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Population and behavioural ecology of two *Eubosmina* species, with emphasis on antipredator traits

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Dissertation

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Abstract

Zooplankton have in many cases developed morphological traits (spines, helmets, unwieldy size or shape), to make it difficult for invertebrate predators to capture and handle the prey. The morphological traits may vary in cycles (cyclomorphosis), and are induced either by abiotic factors, or chemical substances produced by the predator. This thesis focuses on how morphological changes in *Eubosmina* (Cladocera) can be explained from the perspective of predation.

In field studies two coexisting*Eubosmina* populations were described as a normal form (low and elongated, short antennule), and an extreme form (higher carapace, long antennule). The occurence of the extreme form coincided with the hatching of the predaceous *Leptodora kindtii*. The morphological characters of the extreme form was proposed to be effective as a defence against this predator.

Due to confusing taxonomy and nomenclature within the genus *Bosmina* both forms were considered to be *Bosmina coregoni*. After more thorough examination they were classified as *B. (E) longispina* and *B. (E) coregoni*. The specific status was tested and confirmed by using Random Amplified Polymorphic DNA (RAPD) markers. Juveniles with a small cuadal mucro and long antennule were assigned as being juveniles of *B. coregoni.*

In laboratory experiments *B. longispina* swam almost 40% faster than *B. coregoni*, and differences in drag was proposed as an explanation. Drag may influence the swimming speed, which in turn can be correlated to food intake.

Vertical and temporal distribution of prey and predator was studied. *Leptodora* did not exhibit vertical migration despite extensive populations of fish in the lake. *B. coregoni* was more abundant near the surface. The extreme body form may be costly, forcing this species to spend more time in food rich strata. *B. longispina* was more evenly distributed in the water column. Interspecific competition, and vulnerability to *Leptodora* may contribute to this.

B. longispina with low and elongated body, and short antennule, *B. coregoni gibbera* and *B. coregoni retro extensa* with more extreme body shape, and longer antennule, were exposed to *Leptodora* in laboratory experiments. The extreme *Bosmina* forms escaped six times more often than the typical form, when attacked by the predator. Thus, extreme morphological traits in *Bosmina* can be protective against predation from *Leptodora.*

Intermediate specimens of the *B. kessleri* -like morphotype occasionally found among *B. longispina* and *B. coregoni*, have been described as hybrids between the latter two. This was tested by using RAPD technique, but it was not possible to confirm specimens of the *B. kessleri*-morphotype in Lake Östersjön as hybrids. Instead they seemed genetically more similar to *B . coregoni.*

In sum, this study shows that *Eubosmina* species undergoing morphological changes may be less vulnerable to invertebrate predators, which may explain some of the phenotypic variability found in this subgenus.

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LIST OF PAPERS

This thesis is based on the following papers, which will be referred to in the text by their roman numerals.

I. Hellsten, M. E., and. Stenson, J. E. 1995. Cyclomorphosis in a population of *Bosmina coregoni*. Hydrobiologia 312: 1-9.

II. Hellsten, M. E., and Sundberg, P. Genetic variation in two sympatric European populations of *Bosmina* spp. (Cladocera) tested with RAPD. Manuscript.

III. Lagergren, R., Hellsten M. E., and Stenson, J. Increased drag, and thus lower speed, a cost for morphological defence in *Bosmina* (*Eubosmina*) (Crustacea: Cladocera). Functional Ecology (in press).

IV. Hellsten, M. E., Stenson, J. and Lagergren, R. Habitat distribution of two *Eubosmina* species, and *Leptodora* in Lake Östersjön. Manuscript.

V. Hellsten, M. E., Lagergren, R., and Stenson, J. Can extreme morphology in *Eubosmina* reduce the predation risk from *Leptodora*? An experimental test. Manuscript.

VI. Hellsten, M. E. Is the *Bosmina kessleri*-like morphotype a hybrid between *B. longispina* and *B. coregoni*? A molecular test. Manuscript.

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CONTENTS

INTRODUCTION

The environment of open waters is considered relatively uniform (Hutchinson 1961, Hessen 1990), which should favour an opportunistic feeding mode by zooplankton grazers, rather than resource specialization (Hebert and Crease 1980). Grazers therefore compete for food particles (within a certain food range), and their relative success will depend on their own size. For example, larger species are more efficient feeders since they are capable of eating a broader size range of particles, and will therefore be competitively superior to smaller species (Brook and Dodson 1965, Dodson 1974a, Hall et al. 1976). Although there may be a competitive exclusion of species (but see DeMott and Kerfoot 1989, niche overlap is also common (Hebert 1977, Jacobs 1977, Ghilarov 1984, Hessen 1990). In many cases, equally sized or closely related species or clones seem unable to exclude each other, although competition may be intense (Hebert and Crease 1980, Matveev 1985, Bengtsson 1986, Boersma 1995).

Other ecological factors than competition may be equally important for the evolution of zooplankton life. Predation is often a most influencial factor for dynamic population properties, and community structure (Zaret 1980, Sih et al 1985). Predators can control prey populations, in some cases eliminating them, or influence absolute and relative densities in the prey community. Further, predation can also lead to indirect interactions between coexisting species (Abrams 1987). Although larger zooplankton are more efficient feeders , due to size and pigmentation they may also be more exposed to visually feeding predators such as fish. Thereby, predation on large zooplankton will favour smaller and competitively inferior species (Brooks and Dodson 1965, Dodson 1974), making predation a significant selective force.

Predation can also act as a selective force influencing the evolution of defence strategies in the prey (Zaret 1980, Jeffries and Lawton 1984, Lima and Dill 1990). In aquatic communities the environment offers few possibilities to avoid predators by hiding in refugia. Instead prey have evolved adaptations that reduce encounter rate with predators, or hunting success of the predator after encounter. One way for large and more conspicuous zooplankton to decrease encounter rate with visually hunting predators (mainly fish) is to migrate to levels of low light intensity where predation pressure is reduced (Zaret 1980, Lampert 1989). Reduced visible body pigmentation in the prey can also decrease the predator's possibility to detect the prey (Zaret 1980).

While larger zooplankton may be more exposed to visually hunting predators, smaller zooplankton may instead be more vulnerable to invertebrate predators. Many invertebrate predators rely on chemical or hydromechanical cues for prey detection (Pastorok 1980, Kerfoot 1978, Stenson 1987). However, independently of their hunting strategy or how they detect the prey, they have to hold and manipulate the prey item after catch. When exposed to such predators, other antipredator defences will be more effective. For example, detected zooplankton may respond by rapid escape movements (Gerritsen and Strickler 1977, Larsson and Dodson 1993, Svensson in press).

Another way to reduce the possibility of being attacked and captured is to develop morphological traits (spines, helmets, unwieldy size or shape), thereby making it difficult for the predator to capture and handle the prey. Many of the morphological traits vary in cycles (seasonal or aseasonal), so called cyclomorphosis (Black and Slobodkin 1987), and are usually shown by successive generations of animals that reproduce several times a year. Among zooplankton cyclomorphosis occur in rotifers and cladocerans.

Cyclomorphosis in itself is well described (Flössner 1972, Lieder 1983a, 1983b), and was basically explained as subspecies variation without paying any further attention to its origin. However, one proposition was that exhuberant structures might relate to buoyancy. Spines and helmets were thought to reduce the sinking rate in warmer water (see Hutchinson 1967). However, since the late sixties studies have shown that cyclomorphosis in many cases is ultimately related to predation (e.g. Dodson 1974b, O'Brien et al. 1979, Kerfoot 1980, Krueger and Dodson 1981, Havel 1985, 1987, Stenson 1987, Dodson 1988, Walls and Ketola 1989).

The morphological changes are a mixture of succession of genotypes and phenotypic plasticity of individual genotypes, and the cyclomorphic changes are induced either by environmental factors such as temperature, or by chemical substances emanating from the predators (Stenson 1987, Dodson 1988, Larsson and Dodson 1993). Thus, species or populations showing cyclomorphosis seem capable of allocating resources to the morphological changes when needed, *i.e.* under periods of high predation pressure. Hence, the development of morphological traits only in the presence of predators suggests a cost to the production of these defences, as has also been shown in several studies (e. g. Kerfoot 1977, Dodson 1984, Walls and Ketola 1989, Black and Dodson 1990).

One example of morphological changes as antipredator defence is the *Daphnia pulex-Chaoborus* interaction (Krueger and Dodson 1981, Havel and Dodson 1984) where young *D. pulex* are more exposed to the predator *Chaoborus* and therefore produce a neck-tooth. The development of the neck-tooth is triggered by a chemical substance released by the predator (Parejko and Dodson 1990). When the juveniles grow larger and become adults they are no longer vulnerable to *Chaoborus* and the neck-tooth is no longer necessary.

Morphological changes as antipredator defence involve not only traits that develop only in the presence of predators and otherwise disappears. Certain permanent morphological traits may also grow more pronounced in the presence of predators. *B. longirostris* in the presence of certain predatory copepods grows longer antennule and mucro, morphological structures that are always present in this species (Kerfoot 1987). Further, zooplankton species often face different predation pressure at different life stages or periods in the season, and therefore need to have a mixture of defence strategies.

This thesis focuses on how morphological changes in*Eubosmina* (genus *Bosmina*) can be explained from the perspective of predation. In spite of intense research in this field, most studies have focused on morphological defences in *Daphnia* species, and only to a minor extent on *Bosmina* species. European *Bosmina* populations offer good opportunities since species within this genus show a dazzling variety of morphological forms, especially within the subgenus *Eubosmina,* that in many cases also undergo morphological changes.

The first part of the thesis examines a seasonal succession between two morphologically different *Eubosmina* populations, and a possible invertebrate predator (I). The specific status between the two *Eubosmina* populations is also examined (II).

The second part examines how unwieldy size and shape in *Eubosmina* can be costly in terms of increased drag, influence the habitat distribution, and reduce the vulnerability to predators (III, IV, V). Habitat distribution of the predator is also studied (IV). Finally, phenotypic plasticity and hybridization in the studied *Eubosmina* populations is tested and discussed (VI). In the following, I use the term *Bosmina* in a more general sense when discussing features of this zooplankton, otherwise I use the term*Eubosmina* .

The prey species

The family *Bosminidae* is a dominant component of the freshwater cladoceran community, and a complex family with a great number of widely varying forms (Nilssen and Larsson 1980, Lieder 1983a, 1983b, De Melo and Hebert 1994a). The family comprises two genera, *Bosminopsis* and *Bosmina*. The genus *Bosmina* consists of four subgenera *Bosmina*, *Neobosmina*, *Sinobosmina* and *Eubosmina*. In the circumbaltic region of Europe, forms within the subgenus *Eubosmina* showing phenotypical variability in morphological characters like body size and shape, antennule and mucro length are common (Lieder 1983b) (Fig 1.).

