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NORDIC JOURNAL*of* FRESHWATER RESEARCH

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Nordic Journal of Freshwater Research is a modern version of the Report of the Institute of Freshwater Research, DROTTNINGHOLM. The journal is concerned with all aspects of freshwater research in the northern hemisphere including anadromous and catadromous species. Specific topics covered in the journal include: ecology, ethology, evoulution, genetics, limnology, physiology and systematics. The main emphasis of the journal lies both in descriptive and experimental works as well as theoretical models within the field of ecology. Descriptive and monitoring studies will be acceptable if they demonstrate biological principles. Papers describing new techniques, methods and apparatus will also be considered.

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Table 6. Potential management strategies to overcome major yield-limiting factors for cool-water crayfishes. As emphasized in text, populations are often limited by an interaction of factors. Thus, adding shelters may decrease both competition for shelters and predation. See text for literature citations.

Limiting Factor	**Potential Management Strategy**
Low temperature	Add waste heat from power generation or industry
Calcium, pH	Lime lake
Dissolved oxygen	
Summerkill	Aerate in summer
Winterkill	Aerate in winter
Habitat	Add refuges
Food	Fertilize lake
Predation by fishes	Reduce numbers or size of predatory fishes
Intraspecific competition	Increase human exploitation of mature individuals
Disease or parasites	Limit introduction of exotic vectors

Erratum

The table below is missing from (1994) 69: 111-136, page 129.

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Collodictyon triciliatum H.J. Carter (1865) - a Common but Fixation-sensitive Algivorous Flagellate from the Limnopelagial

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Abstract

Collodictyon triciliatum H.J. Carter (1865) was isolated from Lake Årungen near Oslo (Norway), and studied by light and electron microscopy. Its food requirements were tested in cultures. Its particular morphology and strategy for food capture makes it a versatile predator. The smallest particles, bacteria, would not support growth, but many common planktonic algae were acceptable. Large size and food habits of *Collodictyon* makes it a member of the "classical" food chain, not the "microbial loop", in meso- to eutrophic lakes where it may be rather common.

Keywords: *Collodictyon,* flagellate, heterotrophic, laboratory culture, food chain, limnology.

Introduction

As the microbial food web of the marine pelagial has been untangled, new species of phagotrophic protists have been described (e.g. Patterson and Fenchel 1985, Fenchel and Patterson 1988, Vørs 1992) and distinctive strategies for food acquisition have been rediscovered (Jacobson and Anderson 1986). Investigations in the limnic pelagial have confirmed the involvement of protists in food webs here as well (e.g. Nagata 1988, Arndt and Mathes 1991, Laybourn-Parry 1992). Although the diversity of microbial grazers is lower in the freshwater pelagial than in the sea, surprising discoveries were made (e.g. Spero 1982, Klaveness 1984, Bird and Kalff 1986, Nicholls 1987). Several of the limnopelagial protists are of a size that means they are both herbivore grazers and available to crustacean predators as prey (Arndt and Mathes 1991, Mischke 1994).

Some common members of the aquatic food webs are difficult to recognize. *Collodictyon triciliatum* was first described from freshwaters on the Island of Bombay, India (Carter 1865). Its "subpolymorphic" nature was immediately apparent, as well as its feeding habits: "it will frequently enclose part of a body which it is not large enough to enclose entirely ..". Carter (loc.cit.) was inclined "to think that it should be placed among the Rhizopoda". Later studies (Francé 1899, Rhodes 1919, Belar 1921, 1926) shed light upon the cytology of the cell, as revealed by light microscopy techniques. The true number of flagellae is four (e.g. Francé 1899). Cell division and mitosis (a closed orthomitosis) were described in detail (Rhodes 1919, Belar 1921).

Carter depicted *Collodictyon* engulfing an *Oscillatoria*-like trichome. Further observations on food uptake in *Collodictyon* added green algae and flagellates to its menu (Francé 1899). Skuja (1956) confirmed its rather omnivorous habits; green and blue-green algae as well as small diatoms like *Cyclotella* and *Stephanodiscus* were eaten. *Oscillatoria* -like trichomes have been observed inside *Collodictyon* during blooms of the former in Lake Årungen, Norway,

thus verifying the original observations of Carter (1865) and the statements and Skuja (1956).

Collodictyon triciliatum is a rather common pelagic protist in lakes and ponds. It has so far been found in India (Carter 1865), Central Europe (Ettl 1983, Mischke 1994), Sweden (Skuja 1956) and Norway (this paper), but has probably been overlooked elsewhere (Patterson and Hedley 1992).

Collodictyon triciliatum is easily recognized by the LM investigator of live material. It is distinguished from flagellates of similar size by its pyriform to polymorphic shape, the four flagellae with the prominent nucleus located basally, and the vesiculate cytoplasm. Its closest relative is *Aulacomonas* Skuja, which is very similar in cellular morphology with the exception of its size (cf. Mischke 1994), and the fact that *Aulacomonas* has only two flagellae (Brugerolle and Patterson 1990). *Paraphysomonas* De Saedeleer has spines and heterokont flagellae, *Gyromitus* Skuja has a distinctive cell shape, and scales that may appear as a shaggy surface coat at the LM level. Species demarcation within the genus *Collodictyon* has been discussed by several authors, (see Skuja 1956, Pringsheim 1963, Ettl 1983). The presence or absence of a ventral furrow or groove was indicated as one character, but this was found to be variable (Belar 1926, Skuja 1956, Wawrik 1978). Wawrik (loc. cit.) described resting stages from her field observations, and the presence of a stigma in a new variety of the species (*Collodictyon triciliatum* Carter var. *stigmata* Wawrik).

Since Belar's experimental work (1921, 1926), *Collodictyon triciliatum* does not appear to have been held in culture until very recently. Mischke (1994) used modern techniques to study its growth and food uptake, and was able to confirm its herbivory. However, the growth and uptake rates calculated from the experiments were implausible. The fine structure of the organism has never been studied, and its taxonomic position within the Volvocales should therefore be regarded as tentative.

Material and methods

For the present study, *Collodictyon triciliatum* was isolated from Lake Årungen, near Oslo, Norway. *Collodictyon* grew well on the flagellate *Rhodomonas lacustris* Pascher et Ruttner, strain N 750301 (Klaveness 1981), in a simplified version (without organic buffer and no silicate added) of the freshwater medium of Guillard and Lorenzen (1972). Single drops of dense *Collodictyon* culture were transferred to *Rhodomonas* tubes at densities of $5x10^5$ - 10^6 *Rhodomonas* cells per ml. These culture tubes were routinely held at 17 °C at light intensities of 40 µE m^{-2} sec^{-1} for 14 hours a day (≈2 E m^{-2} day^{-1}), and gave rise to a new dense *Collodictyon* culture in less than two weeks. Without a food supply, the culture survived for less than 14 days under these conditions.

For experiments, algae to be tested as food were grown in batch cultures at 17 °C and at higher light intensities (250 µE m^{-2} sec^{-1}, ≈12,6 E m^{-2} day^{-1}). Close to the end of the exponential growth period, *Collodictyon* was inoculated from a healthy, food-depleted stock culture grown on *Rhodomonas* under the same conditions. If the algal culture to be tested supported *Collodictyon* growth, new experiments were reinoculated from the previous generation of test culture. *Collodictyon* and food algae were sampled and counted daily in Palmer-Malloney chambers, growth curves plotted and maximal growth rate estimated from running 3-day linearizations of the natural logarithms of cell densities (Excel™ LINEST).

Collodictyon proved difficult to preserve for microscopy. For counting cells, strong Lugol's solution (10 g KJ and 5 g J_2 to 100 ml aqua dest.) was used at a concentration of two drops per 5 ml culture suspension. The most successful fixation for electron microscopy was carried out on ice; following gentle centrifugation of 10 ml aliquots of culture suspension in tapered tubes, ice-cold electron microscope grade 4% glutaraldehyde (GA) in 0.05 M cacodylate buffer was poured on to the loose pellet (which immediately fell apart into single cells). After 2-4

hours, the cells were rinsed in buffer 3 X, and postfixed in 1% OsO_4 in buffer. Modifications of the fixation procedures (addition of 1.5% $K_3Fe(CN)_6$ to the OsO_4) improved membrane preservation. Embedding and staining techniques were as published elsewhere (Klaveness 1973).

Glutaraldehyde also proved useful for light microscopy. When 5-7% GA was added to the medium, the cell shape was well preserved and the cytoplasm displayed a green fluorescence on excitation with blue light. Various stains were tested, and the results of two is shown here: toluidine blue for cells in plastic-embedded thick (1 µm) sections, and Texas Red conjugated anti-tubulin for glutaraldehyde-preserved cells.

Results

The original drawing of *Collodictyon* published by Carter is reproduced here as Fig. 1a. The best reproductions of living cells have been provided by Francé (1899) and Skuja (1956), see Figs. 1b and c, respectively. The shape of the swimming cells (Fig. 1d) in a clonal culture varied from isodiametrically ovoid to flattened with a slight ventral groove and with a bifid or lobate posterior. The various shapes encountered in the clonal culture agreed well with earlier observations by Francé (1899), Lemmermann (1914) and Skuja (1956). A ventral furrow or groove may be present, but is not permanently present in the strain studied here. Under certain circumstances, for instance when cells adhere to the cover glass under the microscope, viscous cytoplasm may appear floating from one side or from the antapical area, forming lamellipodia and slightly motile filopodia. The cell does not appear to move in amoeboid fashion, but the filopodia may possibly aid in tactile location or identification of particles. Pringsheim (1963) noted that "Sie fangen mit Pseudopodien kleine Algen, die in Nahrungsvakuolen eingeschlossen werden."

Observations of live cells confirm the early observations of vacuolate cytoplasm (Carter 1865, Francé 1899, Rhodes 1919, Belar 1921, and Skuja 1956). The central cytoplasm contains a few large and a number of small vacuoles, which may be distinguished by careful focusing. Some of the vesicles may contain food particles at various stages of digestion. The observation of Wawrik (1973) that the cytoplasm is "dünnflüssig" is pertinent, since the intracellular vesicles are extremely difficult to preserve. Rapid fixation using an ice-cold or concentrated solution of EM-grade glutaraldehyde seems to preserve the cell in a reasonably natural condition, but the alleged intracellular cytoplasm delimiting the vesicles is more difficult to recognize. Staining of glutaraldehyde-preserved cells revealed that the peripheral cytoplasm was of uneven thickness, consisting of thicker areas of cytoplasm interconnected by thin sheets (Fig. 1e).

Fixation appears to alter the intracellular organization of vesicles. After "thick" sectioning of EM-fixed material and staining with toluidine blue for light microscopy, the cellular content of food material is enclosed within one cavity (Fig. 1f) delimited by the peripheral cytoplasm of uneven thickness as described above. An opening is present antapically or slightly ventrally. The cell appears as an inverted sac containing food material at various stages of digestion (Fig. 1f). The cellular organelles are enclosed in the cytoplasm that makes up the sac wall (of various thickness, cf. Fig. 1e) and the nucleus and the flagellar bases are located apically in the area of firm non-vacuolate cytoplasm, at the bottom of the sac.

Electron microscopy of thin sections (Fig. 2a) confirmed the cell structure as interpreted from light microscopy (Fig. 1f). The disagreement between observations of the central cytoplasm in live and fixed cells in the light microscope has led to the inference that the highly vacuolate central cytoplasm may disintegrate on fixation. The more careful fixation procedures employed later in this study (rapid administration of stronger glutaraldehyde solution, fixation on ice, and ferricyanide in the osmium solution) gave a better preserved cell. The central cytoplasm may be spongioform, as indicated in the early drawings (Figs. 1b,c), intercalated by vesicles of vary-

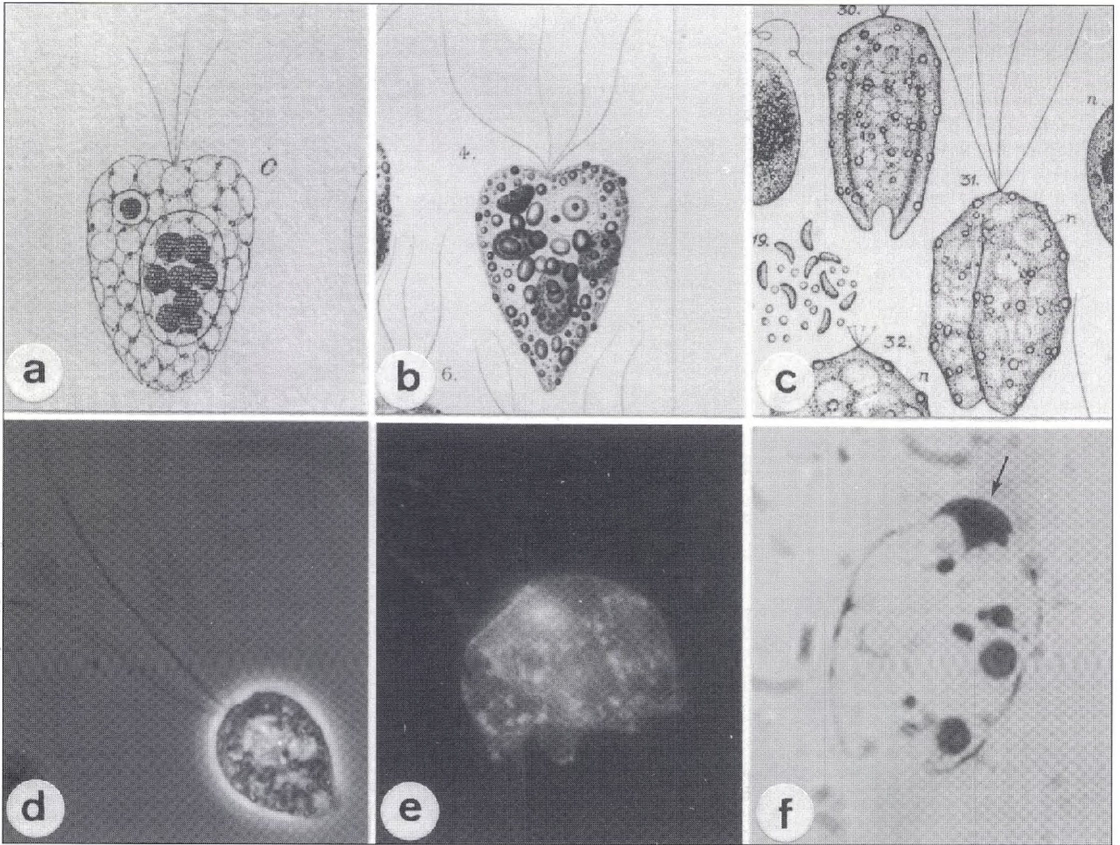

Fig. 1a. *Collodictyon triciliatum* as depicted by Carter (1865; Pl. XII, her fig. 12 c), showing cell with "a digestive space".
Fig. 1b. As depicted by Francé (1899; 1 Tábla, fig. 4), a more realistic rendering of a highly vacuolate cytoplasm and several food vesicles.
Fig. 1c. As depicted by Skuja (1956; Taf. XI, fig. 30-31) giving a very good impression of the fragile vacuolate cytoplasm and the highly refractile peripheral granuli seen in well nourished cells.
Fig. 1d. Phase contrast micrograph of a small, swimming cell of *Collodictyon triciliatum* displaying the length of the flagellae and a cell of pyriform shape. The bifid posterior may be discerned. Flash photograph, 1/1,500 sec. Magnification x 1,000.
Fig. 1e. Glutaraldehyde-fixed cell stained with antitubulin/Texas Red as displayed by epifluorescence microscopy. The non-specific staining reaction shows the cytoplasmic network and internal vacuolization to some extent. The lobate cell shape is well preserved. Magnification X 1,000.
Fig. 1f. 1 µm "thick" section of plastic-embedded cell fixed and embedded for electron microscopy, stained with toluidine blue. From the anterior end of cell (arrow) with its non-vacuolate cytoplasm, a thin sheet of peripheral cytoplasm appears to enclose a large central space where food residues and two cells of *Chlorella* may be discerned. Bright-field micrograph, magnification x 1,700.

ing size. Even when the best fixation methods are used, the plasmatic bridges probably break and appear as cytoplasmic anastomoses, as shown in Figs. 2a-c. The thinly viscous cytoplasm that could be observed as filopodia extending out of the antapical opening in living cells under certain conditions also disappeared after fixation.

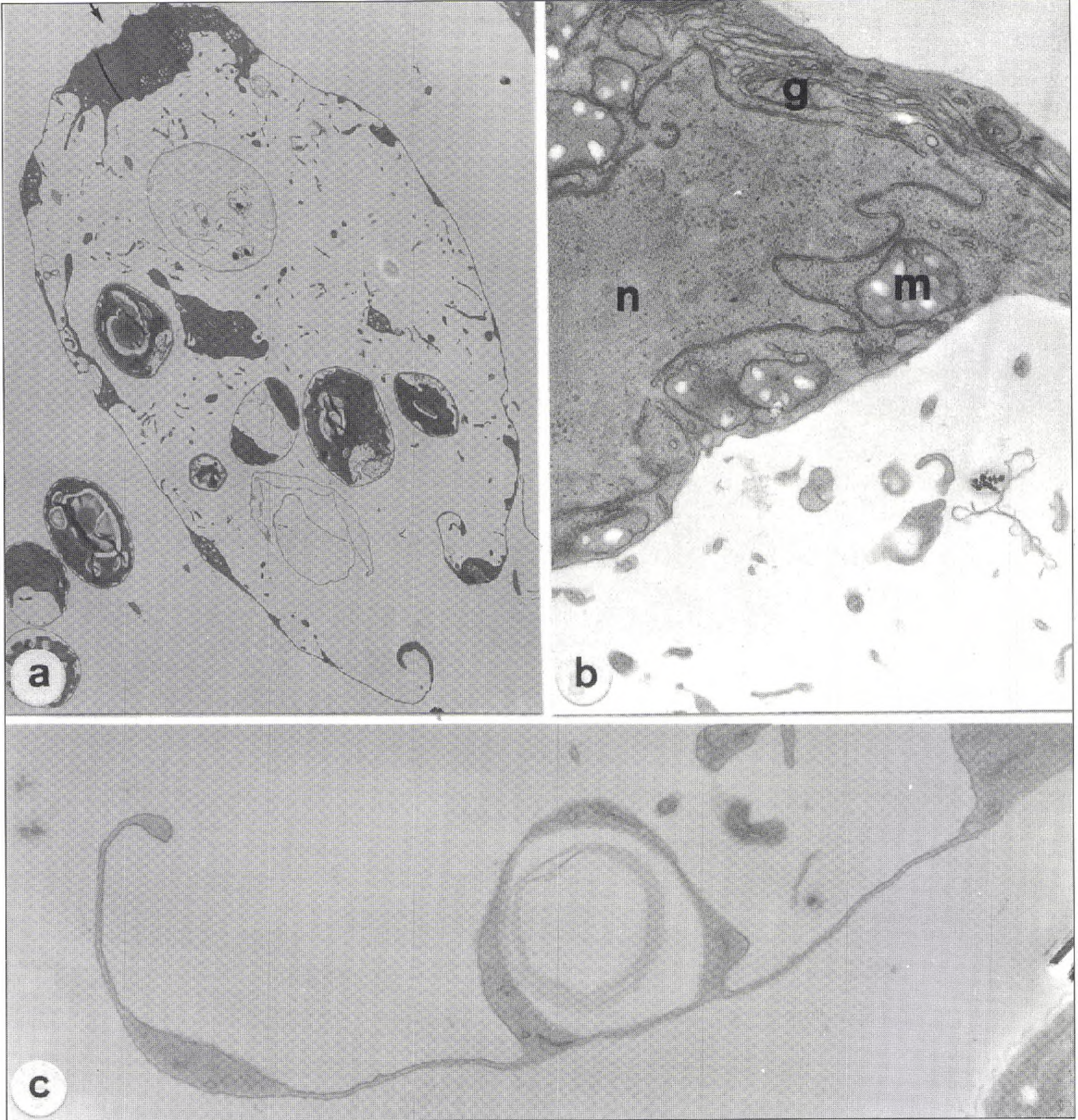

Fig. 2a. Ultrathin section of cell as seen under low magnification (x 5,900) in the electron microscope. The anterior end (arrow) has an area of non-vesiculated cytoplasm where the kinetosomes (flagellar bases), the nucleus and other organelles may be found. The thin sheet of peripheral cytoplasm encloses the central area where residues of the vacuolate cytoplasm are still present as anastomoses and irregular areas, between food cells and their residues.

Fig. 2b. Detail of apical area with part of nucleus (n), Golgi (g) and mitochondria (m). Inside the central cavity (lower part of photograph) anastomoses of the central cytoplasm may be seen. Electron micrograph, x 23,000.

Fig. 2c. Detail of antapical end of cell showing peripheral vesicle and the lip of the oral aperture. No cell wall structures are visible beneath or outside the plasma membrane (a structureless polysaccharide tomentum may be discerned by cytochemical staining (not shown)). Electron micrograph, x 27, 000.

When cells were fixed and stained by conventional techniques, the cell surface membrane displayed no scales or structured glycocalyx. At most, a fine fibrillar material ("tomentum") could be discerned. The cell surface is reactive to ruthenium red (applied as in Klaveness 1973) and a thin polysaccharide glycocalyx may be present.

The mitochondria of *Collodictyon* have tubular, or rather, vesiculate cristae (Fig. 2b). A large dictyosome consisting of flattened vesicles is found close to the apical nucleus, next to the four kinetosomes. The smooth flagellae are of equal length and resemble those of green algae. The fine structure of the flagellar bases may resemble that of its close relative, *Aulacomonas* as shown by Brugerolle and Patterson (1990).

Maximal growth rates in *Collodictyon* were measured during exponential growth in batch cultures, using various food sources (Table 1). The highest growth rate was recorded when the food source was the cryptophycean alga *Rhodomonas lacustris*. A growth rate not significantly different from that on *Rhodomonas* was recorded when *Collodictyon* was fed with a strain of the chlorococcalean green alga *Chlorella vulgaris* Beijerinck, isolated as an endosymbiont from the ciliate *Coleps hirtus* Nitzsch (Klaveness 1984, cf. also Esteve et al. 1988). The survival and growth rates of *Collodictyon* fed on a strain of *Chlorella saccharophila* (Krüger) Migula (my strain μ_0, isolated from the plankton of a lake) were low. When the flagellate was transferred into a culture of the diatom *Cyclotella pseudostelligera* Hustedt, there was an initial burst of growth for the first day or two, followed by a rapid decline to a low growth rate. Diatoms are known to synthesise lipids of high nutritive value (e.g. Groth-Nard and Robert 1993); certain lipids may have been present in minimum amounts in some of the unialgal cultures used.

Blue-green algae such as *Planktothrix* (*Oscillatoria*) *agardhii* (Gomont) Anagnostidis and Komárek, isolated from Lake Årungen where *Collodictyon* was also present, never supported growth under the culture conditions used in this study. This is surprising, as several authors (Carter 1865, Skuja 1956) have noted *Collodictyon* apparently attacking members of this cyanophyte genus. One particular non-colony forming strain (CYA 43) of *Microcystis aeruginosa* Kützing, from the Norwegian Institute of Water Research supported growth quite

Table 1. Growth of *Collodictyon* fed on various unialgal food sources, grown at high light intensities (250 $\mu E\ m^{-2}\ sec^{-1}$, $\approx 12.6\ E\ m^{-2}\ day^{-1}$). The algal cultures were near the end of the exponential growth period when *Collodictyon* was inoculated.

