# Subitaneous eggs of freshwater copepods pass through fish guts: Survival, hatchability, and potential ecological implications

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## Abstract

We measured the survival of subitaneous eggs of two calanoid (*Eudiaptomus gracilis, E. graciloides*) and two cyclopoid (*Cyclops abyssorum, Macrocyclops albidus*) freshwater copepods after they had been consumed by fish. Unexpectedly, over 80% of the calanoid eggs and 30–59% of the cyclopoid eggs were morphologically intact in fish feces. Subitaneous eggs of *E. graciloides* showed similar proportions of gut passage as dormant resting eggs. About 70–80% of the calanoid eggs and 35–50% of the cyclopoid eggs released nauplii within 3 d. Hence, a total of 50–70% of the calanoid eggs and 11–29% of the cyclopoid eggs survived ingestion and gut passage. Survival was slightly higher because of shorter gut passage time when the fish had been prefed natural plankton compared with hungry fish. We interpret digestion resistance of subitaneous eggs in copepods as an adaptation to fish predation on egg-carrying females.

Females of most freshwater copepod species do not spawn their eggs freely, but carry them in egg sacs attached to the genital segment (Einsle 1993). Egg-carrying females are more conspicuous and possibly constrained in their escape ability, which renders them more prone to fish predation than their nonovigerous conspecifics (Brooks and Dodson 1965). Hence, the often-observed skewed sex ratio of adult copepods has been suggested to be a consequence of increased fish predation pressure on females (Sandström 1980; Flinkman et al. 1992). Winfield and Townsend (1983) and Hairston et al. (1983) confirmed this mechanism experimentally when they showed that fish had a greater reaction distance (i.e., higher predation efficiency) for egg-carrying copepod females. Vuorinen et al. (1983) observed a preference of sticklebacks for ovigerous *Eurytemora* at high densities, and inferred selective predation from the more pronounced diel vertical migrations of the ovigerous copepods. Hairston et al. (1983) estimated the costs in terms of population growth rate of carrying subitaneous eggs that develop immediately to be high, and they suggested the production of resting (diapausing) eggs that develop later as an alternative strategy.

Like other zooplankton (rotifers, cladocerans), some copepods can produce diapausing eggs that are resistant to unfavorable environmental conditions and can survive in dormancy for many years (Hairston 1996). Resting eggs have a durable outer shell (Hairston and Olds 1984) or are enclosed in a chitinous case (ephippium of cladocerans). It has been demonstrated that passage through the gut of a fish (Mellors 1975; Hairston and Olds 1984) or invertebrates (Marcus 1984) is among the harsh conditions that they can withstand. On the contrary, subitaneous eggs were not considered to be digestion resistant, as they have a thinner outer

membrane (Hairston and Olds 1984). Population dynamics models (Argentesi et al. 1974; Threlkeld 1979; Taylor and Slatkin 1981) assumed a close correlation between mortalities of females and eggs. However, Marcus (1984) had already noticed that more than 50% of the subitaneous eggs of the marine copepod *Labidocera aestiva* that had been ingested by worms were still viable in the fecal pellets. Various qualitative studies reported the presence of intact copepod eggs in fish guts, although it was not always clear if these were subitaneous or resting eggs (reviewed in Conway et al. 1994). Although the proportions of subitaneous eggs that passed through fish guts were not determined, eggs of brackish water copepods were found to be viable within fish feces. Redden and Daborn (1991) found that 90% of *Eurytemora* eggs that had passed through the gut of *Menidia* successfully were viable and able to hatch, which was not different from the controls. Hatching success was lower (about 60%) in other studies (Flinkman et al. 1994; Saint-Jean and Pagano 1995). The most comprehensive and quantitative study with six marine copepod species fed to larval turbot is by Conway et al. (1994). They found large differences between copepod species. Between 21% and 94% of subitaneous eggs passed through the fish gut undigested. After gut passage, eggs of one group of copepods exhibited 67–92% viability (similar to controls), but only 1.1% and 1.5%, respectively, hatched in two of the species. Although digestion resistance of subitaneous eggs seems to be widespread in marine and brackish copepods, there is little information on freshwater species. Gliwicz and Rowan (1984) found viable subitaneous eggs of *Cyclops abyssorum tatricus* in the hindguts of brook char. They suggested that eggs passed through the fish intestines unharmed, and this mechanism enabled coexistence of the cyclopoids with fish. Some nauplii of *Eudiaptomus gracilis* have been observed hatching from feces of vendace (Flinkman et al. 1994), but quantitative studies are lacking.