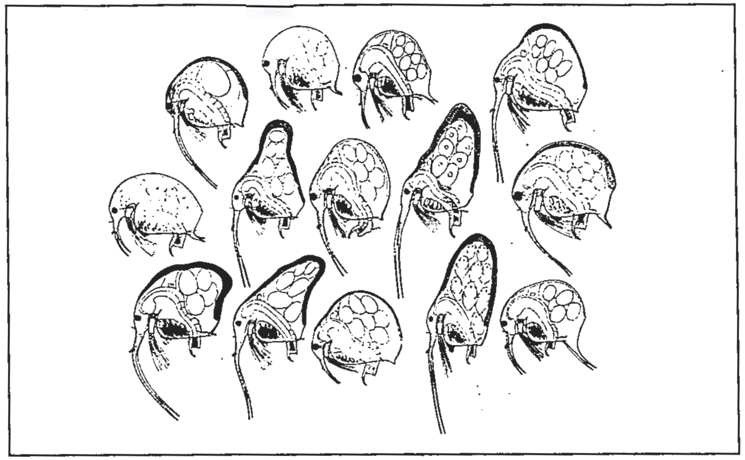

Fig. 1 *Some examples of variable forms within the subgenus Eubosmina. (from Liljeborg 1901).*

Like other cladocerans *Bosmina* have a distinct head, and with few exceptions the body is covered by a bivalve cuticular carapace. The shape of a *Bosmina's* body, much like a bar of soap (Zaret 1980), not only makes the body difficult to handle, but also strong and stress resistant since pressure will be evenly distributed all over the body.They have a large compound eye, and the second pair of antennae are large swimming appendages and constitute the primary organs of locomotion (Wetzel 1983). They are filter-feeders and their primary food is suspended algae and detrital particles. *Bosmina* is a small zooplankton (body length 0.1-0.7 mm) and probably most vulnerable to invertebrate predators, although it may also be exposed to fish predation (Kerfoot 1975, Stenson 1976, Vonder Brink and Vanni 1993).

Like other species within the suborder Cladocera *Bosmina* species exhibit cyclical parthenogenesis, and both asexual and sexual reproduction (via diploid parthenogenesis) can exist in the life cycle. Asexual and sexual reproduction can occur simultaneously in a population.

Usually, females produce eggs that develop into parthenogenetic females. The eggs develop in a brood chamber. Normally, one clutch of eggs is released into the brood chamber during each adult instar. When the production of parthenogenetic eggs declines, some of the eggs develop into males. Sexually produced eggs will then develop into resting eggs that can withstand severe conditions such as freezing and drying. The reproductive shifts seem to be under environmental control such as changes in photoperiod, temperature, food and population density-dependent factors (Lynch 1980, Kleiven 1996). This probably explains why the frequency of sexual reproduction appears to be related to the permanency and size of the habitat (see Mort 1991), being more common in temporary habitats like ponds and less common in lakes (permanent habitats).

The predator

Leptodora kindtii is a large zooplankton (maximum body length 18 mm), one of the few predaceous cladocerans. Unlike grazing cladocerans where juveniles are born like small miniatures of their mothers, *Leptodora* hatch as nauplii larvae which change through metamorphosis to the adult form (Sebastyén 1959). *Leptodora* is one of the few large invertebrate zooplankton that can persist in the presence of fish (Zaret 1980). This probably has to do with *Leptodora*'s lack of hemoglobin and carotenoid pigments (see Zaret 1980). It is therefore highly transparent, which should reduce the vulnerability to fish predation. It is a tactile predator and swims continuously, preying upon a range of zooplankton species (e. g. Edmondson and Litt 1987, Kerzig and Koste 1989, Branstrator and Lehman 1991). The setae of the first thoracic limbs acting as mechanoreceptors, the others thoracic limbs, thorax and head together form an open "feeding basket", into which the prey is pushed. The size of the feeding basket is proportional to the size of the animal, and limits the size of the prey. *Leptodora* prefer small prey items < 1.5 mm. There are few reports of how *Leptodora* eats the prey. Apparently, it is mainly a fluid feeder. After piercing the carapace of its prey near the head it sucks out the juices (Mordukhai-Boltovskaia 1958), but it can also tear a hole in the carapace and then suck out the juices (see Browman et al. 1989).

The lake

Prey and predator species were studied in, and collected from Lake Östersjön, SW Sweden (57° 93'N, 12° 79'E). Lake Östersjön is a rather small (1.5 km2, max depth 32, mean depth 10 m) eutrophic lake. During the summer the upper strata contain high densities of blue-green algae, and the deepest parts of the lake suffer from oxygen depletion. The lake has extensive populations of fish such as: ruff (*Acerina cernua*), roach (*Rutilus rutilus*), white bream (*Blicca björkna*), perch (*Perca fluviatilis*), pike (*Esox lucius*) and brown trout (*Salmo trutta*). The zooplankton community has a diversity comparable to other eutrophic lakes in the region, with many cladoceran species as well as copepods and rotifers.

GENERAL METHODS

The seasonal succession and abundances of the two *Eubosmina* populations were examined by taking field samples once a month from spring to autumn in a succession of three years (I). Body length and height and antennule length were measured on sampled animals, and the variation in the morphological characters between months was tested. The occurence and abundance of *Leptodora* was also monitored during the sampling period.

Habitat distribution of the two *Eubosmina* populations and *Leptodora* was examined by taking field samples from three different depths during a 24 hour period in May, July and August in one year (IV). Mean diel densities among sample dates, and effects of time and depth on the distribution for the two *Eubosmina* populations and *Leptodora* were tested.

Costs in terms of increased drag and predator vulnerability were examined by laboratory experiments (III, V). In the first case, swimming speed of the two *Eubosmina* species was estimated by videofilming specimens put in a cubic glass chamber (III). Body length (L) and height (H) of each individual was measured and the H/L-value and swimming speed were then used in the calculations of drag. In the second case, prey and predator were put together in Petri dishes, and escape efficiency of the prey and handling time of the predator were measured in direct observations.

Specific status and possible hybridization was tested using the Random Amplified Polymorphic DNA technique on animals collected in the field (II, VI).

RESULTS AND DISCUSSION

Field observations and the specific status of two *Eubosmina* populations in Lake Östersjön (I, II).

In the cladoceran family *Daphnidae*, temporal phenotypic variation has been shown to be an adaptation to reduce the risk of predation by invertebrate predators (e. g. Dodson 1988, 1989, Havel 1987, Harvell 1990). In *Bosmina* species however, the reason for morphological changes are less clear. While some studies point to predator defence as an important factor (Black and Hairston 1983, Kerfoot 1987, De Stasio et al. 1990), others have failed to document this (Gerritsen 1983, Johnsen and Raddum 1987). Further, most studies are based on North American populations (but see Johnsen and Raddum 1987), and as a far as I know, it has not been tested whether European *Eubosmina* populations showing cyclomorphosis are less exposed to invertebrate predators.

In Lake Östersjön the succession of two *Eubosmina* populations were monitored from spring to autumn in 1988-1991. The first population can be described as a normal form (in paper I the term morph was used) with short antennule and a caudal mucro, and the second as an extreme form with higher carapace, long antennule, and no mucro. They were both considered to be *B. coregoni*.

During the sampled periods the normal and extreme form succeed each other from early spring to autumn (September). In early spring only the normal form occurred in the lake. In May the normal and extreme form were both found, although the former was more abundant. In July, only the extreme form was found, but in September both forms were again present in the samples. The same pattern of succession is observed all three years.

The occurrence of the extreme form coincided with the hatching of *Leptodora kindtii*, the dominant invertebrate predator in this lake. *Leptodora* is a potent predator with preference for small prey items, including *Bosmina* spp (Mordukhai-Boltovskaia 1958, Cummins et al. 1969, Lunte and Luecke 1990, Branstrator and Lehman 1991). Other invertebrate predators found in the samples were the calanoid copepod *Heterocope appendiculata*, and cyclopoid copepods. *Heterocope* was found in low numbers, and cyclopoid copepods are present all year round, so these predators should be of minor importance for the observed succession between the *Eubosmina* forms.

The extreme form grew a higher carapace and a longer antennule during the season, coinciding with high densities of *Leptodora*, and specimens collected in July and September were overall larger than specimens collected in May and June. *Leptodora* is a size limited predator (see above). Therefore, large body size, extreme body shape, and long antennule may be effective as a defence against this predator.

In all three years no specimens of the normal form were found in July. Juveniles with long antennule and a small caudal mucro were also found, probably being juveniles of the extreme form.

Since the extreme form declined in abundance and the normal form reappeared in autumn, a cost related to the more extreme features was suggested. However, the field study did not reveal whether the two forms are two genetically distinct populations or a succession of genotypes within one population.

The cosmopolitan genus *Bosmina* has a notorius reputation of being taxonomically complex, and much of the difficulties are due to phenotypic variability in morphological characters like body size and shape, antennule and mucro length (Nilssen and Larsson 1980, Lieder 1983a, De Melo and Hebert 1994a). Particularly at the species level there are still difficulties, especially when morphological characters vary in a cyclical manner.

Morphological characters may thus not be sufficient to distinguish between intra- and interspecific variation. Classification of European *Bosmina* species has mainly been based on morphological characters (Nilssen and Larsson 1980, Lieder 1983a, but see De Melo and Hebert 1994c).

The observed succession of the two forms, and the taxonomic uncertainty concerning the species identification of *Eubosmina* forms led to an earlier conclusion that both forms might have been *B. coregoni*. However, after more thorough examination of morphological characters the two forms were classified as being *B. (E) longispina* (with mucro) and *B. (E) coregoni* (without mucro) (see Flössner 1972, Lieder 1983a).

By using the Random Amplified Polymorphic DNA technique I tested whether there is any extensive geneflow between the two *Eubosmina* populations. I also tested whether juveniles with long antennule and mucro could be identified as a certain species.

The RAPD markers distinguished the two populations and confirmed the specific status of *B. longispina* and *B. coregoni* . Juveniles with long antennule and mucro were classified as *B. coregoni*, confirming the suggestion in the field study. I have not found any description of juveniles in the literature, but the lack of mucro is one of the morphological features distinguishing adult *B. coregoni* from other species (Flössner 1972, Lieder 1983a). The results demonstrates that juveniles of *B. coregoni* in Lake Östersjön undergo morphological changes when becoming adults, *i.e.* they develop higher and more pronounced carapace and longer antennule, and the mucro disappears.

Further, the banding pattern differed not only between but also within populations, suggesting that several clones are present within each population. Since *Bosmina* reproduces mainly by parthenogenesis, low genetic variation might have been expected. However, the observation is consistent with studies of *Daphnia*, where populations in lakes are genetically diverse and consist of several clones (Jacobs 1990, Mort 1991).

The study demonstrated RAPD as a possible technique for testing genetic differences between the *Eubosmina* populations, confirming earlier classification based on morphological traits. Furthermore, juveniles with long antennule and mucro were classified as *B. coregoni.*

Different morphology in the two *Eubosmina* populations may lead to differences in drag (III)

Since changes in shape and size often occur in cycles, the animals seem to allocate resources to the extreme morphology only under periods of high predation pressure. Thus, the development of the morphological features are probably associated with costs, as shown in several studies (e. g. Stenson 1987, Walls and Ketola 1989, Black and Dodson 1990). Most studies of these costs have focused on reproductive parameters (Riessen 1984, Black and Dodson 1990), but it is also important to study how antipredator features *per se* can affect fitness negatively.

Direct costs can be increased time and resources needed for body development, proposed to be major cost of *Daphnia* possessing neck- and tailspines (Riessen 1992), and of long-featured forms of *Bosmina longirostris* (Kerfoot 1977, 1987). Another cost could be increased drag resulting in higher energy expenditure for locomotion. The swimming speed of a *Bosmina* may influence filtering rate and the frequency of encountering edible food particles (DeMott 1982, Gerritsen and Strickler 1977). We examined the swimming speed of the two *Eubosmina* species with video technique, and developed a mathematical hydrodynamic model to calculate how drag might affect swimming speed.