Prey	Prey size μm^3 (median)	Growth rate d^{-1}	SE	*N*
Rhodomonas	118	0.81	0.051	7
Chlorella vulgaris	16.7	0.78	0.147	3
Chlorella saccharophila	5.33	0.17	-	3 *
Cyclotella	43.4	0.15	0.034	3 ***
Synechococcus	1.45	0.39	0.245	3 **
Microcystis	37.0	0.36	0.045	3
Planktothrix	>1,000	0.00	-	2

* Only one of three experiments resulted in growth. ** Only two of three experiments were successful. *** *Cyclotella* gave rise to an initial burst of very rapid growth, that levelled out at this rate as long as food was abundant.

well but never gave rise to dense cultures of *Collodictyon*. *Synechococcus* sp., also isolated from Lake Årungen, supported growth of *Collodictyon* to a limited extent, but the cells were small and mis-shapen and did not grow well after 2-3 transfers. None of the cultures was axenic, since I did not succeed in separating *Collodictyon* from the associated bacteria in the culture medium at this stage. Bacteria may therefore have supported the growth of *Collodictyon* by providing growth factors not present in the algae. *Collodictyon* never grew upon mixed cultures of bacteria alone, even at high densities of bacteria.

Fig. 3 shows growth curves (2 parallels of each) of *Collodictyon* fed on *Rhodomonas* or *Microcystis* at non-limiting densities. *Collodictyon* showed a decreasing exponential rate of growth when fed on *Rhodomonas* (Fig. 3), even at the constant non-limiting concentration of prey used here (about 250,000 cells ml^{-1}). The plot in Fig. 4 suggests that growth in *Collodictyon* (or its "functional response") may be a simple function of the ratio of consumer and resource. Ratio-dependent consumer-resource models are an alternative to resource-dependent models, and are currently being discussed in the literature (see Diehl et al. 1993).

The size of *Collodictyon* appears to vary according to the availability of food. When *Collodictyon* is fed on *Rhodomonas*, its cell body length are 13-30 µm (*N*=90) and cell width is 8-22 µm (*N*=90). Cell volume ranges from 561 μm^3 to 4769 μm^3 (*N*=90). The cell size of my strain agrees well with that observed by Mischke (1994).

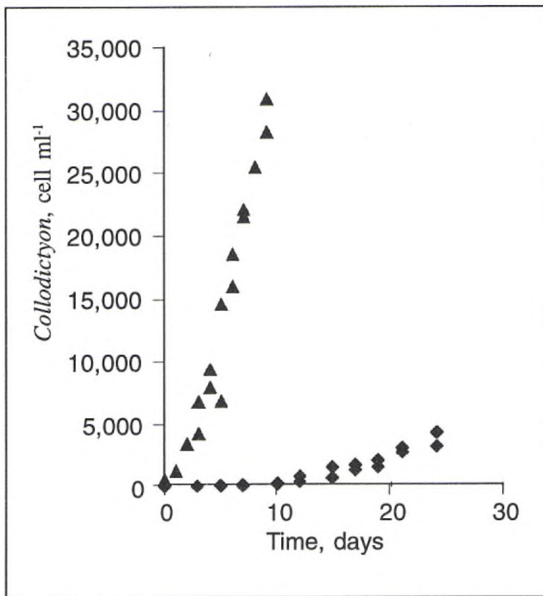

Fig. 10. Concentrations (cells ml^{-1}) of *Collodictyon* cells as a function of time (days) when growing on *Rhodomonas* (triangles) held at a constant concentration of about 200,000 cells ml^{-1}, or on *Microcystis* (diamonds) at concentrations exceeding 500,000 cells ml^{-1}. Note the linear scale of the ordinate, and the fact that the curve for growth on *Rhodomonas* is not truly linear. Two parallels of each.

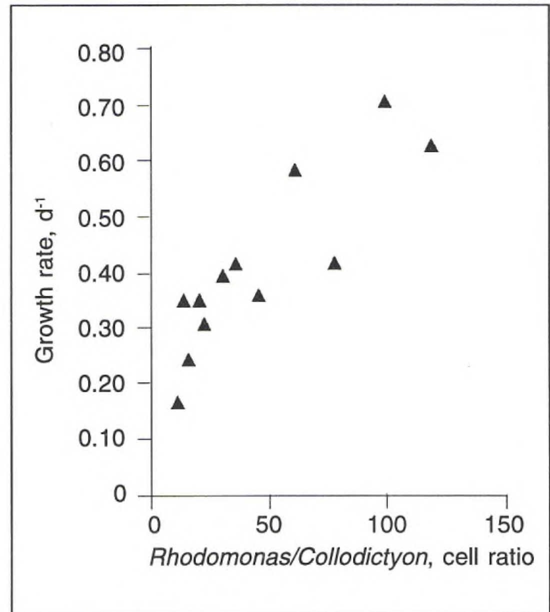

Fig. 11. The growth rate of *Collodictyon* fed on *Rhodomonas* at a constant density of about 250,000 cells ml^{-1}, plotted as a function of the ratio of their concentrations. This figure shows the different growth rates of *Collodictyon* recorded during 5-day periods for all experiments at this food concentration. There appears to be a pronounced effect of increased competition in spite of the food surplus: 250,000 cells ml^{-1} of *Rhodomonas* is almost 30 mg biomass per litre water.

Discussion

The results of this study have implications both for the evolutionary origin and taxonomic position of *Collodictyon triciliatum* and for the role of *Collodictyon* and other planktivorous flagellates in the lacustrine food web.

The cytoplasmatic structure of *Collodictyon* is distinctive, and the combination of vesiculate mitochondria, isokont flagellation and smooth flagellae is unusual. Vacuolated cytoplasm resembling that of *Collodictyon* at the light microscopy level is found among some Heliozoea and probably also in *Aulacomonas* (cf. Swale and Belcher 1973, Brugerolle and Patterson 1990), a very close relative of *Collodictyon*. Mitochondria with vesicular or tubular cristae are found in some members of the Rhizopoda and Heliozoea (cf. Page and Simensmaa 1991) and more generally in the Chromista (for instance compare those of *Chrysolepidomonas* (Peters and Andersen 1993) and *Collodictyon*). The smooth isokont flagellae resemble those found in the chlorophycean line of green algae. However, the structure of the mitochondriae indicates that *Collodictyon* should no longer be classified as a colourless member of the Volvocales (e.g. Pascher 1927, 1931, Fritsch 1935, Fott 1959, Huber-Pestalozzi 1961, Pringsheim 1963, Bourrelly 1972, Ettl 1983).

There are no traces of a reduced plastid in *Collodictyon*, in contrast, for example, to the phagotrophic chromist genus *Paraphysomonas*, in which all the species investigated have vestigial plastids (Preisig and Hibberd 1983). *Collodictyon* seems to be a quite undifferentiated type of unicellular organism (cf. Francé 1899), without a cytopharynx but with a food capture mechanism of amoeboid type based on microfilament mediated motility.

Collodictyon has developed active phagotrophy and eats a range of prey organisms of different sizes. Bacteria alone are unable to support *Collodictyon*. This observation agrees with the recent results of Mischke (1994), who found low uptake rates and no growth in *Collodictyon* fed on bacteria The preferred food particle size range and the cell size of *Collodictyon* itself (well within the prey size range of crustacean zooplankton) locate it as an intermediate member in the classical food chain rather than in the microbial loop. However, there are numerous unanswered questions concerning the life of *Collodictyon* and similar organisms that fill related niches in the limnic food webs. Although frequently found in the pelagial of lakes, it may equally well originate in the sediment surface or sapropel (Ettl 1978), from which it may emerge when conditions in the water become favourable. Similar behaviour has been postulated for the saprotrophic ciliate *Coleps hirtus* Nitzsch, which may detect prey by chemotaxis and enter the pelagic water masses and there reproduce to reach bloom proportions. The role of *Collodictyon* and similar phagotrophic flagellates (such as *Aulacomonas*, *Paraphysomonas* and *Gyromitus*) in the limnopelagic food webs still needs to be clarified.

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Dr. Tom Andersen suggested that there might be a ratio-dependent effect in *Collodictyon* grazing on *Rhodomonas*, and provided literature. Tove Bakar made sections for electron microscopy, which was carried out at the Electron Microscopy Unit for the Biological Sciences, University of Oslo. The author is particularly grateful to two anonymous referees for advices, to Alison Coulthard for linguistic improvements, and to the Royal Society of Sciences, Uppsala, for permission to reproduce Skuja's drawing (Fig. 3).

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Macroinvertebrate Effects on Leaf Pack Decomposition in a Lake Outlet Stream in Northern Sweden

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Abstract

Benthic sampling and a field experiment were used to study the availability of leaf litter to benthic insects and its processing in a section of a north Swedish lake outlet stream. We found that leaves accumulated during a short period of time in autumn and were invaded by invertebrates, primarily insects. In experimental leaf packs, many insects, incl. nemourid and limnephilid larvae preferred and processed alder (*Alnus incana*) leaves at a higher rate than birch (*Betula* spp.) leaves. This preference was reflected in 54% and 64% higher animal concentrations in alder than birch leaf packs after 56 and 198 days, respectively. Alder leaves appeared to represent a vanishing food resource of higher value than the birch leaves. Animal density (numbers/g leaf mass) was higher in spring than in autumn. Differences in processing rates and the relative composition of riparian vegetation are predicted to have strong influence on the structure of the benthic invertebrate communities. Artificial, non-edible leaf packs attracted other species than natural leaf packs, in particular filter feeding blackfly and caddis larvae.

Keywords: Detritus, shredder, stream, experiment, benthos.

Introduction

Small streams are frequently under a strong influence of the riparian vegetation. The canopy reduces sunlight and thereby instream primary production. Instead the periodic, massive input of autumnal litter drives the system energetically. The fauna is phenologically and physiologically well adapted to these conditions (Short et al. 1980, Short and Ward 1981. Cummins et al. 1989). Macroinvertebrate shredders are important actors on this scene (e.g. Cummins 1974, Cummins and Klug 1979, Cuffney et al. 1990), and their presence is likely to influence other trophic groups, especially collectors and predators (Short and Maslin 1977, Wallace et al. 1977, Cummins and Klug 1979, Malmqvist 1993).

Although considerable insight into detritus-animal relationships has been gained over the last couple of decades, many aspects still need to be considered. For example, only recently has the importance of food limitation to detritivores in streams been experimentally demonstrated (Smock et al. 1989, Richardson 1991). This has considerable ecological implications, but far more information from various regions and for further taxa is needed for an understanding of how common and widespread such food shortage is. The issue of quality versus quantity is another important question. Are, for instance, different leaf species equal of quality to shredders once they have been conditioned by microbes (cf. Cummins et al. 1989)? Also, further studies of detritus-shredder relations are justified because processes vary with type of biome (Corkum 1992), and little information is available from boreal parts of Scandinavia (however, see Karlström 1978).

In this paper, we attempt to answer the following three questions: 1) What are the major taxa and what are their numerical relationships in natural leaf packs of a northern Swedish stream? 2) To what extent does leaf material function as food and substrate, respectively? 3) How does the relative availability of different leaf species vary with season, and how do the leaf pack inhabitants track their food/habitat? We approached these problems in a lake outlet stream by sampling natural accumulations of instream leaf litter, using implanted leaf packs of birch, alder, and inert synthetic leaves, as well as studying the colonization by shredders and other invertebrates, and the mass loss rates of the natural leaf substrates.

Site description

The field experiment was conducted c. 500 m downstream the outlet of Lake Bjänsjön, 15 km west of Umeå (63°46'N, 20°02'E), northern Sweden. The studied section of the stream was an approximately 100 m long riffle, 4-5 m wide, with a stony substratum (ranging from sandy to boulder and bedrock sections, cobbles being the predominant material) having substantial retentive capacity (cf. Speaker et al. 1984). Dominant riparian species included the deciduous trees *Alnus incana*, *Betula* spp., *Salix* spp., *Sorbus aucuparia, Frangula alnus, Populus tremula,* and the conifers *Juniperus communis, Picea abies* and *Pinus sylvestris*. Several species of Poaceae, Ericaceae (*Vaccinium myrtillus, Vaccinium vitis-idaea*), *Myrica gale, Viola* sp., *Melampyrum pratense*, *Maianthemum bifolium*, *Potentilla palustris*, *Cornus suecica*, *Epilobium angustifolium*, *Eriophorum vaginatum*, *Rubus arcticus*, *Calluna vulgaris*, *Lysmachia thyrsifolia*, *Galium* sp., *Equisetum pratense*, *Caltha palustris* and *Drosera* sp. were found on the stream banks. In the stream there were local stands of *Carex rostrata*. Aquatic mosses were common.

Water chemistry was estimated in autumn 1990 (Malmqvist and Mäki 1994) as: pH 6.6, alkalinity 0.044 mekv L^{-1}, conductivity 2.8 S m^{-1}, total phosphorus 23 µg L^{-1} and total nitrogen 390 µg L^{-1}.

Abscission in alder began in late August. Peak leaf fall of this species took place in the period 29 Sep-12 Oct 1991, and that of birch and willow species 7-21 Oct. There were heavy rains on 1-4 and 16-19 Oct, accompanied by winds of high velocities. Those weather conditions favoured abscission so that the autumnal leaf fall was virtually completed by 25 Oct. Although litterfall started in the last week of August, natural leaf packs in the stream were not visible prior to 17 Sep. By the end of September the stream bed was almost completely covered with leaves that had accumulated in front of obstacles. Leaf packs were abundant in October, decreasing gradually from early November onwards. After ice break up in April, leaf packs were virtually absent.

Materials and methods

Ten 1 m^2 quadrates were randomly selected and marked on the streambed of the study section. Each quadrate was repeatedly sampled to estimate the accumulation of leaf litter. A handnet (mesh 0.5 mm) was used to sample the leaf litter within each quadrate beginning at the downstream side of an area working upstream.

Sampling of the quadrates began on 26 Sep 1991, and was carried out weekly until 7 Nov 1991. The leaf material was transferred to plastic bags and deep-frozen for later analyses. In the laboratory, after thawing, the content of each plastic bag was moved into a Petri dish filled with water. Leaf litter was removed, washed, dried for 48 hours at 55°C, and weighed. The remaining FPOM (fine particulate organic material) and invertebrates were washed through a net with a mesh width of 0.5 mm, preserved in ethanol (96%) and stored for later analyses. All invertebrates were identified and counted from 2 of the 7 sampling dates: 26 Sep and 7 Nov 1991.

A relationship between fresh and dry mass for both alder and birch leaves was established in a process of weighing and drying (48 h at 55 °C).

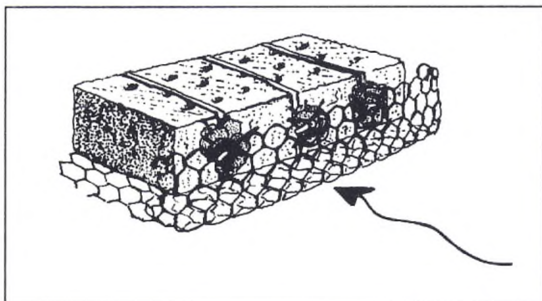

Fig. 1. One of 30 experimental stones consisting of a building brick equipped with three packs of birch, alder, and artificial (polyester) leaves, placed in its chicken net tray to prevent drifting material to interfere with the experiment.

Single species leaf packs of 2.5 g dry alder and birch leaves, collected on the stream banks, and correspondıng to fresh weights of 7.00 g (alder) and 4.72 g (birch), were fabricated and held together with plastic vivets. Artificial leaves were made of polyester cloth cut into shapes resembling leaves with a surface area corresponding to the average of those of alder and birch leaves. One leaf pack of each type was, randomly amongst themselves, fastened to each of 30 bricks using rubber bands attached to the plastic vivets. To stop drifting leaves from clogging the upstream faces of the bricks each brick was placed into a chicken net tray with the upstream side bent upwards (Fig. 1). The upstream faces of the baskets were cleaned every second day to maintain natural flow conditions over the experimental units. The bricks were placed randomly into the stream. Fifteen bricks with their leaf packs were retrieved from the stream on 19 Nov 1991. The bricks were carefully taken out of the water, the rubber bands cut, and the leaf packs transferred into plastic bags. These were brought to the laboratory and deep-frozen for later analysis. Twelve of the remaining 15 bricks were retrieved on 8 Apr 1992 using the same method as in the autumn. Three of the bricks were not found in their original positions and were therefore excluded. The material retrieved was handled in a procedure identical to that for the quadrate samples.

Results

The temperature ranged between 4 and 7 °C from the beginning of the experiment until 19 Oct, dropped to 1 °C on 21 Oct, and slowly increased again until 6 Nov (to 2.8 °C), when it gradually fell to 0.4 °C until 15 Nov (Fig. 2). During most of the experiment discharge was near 0.3 $m^3 s^{-1}$, but in the last two weeks it gradually increased to reach a peak of over 1 $m^3 s^{-1}$ on the terminating day of the first period of the field experiment.

In the quadrates, the average dry mass of the weekly accumulations of leaf material reached a maximum on 3 Oct (Fig. 3). The most abundant invertebrate groups in these accumulations on

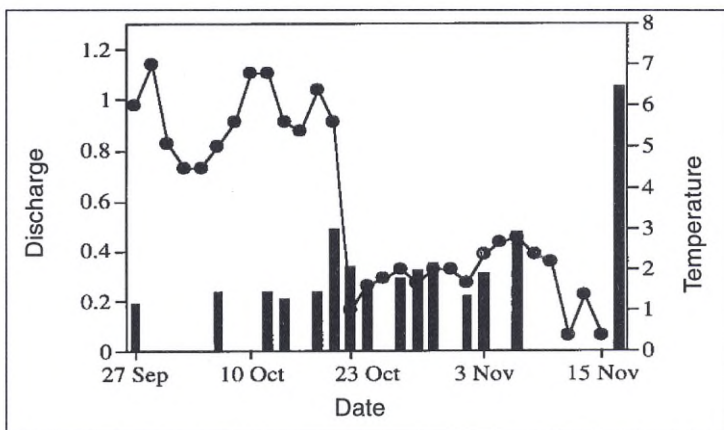

Fig. 2. Temperature (°C; line) and discharge ($m^3 s^{-1}$; bars) during the leaf pack experiment.

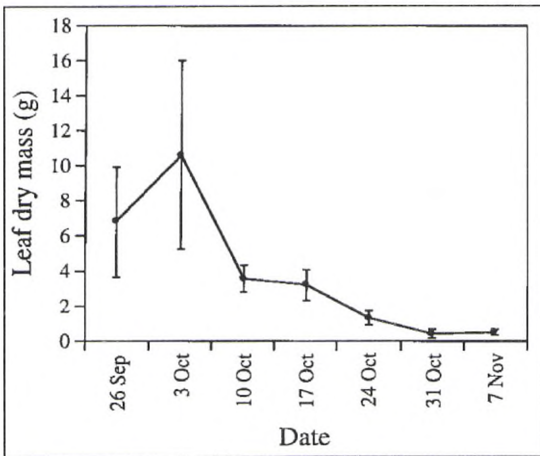

Fig. 3. Average accumulation (g m^{-2}) of leaf detritus (± 1 standard error) of the leaf litter sampled in ten quadrates in the stream on 7 dates.

26 Sep included chironomids, *Hydropsyche siltalai*, *Baetis* spp., simuliids, *Protonemura meyeri* and *Amphinemura* spp. Predatory macroinvertebrates were less abundant. *Isoperla grammatica*, *Diura nanseni*, *Hydropsyche siltalai*, *Polycentropus flavomaculatus* and *Rhyacophila nubila*, and some dipteran species are the most important predators in the stream, whereas Calopterygidae and Corduliidae oc-

Table 1. Mean macroinvertebrate numbers (ind m^{-2}) and densities (ind g^{-1} dry leaf mass) on two dates in natural leaf packs in the outlet stream of Lake Bjänsjön.

Taxon	Numbers		Densities	
	26 Sep	7 Nov	26 Sep	7 Nov
Hydracarina	1.6	0	0.74	0
Cladocera	0.1	0	0.02	0
Baetis spp.	25.4	0	12.24	0
Heptagenia spp.	0.5	0	0.09	0
Leptophlebia spp.	1.0	1.2	0.37	2.23
Taeniopteryx nebulosa	1.3	1.6	0.55	2.03
Amphinemura spp.	11.7	0.5	3.59	0.6
Nemoura spp.	1.7	0.3	0.51	0.44
Protonemura meyeri	17.4	1.4	4.24	4.01
Leuctra spp.	0.2	0	0.04	0
Diura nanseni	2.2	0.2	0.39	0.49
Isoperla grammatica	5.1	0.1	2.00	0.39
Calopteryx virgo	0	0.1	0	0.2
Somatochlora metallica	0.1	0	0.02	0
Rhyacophila nubila	3.6	0.2	1.41	1.44
Hydroptilidae indet.	3.6	0	0.2	0.31
Hydropsyche siltalai	38.1	1.5	11.18	1.58
Polycentropus flavomaculatus	4.2	1	1.07	3.63
Limnephilidae indet	1.5	1.6	0.58	3.59
Silo pallipes	0.1	0	0.02	0
Ceraclea sp.	0.1	0	0.02	0
Sericostoma personatum	0.1	0	0.02	0
Dixidae	0.1	0	0.02	0
Simuliidae	22.4	0.4	8.39	0.82
Chironomidae	100.9	9.1	28.99	15.30
Ceratopogonidae	0.6	0	0.09	0
Empididae	1.1	0	0.26	0
Total number of animals	240.5	19.2	76.9	34.5

Table 2: Mean abundances of animals in experimental alder, birch and artificial leaf packs (ind pack^{-1}) retrieved after 56 days (19 Nov 1991) and 198 days (8 Apr 1992), respectively.

Taxon	Alder		Birch		Artificial leaves	
	56 d	198 d	56 d	198 d	56 d	198 d
Taeniopteryx nebulosa	0.57	0	0.13	0	0.07	0
Amphinemura spp.	8.29	1.73	11.73	8.20	3.87	15.27
Nemoura spp.	2.14	0	1.67	0.13	0.93	0.27
Protonemura meyeri	1.79	0.36	0.73	0.60	0.07	0
Leuctra spp.	2.00	0.14	2.13	1.80	3.33	15.47
Diura nanseni	0.43	0	0.27	0.20	0.33	0.67
Rhyacophila nubila	2.29	0.14	1.87	0.73	0.53	0.93
Hydropsyche siltalai	1.79	0.43	0.73	0.80	1.47	2.00
Polycentropus flavomaculatus	1.00	0.14	0.93	0.40	0.87	1.87
Limnephilidae	0.43	0.29	0.20	0.60	0.07	0
Simuliidae	11.14	5.14	13.20	7.13	15.53	3.13
Chironomidae	106.36	12.00	77.33	32.20	60.27	60.40
Empididae	5.36	1.86	4.27	2.60	1.53	1.47
Others	7.93	0.86	5.60	1.07	9.87	1.93
Total	161.5	23.1	120.8	56.5	98.7	103.4

curred only sparsely (cf. Malmqvist 1994). In the November samples the densities of animals in the quadrates were substantially lower than in September (Table 1). Almost all groups were drastically reduced in numbers, others could no longer be found.

There were significantly (two-tailed *t*-tests) more larvae per g leaf dry mass in September of *Amphinemura* spp. (P=0.012) and *Isoperla grammatica* (P=0.035) than in November (Table 1). Similar trends were obvious also in *Baetis* spp. (P=0.003), Simuliidae, and *Hydropsyche siltalai* (P=0.005). In contrast, Limnephilidae and *Taeniopteryx nebulosa* were more abundant at the end of the sampling period.

In the field experiment, alder leaf packs lost 60.3% and birch leaf packs 56.3% of their initial dry weight in 56 days, corresponding to an average daily loss of 1.08% in alder packs and of 1.01% in birch packs. This difference between leaf species was non-significant (two-tailed, paired *t*-test: P=0.37).

