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# Occurrence and impact of *Heteropolaria* sp. (Protozoa, Ciliophora) on intensively cultured perch (*Perca fluviatilis*)

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This study describes the occurrence and the impact of *Heteropolaria* sp. in intensively cultured perch (*Perca fluviatilis*). *Heteropolaria* sp., in association with *Aeromonas* sp., form a symbiotic complex which may induce severe pathology in intensively cultured perch. This colonial ciliate is most frequently detected in winter when it colonizes the spiny dorsal fin of young perch and spreads all over the body in older individuals. High population density favours the propagation of *Heteropolaria* sp.: the infestation frequency in 40 g perch after two months of rearing at 23°C was 10, 50 and 91%, at stocking densities of 303, 602 and 1 974 fish $m^{-3}$, respectively. High temperatures and repeated handling (e.g. sorting) also increase the risks of infestation.

## 1. Introduction

Rarely observed in natural environments, ciliates such as *Heteropolaria* sp. (Epistylididae) may induce severe epizootic infections on fish in enclosed environment (Roberts 1979). The exact relationship between *Heteropolaria* sp. and its host is still controversial. Depending on the infested species, it ranges from commensalism to parasitism in case of massive infestations (Fauré-Fremiet 1943, Lom & Vàvra 1961, Lom 1966, Rogers 1971, Hazen *et al.* 1978, Kabata 1985, Small & Lynn 1985, Stoskopf 1993). *Heteropolaria* sp. in association with *Aeromonas* sp., form a pathogenic symbiotic complex commonly known as the red sore disease that was reported to cause massive fish kills (primarily striped bass *Morone saxatilis* and other centrarchids) in North Carolina reservoirs (Esch 1976, Miller 1976, Hazen *et al.* 1978).

*Heteropolaria* sp. has been detected on the skin of many fish species (see Lom 1966 and Kabata 1985 for review). Its life cycle is simple; it has single host and reproduces asexually by binary fission. The arborescent colonies constituted by many trophonts (fixed form) are superficially or deeply attached to the integument by a stalk, de-

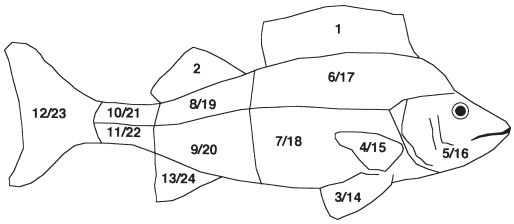


Fig. 1. Reference perch arbitrarily divided into 24 areas (right side/left side) for examination of *Heteropolaria* sp.

pending on the "ecoform" or species (Lom 1973, Stoskopf 1993). *Heteropolaria* sp. spreads through its telotroch stage (free-swimming stage) which uses the fish as a simple fixation surface (Lom 1966, Hazen *et al.* 1978). Esch *et al.* (1976) found a positive correlation between the total length of fish and the infestation frequency in largemouth bass *Micropterus salmoides*. In turbid water, *Heteropolaria* sp. can rapidly spread and form important colonies. High temperature may also favour the emergence and spreading of *Heteropolaria* sp. and associated red sore disease, though infestations are commonly observed in winter and spring in some regions (Lom 1966, Esch *et al.* 1976).

The dearth of knowledge about the biology and the ecology of the symbiosis between *Perca fluviatilis* and *Heteropolaria* sp. in the wild contrasts with its frequent occurrence in culture environments. Since culture of perch will most probably became intensive (Mélard *et al.* 1995ab) and because *Heteropolaria* sp. is more easily propagated at high stocking densities, we studied the occurrence and impact of this ciliate on juvenile perch at different stocking densities.

## 2. Materials and methods

The influence of stocking density on the emergence and spreading of *Heteropolaria* sp. was tested in winter 1993–1994 in four-month-old, non-infested perch. Non-infested perch were stocked in flow-through polyester tanks (4.0 $m^2$, 1.6 $m^3$). Tanks were supplied with automatically-mixed waters from the River Meuse and from the Tihange nuclear power plants in order to regulate temperature around 23°C (± 1°C). Each of the five experimental tanks differed by its stocking density (from 303 to 1 974 fish $m^{-2}$) or mean fish size (mean initial weight from 20 to 62 g). Fish were fed with commercial dry feeds (45% protein, 11% lipid, 2% fibre; 2 mm in diameter) distributed from 9.30 to 21.30 hrs with automatic feeders. Daily food rations (*DFR*) were calculated according to the equation proposed by Mélard and