We found that *B. longispina* swam almost 40% faster than the unwieldy *B. coregoni.* The two species did not differ in body length (L), only in body height (H), *B. coregoni* having much higher carapace, giving a higher H/L-value for this species. The model predicted a relation between the H/L-value and swimming speed due to differences in drag, which was also proposed as a plausible explanation for the observed differences in speed.

There are two kinds of drag, pressure drag (depending on inertial forces), and friction drag (depending on viscous forces). The Reynolds number (*Re*) is an estimate of the relative importance of these forces. Since the *Re* for a swimming *Bosmina* range from 0.8 to 5, depending on size and swimming speed, the most important of the two drags will be viscous drag. Pressure drag becomes important when *Re* >> 1. The model predicts that if the two species use the same amount of energy when swimming, with the mean H/L-ratio of *B. coregoni* and *B. longispina* the difference in swimming speed would be only 6%, much less than the observed difference of 40%. The speed of a *Bosmina* varies during a single stroke (Zaret and Kerfoot 1980), and it may attain a *Re*>10 just after a stroke when speed is highest. In higher *Re*, where pressure drag becomes the dominating force, the shape of the body becomes more important for the drag (Vogel 1981). Thus, the difference in drag between the two *Bosmina* forms is probably greater than predicted by our model.

How do differences in swimming ability influence food intake? As algae are slow moving particles, increased speed in the predator implies higher encounter rates (Gerritsen and Strickler 1977). Thus, swimming speed should be correlated with foraging efficiency, and the lower the speed the lesser the food intake by the filter-feeding zooplankton. It has not been proven that the food intake depends on the swimming speed, but if the concentration of food particles is uneven, optimal foraging should be to swim slowly in high food concentration and more quickly if the concentration is low, in order to find a more profitable patch. In a comparison between slow swimming *Ceriodaphnia dubia* and fast swimming *Daphnia pulex*, only the faster swimmer was able to locate the good food patches (Cuddington and McCauley 1994). Moreover, at low food concentrations the net energy gain falls faster with reduced food concentration for an animal with increased drag (Ware 1975). Therefore, although the more extreme body shape in *B. coregoni* may decrease the vulnerability to predators it also increases drag, and when predation risk declines, selection should favour a hydrodynamically more efficient morphology.

Habitat distribution of the studied species in Lake Östersjön (IV)

The vertical and temporal distribution of the two *Eubosmina* populations and *Leptodora* were investigated in Lake Östersjön.

Leptodora is a large zooplankton, and one problem for this predator is to avoid being discovered by visually dependent fish. Since the lake has extensive populations of fish we predicted that *Leptodora* should migrate to greater depths during light hours in order to reduce predation losses to fish.

Surface waters are usually more rich in food, and grazing zooplankton are therefore expected to spend most time there (Lampert 1989). Therefore, given that fish predation was of minor importance, we also predicted that *B. coregoni* should be more abundant in the upper strata, since the body shape and size may lead to increased drag and thus higher energy expenditure. Variation in body size of the two *Eubosmina* populations in relation to densities of *Leptodora* was also analyzed.

As in the previous field studies *Leptodora* and *B. coregoni* were most abundant in July, while *B. longispina* did not vary among sample dates. Further, in this study *B. longispina* were found also in July samples, which was not case in the previous field studies, probably reflecting some inter-year variation.

Although Lake Östersjön has extensive populations of planktivorous fish, *Leptodora* did not migrate vertically migration as has been found in other studies (Vijverberg 1991, Ketelaars et al 1995). The eutrophic state of the lake leads to high water turbidity (Secchi depth of about 0.5 m), which probably reduces Leptodora's vulnerability to fish predation and tendency to migrate. We also have indications of a more nocturnal feeding behavior of *Leptodora*, which should decrease their susceptibility even more.

As predicted *B. coregoni* was more abundant in the upper two meters of the water column. On the other hand, *B. longispina* did not vary in abundance among depths. Other studies have reported *Bosmina* populations being differently distributed in the water column (Matveev 1985, Hudec 1995), and in one of the studies different temperature optima for the species was proposed as an explanation (Hudec 1995). However, in this study the water column was not stratified at any sample occasion, why other explanations may be more plausible.

Competition may force less competitive species to spend more time in less nutritional parts of the lake. Spatial segregation, aiming to reduce competition, would be advantageous for the inferior competitor (Jakobsen and Johnsen 1987). Most cladocerans do not distinguish between low-quality and high-quality food. *Bosmina* is probably an exeption having the possibility of actively selecting food particles (DeMott and Kerfoot 1982). Therefore, it may be possible that the *Eubosmina* populations in Lake Östersjön are partly separated in food preferences.

Predation was also proposed as an explanation. *Leptodora* is a size limited predator, and cannot handle too large prey items (Herzig and Auer 1990). In Lake Östersjön body size in *B. coregoni* and predator abundance was positively correlated, implying that *B. coregoni* may reduce predation risk from *Leptodora* by growing larger and more extreme, as suggested in the previous field studies. The correlation could also be an effect of size selective predation. As *Leptodora* becomes more abundant, smaller size classes of *B. coregoni* are more susceptible and thus more exposed to predation, resulting in a higher proportion of large mean size in the prey. *B. longispina* being smaller, and with less extreme morphology, may have evolved other adaptations to reduce the predation risk. One possibility for *B. longispina* to reduce the encounter rate with *Leptodora*, is to disperse in the water, and not aggregate in the surface. *Leptodora* is a cruising predator with a relatively high swimming speed (Gerritsen and Strickler 1977). The relatively high swimming speed leads to a higher encounter rate with prey organisms that move around more slowly (according to our measurements).

The results showed that *Leptodora* did not exhibit vertical migration. High water turbidity, and the transparency of *Leptodora* may explain this. Further, *B. coregoni* was more abundant near the surface, indicating that the unwieldy body shape is costly, thus forcing this *Eubosmina* to stay in regions with high food densities. *B. longispina*, on the other hand, was more evenly distributed in water column. Interspecific competition, and different vulnerability to *Leptodora* may have contributed to these results.

Extreme morphology in *Eubosmina* may decrease the vulnerability to Leptodora (V)

To reduce the risk of predation prey may evolve morphological traits such as spines, helmets, unwieldy size or shape, that make it difficult for the predator to capture and handle the prey. *Leptodora* is an efficient predator and can severly influence zooplankton populations during summer (Edmondson and Litt 1987, Kerzig and Koste 1989, Branstrator and Lehman 1991). Although *Leptodora*'s efficiency as a predator is well documented, no studies have investigated how morphological traits in the prey may influence the handling efficiency of the predator.

In this study we tested whether *Eubosmina* with different morphology were equally vulnerable when exposed to *Leptodora*, or if unwieldy size or shape increases the escape chances of the prey. Three different *Eubosmina* forms were tested, each showing differences in morphology. *B. longispina* (Leydig) has a relatively low and elongated body and short antennule, *i.e.* a typical *Bosmina* morphology. *B. coregoni gibbera* (Shoedler 1863) and *B. coregoni retro extensa* (Liljeborg 1901) both have a more extreme body shape, no mucro, and much longer antennule. Further, during summer *B. c. gibbera* and *B. c. retro extensa* grow larger and the carapace becomes more pronounced in shape, and the antennule also grows much longer.

When *B. longispina* and *B. coregoni* were exposed in experimental Petri dishes to *Leptodora* for 12 h, the predator more often ate *B. longispina*, although both species were eaten. Moreover, when the three different *Eubosmina* were exposed to the predator, *B. gibbera* and *B. retro extensa* had a higher escape efficiency than *B. longispina*.

Bosmina is a relatively small zooplankton and cannot outswim the large and faster swimming *Leptodora*, but instead must rely on other defence mechanisms. The higher survival of *B. gibbera*, and the higher escape efficiency of both *B. gibbera* and *B. retro extensa*, suggested that more extreme morphology in *Eubosmina* can act as a defence against a tactile predator like *Leptodora*. The most discriminating characters among the three forms were the shape of the carapace, and antennule length, where the latter discriminated *B. gibbera* and *B. retro extensa* from *B. longispina*. *B. gibbera* and *B. retro extensa* escaped equally well, implying that the length of the antennule was important for the escape efficiency. When copepods capture *Bosmina* they often manipulate the prey to expose the soft underparts, and during this manipulation the antennule may interfere with the predator's handling attempts (see Zaret 1980).

The superiority in escape efficieny was quite large for *B. gibbera* and *B. retro extensa*, almost six times higher than for *B. longispina*. Depending on the prey size, it is possible that the escape chance decreases as the predator becomes larger. Predator body length in relation to prey size is important for how successful the predator will be. In *Leptodora* the size of the feeding basket is strongly correlated to body length (Manca and Comoli 1995), and prey availability is thereby correlated to predator length. This was also shown in the regression analysis where handling time decreased with increasing predator length. Apparently, *Leptodora* with a body length of about four mm (as in this study) would be able to capture prey with a maximum body length of 0.6 mm (mean prey body length in this study was ≈ 0.5 mm) (Herzig and Auer 1990). Therefore, we concluded that the prey used in this study were not too large.

Prey body measurements were strongly correlated, and only the effect of antennule length on handling time was tested, since antennule length was the character significantly discriminating both *B. gibbera* and *B. retro extensa* from *B. longispina*. Although predator length and antennule length both affected the handling time, they only explained about 20% of the variation. Thus suggests that the total form and size of the prey also influenced the handling time, which was supported by the tendency towards longer handling time of the more unwieldy *B. gibbera*. The longer antennule and the higher and more pronounced shape of the body in *B. gibbera* may make it difficult for the predator to turn the prey into position.

In sum, we here show that extreme morphology in *Eubosmina* species can increase the prey's escape efficiency when attacked by an invertebrate predator.

Phenotypic variability and possible hybrids in *Eubosmina* (VI)

Due to cladoceran's capability to reproduce either sexually or asexually via parthenogenesis, hybridization may promote speciation processes because hybrids can circumvent deleterious effects of reduced sexual fertility (see Schwenk 1993).

Although hybrids often have morphological characters intermediate of those found in their parents (Spaak 1994), these characters alone may not be sufficient to identify intermediate specimens as being hybrids, but should also be combined with biochemical methods. In cladocerans large variation in morphological characters is common, making it difficult to distinguish between intra- and interspecific variation in closely related species. Morphological studies have therefore often been combined with allozyme electrophoresis to increase taxonomic resolution (Manning et al 1978, De Melo and Hebert 1994b, 1994c), and to identify putative hybrids (Hebert 1985, Hann 1987, Taylor and Hebert 1992).

Hybridization as a factor in the diversification of the *Bosmina* species complex has been suggested. However, in the most recent revision of North American *Bosmina* species, no evidence of interspecific hybridization could be established (De Melo and Hebert 1994b). In Europe, the classification of *Bosmina* species (Nilssen and Larsson 1980, Lieder 1983a), and identification of hybrids (see Lieder 1983b, 1991) have mainly been based on morphological characters.

Among the two coexisting *Eubosmina* species; *B. longispina* and *B. coregoni* an intermediate form is occasionally found. An intermediate that resembles the *B. kessleri* -like morphotype (Uljanin), by Lieder described as a putative hybrid between *B. longispina* and *B. coregoni* (1983b, 1991). The body of *B. longispina* is relatively low and elongate with a caudal mucro, and the body of *B. coregoni* is more extreme with a round carapace that grows higher and more pronounced during summer. Further, adult *B. coregoni* have no caudal mucro, and the antennule is much longer than in *B. longispina*.The *B. kessleri*-like morphotype has a carapace form and length of the antennule much like *B. coregoni*, and although shorter, a caudal mucro like *B. longispina*.