There was a significant treatment effect on the total number of animals per leaf pack in autumn (ANOVA, F=4.39, P=0.019). Alder leaf packs contained significantly higher numbers of animals than artificial leaf packs (Tukey's HSD post hoc test, P=0.020), whereas the differences in abundances between alder and birch, and between birch and artificial packs were non-significant (Table 2). In spring the abundances in artificial leaf packs were significantly higher than on the packs of alder and birch leaves, that had at that point been greatly reduced.

Animal density (numbers per g dry leaf mass) showed a common trend for several taxa (and also for the total densities, Fig. 4a): 1) densities were higher on alder than on birch, and 2) densities were higher in spring than in autumn. Thus, the densities of *P. meyeri*, *Isoperla grammatica*, *R. nubila*, *H. siltalai*, limnephilids, and chironomids all showed this tendency. Significantly higher densities on alder were, however, only found for *T. nebulosa* (missing in the spring sample due to early emergence), *Amphinemura*, and chironomids (two-tailed, paired *t*-test: P-values<0.05). In contrast, densities of *Leuctra*, simuliids, and empidids were significantly higher in birch packs in the spring (P-values<0.05). Total macroinvertebrate densities were significantly higher in alder than birch leaf packs (P_{autumn}<0.001, P_{spring}<0.05).

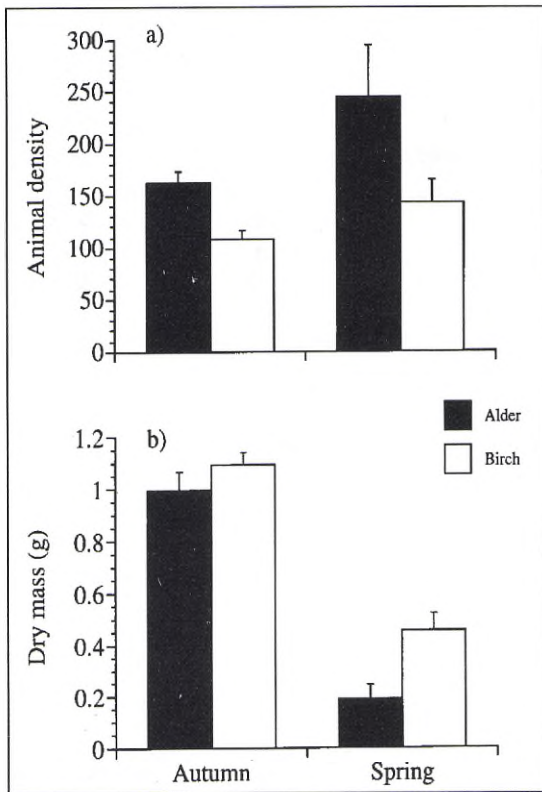

Fig. 4. Densities and remaining leaf mass in the field experiment after 58 (autumn) and 198 (spring) days of exposure. Vertical bars denote one standard error. a) Mean number of animals per g^{-1} dry weight of alder and birch leaves. b) Average dry mass of alder and birch leaves.

The average spring dry mass of alder leaf packs was significantly lower than that of birch leaf packs (two-tailed, paired *t*-test: P=0.001; Fig. 4b). During the period of 198 days alder leaf packs lost 93.3% and birch leaf packs 81.6% of their initial mass, corresponding to an average daily mass loss of 0.47% and 0.41%, respectively. Compensated for temperature, the mass loss was 0.32 and 0.30% per degree day in alder and birch, respectively (or 0.23 and 0.20% if an initial loss of 30% due to leaching is assumed and subtracted), in autumn, and 0.31 and 0.20% per degree day in spring (assuming an average temperature of 2 °C between mid November and mid April).

2

Discussion

Although there are several studies reporting litter production in forest and riparian habitats, those on the standing stock of benthic leaf litter in Nordic streams are scanty. They show considerable geographical variation. Thus, Malmqvist et al. (1978) and Otto and Svensson (1983) found maximum values close to 300 g m^{-2} in three south Swedish (56°N) streams with considerable seasonal variation. In a 30 m wide, western Norwegian (60°N) subalpine birch forest river, Baekken et al. (1981) reported average and maximum standing stocks of leaf detritus of 10.6 g m^{-2} yr^{-1} and 20.9 g m^{-2}, respectively. Corresponding data from a Danish beech forest headwater stream were 127 and 291 g m^{-2}, respectively (Iversen et al. 1982). Obviously, the observations in the present study, 10.6 g m^{-2} (average) and 57.7 g m^{-2} (maximum) on the date with highest quantity (3 Oct) suggest that the amount of leaf litter was comparatively low in the north Swedish stream. This reflects two facts: the relative sparseness of riparian trees at the boreal stream site, and the significant proportion of conifers in the surrounding forest. Another circumstance is the small size of trees at higher latitudes. E.g. *Alnus incana* is a tree species of considerably smaller size than *A. glutinosa*.

Obviously non-edible substrates, such as polyester leaves, attract invertebrates. Several reasons may account for this, including an accumulation of organic particles, growth of benthic algae, provision of a suitable attachment site for filter feeders, including blackfly and *Hydropsyche* larvae, and offering shelters for predation and flow stress. Clearly, the artificial substrate acted as an inferior substrate for shredders, such as limnephilids and *Protonemura meyeri* (cf. Richardson 1992). Relatively high numbers of some other shredder taxa were, however, unexpectedly found in the artificial leaf packs. Thus, both larvae of *Amphinemura* spp. and *Leuctra* spp. had substantial populations suggesting that these animals could successfully subsist on this substrate. A possible explanation,

Table 3: Mean density (ind g^{-1} dry leaf mass) of selected taxa in spring and autumn samples after 56 (autumn) and 198 (spring) days of exposure in experimental leaf packs. Significant effects in two-way ANOVA are indicated with asterisks (NS = non-significant) after Bonferroni post hoc tests.

Taxon	Alder		Birch		Significances		
	Autumn	Spring	Autumn	Spring	Species	Season	Interaction
Taeniopteryx nebulosa	0.57	0.00	0.11	0.00	*	*	NS
Amphinemura spp.	18.03	21.10	10.13	18.44	NS	NS	NS
Nemoura spp.	2.18	0.00	1.38	0.34	NS	***	NS
Protonemura meyeri	1.94	3.22	0.70	1.32	NS	NS	NS
Leuctra spp.	2.03	0.27	1.77	3.53	NS	NS	NS
Diura nanseni	0.42	0.00	0.21	0.69	NS	NS	NS
Isoperla grammatica	0.00	0.76	0.00	0.57	NS	*	NS
Rhyacophila nubila	2.78	3.35	1.81	2.14	NS	NS	NS
Hydropsyche siltalai	1.68	7.84	0.60	1.77	*	NS	NS
Polycentropus flavomaculatus	0.94	0.40	0.76	0.73	NS	NS	NS
Limnephilidae	0.33	6.44	0.17	2.52	NS	*	NS
Simuliidae	11.72	50.03	12.19	19.74	NS	NS	NS
Chironomidae	106.31	137.03	68.77	83.71	**	NS	NS
Empididae	6.11	21.70	3.68	4.91	**	***	*
Total animal densities	163	260	107	142	***	*	NS

at least for the latter genus, could be that small individuals are functionally collectors that switch to shredding at a larger size (Hildrew, Townsend and Henderson 1980). A general overview of ontogenetic shifts in guild classification accomplished through stage-specific diet analyses would be an important contribution to the understanding of lotic food webs.

In autumn, after 56 days of incubation, the daily rates of mass loss differed only slightly between alder and birch leaf packs, whereas after 198 days, alder leaves had lost significantly more mass than birch leaves in the packs. This result was expected, since alder belongs to the group of leaf species which are processed fast (Short et al. 1980). Nitrogen-rich leaf species, such as alder, attract micro-organisms, and animals seem to prefer the leaves colonised by them (Kaushik and Hynes 1971). Anderson and Sedell (1979) reported daily loss rates of 0.5% for 'slow' leaves, 1.5% for 'fast' leaves, respectively. Therefore, the loss rates of dry mass per day found in alder and birch leaf packs in this study suggest that they qualify as slow species. This conclusion does, however, not consider the relatively low temperatures in the study stream. In a graph relating the percentage of remaining leaf mass to the number of degree days Cummins et al. (1989) demonstrated that fast and slow species disintegrated at a rate of approximately 0.16 and 0.10% per degree day, respectively. In this light, the rates of leaf mass loss found in the present study were fast.

The results from the quadrates show that the autumnal leaf litter input to the stream is limited to a rather short period of time. Moreover, from the implantation of leaf packs it was obvious that leaves, alder in particular, rapidly disintegrated. The mass loss is, of course, not only a consequence of the consumption by invertebrates but also of leaching, physical abrasion and microbial mineralization. The higher densities found in a majority of taxa in spring, however, suggest possible food limitation. Not only were densities higher but the size of macroinvertebrates, though not measured, was considerably larger in spring, suggesting further circumstantial evidence for interaction and food

shortage. Our results thereby give support to some recent experimental studies demonstrating resource limitation in stream-living detritivores. Richardson (1991) supplemented leaf litter to streamside channels, thereby increasing the density and size at emergence of shredders, and lowering their emigration. Smock et al. (1989) increased organic matter storage by increasing the number of debris dams. This, in turn, increased macroinvertebrate abundance and the proportion of shredders.

The lower colonisation rate of leaf litter in the quadrates by several invertebrates in November compared to September may partly be attributed to a generally lower mobility, probably caused by lower temperature and by the fact that the surrounding, still abundant, leaf packs were well conditioned, whereas fresh litter dominated inside the quadrates. This is not unexpected since fresh leaves that are not yet conditioned by microorganisms, which are known to modify and improve the food value of the leaves (e.g. Kaushik and Hynes 1968, Cummins et al. 1973), should be less attractive to the invertebrates.

Some macroinvertebrate groups, however, deviated from this pattern and showed unchanged or higher densities in November than in September. The nemourids *Protonemura meyeri* and *Amphinemura* spp. (primarily *A. borealis*) are important inhabitants of the leaf packs of the studied site (Malmqvist 1993). These were rather abundant in the September samples, and their relative abundance was only slightly lower in November. *Taeniopteryx nebulosa* increased strongly from 26 Sep to 7 Nov. Limnephilid larvae also increased in density during this period. It is possible that many limnephilids and stoneflies inceased because they hatched and emerged from diapause, respectively, in the interval between the sampling occasions. Also, some limnephilids are known to not only accept but prefer fresh leaf material (Otto 1974), suggesting the possibility of chemical attraction (Allan and Malmqvist 1989) followed by colonization.

The densities of macroinvertebrates were high in the stream studied; densities (animals per g leaf mass) 5-15 times higher than in three English streams were found here (Dobson 1991). This could probably be attributed to a high retentive capacity of the stream bed, but also to the fact that lake outlet streams in general are very productive habitats (e.g. Brönmark and Malmqvist 1984, Malmqvist 1994).

The significantly higher number of animals per g dry weight in alder compared to birch leaf packs demonstrates that food quality influenced habitat selection. It is interesting that some of the species showing a significant response were not shredders, although it is conceivable that e.g. collectors and predators should also be attracted, since their food resources are probably affected by leaf species too.

Differences in the relative abundance of various leaf species must, on a larger scale, have important consequences for the benthic invertebrate communities. In northern Sweden, *Alnus incana* is a common riparian tree species (in a similar way as *A. glutinosa* is in southern Sweden), which supplies the streams with high quality food material, but probably limiting quantities. Less preferred species, such as *Betula* spp., provide less attractive food which, on the other hand, is available over a longer period of time.

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Rotenone Tolerance in the Freshwater Pearl Mussel *Margaritifera margaritifera*

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Abstract

In connection with rotenone treatments of Norwegian rivers against the salmon parasite *Gyrodactylus salaris*, knowledge of the toxic effect of rotenone on the vulnerable freshwater pearl mussel *Margaritifera margaritifera* was needed. In a field experiment the mussels survived treatments with 5 ppm rotenone solution for 12 h. In a laboratory experiment the mussels survived 30 ppm for 12 h. At 40 ppm the mussels survived the treatment, but died less than a week later. The lethal concentration of rotenone for the freshwater pearl mussel, over a 12 h exposure period in the laboratory, is thus estimated at 30-40 ppm. Compared to fish, the freshwater pearl mussel is highly resistant to rotenone. Rotenone treatments, such as those carried out in Norwegian rivers to get rid of the salmon parasite (<5 ppm rotenone solution for <8 h), would not represent a threat to a population of the freshwater pearl mussel.

Keywords: Pearl mussel, *Margaritifera margaritifera,* rotenone, tolerance.

Introduction

The freshwater pearl mussel *Margaritifera margaritifera* (L.) is distributed throughout northern Europe, Eurasia and eastern North America (Wells et al. 1983, Collins and Wells 1986). The species has a many centuries' long tradition in Europe as a source of excellent pearls. For this reason many local populations were on the point of extinction during the latter half of the 18th century, e.g. in southern Norwegian rivers (Kleiven et al. 1989). The mussel shell makes a valuable record of long-term water qualities of the watercourse (Carell et al. 1987). Due to pollution, especially of lotic habitats, but also to over-collecting, it is now considered to be a vulnerable or endangered species in most European countries, and is therefore included in the Berne Convention app. III (Council of Europe 1992, cf. Collins and Wells 1986, United Nations 1991). In Sweden the number of reproductive mussel populations has declined drastically (Grundelius 1987, Bergquist 1993). Although the freshwater pearl mussel has been eradicated over wide areas of southern Norway today, probably because of acid precipitation (Dolmen and Kleiven 1993), its status seems still satisfactory in large parts of this country. Its distribution in Norway has been dealt with by Økland (1976) (cf. Kleiven et al. 1988).

A number of Norwegian rivers have been treated with rotenone (usually around 2 ppm) to exterminate the monogenean salmon parasite *Gyrodactylus salaris* Malmberg (e.g. Johnsen and Jensen 1986, Dolmen 1987, Johnsen et al. 1989, Direktoratet for naturforvaltning 1992). The use and effect of rotenone in fishery management in North America and Scandinavia have been dealt with by e.g. Soleman (1950), Quenild (1977), Tobiasson (1979), Fox (1985), Sousa et al. (ca. 1985-90), Næss et al. 1991, see also Haley (1978) and Ugedal (1986) for literature reviews.

This paper investigates the toxic effect of rotenone on the pearl mussel, which often occurs in Norwegian salmon rivers. Earlier experiments (e.g. Marking and Bills 1976) indicate a very high tolerance to rotenone in molluscs.

Material and methods

Sites of experimentation

Mussels for field experiments were collected from the upper reaches of the River Hustadelva, Fræna. Those used in the laboratory experiment came (for practical reasons) from the River Aureelva, Sykkylven. Both are medium-sized (ca. 2-5 $m^3 s^{-1}$) and unpolluted rivers with populations of the freshwater pearl mussel, locally up to approximately 100 $m^{2\ -1}$. The field experiment was carried out in the River Haukebøelva, Molde, which much higher up in the watercourse (Arstadelva) has a small population of the pearl mussel. The mussels from this experiment were afterwards released some few hundred meters above the study area. The water for the laboratory experiment was tap water from the Lake Jonsvatnet, near Trondheim, a watercourse in which also the mussel is known to live. The surviving mussels from the laboratory experiment were released into the Creek Trollbekken near Trondheim with no known mussel population. The geographical positions and some water quality parameters of the different localities are shown in Table 1.

The field experiment

The experiment in the River Haukebøelva took place in a small side channel on 5 September 1989. The water-flow could be manipulated and was kept constant during the experiment. The water temperature was about 11 °C, which is normal for Norwegian rivers in autumn when most rotenone treatments are carried out. Mussels collected from the River Hustadelva were placed into the River Haukebøelva in two baskets for acclimatization. The mussels (total 111) were

Table 1. Water quality and other biotope characteristics of sites where freshwater pearl mussels were collected, tested or released.

Locality UTM 32V	Date	pH	Cond. K_{25} µS cm^{-1}	Tot. hard. ° dH	Ca^{2+} mg L^{-1}	Alk. µeq L^{-1}	Cl^- mg L^{-1}	Water colour mg Pt L^{-1}	Temp. °C	Biotope: current, substrate etc.
Hustadelva	1986-08-05	7.1	25	1.22	-	0.31	-	-	-	Slowly-flowing;
MQ 080788	1989-09-06	6.9	97	1.3	6.8	-	13.5	20	12.3	sandy bottom; *Potamogeton perfoliatus*, mosses
Haukebøelva	1986-08-05	6.6	31	0.26	-	0.03	-	-	-	Slowly- and
MQ 003577 (release: MQ 011586)	1989-09-05	6.4	35	0.2	1.4	-	6.5	50	11.4	swiftly-flowing; mud- and sandy bottom
Aureelva LQ 775212	1985-11-11	6.8	50	0.48	-	0.10	-	-	-	Slowly-flowing; sand and stones
Trollbekken	1990-12-04	-	60	0.85	4.3	-	6.75	40	-	Slowly-flowing,
NR 649361	1992-09-25	6.9	71	-	-	-	-	10	-	sand and gravel
Tap water (Jonsvatnet) NR 7829	1994-10-24	7.0	50	-	-	-	-	20	-	Source of exper. water

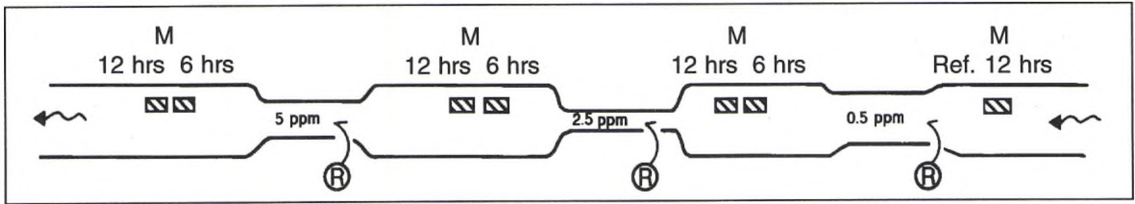

Fig. 1 The position of the baskets of mussels (M), the tanks of rotenone (R) and the different rotenone concentrations along the channel. The river current is from right to left. The distance between the upper and lower baskets is about 100 m.

grouped according to size: 83 large (12-13 cm), 24 medium-sized (9-11 cm) and 4 less than medium (6.5-7 cm). They were then put into seven baskets (cages, 30 x 50 x 25 cm), each containing 15-16 mussels of roughly equal size distribution. The mussels had no possibility to dig down into the substrate. Except for the control group, placed furthest up the river, two baskets were placed at each of three different downstream sites, at a depth of approximately 30-40 cm (Fig. 1). The water velocity varied between approximately 0.2 and 0.5 cm s^{-1} at the different sites. The rotenone solution of known concentration was emptied slowly into the water from tanks fitted with narrow plastic tubes. The river current mixed it well into the water-flow, as shown by help of rhodamine B (see later). The rotenone concentration in the channel was calculated on basis of the cross section of the channel, the water velocity at the site, as measured by help of rhodamine B, and the rate of rotenone release. The mussels were exposed to 0.5, 2.5 and 5.0 ppm of rotenone solution for 6 h (Table 2). After 6 h, one of each pair of baskets was removed from the channel and transferred to the site of the control group, while the second baskets were exposed to rotenone for a further 6 h. Rotenone treatments of Norwegian rivers last usually for 5-8 h.

Table 2. Response of *Margaritifera margaritifera* exposed to different concentrations of rotenone solution in the field experiment. No data = missing observation. Shaded area = mussels after having been transferred to fresh water.

h from start of exposure	Days after exposure	Number of open - narrow slit/closed mussels and responding mussels (in brackets)						
			Total exposure period 6 h			Total exposure period 12 h		
		Contr	0.5 ppm	2.5 ppm	5 ppm	0.5 ppm	2.5 ppm*	5 ppm
0.25		11- 5 (-)	7- 9 (-)	6-10 (-)	4-12 (-)	6-10 (-)	8- 7 (-)	4-12 (-)
4		-	8- 8 (15)	10- 6 (15)	4-12 (-)	10- 6 (15)	10- 5 (-)	7- 9 (-)
6		-	9- 7 (-)	6-10 (-)	3-13 (-)	-	-	-
8.5		-	-	-	-	6-10 (-)	6- 9 (-)	3-13 (-)
12		10- 6 (10)	14- 2 (14)	8- 8 (8)	4-12 (4)	10- 6 (10)	15- 0 (15)	12- 4 (12)
	1	13- 3 (16)	10- 6 (15)	5-11 (15)	7- 9 (15)	3-13 (16)	11- 4 (10)	4-12 (16)
	3	8- 8 (15)	8- 8 (14)	1-15 (16)	0-16 (16)	1-15 (16)	0-15 (15)	0-16 (16)
	7	6-10 (16)	0-16 (16)	0-16 (16)	3-13 (16)	3-13 (16)	8- 7 (15)	3-13 (16)
	11	6-10 (16)	5-11 (16)	8- 8 (16)	5-11 (16)	4-12 (16)	4-11 (15)	10- 6 (16)
	25	6-10 (16)	4-12 (16)	4-12 (16)	3-13 (16)	7- 9 (16)	8- 7 (15)	6-10 (16)
	55	4-12 (16)	0-16 (16)	3-13 (16)	2-14 (16)	3-13 (16)	4-11 (15)	3-13 (16)

* 15 mussels used in this group, 16 in all other groups

After the rotenone exposure, the mussels were kept in the cages and examined after 1, 3, 7, 11, 25 and 55 days. They were then released into the river and re-examined after 1 and 3 years in the river by divers.

During the experiment the mussels were observed at regular intervals, and the number of wide-open shells (the whole shell open, at mid-body >2-3 mm), supposed to indicate healthy condition, and narrow-slit/closed shells (gap at mid-body <2-3 mm), were counted. They were then prodded to control closure response. If open shells did not respond to touch by closing, they were considered to be much weakened (Burress 1982). Any differences observed in the number of open (or responding) mussels between the groups were tested in a chi-square test with two variables and without expected values.

The laboratory experiment

Eight 15 L aquaria, with stagnant tap water from the Lake Jonsvatnet were kept at 10 °C, with continuous light and air-bubbling. After an acclimatization period of 4.5 days to the experimental conditions, on 16 October 1990, 9 medium-sized to large mussels were placed into each aquarium and allowed to remain undisturbed for 1 h before the experiment started. The rotenone solution was then mixed into the water, and the mussels were exposed to 5, 10, 15, 20, 30, 40, and 50 ppm solution for 12 h. One aquarium was kept as a control. Every hour the mussels were examined for shell opening, more detailed in this experiment, since the mussels were easier to observe (wide-open: >1-3 mm, narrow-slit: 0.1-2 mm, closed: 0) and response to touch (strong: immediate response, not so strong: clearly delayed response, weak: almost no response at all) (Table 3). After 12 h of exposure the aquaria were replenished with fresh water, and shell opening and touch response were observed after 2 and 7 days. At the end of the experiment the mussels were marked and then released into the Creek Trollbekken and re-examined after 2 and 3 years.

The rotenone solution used for these experiments was "Gullvik's rotenone" manufactured in Sweden, and which is almost identical to the American Pro-Noxfish, a rotenone solution containing 2.5% rotenone and 2.5% of a synergist (sulfoxide); the overall effect is that of a 5% solution of rotenone. Gullvik's rotenone has been the most widely used rotenone product in fishery management in Scandinavia during the past few years, and is also the one used so far to exterminate *G. salaris* in infested Norwegian rivers.

The water temperature and light intensity used in these experiments were not much different from those found in Norwegian rivers by daytime in autumn, when most rotenone treatments take place. The water quality also lay within the range preferred by the freshwater pearl mussel (Table 1, cf. Grundelius 1987).

Results

The field experiment

During the rotenone treatment of the channel, sticklebacks *Gasterosteus aculeatus* were first affected, then after 20 min trouts *Salmo trutta* were also seen dying; and at last, after approximately 2 h, two eels *Anguilla anguilla* came creeping up from the water and going on land.