Digestion resistance of marine copepod eggs has been interpreted as an important energy loss to larval fish feeding on copepods (Conway et al. 1994). This may not be so important in freshwaters, as young fish there have sufficient alternative (and preferred) prey, e.g., cladocerans. However,

Table 1. Origin of the copepods. Copepods carried subitaneous (sub) or resting (rest) eggs. Origin of predators was Schöhsee for *Perca fluviatilis* and Vierer See for *Gasterosteus aculeatus.*

| Copepod species | Origin | Experimental period (2003) | Egg type |
|---|---|---|---|
| *Eudiaptomus gracilis* | Schöhsee/Plußsee | Aug–Nov | sub |
| *E. graciloides* | Schöhsee | Sep–Oct | sub |
| *E. graciloides* | Selenter See | Nov | rest |
| *Cyclops abyssorum* | Schöhsee | Jun–Aug | sub |
| *Macrocyclops albidus* | laboratory culture | Nov | sub |

if digestion resistance of subitaneous eggs is also common among freshwater copepods, this will have consequences for our understanding of zooplankton dynamics and life histories: (1) Present population dynamics models may underestimate copepod birth rates. (2) Competitive relationships between cladocerans and copepods may be altered if copepod eggs survive gut passage, but cladoceran eggs do not. Gliwicz and Rowan (1984) may be reporting an extreme example for high-mountain lakes dominated by *Daphnia* in the absence of fish, but by *Cyclops* in the presence of fish. (3) Subitaneous eggs may play a role as dispersal agents being transported in the guts of vertebrates.

Fish predation is important both in the sea and in freshwaters. Hence, egg-carrying copepods should have evolved similar strategies, one of them being digestion resistance of their eggs. We tested the hypothesis that digestion resistance of subitaneous eggs is important in freshwater copepods. We predicted that investment into egg resistance will vary between copepod species in relation to their exposure to predators and to other life-history traits. Therefore, we studied pelagic and littoral species, as well as species with small and large clutches. As an "internal standard" we compared subitaneous and resting eggs of the same species.

## Materials and methods

We offered egg-carrying females of two calanoid and two cyclopoid freshwater copepods to their natural fish predators. Three pelagic copepods, the calanoids *E. gracilis* (Sars) and *E. graciloides* (Lilljeborg) and the cyclopoid *C. abyssorum* (Sars), were fed to juvenile (8–10 cm) perch (*Perca fluviatilis* L.). The littoral cyclopoid copepod *Macrocyclops albidus* (Jurine) was fed to adult (3–5 cm) three-spined stickleback (*Gasterosteus aculeatus* L.). All copepods and fish originated from meso- to eutrophic lakes in the vicinity of Plön (Schleswig-Holstein, Germany) where they coexist (Table 1). With the exception of *M. albidus*, which had been maintained as a laboratory culture at the Max Planck Institute for Limnology by I. van der Veen, all copepods were obtained from the lakes shortly before the experiments. Juvenile perch were caught in May 2003 and acclimated to the experimental conditions in a 200-liter aquarium in a temperature-controlled room (18°C, 12:12 h light:dark cycle) where all handling, feeding trials, and hatching tests were carried out under identical conditions. Fish were fed *Chironomus* larvae ad libitum every second day. Sticklebacks were collected at the end of 2003 and kept in a 30-liter aquarium under the same conditions as perch. Pelagic

copepods were freshly collected by a plankton net when they were abundant and carried eggs in the field (Table 1). Plankton samples were brought to the laboratory immediately and were sorted under a dissecting microscope. Egg-carrying females were kept in 1-liter glass jars in the same temperature controlled room as the fish. They were kept in the laboratory for a maximum of 6 d, fed cultured flagellates (*Cryptomonas* spec.), and transferred to fresh, filtered lake water every other day. *E. graciloides* were collected from two sites at different dates to obtain females with both subitaneous and resting eggs. An earlier study by Zeller et al. (2004) had shown that the eggs carried by *E. graciloides* in Selenter See in November were almost exclusively resting eggs. Although the two egg types cannot be discriminated microscopically, the later hatching experiments confirmed that the eggs collected in November were in fact resting eggs.