In this study I used the Random Amplified Polymorphic DNA (RAPD) technique to test if the morphologically intermediate *B. kessleri*- like morphotype is an interspecific hybrid between *B. longispina* and *B. coregoni*. Morphological structures used in previous studies to describe the *B. kessleri*-like morphotype as a hybrid (Lieder 1991) were also examined.

The RAPD technique distinguished *B. longispina* and *B. coregoni* from each other, but did not identify the *B. kessleri*-like morphotype individuals as hybrids. Rather, individuals of the *B. kessleri*-like morphotype seemed genetically more similar to *B. coregoni* than to *B. longispina*. Moreover, the morphological characters examined (eye diameter, sutures on the antennule, and number of spines on the post abdominal claw), were not useful for testing whether the *B. kessleri*-like morphotype is a hybrid. For example, eye size may vary due to fish predation (Nilssen and Larsson 1980), and therefore is probably not a good morphological character for identifying hybrids.

In RAPD there is a tendency to get dominant markers (Burke 1994), *i.e.* no distinction between homozygotes and heterozygotes, which may be a problem when identifying hybrids. This technique may therefore not be ideal for this particular question. However, in a study by Heun and Helentjaris (1994) on F_1-hybrids of corn, 90% of the RAPD markers appeared to be inherited as expected in the F_1 generation.

In the studied lake, *B. coregoni* during spring-autumn grow a higher and more pronounced carapace, and longer antennule. Further, juveniles of *B. coregoni* have a cuadal mucro, which disappears as they become adults. Although having a small mucro, the *B. kessleri*-like morphotype more resembles adult *B. coregoni* which has not yet developed a very high carapace. It was therefore suggested that the *B. kessleri* morphotype in Lake Östersjön is not a hybrid, but instead a phenotypic variable form of *B. coregoni*i. Further studies based on genetic analyses are needed tothis this hypothesis.

Concluding remarks

This study suggests that extreme forms of*Eubosmina* species with cyclomorphic traits like carapace height and antennule length may be less vulnerable to invertebrate predators. On the other hand, a more extreme body form may also lead to costs such as increased drag, forcing these *Eubosmina* to spend more time in regions with higher food density. Such costs could also explain why these *Eubosmina* only exhibit the most pronounced body shape and antennule length under periods of high predator abundance.

Phenotypically variable forms within the subgenus *Eubosmina* are very common in Europe, and in some cases this may be a consequence of predation. Little is found in literature on the morphology of juvenile *Eubosmina* and how they develop through their different life stages. As shown in this study, juveniles can look quite different from adults. More thorough studies are needed in cyclomorphic populations from juvenile to adult stages. In combination with molecular methods and studies of the co-occurence of invertebrate predators, this should give more detailed information about the amount of intra- and interspecific variation, and to what extent morphological variation in this subgenus is related to invertebrate predation.

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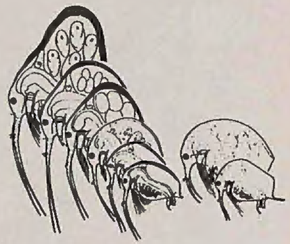

Cyclomorphosis in a population of *Bosmina coregoni*

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Abstract

A population of cyclomorphic *Bosmina coregoni* was studied in Lake Östersjön, southwestern Sweden and results from field samples collected in 1988, 1989, 1990 and 1991 are presented. Animals collected in summer have remarkably higher carapace and prolonged antennule compared to what we call the normal morph. In 1991 the extreme morph reach its maximum body length, body height and antennule length in July to September. The occurrence of the extreme morph coincide with the hatching of the predaceous cladoceran *Leptodora kindtii.*

The two morphs fluctuate in abundance and in relation to each other. In early spring only the normal morph occurred in the samples followed by a period of about two months when the two morphs were found together, in July only the extreme morph was found. In September the two morphs were again present in the lake. As has been shown for other cladoceran, the conspicuous carapace and antennule could be an adaptive response that decreases mortality due to invertebrate predation. Spectacular features like these are likely also accompanied by some sort of costs.

Introduction

One common phenomenon among freshwater plankton is cyclomorphosis i.e. a temporal, cyclic morphological change (Black & Slobodkin, 1987). Although most studies have focused on *Daphnia* and *Bosmina* (Havel, 1987), other freshwater plankton, such as the rotifer *Keratella testudo*, also undergo cyclomorphosis (Stemberger, 1988). Several authors have discussed the causes of cyclomorphosis, and there is now abundant evidence that the phenomenon is ultimately related to predation. This conclusion has been made since recent studies of defence systems in freshwater zooplankton have shown that morphological structures can reduce the predator's efficiency in catching and manipulating the prey (Stemberger & Gilbert, 1987; Harvell, 1990). By enlargement of certain body structures and hence enlarging the total body mass it would be more difficult for a tactile predator to handle the prey (Kerfoot, 1980; Kerfoot & Peterson, 1980; Stenson, 1985, 1987).

The morphological change can be trigged by changes in the abiotic environment e.g. temperature and turbulence (Havel & Dodson, 1985). Such changes can also be triggered by chemical signals produced by the predator (Dodson, 1988; Walls & Ketola, 1989). *Bosmina longirostris* exposed to *Epischura lacustris* resulted in increased mucro length and antennule length, and egg length as well. The increase of these features was a joint effect of selective responses and chemical induction (Kerfoot, 1987).

Since predation may vary in time and space and the development of defence structures may lead to costs which reduce fitness (Kerfoot, 1977; Riessen, 1984; Stenson, 1987; Black & Dodson, 1990), then genotypes which develop such structures only when needed should have higher fitness than those with a constant defence (Havel, 1987). Such conditions should promote the evolution of temporary defences.

Studies made on *Bosmina* and *Eubosmina* species have reported morphological changes in body length as well as in mucro length and antennule length (Black & Hairston, 1983; De Stasio *et al.*, 1990) and these changes can best be explained by the hypothesis that cyclomorphosis has evolved as a defence against predation. Others found no cyclomorphosis in *Bosmina*

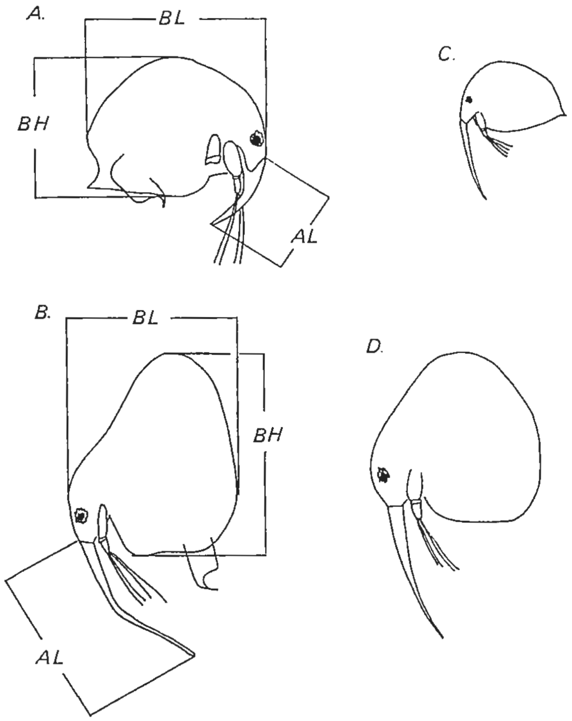

Fig. 1. Diagrams showing features measured: body length (BL), body heigth (BH) and antennule length (AL). A = normal morph, B = extreme morph, C = juvenile of extreme morph, D = extreme morph, spring specimen.

species when exposed to different predators (Gerritsen, 1983; Johnsen & Raddum, 1987).

A population of *Bosmina coregoni* which show cyclomorphosis is found in a lake in southwestern Sweden and we here present results from field samples collected in 1988, 1989, 1990 and 1991. One can describe the cyclomorphic change in this population as a succession of two different morphs, a normal morph and an extreme morph and throughout the text we refer to this succession as a morphological change. The two morphs are quite distinctive from each other and animals collected in summer exhibit a remarkably higher carapace which is also different in shape compared to the normal morph. These animals also have a prolonged antennule. The study includes a description of the relative abundance between the two morphs and the development of certain body measures during the sampling period. The study also presents evidence that the cladoceran *Leptodora kindtii* is linked to these shifts in abundance.

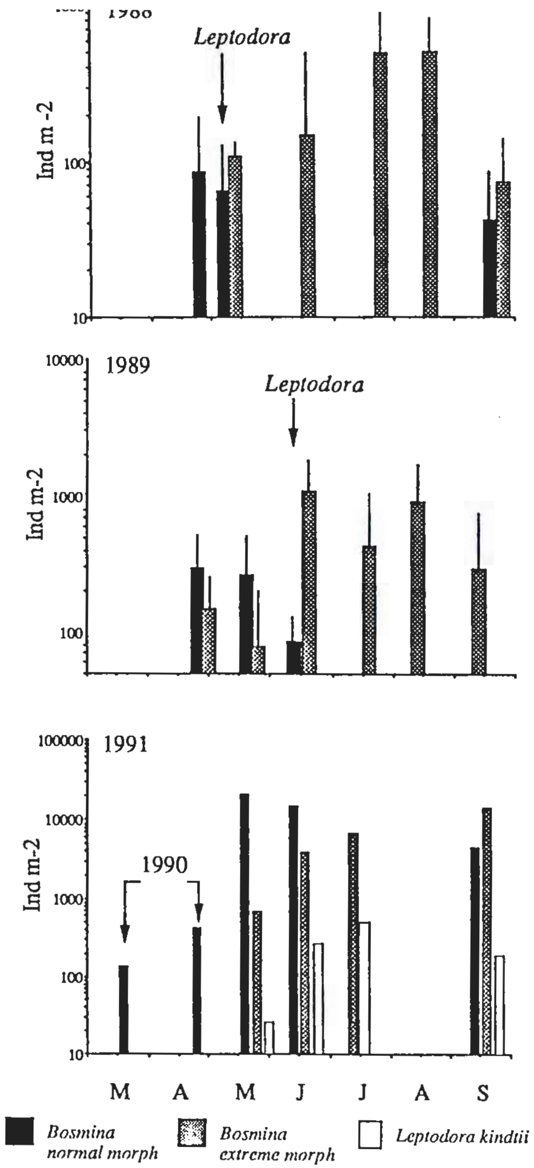

Fig. 2. Densities and the succession of normal morph and of extreme morph of *Bosmina coregoni* and *Leptodora kindtii* collected in Lake Östersjön. Samples collected in 1988 and 1989 represent mean densities from five different regions in the lake (n = 5, ±1SD). Samples in 1990 and 1991 are collected in the western parts of the lake.

Materials and methods

The samples of *Bosmina coregoni* were collected in Lake Östersjön, a rather small lake (1.5 km^2, max depth 32 meter, mean depth 10 meter) in the southwestern part of Sweden (57 ° 93′ N, 12 ° 79′ E). The lake appears to be eutrophic since during the summer the upper strata contain high densities of blue-green algae and the deepest parts of the lake suffer from oxygen depletion. The lake has extensive populations of fish such as: ruff (*Acerina cernua*), roach (*Rutilus rutilus*), white beam (*Blicca björkna*), perch (*Perca fluviatilis*), pike (*Esox lucius*) and brown trout (*Salmo trutta*). Further information about the lake is given elsewhere (Dahlbäck, 1991).