Before the rotenone treatment started, approximately the same number of open and narrow slit/closed shells was recorded in all the baskets. After only 15 min (Table 2), about one third of the mussels in the 5 ppm groups were narrow-slit/closed ($P<0.01$, pooled data) compared to the control group. After 4 h and 6 h a higher number of mussels had more or less closed shells in the 5 ppm groups than in the 2.5 ppm group ($P<0.02$, two baskets) and the 0.5 ppm group ($P<0.05$, one basket).

After 12 h, a greater number in the 5 ppm group of mussels which had been transferred to fresh water 6 h earlier, were narrow-slit/closed than in the control group ($P<0.05$). Besides, a greater number of mussels were narrow-slit/closed, both in the 2.5 ppm group and the 5 ppm group, than in the 0.5 ppm group ($P<0.05$ and $P<0.001$, respectively).

All mussels treated with rotenone, both for 6 h and 12 h, did not keep, or only occasionally

kept, their foot outside the shell during exposure, while most of the mussels in the control group did (all groups, except for 6 h at 0.5 ppm, $P<0.01$). In addition, the response to touch by mussels influenced by rotenone was much slower, especially for the groups exposed to 5 ppm. After 12 h no mussels in the experiment could be diagnosed as dead, however.

During 1-2 days in clean, running water, most of the rotenone-exposed mussels had narrow-slit/closed shells, but almost all still responded to touch (Table 2). Also during the next 2.5 months, with the mussels still in the cages, more narrow-slit/closed mussels than open mussels were usually observed, but this was also the case for the control group (Table 2). Practically all mussels responded to touch.

When the released mussels after 1 and 3 years were examined in the river by divers, 75 and 91, respectively, of the total of 110 mussels, were located. All appeared to be healthy, and no dead ones were discovered.

The laboratory experiment

During 12 h of exposure there were no significant differences in shell gap and response to touch between the 5, 10 and 15 ppm groups and the control group (Table 3), although there was

Table 3. Response of *Margaritifera margaritifera* exposed to rotenone in a laboratory experiment. For each concentration $N = 9$. After 12 h of rotenone treatment the mussels were transferred to clean water. * = dead. Shaded area = control group or mussels after having been transferred to fresh water.

Rotenone concentration	0 ppm control group						5 ppm group 5 ppm				0 ppm	
Time (h)	3	6	9	12	58	168	3	6	9	12	58	168
Shell gap												
Wide-open	9	9	9	8	8	9	9	9	9	9	9	7
Narrow slit				1	1							2
Closed/alm. closed												
Response to touch												
Strong	9	9	9	9	9	9	7	9	8	8		8
Not so strong							2		1	1	9	1
Weak												
No response												

Rotenone concentration	10 ppm group 10 ppm				0 ppm		15 ppm group 15 ppm				0 ppm	
Time (h)	3	6	9	12	58	168	3	6	9	12	58	168
Shell gap												
Wide-open	8	7	7	7	6	9	5	5	7	7	8	9
Narrow slit	1	1	1		3		3	4	2	2	1	
Closed/alm. closed		1	1	2			1					
Response to touch												
Strong	6				9	9	7					
Not so strong	2	7	8	8			1	7	9	9	8	9
Weak	1	1					1	2			1	
No response		1	1	1								

Rotenone concentration	20 ppm group 20 ppm				0 ppm		30 ppm group 30 ppm				0 ppm	
Time (h)	3	6	9	12	58	168	3	6	9	12	58	168
Shell gap												
Wide-open	6	4	6	4	8	9	3		1	1	9	6
Narrow slit	3	2	1	2			3	3	4	1		3
Closed/alm. closed		3	2	3	1		3	6	4	7		
Response to touch												
Strong	4	1				9						5
Not so strong	3	4			8		2					2
Weak	1	2	6	5			4	2	4	2	9	2
No response	1	2	3	4	1		3	7	5	7		

Rotenone concentration	40 ppm group 40 ppm				0 ppm		50 ppm group 50 ppm				0 ppm	
Time (h)	3	6	9	12	58	168	3	6	9	12	58	168
Shell gap												
Wide-open	2		1		9	9						
Narrow slit	6	6	8	4								
Closed/alm. closed	1	3		5			9	9	9	9	9	9
Response to touch												
Strong	1											
Not so strong	4											
Weak	1	2	3	4	6							
No response	3	7	6	5	3*	9*	9	9	9	9	9*	9*

a trend towards an increase in the numbers of less open and slowly-responding mussels with increasing concentration of rotenone. At the concentrations 30, 40 and 50 ppm, at 12 h, however, significantly more mussels were closed than in the control group ($P<0.001$, $P<0.01$, $P<0.001$, respectively). The response to touch decreased in a similar pattern: for both strong and weak responses taken together compared to no response at all; $P<0.05$, $P<0.001$, $P<0.01$ and $P<0.001$ for 20, 30, 40 and 50 ppm, respectively.

After the 12 h exposure period, the mussels exposed to 40 and 50 ppm were dead within one week. The exact time at which the first mussels in the 40 ppm group died is uncertain, but some mussels responded to touch during the whole course of the exposure period, and even two days thereafter. In the 50 ppm group all the mussels remained closed and never responded to touch after 2 h; they probably died at an early stage of the rotenone exposure.

The mussels exposed to ≤30 ppm all recovered completely within one week in clean water.

After 2 and 3 years, 53 of the 54 mussels which survived the 12 h exposure and were placed in the Creek Trollbekken, were located, still alive and appeared healthy (one mussel was not found).

Discussion

The rationale for the present experiment was the recent attempts to get rid of the salmon parasite *G. salaris*. Rotenone treatment of rivers, in order to kill the parasite with the host, could probably also have a devastating effect on the vulnerable freshwater pearl mussel. Mo (1986) showed that at a temperature of 11 °C, salmon (*Salmo salar*) parr died after ca. 15 min following treatment with 1 ppm rotenone under laboratory conditions. The last of the parasites only died after 45-60 min. Without a host, however, *G. salaris* will die eventually within a few days, anyway (Mo 1986). So far, rotenone concentrations of 1.5-2 ppm have been used in Norwegian rivers, and the procedure may in practice last for up to 8 h, and normally 2-5 h. Locally a rotenone concentration of around 5 ppm should also be expected in the river water.

The toxicity and dissipation time of rotenone depends on factors such as temperature, light intensity, alkalinity, oxygen and organic contents of the water (Post 1958, Örn 1962, Schnick 1974, Tobiasson 1979, Dawson et al. 1991). Since rotenone was continuously added to the water in the field experiment, dissipation would be no problem. In the laboratory experiment the rotenone concentration was set at the start of the experiment, and no replenishment of rotenone was made during the 12 h long experiment. With the experimental conditions used, however, i.e. a temperature of 10 °C, a low light intensity and a low organic content of the water, rotenone dissipation will have progressed only slowly (cf. Næss et al. 1991).

The results of both the field and laboratory experiments, show that, compared to fish the freshwater pearl mussel is highly resistant to rotenone. At 11 °C, salmonids are usually killed after only 0.5-1 h at rotenone concentrations of less than 0.5 ppm (even 0.2 ppm) (e.g. Mo 1986). Double concentration (1 ppm) is usually used with cyprinid fish (Burdick et al. 1955, Snekvik 1967, Meadows 1973, Marking and Bills 1976, Søilen 1984, Mo 1986). The lethal concentration (LC) of rotenone for adult freshwater pearl mussels exposed experimentally over a period of 12 h at 10 °C is here shown to lie between 30 and 40 ppm.

Chandler and Marking (1982) also showed that all species of freshwater invertebrates that they investigated (in laboratory), except for the water-fleas, were more tolerant to rotenone than were salmonids. Most tolerant were the molluscs (3 gastropod and 3 bivalve species were used), which tolerated from 100 to 850 times the lethal dose for salmon (cf. Marking and Bills 1976), for 96 h and 24 h, respectively. The bivalve *Corbicula manilensis* died only at a concentration of 7.5 ppm during an experimental period of 96 h, while the gastropod *Helisoma* sp. died at a rotenone concentration of 30 ppm for a 24 h period. Both these species show a very high tolerance, fully comparable to what we found for

the freshwater pearl mussel, with a 12 h LC in the range 30-40 ppm. High resistance of molluscs to rotenone has also been reported from other studies (Chandler and Marking 1982, Holcombe et al. 1987, cf. Sousa et al. ca. 1985-90).

In their natural habitat, both fish and aquatic invertebrates are less affected by rotenone than in laboratory experiments (Marking and Bills 1976, cf. Engstrom-Heg et al. 1978, Chandler and Marking 1982). This may especially be the case for mobile benthic invertebrates, since they are more or less being able to escape the toxicant by digging or hiding in the substrate. The bottom substrate, in itself, also has an inactivating effect on rotenone, at least in lakes (Lindgren 1960, Örn 1962, Andreasson 1963). We therefore presume that the freshwater pearl mussel, in nature dug down in the river bed, tolerates higher concentrations of rotenone than was found by us.

At the lowest rotenone concentration used in the laboratory experiment, 5 ppm, which exceeds the highest concentrations used in Norwegian rivers, the mussels seemed hardly to notice the presence of rotenone at all. However, the number of mussels which remained wide-open, and also those that protruded their foot out of their shells, gradually decreased with increasing rotenone concentration and time. A similar trend was seen in the field experiment at lower concentrations of rotenone.

Lennart Henrikson (pers. comm.) has seen a similar behaviour (keeping their foot inside their shells) in specimens of the freshwater pearl mussel when they were placed into strongly acidic water. Practically all mussels, however, at least among those we have observed in Norwegian rivers, are dug down into the bottom substrate by about two third of their length. This behaviour prevents them from drifting downstream, e.g. during a rotenone treatment.

Adult mussels are thus not seriously affected by rotenone treatments of the kind carried out in Norwegian rivers. However, we have not tested mussels smaller than 5-6 cm, although we presume they are safe at the concentrations used. Mussels in the youngest stage, the parasitic glochidia larva, will die with its host (salmon or trout). Since the river will have a fairly dense population of fish already the year after the rotenone treatment, only one year-class of mussels will be lost, however.

Within the body, rotenone works primarily by inhibiting the electron transport system of the mitochondria (Lindahl and Öberg 1961, Horgan et al. 1968), leading to a slow-down in oxygen transport through the gills and to reduced cell respiration. Lethal rotenone poisoning probably occurs at the time when the uptake exceeds the animal's capacity to break it down metabolically (Gingerich and Rach 1985, cf. Fukami et al. 1969).

Some mussels which did not respond to touch and appeared to be dead even at a relatively early stage of the experiment, recovered after transfer into clean water (Tables 2 and 3). Recovery from high sub-lethal doses of rotenone, and apparent lethargy, is also known from the literature, e.g. by zooplankton (Almquist 1959), by fish (Gilderhus 1972), and by oysters *Ostrea edulis* (Samuelsen et al. 1988). Oysters tolerated about 1 ppm of rotenone solution for 7-8 days. At that time the rotenone concentration within the animals had risen to 7-8 ppm and many of them died. Others survived, however, and as the rotenone concentration outside the animals gradually began to decrease, so did the concentration within the live mussels, through excretion, and they eventually recovered fully (Samuelsen et al. 1988).

The time needed for the freshwater pearl mussel to recover from high sub-lethal concentrations of rotenone (up to 30 ppm rotenone solution) was less than a week, which is comparable to the four days needed by the blue mussel *Mytilus edulis* fully to recover from sub-lethal concentrations of formaldehyde in the experiments of Nordtug et al. (1991).

Although much weakened, no mussels exposed to 40 ppm were classified as dead at the time when the experiment was finished. After being transferred to clean water, however, final mortality occurred after 2 to 6.5 days. A similar

delay, from exposure of fish to a deadly dose of rotenone to final death, has likewise been recorded by Gilderhus (1972).

Many oxygen-demanding invertebrates have been shown to be especially sensitive to rotenone (Morrison 1977, Engstrom-Heg et al. 1978, Arnekleiv 1992). Gill-breathing animals are also highly vulnerable to rotenone poisoning, since the substance is very effectively taken up through the gills (Öberg 1965). Therefore, it seems strange that a gill-breathing animal that lives exclusively in very clean, running and well-oxygenated water, should have such a high tolerance to rotenone.

A reasonable explanation for the high degree of tolerance to rotenone by the freshwater pearl mussel may well lie in a possible capacity for anaerobic respiration. Such facultative respiration is found in the marine blue mussel (Roberts 1976, George et al. 1977, Nordtug et al. 1991), probably an adaptation to surviving in the littoral zone when the shells close during low tide.

The freshwater pearl mussel may in fact very well be adapted to anaerobic conditions, since some of the creeks in which they live dry out almost completely at times during extremely warm summers with low precipitation. In order to test this hypothesis, however, further investigations are needed.

Conclusion

The conclusion is that rotenone treatments such as those carried out in order to get rid of the salmon parasite *G. salaris,* would not seem to represent a threat to adult freshwater pearl mussels. Such treatments usually last for no longer than 5-8 h and with rotenone concentrations of no more than 5 ppm (locally). The tolerance of adult mussels to rotenone appears to be very high. At 10 °C they survived and recovered concentrations of up to 30 ppm (but not 40 ppm) for 12 h under laboratory conditions, and can probably withstand even higher concentrations in nature.

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Foraging under risk of Predation in Wild and Hatchery-reared Juvenile Sea Trout (*Salmo trutta* L.)

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Abstract

In this study, the hypothesis that hatchery-reared sea trout (*Salmo trutta*), progeny from wild-caught parents, are more willing to forage under risk of predation than wild trout was tested. In nine replicate experiments, six hatchery and six wild sea trout juveniles were allowed to choose between foraging in a safe area or an area containing a predatory rainbow trout. The fish were observed during four 25 min periods daily for three consecutive days and food was provided during the first and third period each day. The assessment of predation risk exhibited by hatchery and wild trout was strongly correlated and, overall, hatchery trout did not spend more time in the risky area than did the wild trout. However, wild fish significantly reduced their presence on the risky side during the six non-feeding periods, compared to the six periods when food was provided. Hatchery trout, on the other hand, were equally frequent on the risky side during feeding and non-feeding periods. This suggests that wild trout balance the risk of predation more against the benefits of feeding compared to hatchery-reared trout.

Keywords: *Salmo trutta*, foraging, predation risk, trade-off, behaviour.

Introduction

In Sweden, the sea trout, *Salmo trutta*, spends 1-4 years in small streams before smoltification and subsequent migration to the sea occurs during spring. Sea trout is a popular species for recreational fishing and hatchery production of sea trout smolts is common in Sweden. However, Dellefors (unpubl. data) found that hatchery-reared sea trout had a higher pre-smolt mortality and a lower tendency to migrate than wild trout. Further, Berg and Jörgensen (1991) demonstrated that hatchery-reared sea trout parr, stocked in a Danish stream, had higher mortality rates than wild parr. This increased mortality may be caused by maladaptive anti-predator behaviour in hatchery-reared fish, and may also explain why returns of hatchery-reared salmon smolts are lower than for wild smolts (Österdahl 1964, Toivonen 1977).

Recent studies in behavioural ecology have demonstrated the importance of predation risk as a factor altering foraging behaviour in animals (see Lima and Dill 1990). In an elegant experiment, Gilliam and Fraser (1987) confirmed their theory that juvenile creek chubs, *Semotilus atromaculatus*, select habitats that minimize the ratio of mortality from predation to gross foraging rate. Thus, appropriate trade-offs between mortality risks and energetic gain will be crucial for the fitness of juvenile fish.

The trade-off hypothesis may also have implications for aquaculture and fishery research. Johnsson and Abrahams (1991) demonstrated experimentally that interbreeding with a domestic strain increases foraging under risk of predation in wild steelhead trout (*Oncorhynchus mykiss*), and after further studies Johnsson (1993) suggested that size-selection in hatcheries will favour risk-prone foragers. However, wild and hatchery-reared salmonids could also

differ non-genetically in their foraging behaviour, provided that the hatchery environment does not permit development of behavioural patterns that are important in the wild. More specifically, the absence of predators in the hatchery may increase the tendency to forage under risk of predation, since hatchery fish do not get the opportunity to learn to avoid predators while foraging. For example, Olla and Davis (1989) showed that coho salmon, *Oncorhynchus kisutch*, avoid predators better after conditioning to live predators (Lingcod, *Ophiodon elongatus*) and predation-associated stimuli. Further, the absence of selection by predators in the hatchery environment may keep risk- prone fish, that would not survive in the wild, alive until their release. In this study we test the prediction that one-year old hatchery reared sea trout are more willing to forage under risk of predation than are wild trout of the same age. We also investigate whether food availability affects risky foraging behaviour in hatchery and wild juveniles.

Methods

On 13 August 1990, 100 wild one-year old (1+) sea trout juveniles were caught in the River Norumsån (50 km north of Göteborg, southwest Sweden) by electro-fishing. The fish were transported to the Munkedal Hatchery (120 km north of Göteborg). On 14 August, 100 (1+) hatchery reared sea trout were transported 40 km from the Trollhättan Hatchery to the Munkedal Hatchery. The fish were the progeny of 10 males and 10 females (each female were fertilized by one male) caught in the River Norumsån. In Trollhättan, the trout had been reared under simulated natural photoperiod conditions and at ambient water temperature. During acclimatisation at the Munkedal Hatchery, wild and hatchery juveniles were first fed dry pellets. The commercial food was gradually changed to the experimental food, freeze-dried shrimps (*Gammarus* spp.), which was provided ad lib. 2-3 times daily. Both wild and hatchery fish fed well within ten days.

On 16 September, after 33 days of acclimatisation, the experiment started. The willingness to forage under threat of predation was investigated using a method modified after Johnsson and Abrahams (1991). At 16:00-17:00 hours the day before the start of the experiment, six wild and six hatchery juveniles were randomly selected from the holding tanks. After anaesthetizing with 2-phenoxyethanol (0.4 ml/l), the fish were measured (body weight and fork length) and then marked with two colored wooden beads (4 mm diameter) in one of 12 different colour combinations. The beads were attached to the dorsal musculature using a 0.25 mm nylon thread. The fish recovered within two minutes after handling. After recovery, the 12 juveniles were transferred to the 2,000-litre experimental tank (see Fig. 1). The tank was divided in two parts by a net screen (mesh size 30 mm), through which the juveniles could swim freely, while the predator, a 1,000 g rainbow trout (*Oncorhynchus mykiss*), was constrained to one side of the tank.

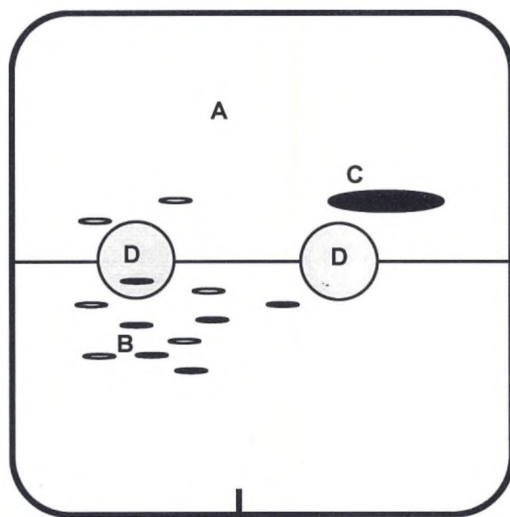

Fig. 1. View from above of the experimental tank (2x2 m) used in the foraging-risk experiment. A= dividing net screen through which the juvenile sea trout (B) could swim freely, while the predatory rainbow trout (C) was constrained to the risky side. D= floating rings inside which the experimental food was provided.

At 09:00 hours the day after transfer, we carefully presented equal amounts of food on both sides of the tank using a mug at the end of a long rod. The total amount of food provided each day was 1.5% dry weight of the total body weight of the 12 tested juveniles. A mirror over the tank allowed us to record the positions of each individual at 30 s interval for 25 min, using binoculars, without disturbing the fish. The procedure was repeated four times per day at 2 h intervals for three days. However, food was only provided during the first and third period each day (see Fig. 2). Generally, the food was depleted on both sides of the tank before the start of the next observation period. Immediately after the termination of the experiment each juvenile was weighed to estimate growth (body weight change/initial body weight). Nine replicate experiments were conducted between 17 September and 3 October using two identical experimental tanks, each with one predator. Four similar sized rainbow trout (about 1,000 g) were alternatively used as the predator in the nine replicates. During the experiments water temperature decreased from 13.4 °C to 10.0 °C.

Since variables were not normally distributed, non-parametric statistics (Wilkinson 1989) were used for analysing differences between two (Wilcoxon signed-ranks test) or more (Friedman two-way analysis of variance) related samples, and between independent samples (Mann-Whitney U-test). Correlations were analysed using the Spearman rank-order correlation coefficient.

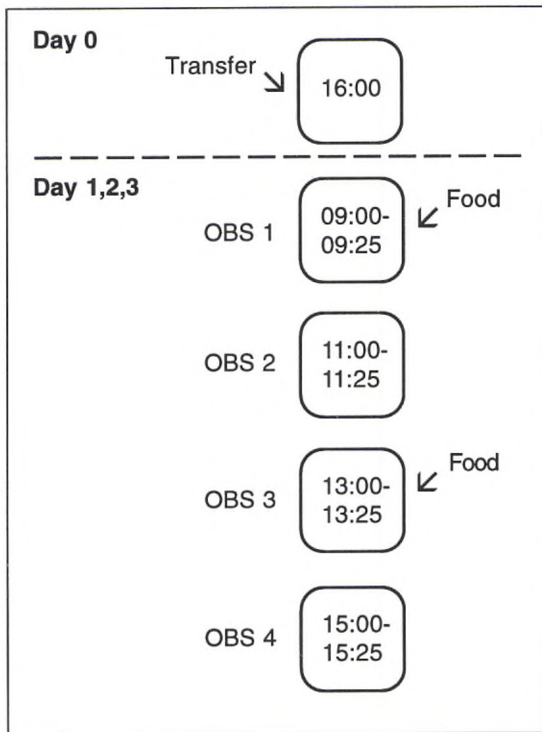

Fig. 2. Scheme describing the general procedure of the foraging-risk experiment. The fish were observed (OBS) during four time periods on three consecutive days. Food was provided at the start of denoted time periods.

Results

Initial mean weights and fork lengths were similar in hatchery and wild trout both within and for pooled replicates (see Table I). However, condition factor was significantly higher in hatchery than in wild fish for pooled replicates. The mean weight of the hatchery trout increased slightly (0.7±0.8% [X±SE]) while the weight of the wild trout decreased (-1.6±1.0%; Wilcoxon signed-ranks test: z=-2.31, N=9, P=0.021), suggesting a higher food intake by the hatchery fish. To get a measure of activity, we calculated the total number of passages between the risky and safe side of the tank for each individual. The mean number of passages were not significantly different between hatchery and wild trout (9.3±2.3 and 8.9±1.9, respectively; Wilcoxon signed-ranks test: z=0.53, N=9, P=0.59).

Foraging under risk of predation

During the experiments one wild juvenile was killed by the rainbow trout predator. The percents of the total time spent by hatchery and wild trout in the risky area were strongly correlated (r_s= 0.82, N=9, P<0.02; see Fig. 3), indicating that wild and hatchery fish experienced the level of risk similarly. Overall, there was no differ-

Table I. Means (±SE) of initial weight, initial fork length and condition factor in nine matched replicates, each consisting of six wild (W) and six hatchery reared (H) juvenile sea trout, respectively.

