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Deviating vertical distribution and increased conspicuousness of parasitized *Calanus*

Abstract—Abnormally colored yellow and red *Calanus* spp. occurred in the Oslofjord (southeast Norway) in late summer. These specimens were infected with an extensive parasitic growth consisting of large branched hyphae-like tubes filled with spores. This parasite has previously been referred to as *Ichthyosporidium* sp. (now *Ichthyophonus*, Ichthyosporidia), and suspected to be a stage in the life cycle of the fish pathogen *Ichthyophonus hoferi*. This assumption was not supported by our examination. Infected copepods were virtually confined to the upper meter, while distribution of the uninfected specimens was much deeper. We argue that the change in color and distribution is induced by the parasite, facilitating near-surface, visual predation, dispersal of spores, and, hence, increasing transmission to new hosts.

During summers 1996–2000 we observed anomalously yellow and red *Calanus* spp. close to the surface in the Oslofjord. They were invariably infected with a parasite resembling earlier descriptions of *Ichthyosporidium* Caullery et Mesnil, 1905 infections in *Calanus* and other copepods (Apstein 1911; Chatton 1920; Jepps 1937a). The genus *Ichthyosporidium* was later fixed as a genus of microsporidians (Sprague 1965), and the fungus-like parasites previously ascribed to it transferred to the genus *Ichthyophonus* Plehn et Mulchow, 1911. *Ichthyophonus hoferi* Plehn et Mulchow, 1911, an important pathogen on numerous fish species, has repeatedly caused significant mortalities in North Atlantic fish stocks, particularly herring (Rahimian and Thulin 1996; Holst et al. 1997, and references in Sindermann 1990). Jepps (1937a) suggested that the infection in *Calanus* might be identical with the *I. hoferi* that infects fish. We wanted to determine whether this infection is identical with *I. hoferi* infecting marine fish and whether the parasite affects the vertical distribution of infected *Calanus*. Vertical distribution, together with color and contrast, may strongly affect susceptibility to predation (e.g., by planktivorous fish), and hence parasite transmission.

Appearance of infected *Calanus* and parasite—Infected *Calanus* spp. were visually identified close to the surface and collected with a bucket for examination in August 1998. *Calanus* spp. were collected for photographing the same way in September 2000 (Fig. 1A). The yellow parasite was highly visible through the exoskeletons, which to a varying extent were covered with deep red lines, covering most of the exoskeleton in some specimens. Transparency of infected individuals was strongly reduced. The transparent uninfected specimens displayed no unusual color. Infected animals were negatively buoyant and swam actively when placed in glass

containers, hence eliminating buoyancy as an explanation for very shallow distributions of infected specimens. Free “spores” were released when the exoskeleton and “hyphae” were ruptured. Both *Calanus helgolandicus* and *C. finmarchicus* occur in the Oslofjord, *C. helgolandicus* being the dominating species (Sars 1903; Bagøien et al. 2000). Ten infected and ten uninfected specimens captured in 1996 were all identified as *C. helgolandicus* from their clearly sharp head profile, a character which has proved adequate for discriminating between *C. helgolandicus* and *C. finmarchicus* in the Oslofjord (Frederik Beyer pers. comm.).

For histological examination, live copepods were fixed in a modified Karnovsky fixative and embedded in Epon 812 (see Nylund et al. 1992). Semithin sections (0.5 μm) were stained with toluidine blue or PAS. Air-dried smears of hyphal material were stained with Diff-Quick (Dade). The infections appeared as branched yellow-brown hyphae, filling much of the available space in the copepod cephalothorax (Fig. 1B). Branches occasionally entered the basal parts of extremities. Central hyphae (in sections) measured 28–68 (mean 52) μm in diameter, while peripheral hyphae (branches) were thinner, 18–34 (27) μm . All hyphae were filled with small spores, measuring 6.4–7.7 (6.9 ± 0.3 SD; $N = 25$) μm in diameter in sections and 8.0–10.4 (9.4 ± 0.5 ; $N = 29$) μm in air-dried smears. The spores show an eccentrically placed indistinct nucleus with a distinct nucleolus and a central aggregate of vacuoles. The vacuoles, up to 23 in sections, form a round or bilobed group; if bilobed it is influenced by the nucleus. In smeared material all or most of these vacuoles have fused into one or two large ones, measuring $3.5\text{--}4.2 \times 2.8\text{--}3.4$ μm . Both hyphae and spores were thin walled.