The samples were taken from April to September in 1988 and 1989, from March to April in 1990 and from May to September in 1991. This was done in order to monitor the morphological changes of *Bosmina coregoni* during the warm season. In winter only few individuals of *Bosmina coregoni* are found and then only the normal morph (Hellsten & Stenson, unpubl.). In 1988 and 1989 samples were taken from five different areas in the lake and 10–15 tows from each area at each occasion. In 1990 and 1991 samples were taken in an eight meter deep zone in the western part of the lake. The lake is long and narrow and this part of the lake constitutes the broadest part with approximately 450 meters across. We assume that the within lake variation in 1990–1991 did not deviate from the proceeding years (1988–1989) as shown in Fig. 2. This assumption justifies the sampling strategy from 1990–1991, which means 10–15 separate tows pooled together already in the field. The samples were collected with a 100 μm mesh plankton net drawn vertically from near the bottom to the surface and then preserved in 4% formalin. At the same time water temperature was measured at three different levels in the water column (7, 3 and 0.5 meter from surface).

The field samples also included potential predators co-occurring with *Bosmina coregoni*, such as the cladoceran predator *Leptodora kindtii* and predatory copepods. All individuals of *Bosmina coregoni* and *Leptodora kindtii* collected were counted except for 1988 and 1989 where the first appearance of *Leptodora kindtii* was just recorded. In 1988 and 1989 all individuals of the two different morphs of *Bosmina coregoni* were measured. In 1990 and 1991 individuals of the two different morphs were chosen haphazardly from the samples so that 25 to 60 animals from each date were measured. The sole exception was the March sample, in which only five animals were found. Individuals were measured in lateral view at 100 × magnification using an occular micrometer fitted to an inverted microscope. We measured body length (BL), body height (BH) and antennulae length (AL) (Figs 1A and 1B). Both morphs were identified to belong to the species *Bosmina coregoni* by looking at the bristles on the claw (Scourfield & Harding, 1966). The variation in the morphological characters for the extreme morph from 1991 were tested between months with one-way ANOVA after checking the assumptions for Analysis of Variance.

Results

The succession from normal to extreme morph of *Bosmina coregoni* from April to September in 1988 and 1989 and from March to September in 1990/1991 are shown in Fig. 2. In March and April, only the normal morph occurred in the lake. In May, the normal morph and the extreme morph were both found, although the former was found in higher abundances. In July, only the extreme morph was found but in September the two morphs were present in the samples. The same pattern is observed for 1988 and 1989 with the exception that in 1989 both morphs are found already in April samples and only the extreme morph in September samples. But overall there was a succession of the two morphs during the season.

The occurrence of the extreme morph coincided with the hatching of *Leptodora kindtii* with exception for 1989 where *Leptodora kindtii* were first seen in June samples (Fig. 2). *Leptodora kindtii* lays resting eggs in the autumn which hatch as nauplius larvae in spring the following year (Sebestyén, 1959). In no year was *Leptodora* found before the extreme morph. Other invertebrate predators found in the samples were the calanoid copepod *Heterocope appendiculata* and cyclopoid copepods. The former was found only occasionally and cyclopoid copepods are present all year round (Stenson, unpubl). The mean temperature for the sampling occasions were in 1988, 6 °C in April, 19.5 °C in July and 15.4 °C in September. In 1989, 7 °C in April, 20.5 °C in July and 12 °C in September. And in 1991, 6 °C in April, 20 °C in July and 14 °C in September.

The mean body length (BL), body height (BH) and antennule length (AL) of the normal and the extreme morph are shown in Figs 3, 4 and 5. There was an overall increase in the characters measured for the two morphs over the sampled period in 1991. Mean BL, BH and AL of the extreme morph were each significantly different between months ($p = 0.0001$ one-way ANOVA). Multiple comparisons between the samples

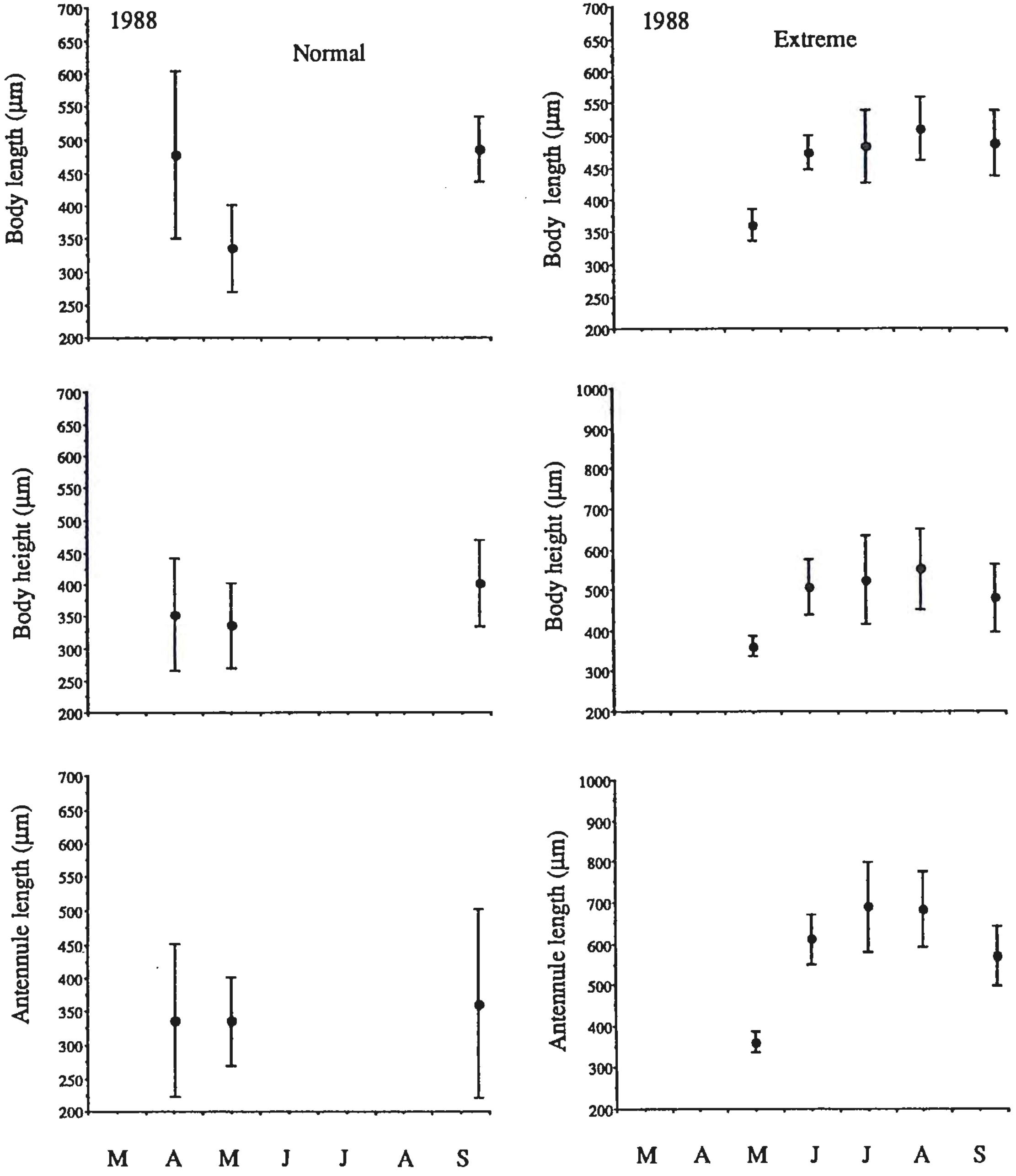

Fig. 3. Mean body length (BL), mean body height (BH) and mean antennule length (AL) for normal and extreme morph in Lake Östersjön in 1988. Sample size for each month as follows: Normal morph, n = 8, n = 6, n = 4. Extreme morph, n = 10, n = 14, n = 54, n = 47, n = 11. Vertical bars represent ±1 standard deviation.

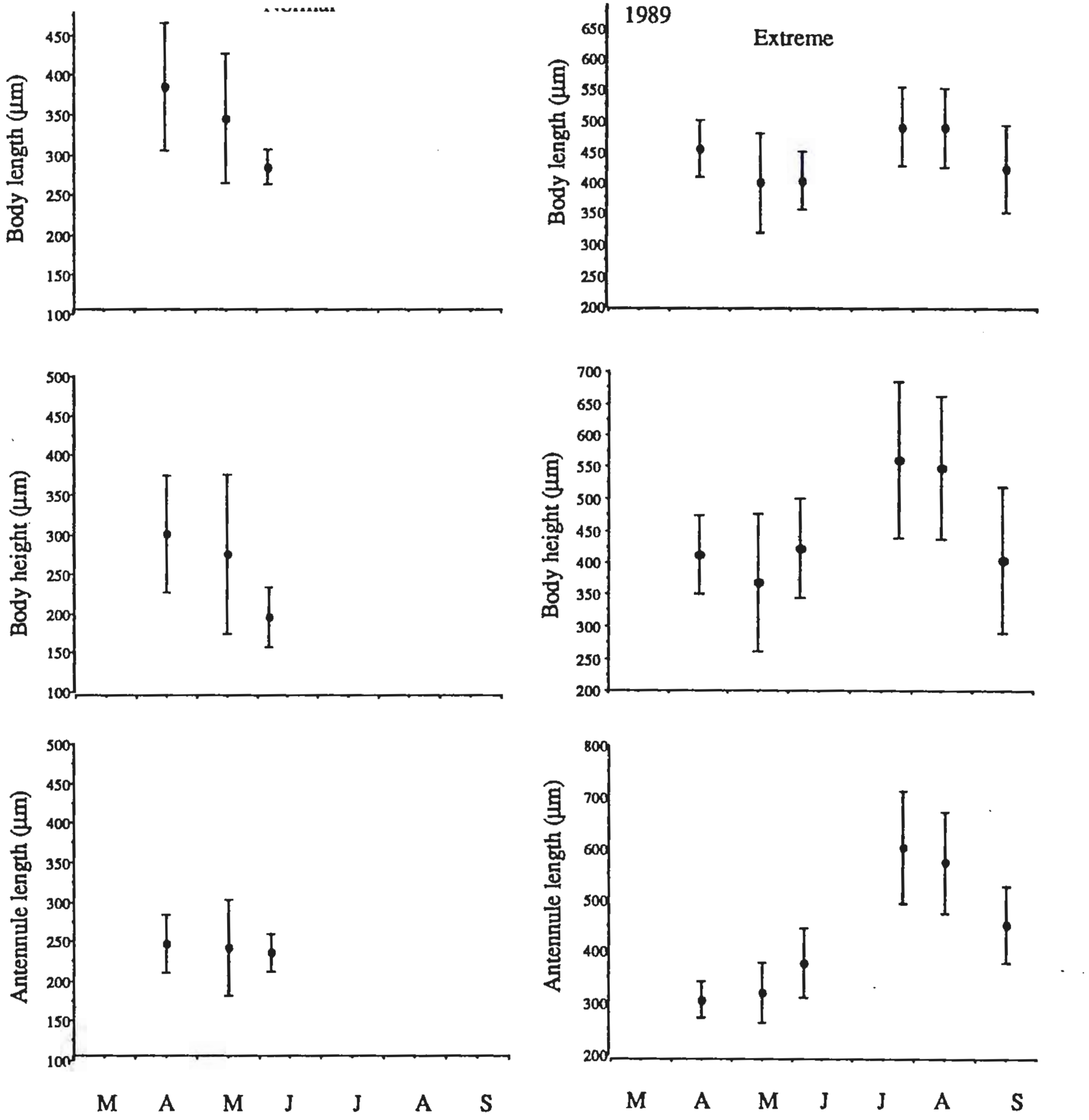

Fig. 4. Mean body length (BL), mean body height (BH) and mean antennule length (AL) for normal and extreme morph in Lake Östersjön in 1989. Sample size for each month as follows: Normal morph, n = 22, n = 18, n = 8. Extreme morph, n = 11, n = 6, n = 102, n = 41, n = 88, n = 28. Vertical bars represent ±1 standard deviation.