Repl.	Mean weight (g)		Fork length (cm)		Condition factor	
	W	H	W	H	W	H
1	9.3 (1.7)	11.2 (1.9)	9.8 (0.6)	10.3 (0.5)	0.95 (0.04)	0.99 (0.01)
2	8.1 (0.9)	6.8 (1.0)	9.3 (0.3)	8.7 (0.3)	0.99 (0.04)	1.00 (0.05)
3	10.6 (1.1)	15.6 (2.4)	10.0 (0.4)	11.3 (0.7)	1.04 (0.02)	1.04 (0.03)
4	8.9 (0.9)	9.7 (0.3)	9.8 (0.4)	9.5 (0.7)	0.95 (0.03)	1.03 (0.02)
5	8.6 (0.6)	8.9 (0.6)	9.8 (0.2)	9.7 (0.2)	0.91 (0.04)	0.98 (0.01)
6	8.3 (1.0)	8.6 (1.1)	9.7 (0.3)	9.6 (0.4)	0.89 (0.03)	0.95 (0.02)
7	11.2 (1.3)	12.6 (3.6)	10.4 (0.4)	10.2 (0.9)	0.96 (0.02) *	1.05 (0.02)
8	12.1 (1.4) *	22.2 (3.0)	10.8 (0.5) *	13.0 (0.5)	0.94 (0.03)	0.97 (0.02)
9	10.4 (1.2)	14.3 (2.6)	10.3 (0.4)	11.2 (0.7)	0.93 (0.03)	0.99 (0.02)
Mean	9.7 (0.4)	12.2 (0.9)	10.0 (0.1)	10.4 (0.2)	0.95 (0.01) **	1.00 (0.01)

* $=P<0.05$ and ** $=P<0.01$; Mann-Whitney U-test.

ence between hatchery and wild trout in their willingness to forage under threat of predation (15.8±6.5% and 13.7±4.8% of the total time being spent in the risky area, respectively; Wilcoxon signed-ranks test: $z=0.53$, $N=9$, $P=0.59$). However, wild fish reduced their presence on the risky side during the six (two periods per day for three days) non-feeding periods (see Fig. 2) compared to the six periods when

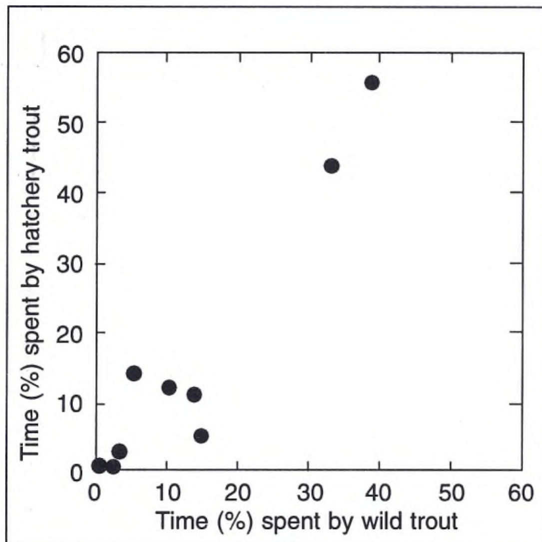

Fig. 3. Percent of total time spent in the risky area by juvenile hatchery (n=6) and wild sea trout (n=6) in nine replicate foraging-risk experiments. Correlation coefficient (r_s) =0.82.

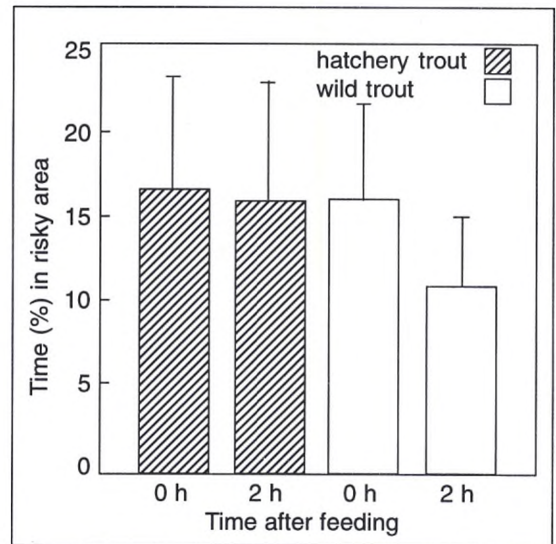

Fig. 4. Percent of total time spent in the risky area by juvenile hatchery and wild sea trout during different food conditions. 0 h denote frequencies during time periods when food was provided. 2 h denote frequencies during periods starting 2 hours after food provision. Error bars denote SE of mean frequencies (N=9). See also Fig. 2.

food was provided (Fig. 4; Wilcoxon signed-ranks test: z=-2.07, N=9, P=0.038), while hatchery trout were equally frequent on the risky side during feeding and non-feeding periods (Wilcoxon signed-ranks test: z=-0.89, N=9, P=0.37).

Since hatchery and wild trout responded differently to feeding and non-feeding conditions, we analysed the effect of observation period (see Fig. 2) on risky foraging separately for feeding (6 periods [two each day for three days] analysed) and non-feeding conditions (6 periods as above). Observation period had no effect on risky foraging, neither between nor within groups (Friedman two-way analysis of variance: N=9, P>0.05 in all cases). Thus, no effect of learning and/or acclimatisation on risky foraging could be demonstrated.

Size and risky foraging

To investigate if size or growth influenced the tendency to forage under threat of predation we estimated, separately for hatchery and wild trout, whether initial weight, fork length and weight change were correlated to the frequency of foraging in the risky area. Correlations within and between replicates were highly variable, and showed no consistent pattern (Spearman rank-order correlation, N=6, P>0.05 for all cases). Thus, no effect of size or growth on the frequency of risky foraging could be demonstrated.

Discussion

The strongly correlated assessment of predation risk exhibited by hatchery and wild trout (Fig. 3) suggests that an inherited adaptive response to the predator is of great importance. This is in agreement with the point made by Slater (1983) that since the first encounter with a predator may be a lethal one, it is also essential that the young animal behaves appropriately without experience of that situation. However, foraging behaviour in animals (Hughes et al. 1992), and predator avoidance in fish (e.g. Olla and Davis 1989, Magurran 1990) can be enhanced by learning.

Even though the overall frequency in the risky area was not different, wild trout reduced their risky foraging during observation periods when food was not presented, while hatchery trout were equally frequent in the risky area regardless of food supply. This suggests that hatchery fish were less sensitive to the variable feeding conditions in the experiment. It appears that wild trout balanced the risk of predation against the benefits of feeding and avoided the predator when benefits were low, while the foraging pattern of the hatchery trout was more indifferent to the feeding conditions (see also Abrahams and Dill 1989). The additional fact that the weight of the hatchery trout increased during the experiment, relative to the weight of the wild trout, suggests an overall higher foraging activity in hatchery fish. This discrepancy could reflect a lower feeding intensity in wild trout caused by the presence of the predator (Dill and Fraser 1984, Lima and Dill 1990).

An alternative explanation is that the hatchery trout were socially dominant over wild fish (Fenderson et al. 1968, Swain and Riddell 1990) and therefore were more successful in their feeding. Social interactions may also have affected the positions of the fish in our experiment, since juvenile sea trout are territorial and compete for profitable positions as well as for food (Jenkins 1969, Fausch 1984). Dominance status and the tendency to forage under risk of predation can also be affected by size differences (Johnsson 1993). However, there were no substantial size differences between groups or correlations between size and risk proneness in the present study.

It is possible that further differences in behaviour between hatchery-reared and wild fish were obscured by schooling tendencies, since the two groups of fish were matched in our experiment. However, methods similar to those in this study have previously demonstrated significant differences in risk-sensitive foraging behaviour between different strains (Johnsson and Abrahams 1991) as well as different size-classes (Johnsson 1993) of rainbow trout.

There are other factors than risky foraging behaviour that may explain the increased mortality rates found in hatchery-reared salmonids (see Berg and Jörgensen1991) released in the wild. Firstly, the absence of selection in the hatchery environment will keep unfit individuals, that would not survive in the wild, alive until their release, causing an artefact when comparing survival rates between hatchery fish and wild smolts which have already been subjected to selection. Secondly, the susceptibility of hatchery fish to predators may increase due to physiological, morphological and behavioural factors. Dellefors (unpubl. data) showed that hatchery-reared sea trout had lower salinity tolerance than wild trout during smoltification, which may increase the susceptibility of hatchery fish to predation. Indeed, Järvi (1989) demonstrated that the risk of predation for Atlantic salmon (*Salmo salar*) smolts pre-adapted to sea water were less than for non-adapted smolts. Further, hatchery salmonids generally have smaller fins than wild fish, mainly because of fin erosion (Mork et al. 1989), which may reduce swimming performance (Webb 1977), and thereby the ability to escape from predators. Finally, unnaturally high levels of aggressiveness in hatchery salmonids could increase their mortality in the wild through loss of feeding time, excessive expenditure of energy, and increased exposure to predators (Fenderson et al. 1968).

In conclusion, the results of the present study suggest that (1) foraging wild and hatchery-reared sea trout assess the risk of predation similarly, but (2) wild trout appear to balance the risk of predation more against the benefits of feeding compared to hatchery-reared trout. Other behavioural components, important for fitness in the wild, may be altered by the hatchery environment. These provide a scope for future behavioural studies.

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Downstream Migration of Atlantic Salmon Smolts in Relation to Water Flow, Water Temperature, Moon Phase and Social Interaction

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Abstract

Atlantic salmon (*Salmo salar)* smolts were caught during their descent in the River Orkla in the period 1980-92. The smolts were mainly caught during May, in traps operated from a bridge. Salmon smolts started their descent in the River Orkla at water temperatures between 1.7 and 4.4 °C. The run usually finished when the temperature reached 10 °C. Smolts aggregated, and most of them migrated in peaks at night. The ultimate decision for smolts to initiate migration seems to be related to the combined effect of several physical stimuli. Daily catches were modelled using generalized linear interactive modeling (GLIM). The smolt run was significantly related to water flow, water temperature, negative change in water temperature, change in water flow and moon phase. The model gave an inadequate fit during peak smolt migration. There was evidence that smolts from upstream areas stimulated migration of smolts situated further down-stream, thereby creating shoals of descending smolts. Interaction during descent may be the reason for the inadequate fit of the model in days of peaking smolt migration.

Keywords: Atlantic salmon, smolt migration, smolt behaviour, physical triggers.

Introduction

Atlantic salmon (*Salmo salar* L.) leave their nursery areas in rivers at ages varying from one to six years (Metcalfe and Thorpe 1990), and migrate to feeding areas in fjords and the sea. The major smolt run is concentrated to about one month in spring (Ruggles 1980). Smolt migration follows smoltification initiated by an increasing photoperiod in the spring (Wagner 1974). Proximate abiotic factors triggering the smolt run may vary among stocks (Heggberget et al. 1993), but water temperature and water flow have been suggested as major influences (Forsythe 1967, 1968, Österdahl 1969, Bagliniere 1976b). Smolts are expected to reach the sea at a time of optimal survival and feeding.

Physical conditions vary in fresh water and in the sea. Therefore each salmon stock has to be investigated separately to identify factors regulating smolt migration (Ruggles 1980). A few quantitative investigations illustrate how these variables regulate the daily descent of Atlantic salmon smolts (Wagner 1974, Forsythe 1967, 1968, Jonsson and Ruud-Hansen 1985). On the basis of material collected in the River Orkla in 1980-81, Hesthagen and Garnås (1986) reported that a significantly greater number of salmon smolts were caught at increasing than at decreasing water flow. In the present study, eleven years of Atlantic salmon smolt descent are analysed in the same river in relation to water flow, water temperature and moon phase. A model predicting daily descent of smolts from

these factors is developed. The hypothesis that migrating smolts from upstream areas initiate run of smolts situated further downstream was analysed by testing dependence between date of recapture of a marked smolt and the distance it has travelled.

Materials and methods

The River Orkla is situated in central Norway at 63°N, 10°E. Ninety-three km of the river is accessible for anadromous fishes. The mean annual discharge is 41 m^3s^{-1}. The peak spring flow in the period 1980-92 was less than 300 m^3s^{-1}, with the exception of 1981, when it exceeded 500 m^3s^{-1}. Water flow was measured at Meldal (Fig. 1) and water temperature at Øyum (Fig. 1) by the Norwegian Water Resources and Energy Administration.

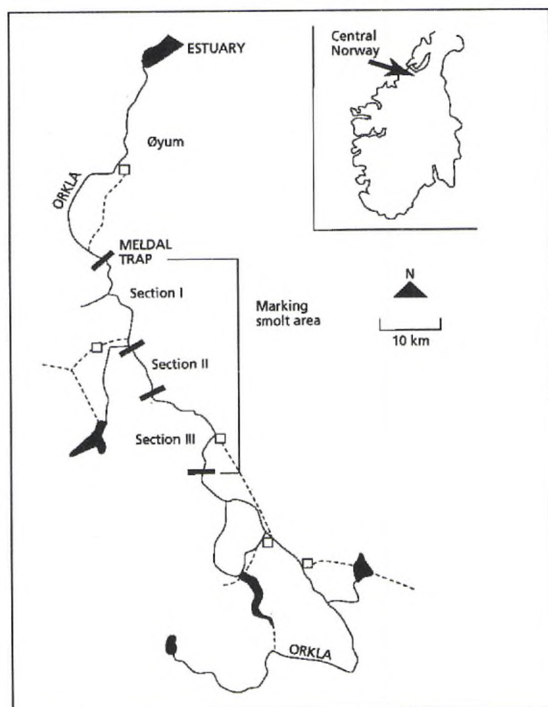

Fig. 1. Map of River Orkla with study area. Atlantic salmon >10.9 cm were prior to smolt descent caught by electrofishing, fin-clipped and released again in section I, II and III. At Meldal a trap which caught downstream migrating smolts was operated.

Prior to the smolt run, in April in the period 1983-92, between 2,000 and 5,000 wild salmon smolts were caught by electric fishing and annually marked by fin-clipping, and released in the same area where they were caught (Table 1). The smolts were marked differently by fin-clipping in each of the three sections of the river. The lengths of section I, II and III were 20, 8, and 19 km) (Fig. 1). The method of cutting fins is not considered to affect migration behaviour (Hvidsten and Ugedal 1991).

Smolts were caught at Meldal during the spring run-off, by two traps according to methods described by Hesthagen and Garnås (1986) and Hvidsten and Ugedal (1991). With the exception of 1981 and 1989, when water levels were too high to operate the traps, sampling of smolts were performed from late April to early June in the years 1980-92. Recapture rates of marked smolts varied between 0.4 and 2.7% in different years (Table 1). The recapture of smolts was correlated with the ratio of recaptured smolts and total number of marked smolts *(R/M)* and the total catch of smolts each year (*C*) (r^2=0.88, P<0.01, df=7). Water discharge, measured as the average flow in the period of the smolt run, was negatively correlated to the total number of smolts caught each year (r^2=0.92, P<0.01, df=7). Hence the smolt catches were taken as an index of the total number of smolts migrating. Catch efficiency is thus a fixed proportion of the smolts passing the sampling site each night. Efficiency was measured as the numbers of smolt caught per hour sampled at night between 21:00 and 03:00 (Hesthagen and Garnås 1986).

A moon phase model using the value one for new and full moon and the value zero for end/start of I/II and III/IV quarters was used. Tidal currents follow moon phases. Therefore we discuss the possibility of reduced predation pressure on smolts reaching the fjord at new and full moon when tidal currents are strongest.

The total number of smolts caught per day was modelled from physical factors by generalized linear modelling, using the GLIM package (Crawley 1993). We assumed that the number A_j of smolts caught each of 402 nights is Poisson

Table 1. Annual numbers of Atlantic salmon (*Salmo salar*) marked by fin-clipping in the three sections of River Orkla, and numbers as well as percentage of smolts which were recaptured in the traps at Meldal. The annual catches of smolts in the traps at Meldal in the period 1980-92 are also given.

Year	Section	Number of fin-clipped smolts	Recaptures		Total number of smolts caught at Meldal
			No.	%	
1980	-	-	-	-	776
1982	-	-	-	-	617
1983	I	1,497	16	1.1	
	II	331	4	1.2	
	III	517	4	0.8	
	total	2,345	24	1.0	1,285
1984	I	1,707	17	1.0	
	II	590	6	1.0	
	III	1,094	9	0.8	
	total	3,391	32	0.9	1,777
1985	I	2,130	10	0.5	
	II	660	5	0.8	
	III	1,420	3	0.2	
	total	4,210	18	0.4	779
1986	I	2,532	10	0.4	
	II	965	6	0.9	
	III	1,592	3	0.2	
	total	5,089	19	0.4	889
1987	I	2,435	26	1.1	
	II	1,173	14	1.2	
	III	1,658	22	1.2	
	total	5,266	62	1.2	2,848
1988	I	2,082	23	1.1	
	II	1,076	13	1.2	
	III	1,620	19	1.2	
	total	4,778	55	1.2	1,778
1990	I	1,502	10	0.7	
	II	912	9	1.0	
	III	1,733	16	0.9	
	total	4,147	35	0.8	2,802
1991	I	2,361	68	2.9	
	II	974	31	3.2	
	III	1,393	27	1.9	
	total	4,728	126	2.7	6,524
1992	I	1,921	20	1.0	
	II	946	7	0.7	
	III	2,077	16	0.8	
	total	4,944	43	0.9	2,335

distributed with expected values $E(A_j)$, $j= 1,....,$ 402. The covariates used were *LNW*, logarithm of water flow (m^3s^{-1}); *CW*, change in water flow (m^3s^{-1}); *T*, water temperature (°C); *CT*, change in water temperature (°C); *M*, moon phase (see above); *Y*, year, a factor with 11 levels for 11 years; *LNI*, logarithm of catch intensity (h). We used the generalized linear model

$$ln\ E(A_j)= ln\ N_j + \beta_1 LNW + \beta_2 CW + \beta_3 T + \beta_4 CT + \beta_5 M + \beta_6 LNI + \gamma_Y$$

where β_i and γ_Y are the effects of the covariates. The term $ln\ N_j$ was used as an offset in the model, where N_j is the sum of A_j and all later catches the same year, divided by the number of catch days left that year.

The parameters were estimated using maximum likelihood. The adequacy of the model was tested using scaled deviance (McCullagh and Nelder 1989), which is approximately χ^2 distributed with *N* degrees of freedom, where *N* equals the total number of observations minus the number of parameters that are estimated (here *N*=402-17=385). High values of scaled deviance (higher than degrees of freedom) indicate inadequate fit. In addition, residuals were examined.

To investigate the possibility of social interaction among migrating smolts, independence of the recapture date of a smolt and the section where it was marked was tested statistically. If there were no interactions among smolts from different sections and migration was initiated only by physical stimuli, one would expect dependence between recapture date and marking section, as smolts in upstream areas need more time to descend to the traps. If, on the other hand, there are social interactions among smolts so that passing migrating smolts from upper parts of the river stimulate the migration of downstream smolts, one would expect that catch date and marking section would be independent. A permutation test was applied instead of a chi-squared test due to small numbers of observations of some combinations of the two variables. The test was performed by permuting the vector of observed recapture dates randomly in relation to the vector of observed marking sections, so that a new data set was obtained. This was repeated 999 times so that 1,000 data sets including the observed data were obtained. For each set the usual chi-squared value was computed. They were sorted in descending order, and the rank of the chi-squared value of the observed data set divided by 1,000 was used as an estimate of the significance probability (Manly 1991, p. 214-216).

Results

Atlantic salmon smolts entered the traps in the River Orkla during the spring flood. The descent period lasted for about one month, and mainly took place in May (Fig. 2). Fifty percent descent

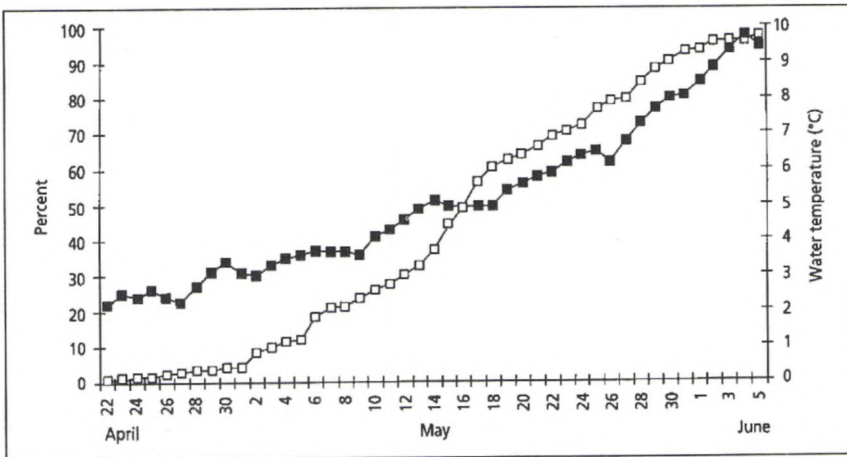

Fig. 2. Cumulative catch of Atlantic salmon smolts and water temperature in the River Orkla. Filled symbols are water temperature.

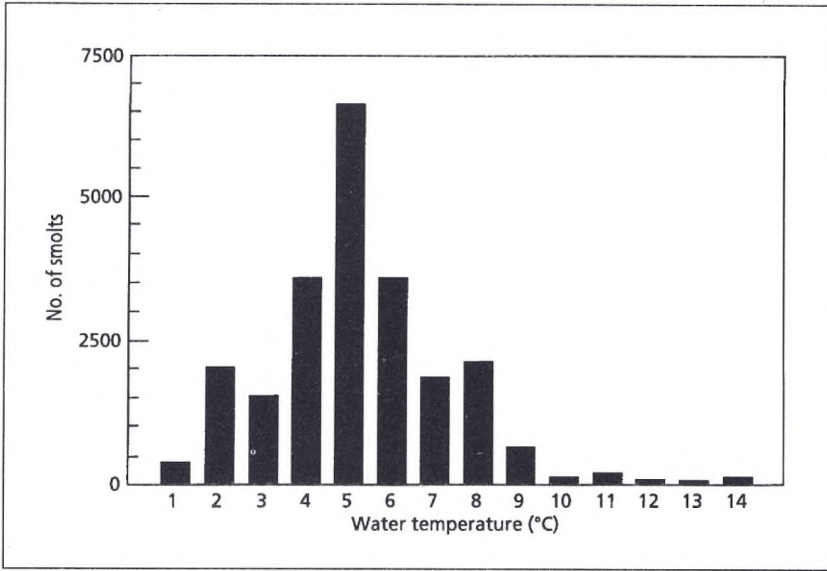

Fig. 3. Frequency of smolts descending at different water temperatures in the River Orkla in the period 1980-92 (N=22,461).

occurred between May 8 and May 28. The smolt run started at temperatures between 1.7 and 4.4 °C. Most of the smolts migrated at temperatures between 2 and 8 °C (Fig. 3).

Peaks in migration lasted until three days and tended to be associated with high and increasing water discharges (Fig. 4). The highest fractions of smolts recorded during one night varied between 12 and 35% of the total number of smolts each year (Table 2).

Most smolts descended in periods near new and full moon (Fig. 5).