The present parasite conforms completely to “*Ichthyosporidium*” sensu Jepps (1937b), originally described by Apstein (1911) in *Calanus* spp. from the North Sea and Kattegat. Apstein (1911) described several parasites (Parasit 6–8,10) that may be related to the present organism (Jepps 1937b), but the descriptions are insufficient. Chatton (1920, p. 285–287) also briefly describes an *Ichthyosporidium* infecting *Acartia*, *Paracalanus*, and *Clausocalanus* in the Mediterranean. Chatton’s form shows branched, yellow-red hyphae 20 μm in diameter, which when mature contain large numbers of 4–5-mm large uninucleate spores. While apparently closely related to “*Ichthyophonus*” from *Calanus* (this study), the Mediterranean parasite differs in dimensions of hyphae and spores.

Both the fish parasitic pathogens *Ichthyophonus hoferi* and the related form *Cyclopterocola marina* Apstein, 1910 can be grown in a bovine serum supplemented MEM Eagle’s

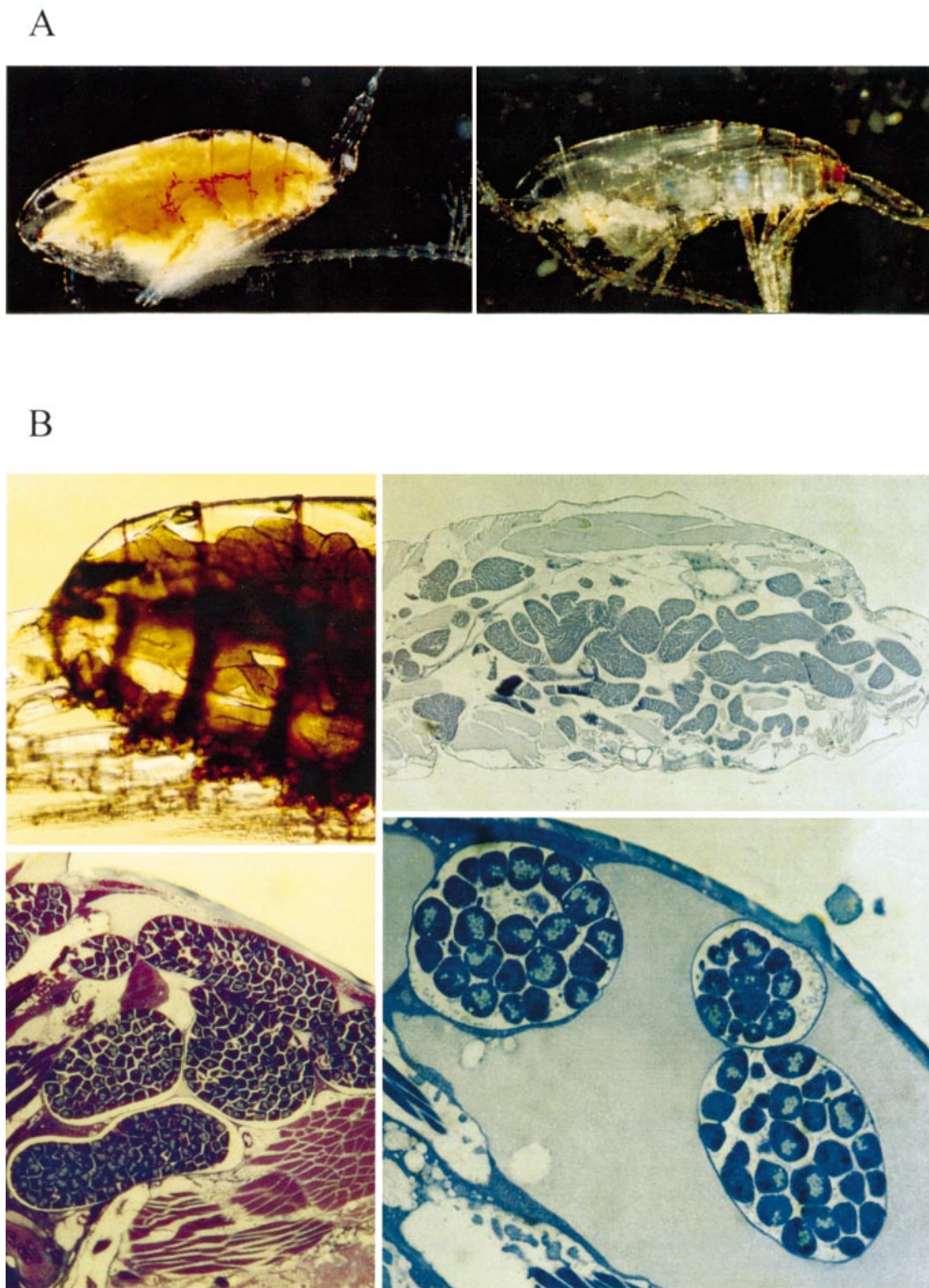


Fig. 1. (A) Infected (left) and uninfected (right) *Calanus helgolandicus* CVI females, photographed through a dissecting microscope. (B) Parasitic organism infecting *C. helgolandicus*; upper left, posterior metasome of live copepod filled with yellow-brown hyphae ($\times 72$); upper right, sagittal section of anterior metasome showing hyphal mass ($\times 72$); bottom left, hyphae ventral in anterior metasome ($\times 445$); bottom right, detail of hyphae showing spores ($\times 1145$).

medium at acid or neutral pH (Okamoto et al. 1985; Hodneland et al. 1997). We have successfully cultured both *I. hoferi* and *C. marina* repeatedly, but we were unable to culture the *Calanus* parasite in this medium at pH 3 and 7. *I. hoferi* from herring was cultured as control. Therefore, and

because of differences in morphology between *I. hoferi* (cf. Rahimian 1998) and *Ichthyophonus* from *Calanus* spp. (this study), it is unlikely that the *Calanus* parasite is *I. hoferi*. We find it more probable that it is a specific *Calanus* pathogen.