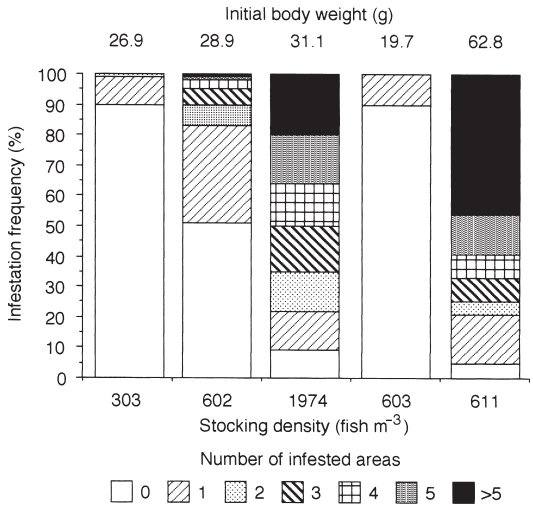


Fig. 2. Variation in the degree of infestation of individual perch (*Perca fluviatilis*) by *Heteropolaria* sp. depending on fish size and stocking density. (Samples of 100, four-month-old fish cultured at 23°C). Areas refer to the body areas defined in Fig. 1.

Kestemont (1994): $DFR\ (\%\ d^{-1}) = 7.11 W_m^{-0.28}$, with $W_m$ corresponding to mean body weight. Food rations were adjusted on a two-week basis when the tanks were controlled and biomass was measured.

At the end of the 41-day experiment, 100 fish were randomly sampled in each of the five experimental tanks. Fish were weighed, measured and examined for infestation by *Heteropolaria* sp. to determine the overall infestation frequency (i.e. occurrence of infested fish among the population). In order to determine the degree of infestation, we arbitrarily divided the body of perch into 24 areas (Fig.1), which were ranked as infested or non-infested depending on the detection at first sight of colonies of *Heteropolaria* sp. (macroscopical observation). This survey also aimed at understanding the propagation of *Heteropolaria* sp. colonies over the body of perch.

## 3. Results

The effects of fish size and stocking density on the development and propagation of *Heteropolaria* sp. in cultured perch juveniles is summarized in Fig. 2. At the end of the 41-day rearing period, the overall infestation frequencies in perch of similar initial weight (27–31 g) differed significantly ($\chi^2 = 131.3$; $df = 2$; $P < 0.001$) depending on stocking density: 10, 49 and 91% at 303, 602 and 1 974 fish $m^{-3}$, respectively. In addition, individual perch were more heavily infested at high stocking density (Fig. 2): the occurrences of fish with six or