We performed two types of experiments with "hungry" and "prefed" fish. In preparation for an experiment, fish were individually kept in a 16-liter aquarium with freshly filtered, aerated lake water. They were fed chironomid larvae and feces were removed frequently. After 2 d, they were transferred again into clean water. One group (hungry) was left unfed for 3 d while the second (prefed) was offered a mixture of *Daphnia,* frozen *Artemia,* copepods without eggs, and small amount of chironomid larvae to mimic a natural diet.

Before each feeding trial, copepod females were isolated and their egg numbers determined. Small subsamples of the copepod populations were transferred into a petri dish and the copepods were gently narcotized with a few drops of carbonated water. Egg-carrying females were then sorted under a dissecting microscope and transferred into small glass beakers, taking care that they recovered quickly. As eggs per female could not be counted on live copepods, a random subsample of 30 females from each population to be offered to the fish was isolated, egg sacs removed by needles, and eggs counted. Average ($\pm 1$ SD) egg numbers per female varied greatly between calanoids and cyclopoids. They were much lower for *E. gracilis* ($6.0 \pm 1.4$) and *E. graciloides* ($4.8 \pm 1.5$) than for *C. abyssorum* ($36.0 \pm 3.5$) and *M. albidus* ($27.8 \pm 3.6$). The total number of eggs offered to each fish was estimated from the mean number of eggs per female and the number of females.

Resting eggs required a special treatment, as they were not recognized as such under the microscope. Of the suspected resting eggs (collected in November), 5 times 10 clutches (200 eggs) were removed from the females and incubated in a petri dish with filtered lake water. They were

monitored during the following 2 weeks for hatched nauplii or decomposed eggs. Eggs that did not hatch or decay within 2 weeks were considered resting eggs (Hairston and Munns 1984; Santer et al. 2000). None of the 200 suspected resting eggs hatched and only two of them decomposed; hence we considered all *E. graciloides* eggs collected in November from Selenter See to be resting eggs.

To start a feeding trial, fish that had been adapted to the respective food conditions were placed into 3 liters of filtered water and the egg-carrying females were added after 15 min. Each fish received either 30 females of a calanoid species or 20 females of a cyclopoid species. After feeding on the copepods for 1 h, each fish was placed into a small intermediate container while the contents of the aquaria were filtered through a 30-$\mu$m gauze to collect all remaining copepods and eggs. The aquaria were then filled with clean filtered water and the fish were put back. Hungry fish were transferred back immediately, whereas prefed fish were offered the food mix again for 30 min before they returned to clean water. The successive treatment was identical for both groups. To stimulate digestion, some chironomid larvae (10–15 per perch, 5–7 per stickleback) were offered after the feeding trial. Feces appeared 5–7 h after the copepod meal when the fish received the food mix, but not until after 17–20 h in the "hunger" treatment. They were frequently collected by a pipette until 48 h after the feeding trial. Fish were then returned to the holding tank, and the contents of the experimental container were again screened through a gauze to collect eggs that might have been released from feces by fish action. The feeding experiments were performed on successive days (i.e., in blocks). There were five blocks per species of five replicates each for the "hungry" treatment and three blocks of three replicates for the "prefed" treatment.