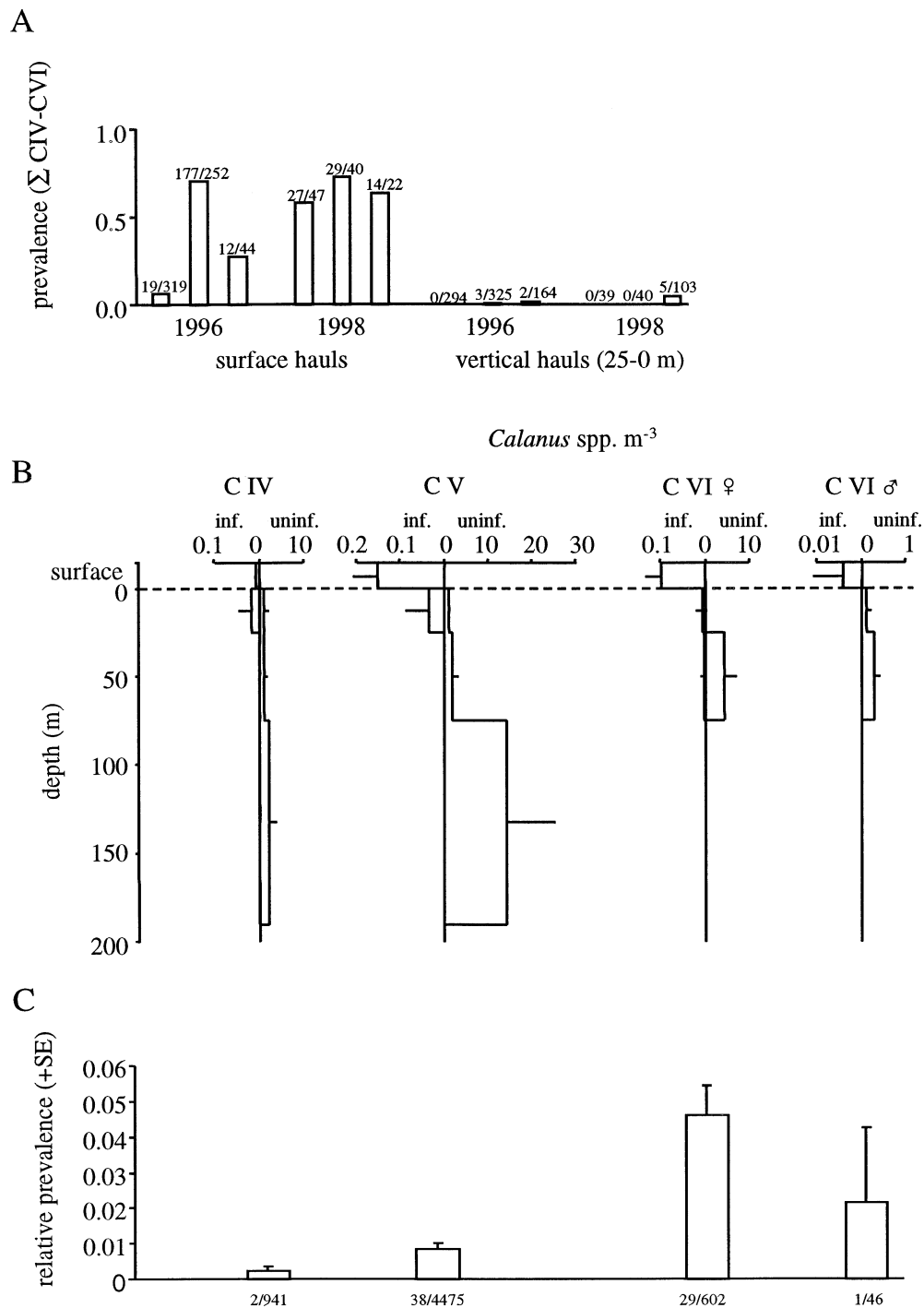


Fig. 2. (A) Prevalences for *Calanus* spp. (Σ CIV–CVI) in the surface (horizontal tows) and at 0–25-m depth (vertical hauls). Y-axis scale denotes proportion of animals diagnosed with the infection. Fractions above bars denote number of infected copepods caught divided by total number of copepods caught. *Calanus* spp. were collected with nets around noon on 28 August 1996 and 7 August 1998. In 1996, the upper 40 cm were sampled with a horizontally towed 150- μm minineuston net (Schram et al. 1981). The upper \sim 25 m was sampled using a 125- μm , 40-cm diameter plankton net, sampling both on the way up and down. In 1998, we sampled with a modified Nansen net (1-m diameter, 500- μm mesh size) equipped with a closing device at a 200-m deep station. The upper meter was sampled by towing the net after the boat for 3 min at \sim 0.7 m s^{-1} and filtering \sim 100 m^3 water and the water column by vertical hauls with randomized depth sequence. The net was closed at the preselected depth by letting a weight glide along the wire to release the closing device. Three replicate series were collected both years. (B) Vertical distribution of infected (left) and uninfected (right) *Calanus* spp. in 1998. 1 SD is shown. Note that scales for infected and uninfected and CVI males and the other stages differ. Description of sampling is given in (A). (C) Estimated stage-specific relative prevalence in 1998. 1 SE is shown. Fractions below bars denote (Σ infected specimens in surface hauls) (Σ uninfected specimens in vertical hauls) $^{-1}$. See text for explanation.