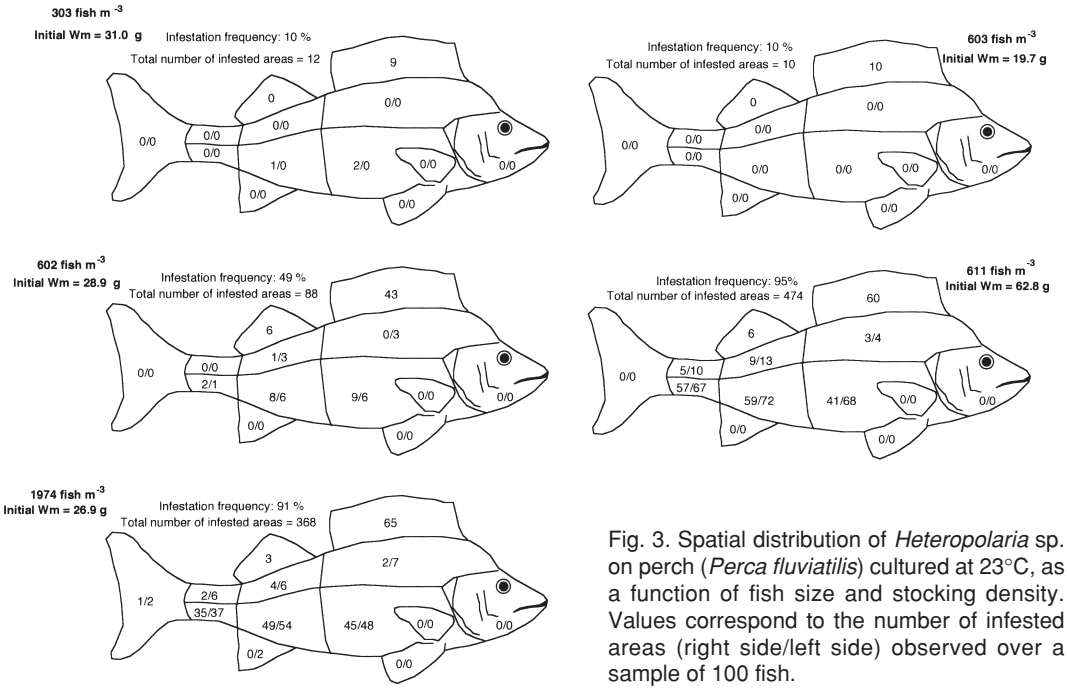


Fig. 3. Spatial distribution of *Heteropolaria* sp. on perch (*Perca fluviatilis*) cultured at 23°C, as a function of fish size and stocking density. Values correspond to the number of infested areas (right side/left side) observed over a sample of 100 fish.

more infested areas were 0, 1 and 20%, respectively. Within each tank, infested fish were larger than non-infested individuals, although the differences were not significant except at the lowest stocking density ($t = 2.37$; $P < 0.05$; $df = 98$) as a result of growth heterogeneity in cultured perch (coefficient of variation of the final body weight around 40%; Table 1).

The effect of fish size on the infestation of perch by *Heteropolaria* sp. emerges more clearly when comparing fish of different initial body weight (19.7, 28.9 and 62.8 g) reared at similar stocking densities (602–611 fish $m^{-3}$): the overall infestation frequencies were 10, 49 and 95%, respectively ($\chi^2 = 148.2$; $df = 2$; $P < 0.001$). Similarly, heavy infestion rates (six or more infested areas) were more frequently observed in larger fish (46%), than among in smaller fish (0 and 1%).

The colonies of *Heteropolaria* sp. were variously distributed over the body of perch, depending on the overall infestation frequency, and thus on fish size and stocking density (Fig. 3). In small perch or in fish reared at low stocking density, the infestation frequency was low and most colonies were observed on the spiny rays of the anterior dorsal fin. In larger fish or at higher stocking density, colonies were observed all over the body of perch except on the head, pelvic and pectoral fins. Similarly, the presence of colonies on the anal and caudal fins was exceptional. The anterior part of the dorsal fin was most frequently colonized by *Heteropolaria* sp. Among body areas covered with scales (areas 6–11 and 17–22, Fig. 1), the infestation was around 10 times as high on the abdominal areas as on the dorsal areas (Fig. 3; $\chi^2$ against theoretical homogeneous distribution = 148.3 and 120.9; $P < 0.001$, for 1974 fish $m^{-3}$ and for 62.8 g perch, respectively). No head-to-tail gradient or heterogeneity in the distribution of *Heteropolaria* sp. colonies were noticed ($\chi^2$ against theoretical homogeneous distribution, $P > 0.10$). Colonies were more frequently observed on the left side than on the right side of perch, though this distribution was significantly non-homogeneous in large fish only ($\chi^2$ against theoretical homogeneous distribution = 4.4; $P > 0.20$).

Growth and survival of perch relative to fish size and stocking density are given in Table 1. The mortality over the 41-day rearing period was low and similar among tanks (0.5 to 3.3%). Growth rates were higher in small fish or at low stocking densities, which had the lower infestation frequency. Still, the effect of size or density on the settlement of *Heteropolaria* sp. colonies does not allow discrimination of the specific influence of the ciliate on perch growth.

## 4. Discussion

According to the spatial distributions of colonies over the body of perch, the settlement of *Heteropolaria* sp. obviously requires the availability of suitable fixation substrata on the host. From early observations by Poirier (1993) and from the results presented in this study, it can be stated that these areas are characterised by the absence of epidermis, as on the rays of the spiny dorsal fin or on scale cteni. Recent experiments of artificial infestation by mechanical tearing of the epidermis (Grignard & Mélard, unpublished) showed that *Heteropolaria* sp. preferentially settled on scales where the epidermis had been damaged. Since larger fish have longer cteni, the probability for *Heteropolaria* sp. colonies to find suitable substrata over the body of the perch would increase with increasing fish size, accounting for the size-related variability evidenced in this study (see also Esch *et al.* 1976 for parallel on centrarchids).