The highest peaks occurred when smolts orginating from different sections of the river were caught at Meldal on the same night (Fig. 6). In years 1984, 1986, 1988, 1990 and 1992,

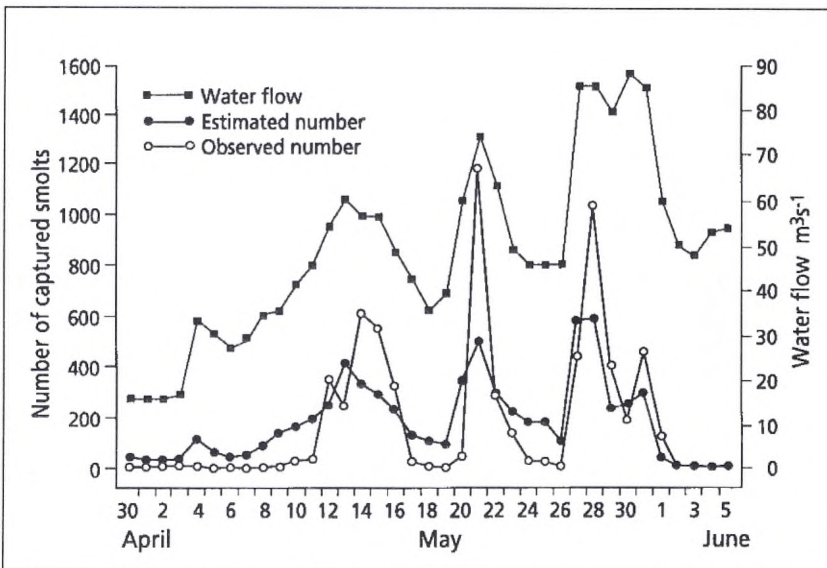

Fig. 4. The observed and predicted numbers of descending Atlantic salmon smolts per night and water discharge in the River Orkla in 1991.

Table 2. Date when the highest percentage of descending smolts was observed in the traps at Meldal, and proportion of total catch. The proportion of marked smolts from the different marking sections the same date are also given. Rank of the years according this proportion, as well as according to recaptures are given in parenthesis.

Year	Date	Percent of total catch and (rank order)		Percent of recaptured marked smolts by section and (rank order)			
				I	II	III	
1983	May 29	12	(7)	0	0	25.0	(8)
1984	May 15	25	(4)	17.7	33.3	22.0	(5)
1985	May 10	12	(7)	20.0	20.0	33.3	(4)
1986	May 3	35	(1)	90.0	33.4	0	(2)
1987	May 17	12	(7)	7.7	7.1	4.5	(9)
1988	May 7	32	(2)	60.9	69.2	15.8	(1)
1990	May 25	25	(4)	10.0	11.1	25.0	(6)
1991	May 14	19	(6)	11.7	14.7	7.4	(7)
1992	May 15	26	(3)	30.0	14.3	31.3	(3)

25% or more of the total catch was observed when wild marked smolts from two or all three sections were caught on the same night (Fig. 6, Table 2). During the 1986 peak migration, smolts from the lower areas (I and II) were recaptured on the same night, while those from the upper section (III) were caught later on. In 1983 and 1990, smolts from the lower area (I) were caught earlier than those from the middle section (II).

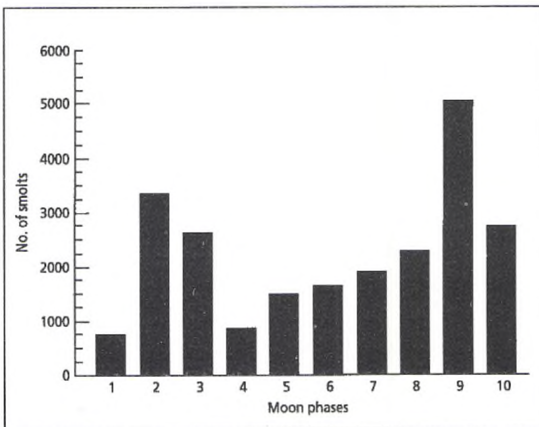

Fig. 5. Catch of descending smolts at different moon phases. Value 1 = start of second and fourth quarter, value 10 = new and full moon, values >1 and <10 are intermediate values, N = 22,461).

In the GLIM analysis, all covariates used in the model significantly explained some of the variation in the catch each night, A_j, and are thus included in the model (Table 3). All covariates explained in total 51.4% of the numbers of descending smolts.

All physical variables tested had a significant influence on salmon smolt migration. Absolute water flow was the most important physical variable influencing smolt descent. Absolute and change in water temperature were more important than change in water flow and moon phase variables.

Scaled deviance (= 18,905 and df = 385) and the deviance residuals plotted against ln *(E(A))* are shown in Fig. 7. The dispersion parameter, *Var(A)/E(A)*, which equals 1 for the Poisson distribution, is estimated to be 68.75, indicative of great overdispersion.

When comparing predicted and observed values of smolt migration there are differences especially during peak migration (Fig 4).

Therefore, the model is incomplete. The chosen physical values do not provide a satisfactory explanation, or we have not chosen the right explanatory variables. The fact that residuals increase with increasing numbers of smolts caught and great overdispersion indicate that the current model has an insufficient predictive capac-

Fig. 6. Cumulative recapture of marked Atlantic salmon *(Salmo salar)* smolts from section I, II and III in the River Orkla.

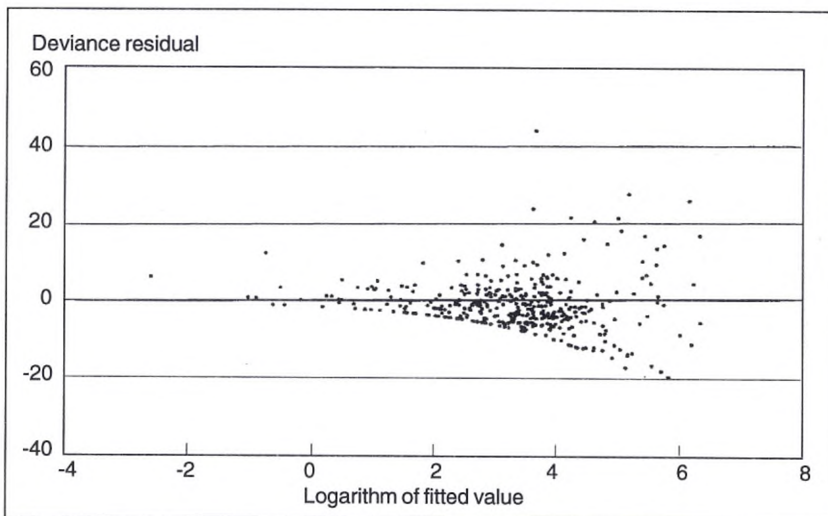

Fig. 7. Deviance residuals for expected number of fish caught (logarithm of fitted value).

Table 3. The parameter estimates and their standard deviation in the GLIM analysis. The chi-squared statistic (χ^2) is used to test the null hypothesis that the parameter for the added effect is zero. The probability of obtaining a greater χ^2 value than that observed, if the null hypothesis is true, is *P*. Small *P*-values are evidence for concluding that the parameter is not equal to zero. The values of the variation explained by the model are shown. * = $P<0.0001$.

Covariate	Year	Parameter estimate (γ_Y, β_i)	SD	χ^2	Std. estimate	Explanation (%)
Y	1980	-10.76	0.155	4,814.96*	-0.2442	10.58
	1982	- 9.87	0.161	3,739.23*	0.6688	
	1983	-10.89	0.162	4,509.11*	-0.3537	
	1984	-11.02	0.169	4,276.80*	-0.4846	
	1985	-10.58	0.169	3,906.68*	-0.0387	
	1986	-10.80	0.172	3,937.78*	-0.2618	
	1987	-10.94	0.163	4,502.86*	-0.4025	
	1988	-10.49	0.163	4,130.39*	0.0522	
	1990	-10.81	0.159	4,624.17*	-0.2736	
	1991	- 9.92	0.156	4,047.26*	0.6131	
	1992	-11.15	0.166	3,738.34*	0.3747	
LNI		1.6173	0.0523	954.82*	0.5076	6.17
LNW		1.2680	0.0190	4,466.35*	0.7020	16.99
T		0.1525	0.0038	1,570.22*	0.3859	5.00
CT		- 0.6178	0.0109	3,232.38*	-0.3734	10.58
CW		0.0048	0.0002	404.12*	0.1266	1.31
M		0.3455	0.0230	224.89*	0.1006	0.77

ity, for high numbers. The covariates used in the model do not explain the occurrence of sudden peaks of smolts.

At the 5% level of confidence, the null hypothesis that recapture date and marking section is independent was only rejected for 1986 and 1988. This gives some evidence that migrating smolts from upper sections of the river may influence the migration of smolts from lower areas.

Discussion

The salmon smolt run starts at low water temperatures in the River Orkla compared to other rivers (Jonsson and Ruud-Hansen 1985, Saksgård et al. 1992). The smolt run started each year at water temperatures below 5 °C, but no indications of a lower threshold temperature for start and maintainance of smolt descent were found. A possible threshold water temperature in the River Orkla for start and maintainance of smolt run is less than 1.7 °C. Fried et al. (1978) reported that the smolt run started when water temperatures increased above 5 °C in the Penobscot River in Maine. In the River Imsa, smolts start their descent at temperatures between 5.8 and 11.2 °C. No threshold temperature was found for the start of smolt descent in Imsa (Jonsson and Ruud-Hansen 1985). Several authors have reported peak smolt migration at water temperatures of 10 °C or higher (Elson 1962, Mills 1964, Solomon 1978, Dempson and Stansbury 1991). Smolt runs in Orkla usually terminated when the water temperature reached 10 °C. This result agrees with Wagner (1974), who concluded that for steelhead (*Salmo gairdneri*) water temperature did not indicate an earlier or later start of the smolting process, but influenced the length of the time the fish were smolts. Jonsson and Ruud-Hansen (1985) concluded that changes in water temperature could change the timing of smolt descent. The different proximate triggers for the smolt run in different stocks of salmon indicate the existence of local adaptations.

In the River Orkla, freshets during winter and in the first part of April did not initiate smolt migration. However from the last week of April, increased water flow initiated smolt migration. In the neighbouring regulated River Nidelva, large fluctuating water flows throughout the winter, do not seem to affect the timing of the smolt run (unpublished observations). Thus water temperature and discharge are proximate triggers, possibly influencing time of descent only within the ultimate "window" for smolt migration.

Moon phases may influence thyroxine levels in both coho salmon (*Oncorhynchus kisutch*) (Nishioka et al. 1989) and Atlantic salmon (Youngson et al. 1989). Releases of coho salmon smolts at the new moon resulted in equal or higher recaptures than releases at other lunar phases. Those released at full moon resulted in increased return rates of adult salmon (Nishioka et al. 1989). Hopkins (1992) found that releases of chinook salmon smolts (*Oncorhynchus tschawytscha)* at the new moon gave increased recapture of adult returns. Releases at full moon gave less returns. Hartmann et al. (1982) discribes several major peak runs of coho salmon fry occuring near and at new and full moon phases over a period of ten years. Atlantic salmon smolt runs in the River Alta were also related to moon phases (Saksgård et al. 1992). It seems that the same environmental cues are used by different salmonid species for timing of smoltification and reaching the sea at periods optimal for survival. In long Norwegian fjords increased tidal currents may result in increased displacement at new and full moon phases, compared to the periods of low ebb tide. Combined high tide and water discharge from the rivers can obviously reduce predation during postsmolt migration (Levings et al. 1994).

The variables describing the daily run of smolts did not provide a complete model. There are at least four different explanations which may have acted separately or in combination;

1) In the first sampling period at peak spring flooding, drifting debris may clog the trap nets, and hence, reduce catch efficiency. Differences in catch efficiency with changing water discharges caused by varying avoidance responses in smolts are not known. However, they are likely to occur at least at the lowest discharges. In total (all years) however, the number of smolts caught was significantly correlated with mean discharge in the total period of catch.

2) Observation of the physical values at the time and place of initiation of the smolt migration might give a better fit. The smolt run and the values of the physical parameters at the time of catch were observed.

3) Within the main period of the smolt run, sometimes no smolts migrated despite supposed favourable environmental conditions. This seems to happen after peak migration, indicating a lack of sufficiently smoltified smolts ready to respond adequately to the physical proximate triggers.

4) The observations may be dependent because of social interaction during migration.

Bohlin et al. (1993) used length as a variable in a model predicting smolt run of brown trout (*Salmo trutta*). In the River Orkla smolt lengths of early and late migrants have no systematic change in length distribution.

The smolt run in the River Orkla was influenced by water discharge, water temperature, negative change in water temperature, change in water flow and moon phase. Water discharge and change in water temperature are correlated ($P<0.05$). Negative change in water temperature indicates increasing water discharge during periods of snow melt. The positive contribution of change in water flow supports this. Water flow and change in water flow were regarded as two different variables for assessing influence of water discharge. Water discharge is the main stimulus for start and maintainance of smolt migration in River Orkla. The spring run-off from snow melt occurs in the period of smolt migration in the River Orkla. In rivers without spring run-off inceased water discharge is reported to increase smolt run (Solomon 1978).

Timing of the Atlantic salmon smolt migration in the spring, has been selected through evolution to provide optimal survival of each individual. Synchrony of oceanic temperatures and feeding conditions with timing of smolt migration from rivers is probably crucial. Thus, the

descent takes place earlier in southern than in northern areas in Europe (range April to late June) (Bagliniere 1976a; Bakshtansky and Barybina 1976; Jonsson and Ruud-Hansen 1985; Hesthagen and Garnås 1986; Heggberget et al. 1993), and is assumed to be regulated by local abiotic factors. As different local, proximate, regulatory mechanisms are reported, it is likely that common ultimate regulatory mechanisms exist (Wagner 1974). Descent at high water discharge in the darkest period of the night is believed to reduce predation in the estuary in the River Orkla (Hvidsten and Lund 1988, Hvidsten and Hansen 1988). The number of smolts predated by cod was estimated at 20% for wild and hatchery reared smolts in the estuary (Hvidsten and Lund 1988). The importance of moon phase has also been suggested to be related to reduced predation. When smolts reach the sea at new or full moon, the water currents are higher than at the start of the second and last quarter in Trondheimsfjord. Postsmolts are reported to follow the tidal current in the top layer (Tytler et al. 1978, Holm et al. 1982). In long and narrow fjords, predation on salmon smolts by saithe (*Gadus virens*) and seagulls (Reitan et al. 1987, Strand et al. 1992) is believed to be serious. Smolts aggregate during descent in the River Orkla and may appear in large shoals. These shoals are detected at night when marked smolts from different sections of the river are caught in the traps at Meldal. There is indirect evidence of social interaction during migration, in the sense that in most years there was no significant dependence between catch date and marking section.

Episodic migration seems to be normal for migrating smolts (Elson 1962, Österdahl 1969, Solomon 1978). In the River Imsa, stocking with hatchery reared smolts seemed to initiate shoal formation during the period of the smolt run (Hansen and Jonsson 1985). Kennedy et al. (1984) found a significant correlation between descent of hatchery reared smolts and wild smolts. Smolts aggregate by joining migrating shoals, as they descend. In the River Orkla, the largest shoals appeared in traps when smolts from different upstream areas were recovered on the same nights. Releases of hatchery reared smolts in a peak smolt run in Orkla gave 6.8% recapture of adult salmon compared to mean recapture of 2.6% after releases when few wild smolts migrated (Hvidsten and Johnsen 1993). Therefore, we assume that wild salmon smolt survival also increases when migrating in peaks.

In the neighbouring rivers Gaula and Surna, increased recaptures of adult salmon were observed for stocked hatchery reared smolts at high water discharges compared to releases at low water discharges within the period of the wild smolt run (Hvidsten and Hansen 1988).

Water discharge, changes in water temperature and moon phases were proximate mechanisms regulating smolt runs in the river Orkla. Smoltification, which is the ultimate requirement for smolt migration, is regulated by increasing day-length (Wagner 1974; Wedemeyer et al. 1980). The social aggregative descent observed in Atlantic and Pacific salmon stocks are important for smolt survival. The above abiotic factors and social interaction are probably major regulatory mechanisms influencing the smolt run.

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Temporal Changes in Parasite Load of Lake Resident Arctic Charr *Salvelinus alpinus* (L.) held in Brackish Water Cage Culture

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Abstract

Parasite abundances were monitored in two groups of lake resident Arctic charr (*Salvelinus alpinus*) from Lake Takvatn, northern Norway, after transfer from the lake to rearing in brackish water. One sample was taken from a batch of about 90,000 fish caught in summer (June) 1988, and the other from a total of 40,000 individuals taken under the ice in the winter (April) of 1989. The fishing gear was baited funnel traps. Ten species of macroparasites were found in the fish samples. The cestode *Diphyllobothrium ditremum* and the nematode *Cystidicola farionis* dominated both in terms of prevalence and abundance. The age-specific densities of *Diphyllobothrium* spp. were significantly higher in the 1988 catch than in the one from 1989, probably because the fish from 1988 were transferred for rearing about two months later in the season. Reduced abundances were demonstrated for the ectoparasitic copepod *Salmincola* sp. and the nematode *Philonema* sp. during the rearing period, while infections with other important species remained unchanged. Parasites obviously may represent a quality problem for the use of landlocked charr as stocks for commercial farming, but this can be minimised by catching the youngest fish possible early in the year before ice breakup.

Keywords: Parasites, Arctic charr, commercial culture.

Introduction

Many large lakes in northern Norway have dense populations of stunted and heavily parasitised Arctic charr *Salvelinus alpinus*. Intensive fishing has been proposed as a measure to improve the status of such stocks. In 1984, an intensive fishing programme with baited funnel traps was initiated in Lake Takvatn (14 km^2) to test this idea (Amundsen 1989, Amundsen et al. 1993). The annual yield between 1984 and 1989 was up to 125,000 live charr. Since this fisheries enhancement method is now common in northern Norway, considerable numbers of live 'surplus' charr are available each year. It has been suggested to use such fish as stocks for commercial farming (Grotnes et al. 1987, Heggberget et al. in press). A premise is that parasites acquired by the fish prior to the capture do not pose a quality problem.

In Lake Takvatn, catches of the first few years were unsuited for farming because old and stunted fish with heavy parasite burdens dominated. Later, young immature fish with a higher growth potential prevailed (Amundsen et al. 1993). For the present study, the macroparasite abundances in Takvatn charr trapped in the summer of 1988 and the winter of 1989 were monitored after transfer to commercial rearing in tanks with brackish water. Since there was no possibility of new infections with freshwater parasites, a decline in parasite burdens was expected. The decline rates should differ among parasite species, both because of varying longevities in the fish, and because the fish were transferred to brackish water conditions. The results are important for assessing the feasibility of using wild charr in farming.

Material and methods

The charr from Lake Takvatn (see Klemetsen et al. (1989) and Amundsen et al. (1993) for a description of the lake, its fish populations and collecting methods) were transferred to the rearing tanks in Jøvik, Ullsfjord, in two batches; 90,000 in early July 1988 and 40,000 in early May 1989. The first batch was a summer catch (mainly caught throughout June), the last was taken under the ice (caught throughout April).

The total sample analysed for parasites consisted of 296 charr, 120 from the 1988 summer catch and 176 from the 1989 winter catch. Parasite abundances were examined after 11 and 16 months under culture conditions for the 1988 sample (June and November 1989) and after 1, 3, and 6 months for the 1989 sample (June, August and November 1989).

The fish were fed commercial dry pellets in indoor tanks where fresh and sea water of ambient temperatures were mixed to a salinity of about 15 ppt. The freshwater source was a steep small mountain stream without any lakes or fish populations. Thus, there was no possibility of new infections with freshwater parasites. At each sampling time, the fish were killed and brought fresh to the laboratory where individual length, weight and sexual status were noted. Fish age was determined from surface reading of otholiths immersed in glycerol under a binocular microscope.

The skin, gills, and body cavity of each fish were inspected macroscopically for parasites, and the swimbladder was preserved in 70% ethanol. The intestine including the pyloric caeca was cut open in a petri dish with physiological saline solution, and examined under a binocular microscope. Parasites were preserved in 4% saline formalin. Encysted *Diphyllobothrium* spp. plerocercoids were recovered by pepsin-HCl digestion of the viscera including the emptied intestine. The digestion process was carried out in petri dishes at room temperature, and the excysted plerocercoids were picked out and preserved in formol-saline after two and twelve hours. Identification and counting of cestode larvae was done under a binocular microscope. Finally, the fixed swimbladders were opened and examined for nematodes.

Results

The catches from summer 1988 were dominated by three and four year old fish, and each of these two age-classes made up about 40% of the total (Fig. 1a). The oldest fish in the sample was six years old. In the May 1989 batch, the proportion of fish older than five years was higher than the preceding year (Fig. 1b). Individuals with ages of six years made up about 12% of the sample, and the oldest individual was eight years. Charr with ages of three years dominated this group, and comprised about 43% of the total.

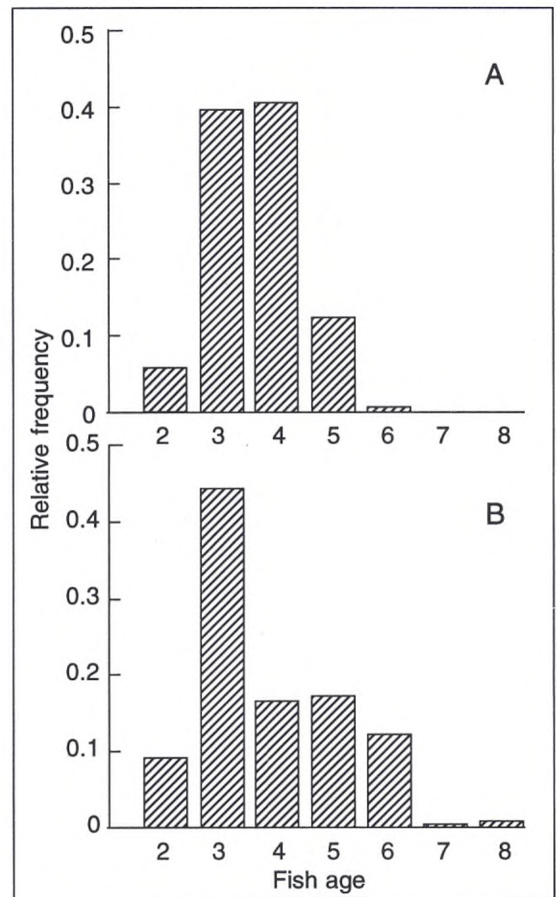

Fig. 1. Age distributions of charr caught in funnel traps in Lake Takvatn. A: Summer 1988 (June; N=120), B: Winter 1989 (April; N=176)

Ten species of macroparasites were encountered in the charr materials (see Tables 1 and 2). The two clearly dominant species both in terms of prevalence, relative density and mean intensity (sensu Margolis et al. 1982) were the cestode *Diphyllobothrium ditremum* and the nematode *Cystidicola farionis.*

Age-specific relative densities of the different parasite species were estimated in the fish samples from 1988 and 1989. The results from the two years are compared in Fig. 2. The total parasite density was consistently lower in the catch from 1989 than in the one from 1988. This difference was mainly due to significantly lower

Table 1. Prevalence and mean intensity (= mean number per infected fish) of macroparasites in Takvatn charr caught June 1988 necropsied in June and November 1989. The fish were reared in brackish water in indoor tanks (N=120). (CP) = Copepod, (D) = Digenean, (C) = Cestode, (N) = Nematode.

Parasite species	Prevalence		Mean intensity (range)	
	Jun	Nov	Jun	Nov
Salmincola sp. (CP)	0	0	0	0
Crepidostomum spp. *) (D)	6.7	0	1.0	0
Cyathocephalus truncatus (C)	3.3	0	1.5 (1-2)	0
Proteocephalus sp. (C)	16.7	6.7	5.5 (1-16)	2.0 (1-5)
Eubothrium salvelini (C)	55.0	60.0	2.5 (1-7)	2.2 (1-12)
Diphyllobothrium dendriticum (C)	38.3	31.7	5.4 (1-23)	2.7 (1-9)
Diphyllobothrium ditremum (C)	98.3	96.7	33.6 (1-441)	26.4 (1-132)
Cystidicola farionis (N)	90.0	88.3	16.7 (1-94)	17.5 (1-241)
Philonema sp. (N)	51.7	21.7	8.9 (1-40)	5.6 (1-15)
Number of fish examined	60	60		

*) = *C. farionis* and *C. metoecus*

Table 2. Prevalence and mean intensity (= mean number per infected fish) of macroparasites in Takvatn charr caught April 1989 necropsied in June, August and November 1989. The fish were reared in brackish water in indoor tanks (N=176). (See Table 1 for explanation of suffixes.)