Feces were checked for intact eggs under the microscope. Eggs were collected with a pipette, counted, and transferred to a petri dish containing filtered lake water. Controls were set up with eggs removed from healthy females before the feeding trial. They were treated in the same way as the eggs removed from the feces. Eggs were monitored once or twice a day for hatched nauplii that are supposed to appear from subtaneous eggs after 2–3 d at 18°C (Einsle 1993). Monitoring was continued until all eggs had released nauplii or had decayed. Eggs were considered viable only when nauplii hatched. *E. gracilis* and *E. graciloides* were used to test for effects of gut passage on the survival of nauplii after hatching. Subsamples of 30 hatched nauplii each of treatments and controls were placed into 1-liter glass jars and fed *Cryptomonas*. Surviving nauplii were counted and transferred into fresh medium every second day for 2 weeks. This experiment was repeated three times (i.e., with 90 nauplii each).

Numbers of eggs consumed by each fish were estimated from the difference between the eggs offered and those found in the aquarium after the feeding trial. They varied, but were always between 100 and 180 for the calanoids and between 370 and 720 for the copepods. We were then able to calculate the proportions of consumed eggs that had passed the gut morphologically intact, the proportion of viable eggs in the feces, and the proportion of consumed eggs

that produced nauplii in treatments and controls. The difference of the latter was defined as egg mortality imposed by the gut passage. Only gut passage could be compared for resting eggs, as they did not hatch by definition. After checking for homogeneity of variances and normality of residuals, we tested for differences between treatments and copepod species by nested analysis of variance (ANOVA) for the arcsin proportions with species and food as fixed factors and block as nested factor. The ANOVA was followed by a Tukey–Kramer post hoc test. The effect of gut passage on survival of nauplii was tested by two-way ANOVA, and the difference in gut passage of subtaneous and resting eggs by one-way ANOVA. All ANOVAs were carried out using the NCSS statistical package (Hines 2000).

## Results

Rather high proportions of consumed subtaneous eggs were recovered morphologically intact from the feces, both in the hungry and the prefed fish (Table 2). Percentages of eggs that passed through the gut ranged from 80% for calanoid eggs to 30% for *M. albidus*. The copepod species had a marked effect (Table 3). A Tukey–Kramer post hoc test showed that both calanoid copepods differed from the cyclopoids, but not from each other, whereas there was a significant difference between the cyclopoids. Gut passage was slightly lower for hungry fish in all copepods. Resting eggs of *E. graciloides* passed through the gut of perch significantly better than subtaneous eggs ($F_{1,38} = 9.45$, mean squares [MS] = 0.14, $p = 0.012$), but there was no effect of the food treatment. The mean ($\pm 1$ SD) proportion of resting eggs recovered in feces ($n = 18$) was 86% $\pm$ 5.1% (cf. Table 2 for subtaneous eggs). Within 2 weeks, no nauplii hatched from 1,961 recovered and 738 control eggs, but 2.5% of the recovered and 0.4% of the control eggs decomposed. This assured us that the females fed to the fish in this experiment carried exclusively resting eggs.

In addition, there was a significant effect of copepod species (but not food level) on the proportion of subtaneous eggs that hatched after gut passage. Eggs of the two calanoid species were significantly more viable than those of the cyclopoid species, but there was no significant difference within the two groups. Differential gut passage and viability resulted in an even more pronounced difference between calanoids and cyclopoids for the proportion of ingested eggs that hatched. They ranged from $\approx$70% in *E. gracilis* to only $\approx$12% in *M. albidus*. The effect of food was not very strong, but marginally significant.

However, hatching proportions differed also in the controls. Over 90% of the isolated eggs hatched in both *Eudiaptomus* species, whereas only $\approx$70% hatched in the cyclopoids. Pooled proportions are given in Table 2, as there was no significant difference between the controls of the two food treatments. The species effect was highly significant ($F_{3,22} = 44.5$, MS = 0.35, $p < 0.001$) and was due to the difference between calanoids and cyclopoids. Because of the species differences in natural mortality of eggs, the differences in hatched proportions of ingested eggs alone do not reflect the true effect of gut passage. Hence, we calculated

Table 2. Gut passage and survival of subitaneous eggs of the four copepod species offered to prefed or hungry fish. Means (±1 SD) of *n* experiments.