revealed that animals collected in July and September showed a significant increase in the characters measured compared to May and June ($p<0.05$, Scheffé's test). Individuals with BH between 200–250 μm were considered juveniles and juveniles of the extreme morph were easily recognized due to their relatively long AL in relation to BH (Fig. 1C). The juveniles also

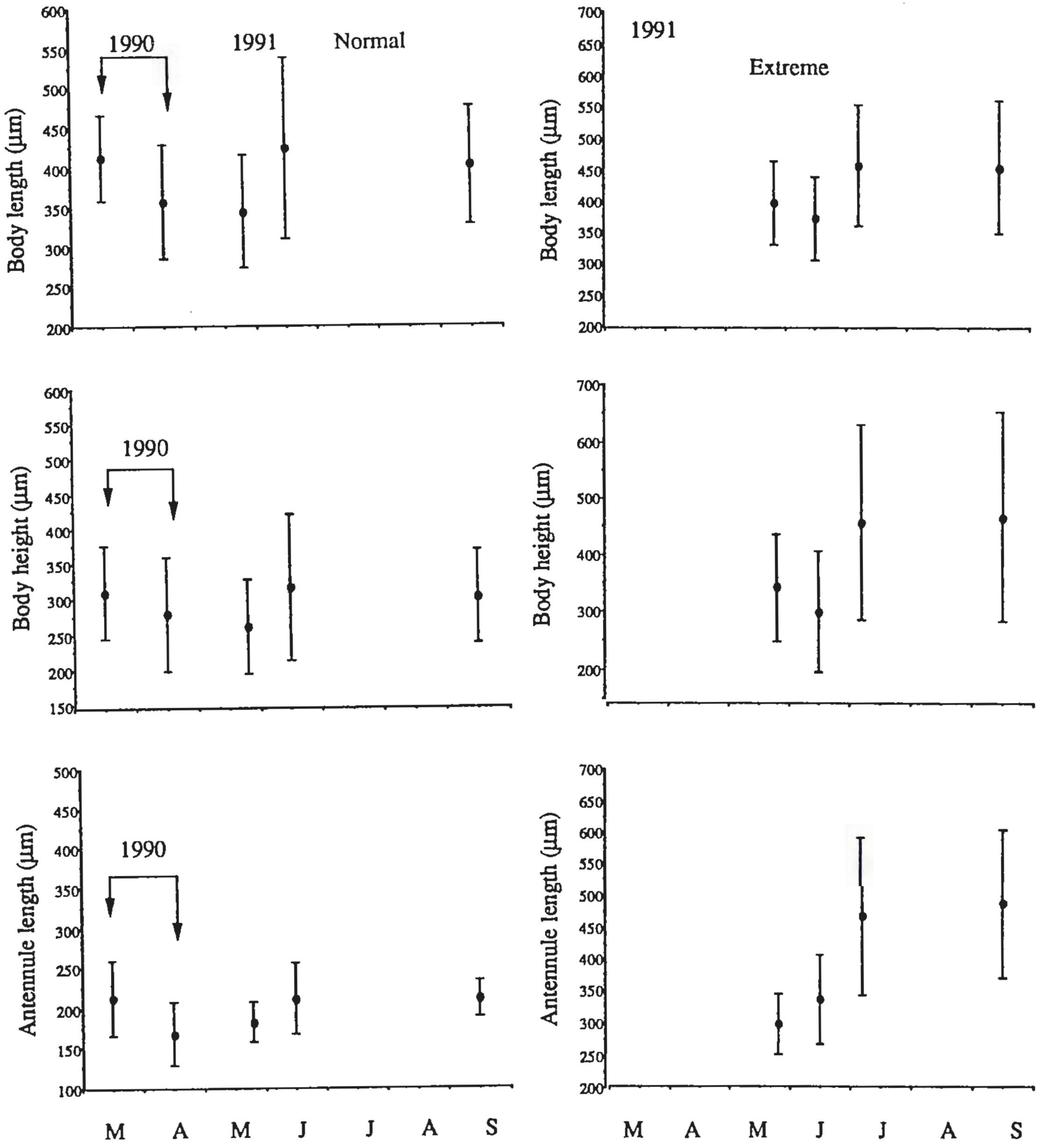

Fig. 5. Mean body length (BL), mean body height (BH) and mean antennule length (AL) for normal and extreme morph in Lake Östersjön, March to April 1990, May to September 1991). Vertical bars represent ±1 standard deviation.

exhibited a small mucro, a feature that was absent in the adult animal.

Discussion

Our field data clearly shows the morphological changes or the succession of the two morphs in this *Bosmina* population (Fig. 2). In 1990/1991 in March, only the normal morph occurs, but in late April–beginning of May both morphs are found although the densities of the normal morph are higher. In July, only the extreme morph occurred in the samples and total density of *Bosmina* was higher than in May and June. The same pattern is observed for 1988 and 1989 except that in 1989 the two morphs are found together already in April samples, and the same year no individuals of the normal morph occurred in the September samples. From these years we do not know anything about the *Leptodora* densitites only when it was first seen. In May samples in 1988 and in June samples in 1989. The succession from normal morph in early spring to extreme morph in summer and then back to normal and extreme morph together in autumn could be a defence against invertebrate predators as has been shown for other cladocerans, bosminids as well as daphnids (Black & Hairston, 1983; Dodson, 1984). We support our statement by arguing that in July 1991 when only the extreme morph was found we also had high densities of *Leptodora kindtii* (Fig. 2). *Leptodora kindtii* is a very potent predator with preference for smaller prey items including *Bosmina* spp. (Mordukhai-Boltovskaia, 1958; Cummins *et al.*, 1969; Lunte & Luecke, 1990). Branstrator & Lehman (1991) showed that the regulation of the size of a population of *B. longirostris* under certain circumstances could be attributed to predation by *Leptodora*. Since *Leptodora* is abundant in the lake during the summer season and with warmer temperatures the individual feeding rates of predators would also be higher, it is reasonable to think that this predator could influence small prey as *Bosmina*. Cyclopid copepods are present all year round and the calanoid copepeod *Heterocope appendiculata* was found only occasionally why we consider these to have minor impact on the *Bosmina* population.

In 1988, 1989 and 1991 the occurrence of *Leptodora* in field samples always coincided with the presence of the extreme morph together with the normal why there could be a link between *Leptodora* and the morphological changes in the *Bosmina* population. In 1991 maximum densities coincided with maximum BL, BH and AL in *Bosmina* (Fig. 5). Although we recognize that this study is based on a single population this could be a more general phenomenon since cyclomorphic *Bosmina* and *Leptodora* populations have been observed in two other lakes situated 150 kilometers south of the studied lake (Hellsten & Stenson, unpubl).

Recent studies on freshwater zooplankton have shown that morphological structures can reduce the predator's efficiency in catching and manipulating the prey (Harvell, 1990). The enlargement of certain body structures in the prey and hence making it more difficult for the predator to handle would then be the ultimate cause for this phenomenon to occur. The extreme morph is very conspicuous with its carapace being extremely high, differently chaped and its antennule extremely long especially in July and September (Fig. 5), why the extreme features could be effective as defence against tactile predators like *Leptodora*. Another problem for the prey is the trade-off between being too large for this kind of predator and stay small enough for a predator like fish (Stephen & Krebs, 1986). The increase in size of the extreme morph is not so dramatic (may not even imply larger visual size) that this would imply a higher risk in being discovered and preyed upon by visually dependent predators such as fish although fish clearly prey upon smaller zooplankton (O'Brien, 1979; O'Brien, 1987).

The juveniles of the extreme morph were recognized due to their relatively long AL and small mucro (Fig. 1C). A plausible explanation to the long AL and mucro found in juveniles is that different size classes of *B. coregoni* face different predation pressures and that juvenile *Bosmina* suffers more from even smaller invertebrate predators such as copepods. According to Kerfoot (1975, 1988) the length of the mucro and the antennule of bosminids are associated with copepod predation.

We can not tell from these results if the successive change from the normal morph to the extreme morph is in fact a change from one genetically distinct morph to another and we in that case are observing a substitution of different genetic lines i.e. subspecies of *B. coregoni*, (Lieder, 1991) or if we observe morphological changes within clonal lines (Kerfoot, 1977; Kerfoot, 1980). If so, intermediates between the two morphs are likely to have been seen. The question is also how one does define intermediates. Early spring specimens of the extreme morph could perhaps be considered as intermediates since they do not have an extremely high carapace and the shape of it look more like that of the normal morph (Fig. 1D) although they have a long antennule and no mucro. In one case, in 1989 May sample, an individual of the extreme morph had a small mucro.

In 1988 and 1991 both morphs were again found in the September samples (Fig. 2) and this could imply a cost of being extreme. With decreasing temperature and shorter day length there is less food available to the *Bosminas* and less food would give less energy to put into extreme features. If we do observe morphological changes within clonal lines the succession could be caused either by changes in the abiotic environment such as temperature and turbulence (Havel & Dodson, 1985) or by chemical cues from predators.

If instead there are distinct populations in the sampled area the disappearance of the normal morph in July could imply heavy mortality due to predation and thus the population should be surpressed almost entirely. On the other hand we then think it is reasonable that some individuals of the normal morph would be found in the samples during this period. A support for the extreme morph to be the only morph in the sampled area in July, in 1988 and 1989 also in August (Fig. 2), is that the same pattern is observed for all three years.

Further studies are necessary to give better insight into the problem and to establish a generality to the observed morphological changes. One way to do so could be comparative studies with other populations, predator/prey experiments and molecular studies to establish eventual genetical likeness between morphs.

Acknowledgments

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II

Genetic variation in two sympatric European populations of *Bosmina* spp (Cladocera) tested with RAPD markers

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Abstract

We used RAPD (Random Amplified Polymorphic DNA) to test genetic divergence between two populations of *Bosmina* spp in Lake Östersjön , Sweden. Previous taxonomic studies on European species within the genus *Bosmina* have been based on morphological characters alone. RAPD markers distinguished the two populations and supported the specific status of *B. coregoni* and *B. longispina* based on morphological characters. Furthermore, juveniles with long antennule and a mucro were classified as *B. coregoni*. The RAPD also revealed genetic differences among the tested individuals, suggesting several clones within each species.