Parasite species	Prevalence			Mean intensity (range)		
	Jun	Aug	Nov	Jun	Aug	Nov
Salmincola sp. (CP)	8.9	5.0	1.7	1.0	1.0	1.0
Crepidostomum spp. *) (D)	0	5.0	3.3	0	1.0	1.5 (1-2)
Cyathocephalus truncatus (C)	1.8	0	0	1.0	0	0
Proteocephalus sp. (C)	1.8	3.3	6.7	1.0	3.0 (1-5)	1.0
Eubothrium salvelini (C)	37.5	56.7	46.7	1.4 (1-4)	1.9 (1-8)	2.6 (1-19)
Diphyllobothrium dendriticum (C)	14.3	28.3	26.7	5.8 (1-11)	7.1 (1-35)	7.9 (1-62)
Diphyllobothrium ditremum (C)	66.1	88.3	91.7	15.2 (1-157)	25.0 (1-195)	28.9 (1-285)
Cystidicola farionis (N)	76.8	86.7	91.7	16.6 (1-207)	41.6 (1-410)	15.0 (1-141)
Philonema sp. (N)	64.3	45.0	46.7	4.8 (1-38)	3.7 (1-12)	2.6 (1-8)
Number of fish examined	56	60	60			

*) = *C. farionis* and *C. metoecus*

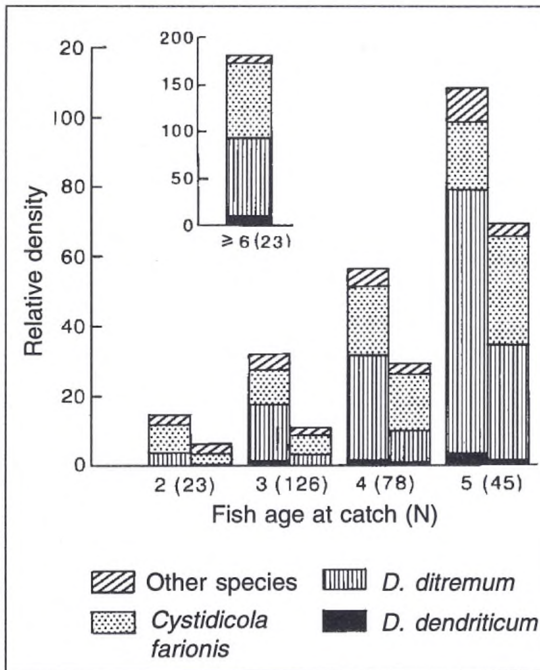

Fig. 2. The age-specific relative densities of the different parasite species in the samples of Takvatn charr caught in funnel traps in summer 1988 (left column) and in winter 1989 (right column). Data from fish of six years and older from the 1989-batch is inserted. (Numbers of observations are given in the figure.)

abundances of *D. ditremum* in all age groups of charr in the sample from the 1989 batch (Mann-Whitney U-tests, $0.013>P>0.000$). There was also a tendency towards lower age-specific densities of *D. dendriticum* and *C. farionis* in 1989, and the difference was significant for both parasite species in age groups 3 and 4 years (Fig. 2; Mann-Whitney U-tests, $0.031>P>0.00$).

The parasites were grouped into two categories; species transmitted by copepods, and species transmitted by the amphipod *Gammarus lacustris*. The age-specific mean increase per fish of parasite numbers was estimated in the catches from 1988 and 1989 for both categories. Infection rates of copepod-transmitted parasites were higher in charr caught in 1988 (Fig. 3). The increase with age of amphipode-transmitted parasites was similar between the two catches, except that there was no increase in parasite numbers from the age of four to five years in the catch from 1988 (Fig. 3).

After almost one year in culture, no *Salmincola* sp. were found on the gills of the 1988 fish. *Crepidostomum* spp. and *C. truncatus* were encountered only in the June sample (Table 1). There was a significant reduction in both prevalence (Chi-square test, $P<0.05$) and intensity (Mann-Whitney U-test, $P=0.001$) of *Philonema* sp. from June to November. For the remaining five parasite species there were no significant differences in prevalence or abundance between the two sampling periods (Table 1; Chi-square and Mann-Whitney U-tests, $P>0.05$).

In charr caught in April 1989 there was a tendency of a reduced prevalence of *Salmincola* sp. throughout the year (Table 2; Chi-square tests, $0.1>P>0.05$). For *Philonema* sp. the prevalences in August and November were significantly

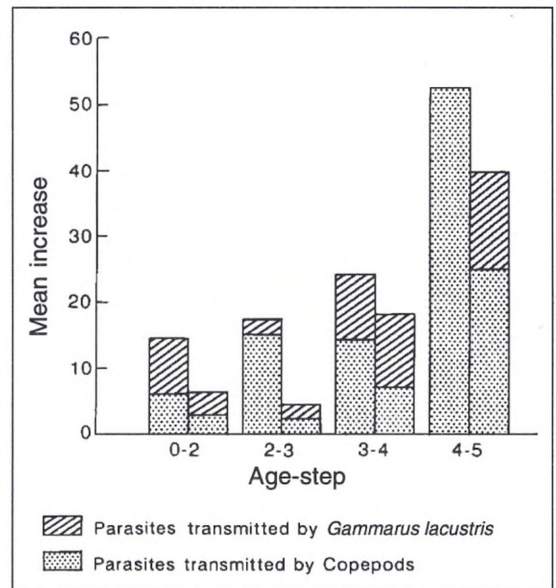

Fig. 3. Mean age-specific increase of parasites transmitted by copepods and by *Gammarus lacustris* estimated as the difference in relative density between subsequent age-groups in the samples of Takvatn charr caught in funnel traps in summer 1988 (left column) and in winter 1989 (right column). (See Fig. 2 for numbers of observations.)

lower than in June (Chi-square tests, $P<0.05$), and there was also a decreasing intensity with time as the estimate from June was significantly higher than the two later (Mann-Whitney U-tests, $0.047>P>0.014$.) *C. truncatus* was recorded in the June sample only.

The prevalences of the two *Diphyllobothrium* species in fish caught in 1989 were significantly higher in the two later sample collections than in June (Table 2; Chi-square tests, $P<0.05$). Furthermore, the abundance of *D. ditremum* was significantly higher in the August and November samples than in the one from June (Mann-Whitney U-tests, $P=0.000$). Finally, the prevalence of *Cystidicola farionis* was significantly higher in November than in June (Chi-square test, $P<0.05$). With no possibility of new infections in the tanks, these differences must have other explanations. There was an increased proportion of fish older than four years in the later samples (June 23%, August 32%, November 35%), and the mean fish age was significantly higher in the November sample than in June (*t*-test, $P<0.05$). It is therefore likely that the lower parasite abundances recorded in June were an effect of a higher proportion of younger fish in this sample.

A comparison between the August and November samples from the 1989 winter catch revealed no significant differences in prevalence or abundance for any parasite species (Table 2; Chi-square and Mann-Whitney U-tests, $P>0.05$).

Discussion

The difference in the age distributions between the catches from 1988 and 1989 reflects seasonal habitat shifts in the Takvatn charr population. During winter, the majority of the charr are located in the littoral zone (Staldvik 1992). The traps were placed in shallow waters under the ice in April 1989, and caught a broad range of age classes. In contrast, the traps were fished much deeper in June 1988 (20-30 m). At this time the youngest fish usually have retreated to the profundal zone of the lake, while the older fish remain in the littoral (Klemetsen et al. 1989, Amundsen et al. 1993). Consequently, the proportion of fish older than four years was significantly lower in the 1988 catches. Since there normally is a positive correlation between fish age and parasite burden, the conclusion from this alone would be that the younger fish from summer catches are more suited for commercial farming than those taken under the ice.

However, the prevalence and mean intensity of most parasite species were comparable between the 1989 winter catches and those from the preceding summer (see Tables 1 and 2), although the former included fish up to eight years old. The reason was significantly lower age-specific abundances of parasites in the charr caught in 1989, most conspicuously for the two *Diphyllobothrium* species. This may reflect that the charr were transferred for rearing about two months later in the year in 1988. During this period (May and June), the infection rate of *Diphyllobothrium* spp. seems to have been very high. The advantage of a higher ratio of young fish in the summer catches therefore appears to be outweighed by their significantly higher age-specific abundances of parasites.

The age-specific densities of *Diphyllobothrium* spp. in charr caught during June 1988 were similar to those from corresponding cohorts taken in April 1989 (see Fig. 2). This indicates that the infection rate of the copepod-transmitted *Diphyllobothrium* species must have been negligible throughout this period. On the other hand, the age-specific infection rates of parasites transmitted by *Gammarus lacustris* (mainly *C. farionis*) were similar in the catches from the two years (see Fig. 3). This corresponds well with earlier studies from Lake Takvatn which indicate that the charr predominantly become infected with these parasites from mid-summer throughout the autumn (Giæver et al. 1991, Knudsen 1991, Staldvik 1992).

The higher infection rate of parasite species transmitted by *G. lacustris* recorded from fish age 4-5 years in the 1989 sample may indicate an increased availability of amphipods to the charr caused by reduced fish densities. A dietary shift from copepods to benthic invertebrates - and, hence, reduced abundances of copepod-transmitted parasites - has been documented in

a dense whitefish (*Coregonus lavaretus*) population after a massive stock reduction (Amundsen and Kristoffersen 1990).

Reduced abundances were demonstrated for *Salmincola* sp. and *Philonema* sp. during the Arctic charr rearing period in brackish water. Knudsen (1991) reported a natural infection of about 20% of *Salmincola* sp. in Lake Takvatn. It seems that *Salmincola* sp. has a poor tolerance for increased salinities since the prevalence was as low as 8.9% after about one month. The adult stage of the related species *Salmincola californiensis* may survive for up to one year under natural freshwater conditions (Bailey et al. 1989).

The reason for the decreasing abundance of *Philonema* sp. seen simultaneously in both charr batches was probably that many females matured and left the fish during the autumn 1989. It takes about four years from the time larvae of *P. oncorhynchi* (probably the species in Takvatn) enter the fish host via copepods until the females mature (Platzer and Adams 1967). At that time, the nematodes are gravid with first-stage larvae, and leave its host. When outside, they burst rapidly and liberate many thousands of larvae.

In charr caught in April 1989 the cestode *Cyathocephalus truncatus* was encountered in the June sample only. This pattern could be expected since Vik (1958) reported a longevity of this species of up to 55 days in its salmonid host. However, the occurrence of infection in two fish after 11 months in rearing was unexpected and indicates that some infections persist longer.

The prevalence and mean intensity of *Eubothrium salvelini* was relatively similar in all the current charr samples and also similar to the infection levels found in the lake (Skogsholm 1990, Knudsen 1991). Thus, the longevity of this species was not significantly influenced by the increased salinity. Smith (1973) reported that *E. salvelini* may remain in the fish host for 24-26 months. Several studies have shown that *Eubothrium*-infections give deleterious effects in salmonid hosts, such as reduced growth and stamina (Boyce 1979, Bristow and Berland 1991), lowered condition index (Hoffman et al. 1986), and poorer hypoosmoregulatory performance (Boyce and Clarke 1983).

Digeneans of the genus *Crepidostomum* may survive for about one year in the intestine of the fish host (Thomas 1958, Awachie 1968). This corresponds well with the present results, and the conclusion regarding these species is thus similar to that for *E. salvelini*. The same might also be said about the cestode *Proteocephalus* sp. since no decreasing trend was seen with time.

Both of the *Diphyllobothrium* species (Henricson 1977, Halvorsen and Andersen 1984, Kristoffersen 1993) and the nematode *C. farionis* (Giæver et al. 1991) may persist in charr for many years. The results from the present study did not give any clear indications of increased mortality rates during the rearing of the fish in brackish water. Furthermore, the age-specific densities of these species estimated in the 1988 catches after more than one year in rearing were comparable with those recorded in the lake in 1987/88 by Knudsen (1991).

The present results show i) that parasite abundances were not very much reduced after more than one year in culture in brackish water, and ii) after several years of intensive fishing the parasite infections in the Takvatn charr were still considerable. This can not be neglected when commercial rearing of the funnel trap catches is considered. It may be argued that almost every parasite is removed when the fish are gutted, and therefore product quality is unaffected. The only parasites to occasionally enter muscle tissue are *Diphyllobothrium* spp. (*D. dendriticum* in particular). On the other hand, the growth capacity and general condition of the fish is questionable, especially since about 50% of the charr maintained *Eubothrium*-infections for a considerable time in rearing. Further, the reputation for quality of the product could be compromised.

Setting an upper limit of acceptable number of parasites for commercial rearing is always debatable. With an arbitrary level of 20 parasites/fish, only about 33% or 29,700 of the 90,000 charr caught in June 1988 would pass. The corresponding numbers for the catches from April 1989 would be 64%, or 25,600 out of the total 40,000 individuals. The conclusion is that it is preferable to catch the youngest fish possible, as early as possible in the year before ice breakup.

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NOTES AND COMMENTS

Hybridization between Atlantic Salmon (*Salmo salar*) and Brown Trout (*S. trutta*) in the Teno and Näätämö River Systems, northernmost Europe

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Teno and Näätämö River systems are subarctic drainages which flow through northern Finland and Norway to the Barents Sea. In both drainages anadromous Atlantic salmon (*Salmo salar*) is the most predominant fish species with coexisting brown trout (*Salmo trutta*). Brown trout has different life history types, but their geographic distribution is unclear. Presently, only few populations are known to contain anadromous, sea-run brown trout. Preliminary studies of brown trout in Teno have suggested that the life history of an individual can be flexible, varying between stream residency and anadromy (Erkinaro and Niemelä, pers. comm.).

Atlantic salmon and brown trout are known to hybridize in nature with varying frequencies (Table 2 and references therein), but the reasons for this and also the factors generally affecting hybridization are not yet fully understood. Here we report hybridization between Atlantic salmon and brown trout in two subarctic rivers draining to the Barents Sea, and discuss the possible reasons affecting the hybridization. Our aim was also to distinguish first-generation hybrids from backcross hybrids. In addition, we give some regional comparisons of hybrid frequencies, and provide further evidence for the view that factors promoting hybridization may be very local.

Atlantic salmon and brown trout were sampled during 1986, 1987 and 1991 (samples 8, 18, 21) from four locations in River Näätämö and 26 locations in the River Teno drainage (Fig. 1, Table 1). Seven of the rivers (locations 7, 8, 9, 18, 20, 21, 24) do not have suitable grounds for spawning of anadromous Atlantic salmon, but other sampling locations are situated on known or possible spawning rivers. Juvenile fish were caught by electrofishing and adults by gillnetting. Most of the fish were juveniles but 81 Atlantic salmon and 20 anadromous brown trout adults were also captured. The fish were identified visually as being Atlantic salmon (N=1541) or brown trout (N=483). Whole juveniles and tissue samples from adult fish were stored at -20 °C for up to two months and afterwards at -75 °C until analysed. Horizontal starch gel electrophoresis was performed on skeletal muscle, liver, and eye extracts as described by

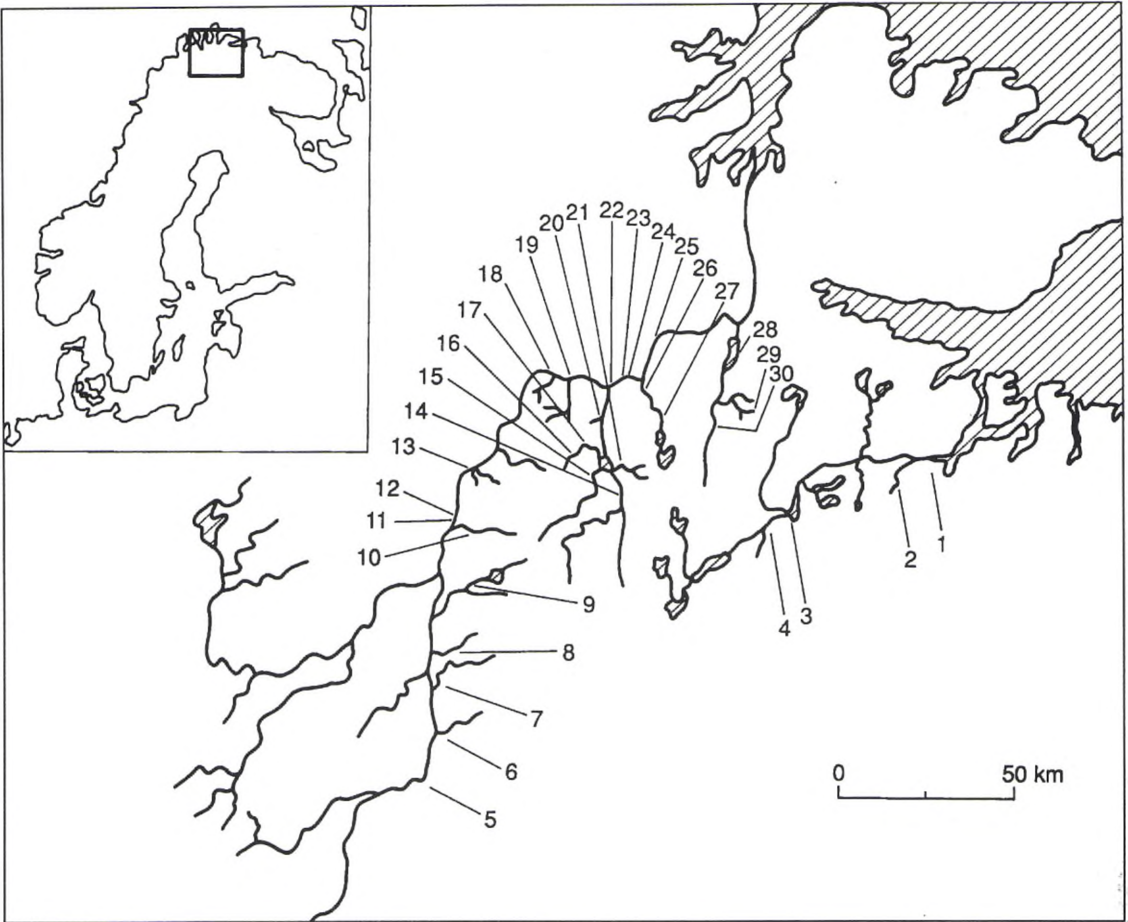

Fig. 1. Location of river systems and sampling sites. Numbers refer to Table 1.

Vuorinen and Berg (1989). The following 15 enzymes were assayed from all specimens (enzyme nomenclature no. in parenthesis): aspartate aminotransferase (2.6.1.1), alcohol dehydrogenase (1.1.1.1), creatine kinase (2.7.3.2), esterase (3.1.1.-), glucose-6-phosphate isomerase (5.3.1.9), glycerol-3-phosphate dehydrogenase (1.1.1.8), L-iditol dehydrogenase (1.1.1.14), NADP$^+$-dependent isocitrate dehydrogenase (1.1.1.42), lactate dehydrogenase (1.1.1.27), malate dehydrogenase (1.1.1.37), NADP$^+$-dependent malic enzyme (1.1.1.40), phosphogluconate dehydrogenase (1.1.1.42), phosphoglucomutase (5.4.2.2), superoxide dismutase (1.15.1.1), and xanthine dehydrogenase (1.1.1.204). Four samples which were exclusively (8, 18, 21) or predominantly (20) of brown trout, were analyzed for four additional enzymes to increase the probability of recognizing introgressive hybridization. The supplementary enzymes were diaphorase (1.8.1.4), fumarate hydratase (4.2.1.2.), hexokinase (2.7.1.1) and mannose-6-phosphate isomerase (5.3.1.8). The number of loci studied was consequently 36 or 40. Of these enzymes, the following five have shown to be diagnostic in the identification of Atlantic salmon, brown trout, and their F_1 hybrids: glucose-6-phosphate isomerase, NADP$^+$-dependent malic enzyme, phosphoglucomutase, superoxide dismutase, and xanthine dehydro-

Table 1. Distribution of brown trout x Atlantic salmon hybrids within Teno and Näätämö river systems. Sampling locations with code 1-4 belong to Näätämö and 5-30 to Teno river system, respectively. Symbols L and U refer to lower and upper parts of the rivers. Percentage of mature precocious Atlantic salmon males was calculated from the total number of juveniles of Atlantic salmon sampled per tributary. If sex ratio is 1:1, maximum percentage should be 50%. Observations on the anadromy of brown trout were classified to groups as follows: P = present, observations on sea trout migrating or spawning or smolts, A = absent, physical restrictions, N = not direct observations on anadromy, but may be present.

Sampling location	Percentage of precocious *S. salar* males	Anadromous *S. trutta* (P/A/N)	Number of individuals studied		Number of hybrids
			S. salar	*S. trutta*	
1 Näätämö L	0	P	54	17	
2 Nuortijoki	4	P	47	58	1
3 Näätämö, Opukas	0	N	67		
4 Näätämö U	3	P	61	1	
5 Teno, Angeli	-	P	8		
6 Vuomajoki	3	N	66	1	
7 Kuoddoveäijoki	5	N	60		
8 Kuoldnajoki	-	N		39	
9 Luomusjoki	17	N	60	4	1
10 Akujoki	11	N	63	14	
11 Outakoski	-	P	14	6	
12 Teno 4	2	P	66		
13 Nil and Nuvvus	2	N	56	10	
14 Utsjoki U	20	P	56	3	
15 Kevojoki	8	P	104	10	
16 Tsarsjoki U	10	P	83	14	
17 Tsarsjoki L	2	P	41	2	
18 Paddajoki	-	P		89	
19 Kuoppilasjoki	5	P	57	15	
20 Yläseitikko	5	N	62	93	
21 Tsieskuljoki	-	A		66	
22 Utsjoki L	1	P	75		
23 Teno, Karnjarga	0	P	36		
24 Vidgaveädji	2	A	44	7	
25 Teno 29	4	P	53		
26 Vetsijoki L	0	P	53		
27 Vetsijoki U	8	P	61		
28 Pulmankijärvi	-	P	62	21	
29 Skiihpajoki	-	N	58	3	
30 Ylä-Pulmankijoki	6	P	72	8	1
Average	6 %	Total	1,540	481	3

genase (Crozier 1984, Vuorinen and Piironen 1984).

From a total of 2,024 fish, three Atlantic salmon x brown trout hybrids were detected by electrophoresis. All hybrids were classified as most probable F_1 hybrids. One hydrid was from a tributary to River Näätämö and two from separate tributaries to River Teno. The proportion of

Table 2. Comparison of naturally occurring hybrids between brown trout and Atlantic salmon among regions. Excluding cases from North America and River Grönån, the hybridization averaged 0.38 % among European regions (89 hybrids or backcrosses in a total of 23,136, notice that the samples of the study f are included in e). Studies b, e, f, g, i, and l give probably underestimates for hybridization because only one of the parent species was analyzed.

Number of hybrids and/or backcrosses	Number of individuals studied	Percentage hybrids	Samples taken (E=eggs, J=juveniles, S=smolts, A=adults)	Location	Reference
0	1,066	0	E/J	Norway	a)
1	1,500	0.07	J	Scandinavia	b)
3	3,200	0.09	S	British Isles	c)
3	2,024	0.15	A/J	Finland and Norway	d)
28	9,166	0.3	A	British Isles	e)
17	4,431	0.4	A	British Isles	f)
8	2,057	0.4	J	Ireland	g)
12	922	0.9*	A/S/J	Canada	h)
34	3,389	1.0	J	British Isles	i)
8	559	1.4	J	England	j)
1	56	1.8*	J	Canada	k)
4	175	2.3	J	Spain	l)
37	792	4.7*	J	Canada	m)
44	332	13.3	J	Sweden	n)

Asterisk in percentage indicates that hybrization estimate is for location where brown trout are not indigenous.

