weis & Weis, 1987; Lenat, 1993; Giesy *et al.*, 1994; Müller & Lloyd, 1994). Such teratologies may be caused by a number of factors, including mutant genes, environmental conditions, infection, drugs and, perhaps most frequently, by interactions between any or all of these causal agents. As stated, a major cause of malformations are hereditary factors, including gene mutations, many of which are due to chance and are not passed on to the offspring. Environmental factors, both external and internal, can also be critical in aquatic organisms. Thus, pollutants can interfere with development by disrupting metabolic process and thereby act as teratogenic substances, albeit fairly non-specific. Although there appear to be several ways in which teratogenetic agents can affect susceptible embryonic cells, the final result is probably either cell impairment or death, or a changed rate of growth. Either of these measures puts local tissue development out of step with adjoining parts and disrupts the co-ordinated schedule of development. Such interference with normal development can be used as a basis for a bioassay, and indeed is used in certain toxicity screening tests (Weis & Weis, 1987; Lenat, 1993).

As far as terminology is concerned, it would seem appropriate to ascribe all recorded morphological changes of attachment clamps of diplozoons as 'abnormalities'. In the case of the first type of abnormal change (see list in Results), it means that changes in the size of clamps without any accompanying changes in their structure may be interpreted as a 'teratological change', provided the genetic cause of these abnormal changes can be verified. This requirement is also necessary for an assessment of an abnormal quantitative change (see the third type listed above); this involves the absence of some clamps so that the usual 4:4 complement in longitudinal rows is not present. As for the second type of abnormality listed, perhaps the least controversial interpretation would be to consider it as a 'deformation' of the sclerites or a 'malformation' of the clamp.

Monogeneans represent more than 50% of species richness of metazoan parasites of fish in Europe (Gelnar *et al.*, 1994, 1997; Dušek *et al.*, 1998) and would seem to be a very useful group of organisms for environmental risk assessment. Parasites are ubiquitous components of animal communities and most have a wide geographical range, reflecting the distribution of their hosts. In general, they are also very abundant, more so than their hosts, and this can be related to the trophic structure of certain ecosystems. They move through the food web and are usually situated at the top of the trophic structure of the entire ecosystem. As a result, parasites help integrate the adverse effects of complex and varying environmental pollutants and stresses. Currently, there are three different levels at which parasites could perhaps be used as potential biological indicators: those of the individual, the population, and the community. From the present study, the individual level approach can be further divided into: (i) an investigation of morphological abnormalities and structural malformations that relate to the level of pollution in the aquatic environment; and (ii) a study of the ability of parasites to accumulate contaminants, especially heavy metals.

Unfortunately, current knowledge on monogenean ecotoxicology is very limited. Thus far, there is an absence of information on the distribution of contaminants within the body of the individual parasite and the potential effect of pollutants and accumulated contaminants on parasite ontogenetic development and life-cycle, including lifespan, reproductive potential and fitness. For this reason, future research should focus on investigating the relationship between parasite morphological abnormalities and the ability of parasites to accumulate toxins. There is a need both for further field studies to assess the comparability of model parasite–host systems originating from different sites, and for experimental studies to verify any correlation between levels of teratological change in the parasite and disturbances in its freshwater environment. This calls for a multidisciplinary approach to the problem, involving closer collaboration between fish parasitologists, ecotoxicologists and environmental scientists.

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