| | | Recovered in feces | | Proportion hatched | | |
| | | of ingested | viable | of ingested | of controls | |
| Species | *n* | (%) | (%) | (%) | *n* | (%) |
|---|---|---|---|---|---|---|
| *Eudiaptomus gracilis* | | | | | 8 | 91.0 (4.5) |
| prefed | 9 | 83.2 (10.5) | 82.9 (5.4) | 68.9 (9.8) | | |
| hungry | 25 | 81.3 (11.0) | 71.9 (12.2) | 58.9 (14.3) | | |
| *E. graciloides* | | | | | 8 | 91.5 (2.4) |
| prefed | 9 | 82.6 (6.7) | 72.5 (19.9) | 59.9 (10.2) | | |
| hungry | 25 | 76.2 (6.7) | 67.0 (14.8) | 51.3 (13.1) | | |
| *Cyclops abyssorum* | | | | | 8 | 71.3 (7.1) |
| prefed | 9 | 58.5 (7.8) | 50.5 (12.8) | 29.4 (8.2) | | |
| hungry | 23 | 38.3 (11.4) | 35.1 (12.5) | 14.0 (6.8) | | |
| *Macrocyclops albidus* | | | | | 6 | 65.9 (5.9) |
| prefed | 9 | 34.1 (11.9) | 35.7 (15.9) | 11.4 (4.5) | | |
| hungry | 9 | 30.2 (8.3) | 39.4 (10.4) | 12.6 (6.7) | | |

the mortality imposed by gut passage as the difference between proportions in treatments and respective controls. There is still a significant species effect due to higher mortality in cyclopoids, but the food effect is no longer significant (Table 3). The treatments were, therefore, pooled to demonstrate the impact of gut passage on hatching success (Fig. 1). The smaller the negative impact of gut passage, the higher the digestion resistance of the eggs. Gut passage reduced hatching success by only 27% in *E. gracilis* and 37% in *E. graciloides,* but by 53% in *C. abyssorum* and 58% in *M. albidus.* Subitaneous eggs of calanoids are clearly more resistant than those of cyclopoids.

Once the calanoid nauplii had hatched, gut passage had no further impact on their survival during the following 2 weeks. There was no significant difference between nauplii hatched from eggs that had passed through the fish guts and controls. In addition, survival rates of *E. gracilis* and *E. graciloides* nauplii did not differ significantly. Approximately 70% of both species survived in treatments as well as controls.

## Discussion

As in marine and brackish water copepods (Redden and Daborn 1991; Conway et al. 1994; Flinkman et al. 1994;

Saint-Jean and Pagano 1995), subitaneous eggs of all four freshwater copepod species passed through the gut of fish in considerable proportions. The range of proportions passing through the gut morphologically intact (83–30%) is similar to the results obtained by Conway et al. (1994) for marine species. The study by Conway et al. (1994) is the only one that estimated ingestion rates of eggs by the fish, and thus has quantitative data to compare with ours. All other studies tested the viability of recovered eggs, but did not know how many were lost during gut passage. To evaluate the ecological impact of gut passage, however, it is necessary to know how many of the ingested eggs finally produce nauplii, and if these nauplii survive as well as those hatching directly. Nauplii must not only hatch, they must also be able to escape the feces that are initially covered by a peritrophic membrane. All studies so far removed the eggs from gut contents or feces; hence, hatching rates may be overestimated. If remaining inside the peritrophic membrane, hatched nauplii may be trapped and die. The peritrophic membrane dissolves depending on environmental conditions and bacterial colonization. Nauplii have been observed escaping from broken ends (Conway et al. 1994) as well as from central parts (pers. observ.) of the fecal pellets. It seems justified to assume that feces dissolve faster in the field than in an aquarium, but

Table 3. Subitaneous eggs. Results of nested ANOVAs for arcsin-transformed experimental variables with copepod species (spec) and hunger level (food) of fish as fixed factors and experimental block as nested factor. Interactions not shown, as none of them was significant.