Introduction

The cosmopolitan cladoceran genus *Bosmina* (family Bosminidae) is regarded as being taxonomically complex (Goulden and Frey 1963), and much of the difficulties are due to phenotypic variability in morphological characters like body size and shape, antennule and mucro length (Nilssen and Larsson 1980; Lieder 1983a; De Melo and Hebert 1994a). Most of this variability was earlier explained by species and subspecies differences, and by the turn of the century around 30 species and numerous varieties were described within the genus (Lieder 1983a). The genus has been revised several times since then. Deevey and Deevey (1971) proposed two genera (*Bosmina* and *Eubosmina*), and Lieder (1983a, 1983b) classified the species into one genus (*Bosmina*) and four subgenera (*Bosmina*, *Eubosmina*, *Neobosmina* and *Sinobosmina*). Lieder´s classification is now widely accepted (De Melo and Hebert 1994a). There are, however, still some difficulties at the species level, especially when morphological characters vary in a cyclical manner, (cyclomorphosis; Black and Slobodkin 1987). This may lead to problems in finding good morphological characters for species identification, resulting in taxonomists using different names for the same species, subspecies or varieties (Nilssen and Larsson 1980, Lieder 1983a). Although phenotypical variation within species has been recognized for a long time in certain species, it was basically explained as subspecies variation without paying any further attention to its origins. In the cladoceran family Daphnidae with similar taxonomic problems (Hebert and Wilson 1994; Lehman et al. 1995), temporal phenotypic variation seems to be an adaptation to avoid or reduce predation from invertebrate predators (Dodson 1988, 1989; Havel 1987; Harvell 1990). In *Bosmina* species, however, the reason for morphological changes are less clear. While some studies point to predator defence as an important factor (Black and Hairston, 1983; Kerfoot 1987; De Stasio et al. 1990), others have failed to document an effect (Gerritsen 1983; Johnsen and Raddum 1987).

Morphological characters may thus not be sufficient to distinguish between intra- and interspecific variation. Morphological studies of *Bosmina* in North America have therefore been combined with allozyme electrophoresis to increase taxonomic resolution (Manning et al. 1978, De Melo and Hebert 1994b, 1994c). The most recent revision of North American species, based on both morphological and allozymic characters (De Melo and Hebert 1994a, 1994b), established 10 good species within the genera. Classification on European *Bosmina* species has, however, mainly been restricted to morphological characters (Nilssen and Larsson 1980; Lieder 1983c; but see De Melo and Hebert 1994c).

Two forms of *Bosmina* are found in Lake Östersjön, Sweden (Hellsten and Stenson 1995), corresponding to *B. longispina* and *B. coregoni*, respectively (see Flössner 1972; Lieder 1983a). The specific status is, however, uncertain, because in an earlier study, Hellsten and Stenson (1995) found a temporal succession of the two forms as well as morphological changes within one of the forms during spring-autumn. They proposed that the observed pattern may have been intraspecific changes due to temporal differences in predation pressure. However, they also discussed the possibility that the pattern may have been caused by a succession of species.

Using Random Amplified Polymorphic DNA (RAPD) technique (Williams et al. 1990, Welsh and McClelland 1990), we here examine whether there is any extensive gene flow between the two *Bosmina* forms, or if they represent two distinct species. The RAPD technique generates DNA fragments, called markers, consisting of relatively short fragments (about 200-2000 base pairs long). The sequence of nucleotides amplified is conventionally called a RAPD locus. The amplification product(s) are separated (by size) electrophoretically on a gel, and the presence/absence of a band of a specific molecular weight is used to assay genetic variation within and between species/populations (Grosberg et al. 1996). RAPD analysis does not require any prior knowledge of the target genome (Burke 1994), and since it is based on the polymerase chain reaction (PCR) technique it requires only a small amount of DNA, making it particularly suitable for small organisms.

Materials and methods

Animals were sampled from lake Östersjön, SW Sweden (57° 93' N, 12° 79'E), at the beginning of June in 1994, and in mid-July in 1995. All samples were preserved in 70% ethanol. Adult individuals (20-30 specimens from each population) without eggs or embryos in the brood chamber were randomly subsampled from the 1994 and 1995 samples.

Adult *B. longispina* have a relatively short antennule and a mucro, whereas adult *B. coregoni* have a long antennule but no mucro. Two forms of juveniles are also found, one being a miniature of *B. longispina* but the other being more intermediate, with long antennule and a mucro (Fig. 1). To identify intermediate juveniles as a certain species, we randomly subsampled 20 specimens from the 1995 sample.

DNA was extracted individually according to the procedures in Sundberg and Andersson (1995), and measured with a Hoefer 102 flourometer (DNA ranged between 2 and 20 ng/µL). Table 1 lists the protocol we used for PCR amplification; 5 µL x loading buffer (50% sucrose, w/v, 0,10% SDS and 0,15% bromophenolblue in 6xTBE) was added to each sample after amplification, and 5 µL of this mixture was loaded onto a 2% agarose gel (MetaPhorTM, FMC Bio-products) stained with ethidium bromide (1 mgml-1H2O) and run at 90V for 1 h and at 80V for 1/2 h. The gels were electronically captured in UV-light by a videocamera (Ikegami ICD-42E Type F/L) attached to a video copy processor (Mitsubishi P67E).

Primers were obtained from Operon Techniques (Alameda, California). We started by testing a total of 50 primers on four individuals from each population to find primers that yielded scorable bands and showed polymorphism. Of those 11 primers were chosen with sequences presented in Table 2.

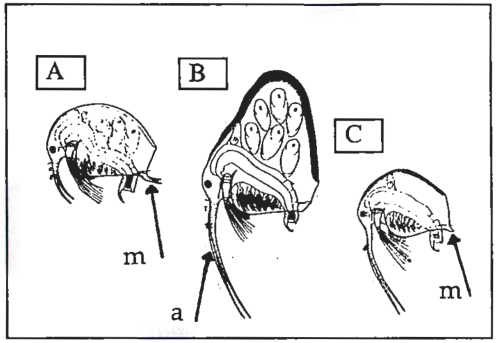

Fig. 1. Lateral view of adult females of B. longispina (A), B. coregoni (B) and Intermediate juveniles (C). Antennule and mucro are indicated with arrows.

Table 1. The PCR-protocol for the RAPD analysis of the two *Bosmina* populations in lake Östersjön

MgCl2 (25 mM) (Perkin-Elmer)	2 µL
10x Stoffel Buffer (Perkin-Elmer)	1,25 µL
Primer[1]	2,5 µL
dNTP (2,5 mM each)	5,2 µL
Ampli Taq®DNA Polymerase Stoffel fragment (Perkin -Elmer)	0,1 µL (0,1 U)
dd H2O	0,45 µL
Template (2-20 ng µl-1)	1,0 µL

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[1] Primers (from Operon Technologies, Alameda, California), OPA-04 (5,2 mM), OPA-07 (4,8 mM), OPB-08 (5,4 mM), OPB-12 (5,5 mM), OPC-05 (5,4 mM), OPC-11 (4,8 mM), OPC-14 (5,8 mM), OPC-15 (4,8 mM), OPC-20 (5,5 mM), OPD-06 (5,5 mM), OPD-13 (5,5 mM). Temperature profiles, for all primers: 94°C, 3 min, followed by 46 cycles of 94°C, 20 s: 42°C, 30 s, 72°C, 1 min: extension at 72°C for 10 min. Amplification products were kept at 4°C until loaded onto a gel.

Table 2. Sequences of primers used in the RAPD analysis. Sequences read from 5' end. Primers marked with asterisk were not used in the third test group

A-04=AATCGGGCTG
A-07=GAAACGGGTG
B-08=GTCCACACGG
B-12=CCTTGACGCA *
C-05=GATGACCGCC*
C-11=AAAGCTGCGG*
C-14=TGCGTGCTTG
C-15=GACGGATCAG
C-20=ACTTCGCCAC
D-06=ACCTGAACGG
D-13=GGGGTGACGA

Three groups of animals were tested. The first group consisted of 12 individuals from *B. longispina* and 11 from *B. coregoni* (1994 sample). A second group, seven *B. longispina* and eight *B. coregoni*, acted as control group to test for the reliability of the method (1994 sample). In a third group, we tested intermediate juveniles along with adults from the two populations (1995 sample). In the last group we used eight primers. Bands were scored as present or absent at a given site, using only the positions where bands could be unambiguously scored over all 11 and 8 primers respectively.

We tested for stringency by replicating the RAPD analyses on six individuals from group one with primers A-07, C-15, B-08 and C-20, on six individuals from group two with primers A-07, C-14, C-15 and C-20, and on nine individuals from group three with primers B-12, C14 and D-13. These primers were selected because they provided distinct bands that were easy to score.

Pairwise similarities between specimens were calculated based on presence and absence of RAPD bands using the Dice coefficient (S) (Rohlf 1993): $S = 2a/(2a + b + c)$, where a is the presence of a fragment in both individuals, b the presence in one but not the other, and c opposite of b. We have not used a common absence of fragments as an indication of similarity, taking a conservative approach which was further emphasized by doubling the common presence of fragments. To reveal possible groupings of the specimen (corresponding to genetic units), non-metrical multidimensional scaling (Kruskal, 1964) was used for ordination of the similarity matrix into two dimensions. This is an ordination technique (like principal coordinates analysis, PCA) for representing similarities between objects in a two- or three dimensional space, where similar objects are placed closer together and dissimilar objects further apart (Rohlf 1993). Similarity calculation and ordination was done with NTSYS. pc ver. 1.80 (Rohlf, 1993).

Sufficient genetic differences between the populations, indicating different species, should show up as a grouping of the individual specimens in agreement with the two forms. To test for grouping, we compared our original results with 12 randomized populations (using MacClade 3.0), each with the same frequencies of absences and presences as in the original sample. Each of the randomized populations was analysed in the same way as the original data set. We presented the original ordination, together with one ordination based on the randomized data, to 12 colleagues, asking them which of the two ordinations (if any) showed a concordant grouping. Each colleague was presented a unique randomized ordination together with the original. Assuming no difference in degree of grouping between the original and the randomized populations, the null hypothesis was that both populations had the same probability (p=0.5) of being scored as most clearly grouped. We then tested the outcome of the twelve comparisons by sign test (Siegel and Castellan 1988).

Results

Considering primers together the first group had a mean of 16.1 ± 4.5 (SE) bands per primer. The second group showed a similar pattern with a mean of 15.2 ± 3.5 (SE), and the third group with a mean of 17 ± 4 (SE) bands per primer. The mean number of bands per individual and primer was lower (Table 3). No primer was monomorphic, that is no primer produced exactly the same amount or position of bands in all individuals.

Table 3. Mean number (± SE) of bands per individual and primer in the three test groups.