The comparison between the distributions of colonies on perch reared at different densities suggests that *Heteropolaria* sp. initially settles on the spiny dorsal fin then further spreads over the body, depending on the availability of suitable areas. As suggested by the experiments on artificial infestation, suitable areas may correspond to places where the epidermis was damaged as a result of handling or frequent contact with any cutting or abrasive surface (e.g. spiny rays of other fish, sides of polyester tanks). Obviously, this probability is higher at high stocking density, accounting for the density dependent infestation frequency and degree observed in our study. This hypothesis is supported by the localization of *Heteropolaria* colonies in areas corresponding to the maximum body width of perch (below the lateral line), where the probability of contact with the sides of the tanks is highest. Similarly, the side-related distribution of colonies over the body of perch probably reflects a typical behaviour of perch preferentially swimming against the current in tanks (i.e. clockwise in our tanks) and thus having a higher probability of damaging their epidermis on one side (i.e. left side) than on the other.

Independently of fish size and stocking density, the settlement and spreading of *Heteropolaria* sp. will be intimately dependent on the environmental conditions that favour the initial contamination of the culture environment by telotrochs (free-swimming stage). Since perch were reared in a flow-through system, this contamination was directly dependent on the input of telotrochs from the River Meuse. These free swimming stages are plausibly present all year round. Although their density can not be easily assessed as a result of their small size (≤ 120 µm) with respect to the sediment load, it probably undergoes seasonal fluctuations and peaks during autumn or winter, when high water velocities modify the arrangement of the substratum and move higher loads of mineral and organic material. The initial settlement of *Heteropolaria* sp. on perch would thus be dependent on the probability of meeting a substratum, and thus would be favoured by high stocking density and large fish size. High rearing temperatures also would favour the spreading of

Table 1. Effect of population density and fish size on the development of *Heteropolaria* sp. in perch *Perca fluviatilis* reared in flow-through tanks (4.0 $m^2$, 1.6 $m^3$) at 23°C (initial age: 4 months). Standard production variables also are given.

| Stocking density (fish $m^{-3}$) | 303 | 602 | 1 974 | 603 | 611 |
|---|---|---|---|---|---|
| Mean initial weight (g) | 31.0 | 28.9 | 26.9 | 19.7 | 62.8 |
| Mean final weight (g) | 43.4 | 41.7 | 36.7 | 37.6 | 84.5 |
| Coefficient of variation (%) | 41.7 | 40.6 | 44.8 | 40.4 | 35.0 |
| Growth (g fish $d^{-1}$) | 0.48 | 0.47 | 0.32 | 0.44 | 0.54 |
| Production (g $m^{-3}$ $d^{-1}$) | 146 | 285 | 626 | 264 | 327 |
| Food conversion ratio | 1.90 | 1.87 | 2.63 | 1.66 | 2.56 |
| Survival rate (%) | 97.8 | 98.3 | 96.7 | 98.3 | 99.5 |
| Infestation frequency (%) | 10 | 49 | 91 | 10 | 95 |

trophonts. Recent experiments on initially non-infested 52 g perch reared at 300 fish $m^{-3}$ for 41 days (Grignard *et al*., unpublished) demonstrated that the infestation frequency at 14°C was much lower than at 23°C (26.5 vs 65.7%, respectively).

Over the course of the 41-day experiments, no specific effect of *Heteropolaria* sp. on fish growth and survival could be distinguished from the direct influence of fish size and stocking density. The study period may have been too short with respect to the assessment of such production variables. During the following weeks and consecutively to an increase of sediment load in the River Meuse, we recorded significant mortalities similar to those reported for the red sore disease caused by the *Heteropolaria* sp.–*Aeromonas* sp. complex (Rogers 1971, Esch *et al*. 1976, Miller & Chapman 1976).

These preliminary results stress the need for more detailed investigations on the relationship between this symbiotic complex and perch, that could restrict the development of the commercial intensive production of this promising species for aquaculture (Tamazouzt *et al*. 1994). Zootechnical adjustments (e.g. tank shape, light intensity, food distribution) should be sought on the base of behavioural studies to minimise the potential damages to fish epidermis as result of aggression between fish or abrasion by the tanks. Special care also should be dedicated to prophylactic strategies using chemical substances authorized in food aquaculture to prevent the settlement or spreading of *Heteropolaria* sp. Similarly, handling procedures such as size grading (see advantages in Mélard *et al*. 1995bc) should be minimized beyond the size (around 20 g) and age when the probability of infestation by *Heteropolaria* is high. Finally, since infestation is dependent on the initial contamination by free swimming stages in the water supply, the use of recirculating systems should be seriously considered.

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