| Variable | Factor | Degrees of freedom | Mean squares | *F* | *p* |
|---|---|---|---|---|---|
| PASS* | spec | 3, 88 | 2.29 | 48.46 | <0.001 |
| | food | 1, 88 | 0.25 | 5.31 | 0.031 |
| VIABLE | spec | 3, 88 | 1.47 | 26.99 | <0.001 |
| | food | 1, 88 | 0.20 | 3.66 | 0.069 |
| HATCH | spec | 3, 88 | 1.86 | 49.84 | <0.001 |
| | food | 1, 88 | 0.21 | 5.58 | 0.027 |
| MORT | spec | 3, 88 | 0.49 | 10.37 | <0.001 |
| | food | 1, 88 | 0.10 | 2.02 | 0.169 |

* PASS, proportion of eggs recovered after gut passage; VIABLE, viable proportion of recovered eggs; HATCH, proportion of ingested eggs that hatched after gut passage; MORT, egg mortality caused by gut passage in addition to natural (control) mortality.
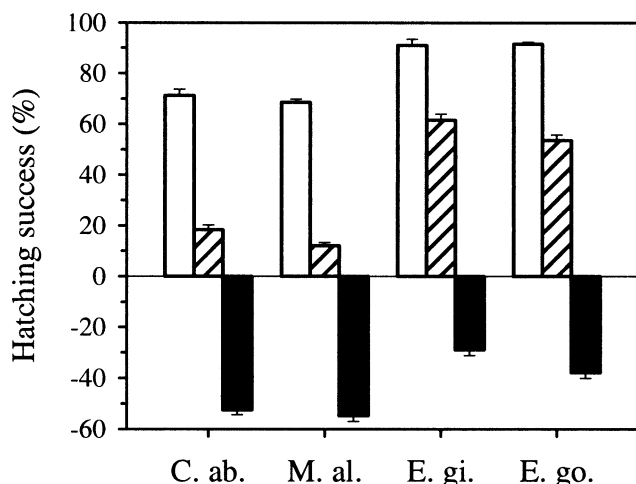
Fig. 1. Hatching success of subitaneous eggs of *C. abyssorum* (C. ab.), *M. albidus* (M. al.), *E. gracilis* (E. gi.), and *E. graciloides* (E. go.) after gut passage (white bars = controls, hatched bars = treatments) and impact of gut passage on hatching (black bars). Means (±SE) of prefed and hungry fish.

there may be nevertheless some nauplii mortality, in particular among eggs ingested at late developmental stages. The feeding status of the fish may be important in that respect. Fish fed natural plankton in our experiments produced feces much faster than starving fish. Also, the peritrophic membranes of feces from fed fish were already perforated when they were collected. As this is the natural condition, our estimates of hatching rates are probably not too far from reality.

Feeding history had a significant effect on gut passage, but not on the viability of passed eggs. Egg recovery was always lower when the fish had been starved. As eggs stayed much longer in the guts of hungry fish, there may have been some direct egg loss due to digestion. It is possible, however, that more nauplii hatched from eggs at late developmental stages during the prolonged gut residence and were successively digested. Although inspection of the feces showed that egg sacs were often dislodged from the remaining chitinous carapaces of the females and eggs were separated, mechanical destruction of eggs during feeding or in the gut seems to be of minor importance, at least in calanoids. Of the four copepods tested, only *E. graciloides* produces resting eggs that could be compared to subitaneous eggs. The proportion of resting eggs passing through the gut was significantly larger for resting eggs than for subitaneous eggs, but the difference was small with prefed fish. However, there was no significant effect of the food treatment on gut passage of resting eggs, which suggests that the increased losses of subitaneous eggs in hungry fish are due to longer digestion time. Although there are no quantitative data on gut survival of resting eggs, they are usually considered digestion resistant (Hairston and Munns 1984; Marcus 1984). Hence the small difference between resting eggs and subitaneous eggs is surprising.