Distribution—We visually detected infected *Calanus* in the upper 10 cm on many occasions during the period 1996–2000, with abundances peaking during late summer. We never observed infected specimens from October until May. Particularly dense aggregations, sometimes >10 infected *Calanus* L^{-1} , were repeatedly observed in surface convergences, both close to land (<1 m) and in Langmuir rows and other open water convergence zones. Within-day and day-to-day variations in abundance and local distribution were great, apparently depending on meteorological conditions and tides. Generally, infected copepods seemed to appear in the area during and after periods of southern winds that caused advection of surface water into the fjord.

The infected specimen's affinity for the surface was confirmed by consistently much higher prevalences (pooled for stages IV–VI (CIV–CVI)) in horizontal surface tows than in vertical tows from the upper ~ 25 m, both in 1996 and 1998 (Fig. 2A). In 1998, when the entire water column was sampled, virtually all infected specimens were caught in the surface tows (Fig. 2B, details about sampling in legend). This pattern was consistent across the stages, although very few infected CIV and CVI males were caught (two and one respectively). Uninfected *Calanus* spp. were distributed deeper. CIV and CV were caught mainly below 75 m, while the adults were concentrated in the 25–75-m interval.

Few plankton investigations include horizontal, surface plankton hauls. This, together with the surface affinity of *Ichthyophonus*-infected *Calanus* spp., their very horizontally patchy distribution, and the ephemeral nature of their presence, probably explains why the very obvious effects of the infection on *Calanus* distribution have not been previously described.