References: a) Heggberget et al. (1988), b) Gunnar Ståhl, personal communication in Verspoor (1988), c) Solomon and Child (1978), d) this study, e) Solomon and Child (1978), f) Payne et al. (1972), g) Crozier (1984), h) Verspoor (1988), i) Jordan and Verspoor (1993), j) Hurrell and Price (1991), k) Beland et al. (1981), l) Garcia de Léaniz and Verspoor (1989), m) McGowan and Davidson (1992) and n) Jansson et al. (1991).

hybrids was 0.15%, or 0.1% in Teno and 0.3% in Näätämö correspondingly. Among tributaries the proportion of hybrids varied from 0% to 1.6% (Table 1). Hybrid frequencies in the Rivers Teno and Näätämö were quite similar to the value of 0.61% from other European river systems (pooled data from studies a, b, c, e, g, i, j and l in Table 2). If the exceptionally high frequency of 13% in the Swedish River Grönån is excluded, the hybrid frequency for Europe decreases down to 0.41%. In River Grönån the impact of hatchery-reared fish cannot be totally ruled out (Jansson et al. 1991), although the authors assert that the hybrids were of natural origin. Based on this possible impact of hatchery-reared fish, we excluded River Grönån from following comparisons of hybrid frequencies.

A variety of ecological mechanisms can influence the frequency of natural hybridization (see Hammar et al. 1991 and references therein). Hybrid frequency comparisons between populations and geographical regions may provide new information on these factors. The pooled frequency for Scandinavia (including this study) is significantly lower than for the rest of Europe (t=284.9, df=∞, P<0.001). The same conclusions are drawn also by comparing the confidence intervals based on Poisson distribution of the frequencies. For 99% probability the confidence intervals were obtained from the statistical tables of Diem and Lentner (1970) if Nf<101, where N is the sample size and f is the average hybrid frequency. If $Nf \geq 101$ the confidence intervals were calculated as follows: the

lower limit is $(1.288 - (Nf)^{0.5})^2$ and the upper limit $(1.288 + (Nf+1)^{0.5})^2$. The confidence intervals for Scandinavia (0.015-0.27%) and for the rest of Europe (0.34-0.60%) do not overlap. The same conclusions are obtained even if the hybrid frequencies are weighted by sample size.

Verspoor (1988) reported that the hybrid frequency for North America is significantly higher than for Europe. He suggests that this difference could be a consequence of the late introduction of brown trout to North America, compared to the long-time sympatry of the species in Europe. McGowan and Davidson (1992) present further data on hybrid frequencies for North America and doubt the inferences made by Verspoor (1988). Based on Table 2, the 99% confidence intervals for hybrid frequencies in Europe (0.28-0.50%) and Canada (1.9-4.0%) do not overlap if River Grönån is excluded. The finding made by Verspoor (1988) is further supported by the weighted hybrid frequencies, since the confidence intervals are 1.2-3.3% for Canadian populations and 0.20-1.7% for European populations.

The hybrid frequencies in Table 2 were obtained from different age-groups, which may reduce the reliability of our comparisons. It is possible, for example, that the viability of hybrids is lower than that of parental species, so that the proportion of hybrids among adults or smolts may be lower than among juveniles.

The main mechanism segregating Atlantic salmon and brown trout may be the difference in their spawning time (Heggberget et al. 1988). But neither temporal nor physical factors or habitat selection did segregate the spawners of the two species completely (Heggberget et al. 1988). In the Rivers Teno and Näätämö the spawning of Atlantic salmon and brown trout extends through four to five weeks during September and October, but it is not known whether there is temporal segregation in their spawning times.

One of the most evident causes for interspecific hybridization is environmental perturbation, e.g. construction of habitats, transplantation of stocks, and introduction of new species (Campton 1987; Verspoor 1988; Vuorinen 1988).The Teno and Näätämö River systems have both been in their natural condition with fishing being the only prominent human activity. But recently, ever increasing fish farming has become a threat, and some Atlantic salmon escapees have been recognized in River Teno since the sampling for this study was completed.

In non-disturbed environments the most important causes promoting hybridization may be local factors. In the Rivers Teno and Näätämö these could include precocious maturation together with high age of smoltification, biased sex ratio, and coexistence of different life-history types of salmon and trout on the same spawning grounds. Garcia de Léaniz and Verspoor (1989) found 2.3% hybrids among Atlantic salmon parr from Spanish rivers. One of the factors to explain this high frequency was that almost all salmon males became precociously mature. McGowan and Davidson (1992) reported from Newfoundland, based on mtDNA analysis, that the direction of hybridization is solely unidirectional, Atlantic salmon males fertilizing brown trout females.

These observations support the view that precocious maturation of Atlantic salmon males have impact on incidence of hybrids. Percentage of precocious males in Atlantic salmon sample from Rivers Teno and Näätämö varied from 0% to 20%, and among locations with hybrids occurring the frequency of precocious males was 4-17% from the total number of sampled juveniles of Atlantic salmon (Table 1).

All hybrids were found in tributaries where anadromous brown trout most probably coexists with Atlantic salmon. Females of anadromous brown trout are known to be four times more numerous than males in River Teno (Niemelä and McComas 1986). According to subarctic conditions, the age at smoltification is 5-7 years for brown trout (Niemelä and McComas 1986) and 3-6 years for Atlantic salmon within the Teno water system. Sexual maturation before smoltification can increase the probability of hybridization, especially, if precocious males are able to maturate in successive years. Presently there is a lack of evidence of multiple maturations among juveniles of Atlantic salmon.

The relationship between these specific factors and hybridization needs further studies. It is suggested that the incidence of hybrids within the Rivers Teno and Näätämö should be monitored with non-invasive methods, e.g. using adipose fin or blood samples and diagnostic protein loci or DNA markers. More detailed information on brown trout life-history and within water course migrations should be collected. Precocious maturation frequencies of Atlantic salmon are based on small sample sizes per tributary, and to get more reliable estimates, more individuals should be studied.

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Evolution of Morphological Traits in Sea Trout (*Salmo trutta*) Parr (0+) through Sea-Ranching

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During the past five to six decades anadromous species of salmon and trout have been artificially reared and released in nature to such a extent that the number of artificially reared fish may now approach, or even exceed the number of the naturally spawned (wild) fish in certain places. There is concern that such a practice will result in the developmental and evolutionary divergence of the reared strain away from the wild phenotypic norm (e.g., Fleming and Gross 1992), and that interactions will occur in the wild between the reared and the wild stocks in respect of diseases, parasites, behaviour and genetics (Saunders 1991). The rearing procedure probably involves selection for new phenotypes capable of succeeding in this new environment. Selection for traits previously advantageous in the wild may also be relaxed. This will result in a divergence of reared fish from their wild conspecifics and will probably render the former inferior under natural condition (e.g. Fleming and Gross 1992, Petersson and Järvi 1993).

The shape of the fish body is generally supposed to be subject to conflicting selectional pressures (e.g. Riddell and Leggett 1981, Taylor and McPhail 1985a, Fleming and Gross 1989, Swain and Holtby 1989, Swain et al. 1991). For example, the most adaptive shapes for burst and for sustained modes of swimming are different (Webb 1978, Taylor and McPhail 1985b). Burst swimming requires "thrust," which is maximised by increased depth in each propulsive element (such as body height, caudal-peduncle depth, and median fin sizes). Such a body shape is superior because it facilitates escape from predators and confers an advantage in intraspecific interactions. A fusiform body shape, on the other hand, is superior for sustained swimming. In nature, the frequency of intraspecific interactions probably is lower than in a hatchery, but a hatchery contains no predators. Salmon live in running water, both in nature and in hatcheries, but the water speed may be greater and more variable in nature.

Many studies on dominance and agonistic behaviour in salmonid fishes have indicated that sea-ranched populations are more aggressive and more often attain dominance than their wild counterparts (e.g., Fenderson et al. 1968, Moyle 1969, Swain and Riddell 1990). However, game theory predicts the opposite: at extremely high densities (such those occurring in hatcheries) there should be a strong selection pressure for less aggressive individuals, because the number of interactions with conspecifics would make it almost impossible to maintain territories (cf. Doyle and Talbot 1986, see also Fenderson and Carpenter 1971 for discussion of crowding effects). Selection experiment carried out by Ruzzante and Doyle (1991, 1993) suggest that

there is a negative correlation between growth and aggressiveness in food-rich environments, individuals having high grow rate show lower levels of aggressiveness than those growing slower. Ruzzante (1994) concluded that indirect selection on agonistic behaviour as a result of direct selection on growth will be significant if selection is conducted under competitive conditions that promote aggressive interactions, and will be positive or negative depending on whether food is limited or available in excess. Although those later mentioned studies do not discussion body form, rather body size, they indicate that there are no absolute relations between aggressiveness and morphological variables. It is, however, reasonable to suggest that if selection for aggressiveness associates with the body shape, a more robust (deeper) body shape of the more aggressive population (probably the sea-ranched one) is to be expected. However, as noted above, there are also selectional pressures that may lead to streamlining of the body of sea-ranched fish.

In a previous study, we showed that the body morphology of wild and sea-ranched adult sea trout, *Salmo trutta* (L.), differs (Petersson and Järvi 1993). In the present study we compared parr (0+) of these two types of sea trout using fish with a common genetic origin.

The study was made in 1992 at the Fishery Research Station at Älvkarleby, in central Sweden. The research station is situated on the River Dalälven that flows out into the Baltic Sea. Diadromous fishes are hindered from following their natural migration routes, due to the hydroelectrical power plant at Älvkarleby. Adult salmon and sea trout migrating upstream are caught by using a catching case (an underwater trap with a vertically movable base) and transported to a sorting hall, where they are kept and used for artificial breeding.

Two strains originating from the River Dalälven occur in the river today. One strain was established in 1965 when a large number of these fish were caught and used for artificial breeding and cultivation. The released offspring of these fish were marked by cutting the left pelvic fin. This strain is designed the "sea-ranched" strain. The other strain is a mixed one, consisting of all sea trout that have both their pelvic fins intact. Since 1986 this strain has also been used for artificial breeding and their offspring released, without being marked. In addition, this strain has most likely been diluted by the sea-ranched strain during the course of spawning in nature and by sea trout from other rivers introduced to River Dalälven. These other strains originate from the River Weichel (Poland) and the River Klarälven (southern Sweden). The numbers of offspring of these two introduced strains released have never made up more than ten perceant of the strain originating from the River Dalälven itself. All artificially reared fish have been released as smolts (2+). It should be noted that other artificially reared sea trout had been released into the river long before 1965 (since 1872 in fact), but then mainly as fry or as one-year old parr and without being marked. Nevertheless, the offspring of all the unmarked sea trout studied will be referred to as the "wild" strain. In addition, electrophores has been carried out in 1989 on the offspring to marked and unmarked parent fish (H. Jansson, pers. comm.). Eight loci were investigated, and significant differences in gene frequencies between the two strains were found for four loci. Whether this difference were due to a genetic divergence between the two strains or were an effect of the relative small sample size (the fish were sample from the offspring of 25-30 parent pairs). Jansson's study was initially conducted to survey for alleles from the River Weichel strain, but any influence from this foreign strain could not be demonstrated.

In this study we used 100 fish, 50 wild and 50 sea-ranched, for the morphological measurement. Both wild and the sea-ranched fish were a mixture of offspring from several parent pairs (31 wild and 94 sea-ranched pairs, respectively). All parent fish were stripped for eggs and milt, and the fertilised eggs and the hatched juveniles were raised in the hatchery under the same conditions. Each fish used in this study was weighed, and total length, body depth and width, and length of bases of the dorsal, anal and adipose fins measured (Fig. 1). After that, each fish was

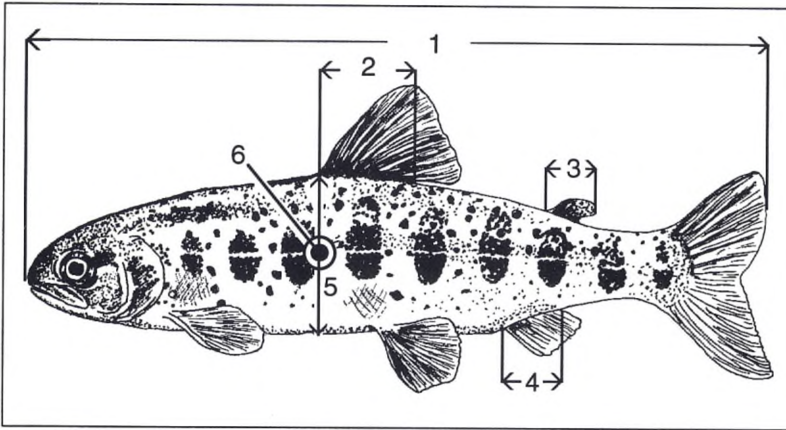

Fig. 1. Measurements made on juvenile sea trout (*Salmo trutta*). 1) Total length, 2) length of dorsal fin, 3) length of adipose fin, 4) length of anal fin, 5) depth, and 6) width (measured as the distance from the centre of the cross to the corresponding spot on the other side of the fish).

dissected for sex determination and the heart removed, and weighed. The comparisons between the two strains were made as follows. First, the means for all variables were compared using Bonferonni's method (Table 1). Second, homogenety-of-slopes (HOS) for variables on body size of sexes within strains were compared. No significant differences could be demonstrated. Thereafter, an analysis of covariance (ANCOVA) between sexes within strains was made for each variable on body size. No significant differences were found, thus the sexes within strains were pooled in the subsequent analyses. Third, a HOS of strains was made for each variable on body size. If the strains showed no difference in slopes for a variable, an ANCOVA was calculated (see Table 2). In all cases multiplicative regressions ($y=a \cdot x^b$, x being the body size, y the trait and a, b constants) have been used. Residuals from separate within-group multiplicative regressions were used in a canonical discriminant analysis. This analysis, however, did not demonstrated any overall differences between the two strains.

On average the sea-ranched fish were larger than the wild ones (Table 1), despite being of the same age and given the same amount of food per total mass of fish in each rearing containment. This probably reflects the fact that sea-ranched fish have been selected for fast growth. Because the hatchery is a risk free environment, individuals that have a high feeding rate might be superior in a hatchery environment. In nature an extraordinarily high growth rate might be disadvantageous, because such individuals will be obliged to search very intensively for food, which might lead to more intra- and interspecific interactions and a to higher risk of exposure to predators (cf. Bennett and Houston 1989). The two strains had significant different slopes for two variables, body depth and base length of the anal fin (Table 2). In both cases the wild strain had a steeper slope than the sea-ranched one. Because the regression lines cross each other in both cases, this might indicate that the wild strain will have a greater body depth and larger anal fin when obtaining the same size as the sea-ranched one. Alternatively, these effects might be due to ontogenetic changes in allometric growth, i.e. the wild strain will look like the sea-ranched one when it develops to a larger size. Further studies have to be done to investigate these questions. Finally, the wild strain had a larger body width than the sea-ranched one (Table 2), and this effect is constant throughout all investigated body sizes. This means that wild fish had a more "cylindrical" body shape. However, whether this is related to swimming performance is unclear.

Swain et al. (1991) found both genetic and environmental differences between wild and sea-ranched juveniles of coho salmon (*Oncorhynchus kisutch*). In their study, the wild-reared fish tended to have relatively longer and deeper heads, relatively shorter midbody lengths, rela-

Table 1. Comparison of different morphological traits of male and female sea trout (*Salmo trutta*) parr (0+) raised in the hatchery, but having parents of wild or sea-ranched origin. Sample size; Sea-ranched females=25, sea-ranched males=25, wild females=27, and wild males=23. The mean values designated by the same letter did not differ significantly at the $P<0.05$-level according to Bonferonni's multiple comparison method.

Variable	Origin/Sex	Mean ± SD
Depth (mm)	Sea-ranched females	25.728 ± 4.200 a
	Sea-ranched males	25.136 ± 3.596 a
	Wild females	21.389 ± 4.191 b
	Wild males	20.435 ± 4.481 b
Width (mm)	Sea-ranched females	14.588 ± 2.602 a
	Sea-ranched males	14.036 ± 2.052 a
	Wild females	12.470 ± 2.401 ab
	Wild males	11.839 ± 2.653 b
Heart (Wet weight, g)	Sea-ranched females	0.040 ± 0.019 a
	Sea-ranched males	0.036 ± 0.015 a
	Wild females	0.024 ± 0.011 b
	Wild males	0.022 ± 0.012 b
Body weight (g)	Sea-ranched females	25.694 ± 12.112 a
	Sea-ranched males	22.823 ± 9.007 a
	Wild females	15.226 ± 7.496 b
	Wild males	13.899 ± 7.243 b
Body length (mm)	Sea-ranched females	128.360 ± 19.451 a
	Sea-ranched males	123.840 ± 14.812 a
	Wild females	106.185 ± 17.079 b
	Wild males	103.217 ± 17.772 b
Adipose fin (mm)	Sea-ranched females	4.592 ± 0.495 a
	Sea-ranched males	4.636 ± 0.588 a
	Wild females	3.996 ± 0.533 b
	Wild males	4.148 ± 0.935 ab
Dorsal fin (mm)	Sea-ranched females	15.576 ± 2.263 a
	Sea-ranched males	15.676 ± 1.980 a
	Wild females	13.415 ± 2.178 b
	Wild males	12.817 ± 2.455 b
Anal fin (mm)	Sea-ranched females	10.876 ± 1.439 a
	Sea-ranched males	10.612 ± 1.474 a
	Wild females	9.137 ± 1.756 b
	Wild males	8.943 ± 1.608 b

Table 2. Differences between strains (of wild or sea-ranched origin) of sea trout (*Salmo trutta*) in regressions of different morphological measurements on body size (i.e. body length, except for heart wet weight were x=body weight). In all cases a multiplicative regression have been used. First a homogeneity-of-slope analysis (HOS) was performed, if this analysis did not reveal any significant difference, an analysis of covariance was performed. The corresponding F-value (for ANCOVA t-value) and level of significance are indicated, NS means that neither analysis revealed any significant difference between the two strains.

Variable	Origin	Regression	Analysis	F/t	*P*
Depth (mm)	Sea-ranched	$0.132 \cdot x^{1.232}$	HOS	4.67	<0.035
	Wild	$0.068 \cdot x^{1.087}$			
Width (mm)	Sea-ranched	$0.064 \cdot x^{1.119}$	ANCOVA	3.69	<0.001
	Wild	$0.040 \cdot x^{1.229}$			
Heart (Wet weight, g)	Sea-ranched	$0.0018 \cdot x^{0.943}$			NS
	Wild	$0.0017 \cdot x^{0.955}$			
Body weight (g)	Sea-ranched	$(5.58 \cdot 10^{-6}) \cdot x^{3.147}$	ANCOVA	3.51	<0.001
	Wild	$(4.11 \cdot 10^{-6}) \cdot x^{3.222}$			
Adipose fin (mm)	Sea-ranched	$0.263 \cdot x^{0.592}$			NS
	Wild	$0.357 \cdot x^{0.521}$			
Dorsal fin (mm)	Sea-ranched	$0.217 \cdot x^{0.884}$			NS
	Wild	$0.126 \cdot x^{0.999}$			
Anal fin (mm)	Sea-ranched	$0.228 \cdot x^{0.796}$	HOS	3.97	<0.050
	Wild	$0.078 \cdot x^{1.022}$			

tively deeper bodies, and relatively larger median fins than the sea-ranched fish did. These results are somewhat in accordance with ours, but our main conclusion have to be similar to theirs: the rearing environment probably affect the body shape of the fish more than the possible differences in genetics. Morphological plasticity in salmonids has been demonstrated in several studies (e.g. Currens et al. 1989, Beacham 1990). The largest difference between the two strains of the sea trout is growth rate, but no conclusions can be drawn concerning aggressiveness. In order to investigate this a closer examination of the effect of domestication on behaviour has to be done. If the aim is to restore, or maintain, a wild population, then the main concern must be to study just how hatchery-rearing should be done so as to result in fish that are as close to wild populations as possible.

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Time

A colon should be used as the separator between hour and minute and between minute and second. The symbols "h", "min", and "s" are not used, since they are the symbols for hour, minute, and second in the sense of duration or the length of time. Thus "12 h 30 min" expresses a measured time of twelve hours and thirty minutes duration whereas 12:30 refers to the time of day.

Prefixes

giga (10^9)	G
mega (10^6)	M
kilo (10^3)	k
milli (10^{-3})	m
micro (10^{-6})	μ
nano (10^{-9})	n
pico (10^{-12})	p

Time and Temperature

day	d
degrees Celsius	°C
hour (spell out for diel time)	h
kelvin	K
minute	min
second	s

Spell out year, month, and week.

In Table and Fig.:

year	yr
month	mo
week	wk

Weights and Measures

centimeter	cm
gram	g
kilogram	kg
kilometer	km
liter (exception to SI)	L
meter	m

Spell out hectare and tonne.

Mathematics and Statistics

all standard mathematical signs, symbols, and abbreviations base of natural logarithm	*e*
common test statistics (*F, t,* etc.)	
correlation or regression coefficient (multiple)	*R*
correlation or regression coefficient (simple)	*r*
degree (angular)	°
degrees of freedom	df
expected value	*E*
intercept	α
logarithm (specify base)	log
minute (angular)	'
not significant	NS
percent	%
probability	*P*
probability of type I error (false rejection of null hypothesis)	*Pα*

probability of type II error (false acceptance of null hypothesis)	$P\beta$
radian	rad
sample size	N
second (angular)	''
standard deviation	SD
standard error	SE
variance	V or var

Physics and Chemistry

all atomic symbols	
alternating current	AC
ampere	A
becquerel	Bq
candela	cd
chemical acronyms listed in Webster's dictionaries (DDT, EDTA, etc.)	
coulomb	C
dextro	D
direct current	DC
electron volt	eV
equivalent	eq
farad	F
gray	Gy
hertz	Hz
hydrogen ion activity (negative log of)	pH
joule	J
levo	L
lumen	lm
lux	lx
molar	M
mole	mol
newton	N
normal	N
ohm	Ω
ortho	*o*
para	*p*
pascal	Pa
per mille (per thousand)	‰
siemens	S
tesla	T
trishydroxymethyl-aminomethane	tris
volt	V
watt	W
weber	Wb

General (some are restricted)

compass directions (maps and coordinates): east	E
north	N
south	S
west	W
et alii	et al.
et cetera	etc.
filial generation	F
for example	e.g.,
international unit	IU
months (tables, figures): first three letters (Feb, Jun, etc.)	
ploidy	n
sex (tables, figures, hybrid crosses): female	♀
male	♂
that is	i.e.,

Word List

The spelling of the following words is frequently inconsisten in submitted manuscripts. We prefer that authors adhere to the Journal's house style for these commonly used terms:

age-class (n.)
age-group (n.)
aquaculture (n.)
Arctic char (n.)
brackish water (n.)
brackish-water (adj.)
chi-square (n., adj.)
cold water (n.)
cold-water (adj.)
deep sea (n.)
deep-sea (adj.)
deep water (n.)
deepwater (adj.)
freshwater (n., adj.)
fresh water (n.)
groundwater (n., adj.)
hard water (n.)
hardwater (adj.)
headwater (n., adj.)
lake water (n., adj.)
meltwater (n., adj.)
open water (n.)
open-water (adj.)
percent (n.)
salt water (n.)
saltwater (adj.)
sea-run (adj.)
seawater (n., adj.)
shallow water (n.)
shallow-water (adj.)
short term (n.)
size-class (n.)
snowmelt (n.)
soft water (n.)
softwater (adj.)
tidewater (n., adj.)
t-test (n., adj.)
warm water (n.)
warmwater (adj.)
year-class (n.)
young-of-the-year (n., adj.)

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