Subitaneous eggs of the two calanoid species were clearly more resistant than those of the cyclopoids. Higher proportions of the calanoid eggs passed through the guts, and a

higher proportion of the recovered eggs was viable. This resulted in large differences in proportions of eggs that finally hatched after ingestion; whereas 60–70% of the eggs of *Eudiaptomus* produced healthy nauplii, the figures for the cyclopoids were only between 12% and 30%. It is not clear, however, if this is really specific for the different taxonomic orders or for the larger clutches carried by the cyclopoids. A comparison with the literature does not yield a decisive result. In the study of Conway et al. (1994), none of the eggs of the marine cyclopoid *Corycaeus anglicus* survived the gut passage, but large differences were found among the calanoids. Whereas over 60% survived in *Eurytemora,* survival was less than 1% in *Pseudocalanus,* although *Pseudocalanus* had fewer eggs than *Eurytemora.* Only the hatching rates of nauplii from eggs recovered from guts or feces can be compared with the remaining studies. Egg viabilities of *Eurytemora* (Redden and Daborn 1991; Conway et al. 1994; Flinkman et al. 1994) were very similar to our *Eudiaptomus* results, and the slightly lower values for *C. abyssorum* are consistent with the observations of Gliwicz and Rowan (1984).

*M. albidus* showed the lowest survival of all four species. Only about 12% of the ingested eggs released nauplii. This cyclopoid is a littoral species and, thus, it probably experiences a predation pressure different from the pelagic species. To make the predation more realistic, we confronted *M. albidus* with a littoral predator, stickleback. However, the comparison of *M. albidus* with the other copepods is now hampered by the use of a different predator. There is the possibility that sticklebacks are more efficient in digesting eggs than perch. On the other hand, fish predation may be less important as a selection factor for *M. albidus* as they can hide in the littoral. The large, conspicuous clutches point to the same direction. Despite the low survival rate of cyclopoid eggs, there are still a reasonable number of nauplii produced after gut passage. Because of the much larger clutch sizes of the cyclopoids, the absolute numbers of nauplii produced per ingested female is rather similar in calanoids and cyclopoids. We did not test the survival of cyclopoid nauplii after hatching, but we have no reason to believe that they were less healthy than the calanoid nauplii. The relation between clutch size and digestion resistance should be studied in more detail within and between species. Clutch sizes vary with food abundance and the trophic state of a lake (Elster 1954; Czeczuga 1959; Santer 1994); hence a trade-off between egg number and digestion resistance may be important for the distribution of copepod species.

There are also significant differences between the hatching proportions of calanoids and cyclopoids in the controls. Hatching success of about 70% for detached eggs has also been found for *Apocyclops panamensis* (Saint-Jean and Pagano 1995) and for *C. abyssorum tatricus* (Gliwicz and Rowan 1984), whereas 99% were reported for *Eurytemora* (Redden and Daborn 1991). Cyclopoids are generally not more sensitive to handling and egg removal than calanoids (B. Santer pers. comm.); thus the lower hatching success must reflect the specific experimental conditions.

Therefore, we have calculated the additional egg mortality caused by gut passage as the difference between treatments and controls (Fig. 1). This characteristic is more important

from a physiological than from an ecological point of view. Although there is no longer a difference between food treatments, the clear distinction between calanoids and cyclopoids is retained, but the difference between *C. abyssorum* and *M. albidus* is no longer significant. Gut passage causes only 29–38% mortality in the calanoids and 53–55% in the cyclopoids. Considering that the cyclopoid hatching success in the controls may have been underestimated, these are conservative results, i.e., the difference between the two groups may even be larger. Taking all this information together, we conclude that a significant part of subitaneous eggs of freshwater copepods, in particular in *Eudiaptomus,* the most abundant calanoid genus in European lakes, will survive gut passage in fish.

Although it may be important for zooplankton ecology and evolution, this aspect has rarely been considered until now. Whenever predation on egg-carrying copepod females is taken into account in population dynamics models (e.g., Argentesi et al. 1974), they should be modified, as not all eggs are lost. However, one has to be cautious, as the impact of gut passage on population dynamics can be easily overestimated. Most copepods carry multiple clutches. The total gain is low if a female is being consumed when it carries a late clutch. If it is killed when carrying an early clutch, the actual clutch is saved, but all possible successive clutches of this female are lost anyway.