Prevalence—Overall prevalence (stages CIV–CVI, based on vertical tows) from the 1998 sampling was 1.0‰. The population's prevalence is certainly even lower, since our investigation apparently was conducted in spatiotemporal aggregations of infected specimens.

Assuming that the surface-integrated abundances of healthy copepods of each stage at the location of the 1998 investigation reflected the demography of the population, relative prevalence, measured as $(\sum \text{infected specimens in surface hauls}) / (\sum \text{uninfected specimens in vertical hauls})^{-1}$ increased from CIV, through CV, to CVI females (Fig. 2C), with nonoverlapping binomial 95% CIs. This is in accordance with the general pattern of parasite prevalence of *Calanus*, described by Marshall and Orr (1955).

General discussion—The shallow distribution of infected *Calanus* spp. dramatically increases their risk of being seen by visual planktivores (Aksnes and Giske 1993). Furthermore, the parasite itself makes *Calanus* spp. much less transparent, and hence more visible to predators, as does the red color at the very shallow depths where red light penetrates. Fish kept in aquaria willingly ate infected *Calanus* spp., rendering the possibility of the infection causing bad taste or noxiousness unlikely. The accumulation in convergences resulting from their surface affinity may also increase their risk of predation, since fish often feed in these zones. Sims and Quayle (1998), studying the distribution of filter feeding

basking sharks, also reported that many species of visually hunting, planktivorous teleosts concentrated their feeding in small-scale, plankton-accumulating fronts.

Since the introduction by Holmes and Bethel (1972) of the manipulation hypothesis, that parasites induce behavioral changes in their hosts that increase probability of transmission, numerous examples of this phenomenon have been reported in the literature. However, not all behavioral abnormalities of infected animals are beneficial to or induced by the parasite (Moore and Gotelli 1990; Poulin 1995). The vertical distributions of uninfected *Calanus* spp. differed strongly between stages. The CIVs and CVs had already descended for their winter dormancy (see Bagøien et al. 2000), the females were probably active and feeding at intermediate depths, while the distribution of males closely resembled that of females. That stages with highly differing innate needs and behaviors respond identically to infection and that the upper decimeters probably did not offer significantly higher profitability in any respect than deeper strata indicate that infected *Calanus*'s strong orientation toward the surface is directly induced by the parasite and is not a result of altered needs of the host for some resource as a result of the infection.

If the parasite is a specific *Calanus* pathogen, the general advantage to the parasite from predation may simply be that infectious spores are dispersed by the fish, either by release when ruptured in their mouth or by defecation, hence enhancing horizontal transmission to new copepod hosts. Even when filling much of the host, the parasite mass does not protrude through its exoskeleton. Therefore, if the host is not eaten, it would ultimately die and sink to the bottom before the parasite spores are released. There the probability of transmission to new hosts is likely low.

Predation-risk enhancing changes in host appearance and behavior may be common in parasitized copepods. Marshall and Orr (1955) write, "it is a curious fact that several [parasites on *Calanus*] induce in their host a bright red color. The color is sometimes in the parasite itself (. . .) but is often an unusual development of the pigment always found in *Calanus*." There is also some evidence that parasite-induced change of color is accompanied by shallower vertical distribution of copepods when caused by other parasites than the one described here. Together with the *Ichthyophonus*-infected *Calanus* spp. collected in 1998 in the upper 10 cm for inspection, we collected three *Temora longicornis*, all infected with larvae of a didymozoid trematode. Inspections of hundreds of *T. longicornis* from other collections have revealed no didymozoid larvae (unpubl. data). Also, together with the *Calanus* spp. collected at the surface for photographing in September 2000, three unusually orange *Centropages typicus* were captured, each being parasitized by a trematode metacercaria. Hence, induced elevated distribution accompanied by red coloration may be a general transmission-enhancing mechanism of parasites infecting zooplankton copepods. It is, however, possible that the red color of the hosts is induced by the stronger UV radiation experienced at their shallow depths, rather than by the infection directly. Copepods tend to display increased concentrations of the red carotenoid astaxanthin, acting as an antioxidant, neutralizing free radicals, in environments of high UV ra-

diation (Hansson 2000, and references therein). Irrespective of the mechanism causing the coloration, the shallow distribution of highly visible copepods will facilitate near-surface predation. Although the life cycle of the *Calanus* parasite is unknown, our interpretation of these observations is that they reveal a parasite-mediated manipulation that promotes parasite transmission.

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