Small fitness differences, however, may have implications for the evolution of egg-carrying in copepods compared to free spawning (Hairston et al. 1983; Webb and Weaver 1988). Costs of egg-carrying are associated with increased predation mortality, and resistance of eggs to digestion would lower these costs considerably, favoring the carrying of eggs. The model will probably have to be modified with the new information, or made species specific. Hairston et al. (1983) based their calculations on complete mortality of subitaneous eggs of *Diaptomus sanguineus,* as they found no subitaneous but most of the diapausing eggs to pass through the gut of sunfish (N. G. Hairston Jr. pers. comm.). As they found egg-carrying in the presence of fish very costly, they suggested the production of diapausing eggs as an alternative to free spawning. Hairston and Munns (1984) followed this line, presenting convincing evidence for timing of diapause in *D. sanguineus* as an evolutionarily stable strategy. Digestion resistance of subitaneous eggs in *E. graciloides* may be the reason why resting egg production in this species is not related to fish predation. Instead, *E. graciloides* produces resting eggs in late fall when fish predation ceases (Pasternak and Arashkevich 1999; Santer et al. 2000). Costs of egg mortality may not be sufficient to make switching to resting eggs profitable as a predator avoidance strategy. The remaining function of the resting eggs may be the avoidance of harsh environmental conditions, contribution to the resting egg bank (Hairston 1996), and dispersal.

Our results support the idea of Gliwicz and Rowan (1984) that gut passage of subitaneous copepod eggs facilitates the balance between cladocerans and copepods. Cladocerans, especially *Daphnia,* are strong competitors of copepod nauplii and may force copepods to enter diapause (Santer and Lampert 1995). To our knowledge, subitaneous cladoceran eggs have never been reported to pass through a fish gut. Saint-

Jean and Pagano (1995) found complete digestion of the eggs of *Moina* and *Diaphanosoma.* We performed preliminary experiments with *Daphnia* to test for egg survival, but contrary to ephippia, not a single subitaneous *Daphnia* egg survived gut passage. In our main experiments, fish were fed *Daphnia* in the prefed treatments, but no intact egg was detected in the feces. Hence, under fish predation, copepods may have an advantage over *Daphnia* not only because they are better evaders. In the rather extreme high-mountain lakes (Gliwicz and Rowan 1984), large *Daphnia* monopolized the resources in the absence of fish, while only *C. abyssorum tatricus* coexisted with fish. Viable gut passage in copepods, but not in *Daphnia*, may also provide an explanation for the greater recolonization success of copepods compared to *Daphnia* in lakes with heavy fish predation (Yan et al. 2004).

Finally, gut passage of subitaneous eggs can be involved in dispersal of copepods and the colonization of new habitats. Resting eggs, in particular of cladocerans, have long been suggested to be dispersal agents after they had been found intact in the hindguts of waterfowl (Proctor 1964). Many copepods (cyclopoids, various calanoids), however, do not produce resting stages, but nevertheless colonize small, isolated new water bodies quickly. This has been explained by attachment of copepodids and adults to the plumage of birds (Einsle 1993). Though not explicitly stated, gut passage of subitaneous eggs in birds is implied in the experiments of Proctor et al. (1967), as they report *C. vernalis* hatching from duck feces. As birds have been observed feeding on zooplankton (Dodson and Egger 1980), the possible transport of viable copepod eggs in bird guts might be worth reinvestigation. It would be particularly interesting to know if a double gut passage through fish and fish-eating birds is possible.

In connected water bodies, fish themselves can act as dispersal vector, as has been observed frequently for seeds (Chick et al. 2003). Jarnagin et al. (2000) have studied the dispersal of *Bythotrephes* through resting eggs in fish, but the same should also apply for subitaneous copepod eggs. Using fish as vectors, copepods could easily colonize otherwise inaccessible upstream lakes. They would even be transported across watersheds in the guts of bait fish.

Our experiments and comparisons to the literature suggest species-specific differences in digestion resistance of subitaneous copepod eggs, but the phenomenon seems to be more widespread than previously believed. We have demonstrated various ecological topics where this might be important. A survey including more copepod species should explore patterns in digestion resistance related to taxonomic units, predator type, life-history strategies, or habitat preferences. We may even find local adaptation to the predator regime.

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