	Group one:		**Group two:**	
	B. longispina (n=11)	*B. coregoni* (n=12)	*B. longispina* (n=7)	*B. coregoni* (n=8)
Primers:				
A04	5.4 ± 2.4	8.7 ± 1.6	8.7 ± 1.6	6.5 ± 1.8
A07	5.8 ± 1.5	6.3 ± 2.4	6.3 ± 2.4	6.6 ± 1.2
B08	7.4 ± 1.9	6.7 ± 2.1	6.7 ± 2.1	5.8 ± 1.0
B12	6.8 ± 2.0	6.3 ± 1.1	6.3 ± 1.1	5.8 ± 2.0
C05	7.4 ± 1.6	7.3 ± 1.1	7.3 ± 1.1	6.9 ± 1.6
C11	6.5 ± 1.4	9.4 ± 1.3	9.4 ± 1.3	7.9 ± 2.1
C14	5.9 ± 1.6	6.6 ± 2.2	6.6 ± 2.2	4.5 ± 2.0
C15	5.4 ± 1.3	2.1 ± 1.2	2.1 ± 1.2	2.5 ± 1.1
C20	9.2 ± 1.1	6.3 ± 1.8	6.3 ± 1.8	5.6 ± 1.1
D06	6.3 ± 1.5	3.1 ± 2.1	3.1 ± 2.1	4.2 ± 1.0
D13	7.8 ± 2.0	8.3 ± 2.1	8.3 ± 2.1	5.5 ± 1.6

	Group three:		
	B. longispina (n=9)	*B. coregoni* (n=8)	Juveniles (n=10)
Primers:			
A04	1.9 ± 1.0	4.0 ± 0.9	4.9 ± 0.9
A07	1.7 ± 0.7	5.4 ± 2.0	4.2 ± 1.4
B08	2.3 ± 1.3	6.4 ± 3.2	6.8 ± 1.9
C14	4.2 ± 1.0	6.5 ± 1.3	6.4 ± 1.4
C15	1.6 ± 0.7	3.2 ± 0.9	5.0 ± 1.8
C20	3.4 ± 1.1	3.8 ± 0.7	4.9 ± 1.1
D06	2.1 ± 0.8	4.9 ± 2.3	4.1 ± 1.4
D13	3.6 ± 1.2	3.9 ± 0.6	3.9 ± 1.0

The average reproducibility, measured as the same bands appearing in replicates and tests on the gel, was 83% for the primers tested in group one, and for group two and three 85%. No differences in position of bands between replicates and tests were found. In some cases a band appeared in tests but not in replicates and vice versa.
The ordination analysis based on the similarity matrix separated the two species in the case of the first group (Fig. 2A). This was confirmed by the results in the control group, also separating the two species (Fig. 2B). In the third group, where juveniles with long antennule and mucro were included, juveniles and adults of *B. coregoni* were grouped together, but separated from *B. longispina* (Fig. 2C). When testing the combined outcome of original data and randomized data, the null-hypothesis was rejected ($p<0.0005$). One of the 12 ordination analyses based on randomized data is presented in figure 3.

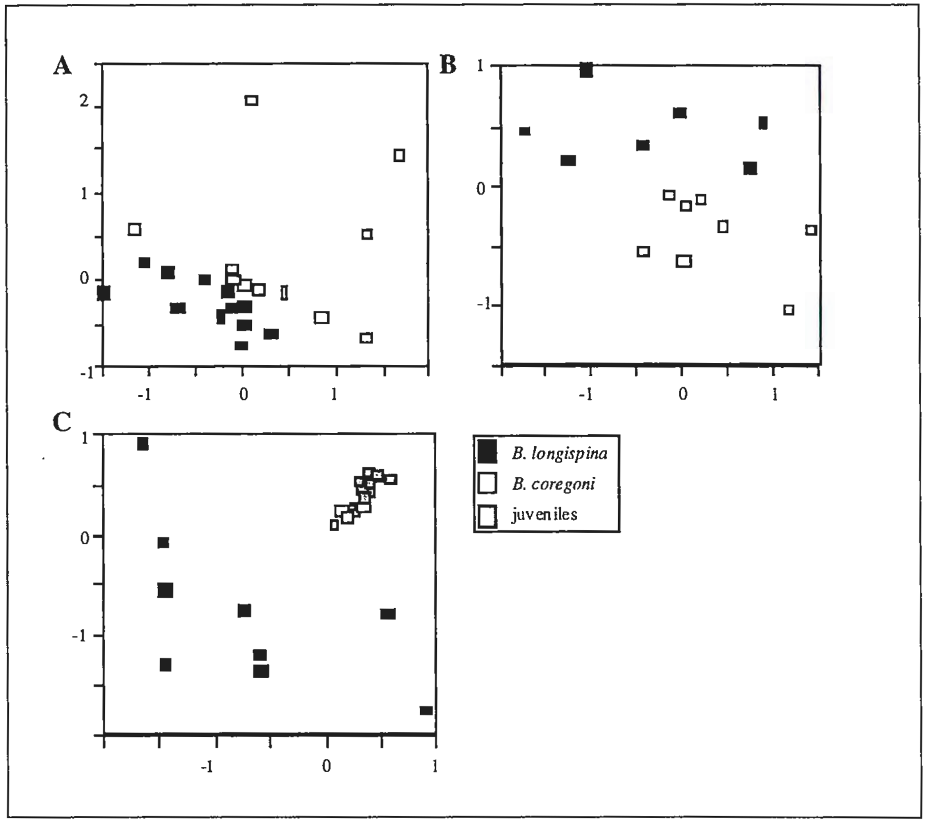

Fig. 2. *Ordination of the specimens in two dimensions based on the Dice similarity between individuals. A: group one, B. group two, and C: group three.*

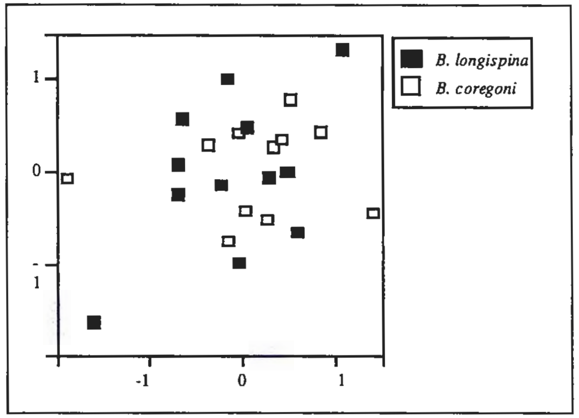

Fig. 3. Example of one ordination of randomized specimens in two dimensions based on the Dice similarity between individuals, mimicing the two species B. longispina and B. coregoni. The expected frequency of RAPD markers were estimated from the data underlying Fig. 2A and 2B.

Discussion

RAPD has been applied to identify genetic markers linked to certain phenotypes (Martin et al. 1991; Michelmore et al. 1991; Paran et al. 1991), and to identify species (Crossland et al. 1993; Dinesh et al. 1993). In many respects, RAPD has replaced or complemented enzyme electrophoresis studies. One distinct advantage is that it requires such small amounts of DNA that even a tiny single specimen can be used for a number of analyses. However, not all variation at RAPD loci can be detected, and not all primers produce polymorphism. In our study, the RAPD markers turned out to be polymorphic, and we used primers that produced an appropriate number of bands (Crossland et al. 1993). Several such primers were applied to increase the number of bands used for assessing the genetical differentiation.

The RAPD showed a clear genetic difference between the populations indicating that they are reproductively isolated from each other, and hence belong to different species. The results are in accordance with the classification based on morphological characters (Flössner 1972; Lieder 1983a; De Melo & Hebert 1994a). It is thus, not a question of different forms, but different species replacing each other during the season. Also, juveniles with long antennule and mucro could be assigned as juveniles of *B. coregoni*. We have not found any description of juveniles in the literature, but the lack of mucro is one of the morphological features distinguishing adult *B. coregoni* from other species (Flössner 1972; Leider 1983a). Our results clearly demonstrate that juveniles of *B. coregoni* in lake Östersjön undergo morphological changes when becoming adults, i.e. they develop a higher carapace, and longer antennule, and the mucro disappears. Thus, morphological changes in this population occurs in juveniles as well as in adults.

The banding patterns differed not only between but also within the populations. This is somewhat unexpected for two reasons. First, *Bosmina* like other cladocerans reproduce mainly by parthenogenesis (Manning et al. 1978) which should result in lower genetic variation (Mort 1991). Studies on *Daphnia* and rotifers have, however, revealed genetic variation comparable to sexual populations, such as copepods (Mort 1991; Hebert et al. 1989). Second, we expected to find members of the same clone in the samples

(samples were taken within a limited area in the lake). We suggest that the observed variability may have been due to several clones being present in the lake (though we cannot rule out technical artefacts, e.g. differences in the PCR amplifications). However, the observations are consistent with studies on *Daphnia,* where populations in large lakes are genetically diverse and consists of several clones (Jacobs 1990; Mort 1991).

We have demonstrated that RAPD is a relatively easy technique for revealing genetic differentiation among *Bosmina* populations, confirming earlier classifications based on morphological traits. Phenotypic variation is common within the *B.coregoni* complex, and a variety of subspecies have been described (Lieder 1983b). More extensive studies are, however, needed to determine the extent of genetic differences among these subspecies. As documented here, the RAPD method may prove useful in detecting such differences.

Acknowledgments

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III

Increased drag, and thus lower speed, a cost for morphological defence in *Bosmina (Eubosmina)* (Crustacea: Cladocera)

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Abstract

The swimming speed of two forms, an extreme and a typical, within the cladoceran subgenus *Eubosmina* were examined using three-dimensional video-technique. The extreme form has a very high carapace and extremely long antennule, features probably involved in predator defence. We found that the extreme form swam almost 40% slower than the typical form. Calculations show that the extreme form had to work at least 12% harder to swim at the same speed, or if they used the same amount of energy to swim, the extreme form should swim 6% slower. Increased drag, because of its distinguishing carapace and antennule, is thus the most likely explanation for the slower swimming speed of the extreme form assuming they select the same power output. Swimming speed can be correlated to food intake either by the frequency of hits to eatable food particles or the time to swim from a poor food patch to a good one. So the reduced speed is probably a great cost for the extreme form.

Introduction

Predation is one of the most important evolutionary forces in zooplankton communities. Small planktonic species suffer from the predation of larger carnivorous invertebrates which has led to the evolution of defensive morphological structures such as spines, helmets and gelatinous capsules, that make it more difficult for the invertebrate predators to handle them (Havel & Dodson 1984; O'Brian, Kettle & Reissen 1979).

Since it is often the case that defended morphs are replaced by undefended morphs when the predation pressure declines (Kerfoot & Peterson 1980), it is reasonable to assume that there should be some cost to develop and maintain a morphological defence (Harvell 1990). Most studies of the costs of morphological defences have focused on measurements of reproductive parameters (e. g. Reissen 1984; Black & Dodson 1990) but an important question is the way in which the structure of these traits can affect fitness negatively. Lüning (1994) proposed that the reproductive costs did not necessarily have to be directly associated with the defences but argued instead that induced life history shifts may present another explanation to the decreased reproductive rates observed in some experiments. Direct costs might be increased time and resources needed for the body development - which has been proposed to be the major cost of *Daphnia* possessing neck and tailspines (Reissen 1992) and in the long-featured morphs of *Bosmina longirostris* (Kerfoot 1977; 1987). Another cost could be increased drag and a higher energy expenditure for motion or a lower speed. The swimming speed may influence filtering rate and the frequency of hits to eatable food particles (DeMott 1982; Gerritsen & Strickler 1977). Costs in this sense have not been investigated to any large extent but it has been suggested as a cost for gelatinous capsules in *Holopedium gibberum* (Stenson 1987).

In Lake Östersjön, SW Sweden, two forms appear within the cladoceran subgenus *Eubosmina*, one typical *E. longispina longispina* (Leydig) and one extreme *E. coregoni gibbera* (Schoedler), the latter with a very high carapace and long antennule. Hellsten & Stenson (1995) have described a replacement cycle wherein the typical form is replaced by the extreme, from early spring to autumn, coinciding with the hatching of the cladoceran predator *Leptodora kindtii* Focke. In an experimental study the extreme form survived significantly better than the typical form when exposed to the cladoceran predator *Leptodora kindtii* (Hellsten & Lagergren unpublished).

These very different forms (Fig 1) of continuously swimming *Eubosmina* provide suitable material, with which to study the costs of morphological defences in terms of increased drag. This study includes a model of how the drag could affect the two forms and an experiment wherein the swimming speed of the two forms was measured with the help of video technique.

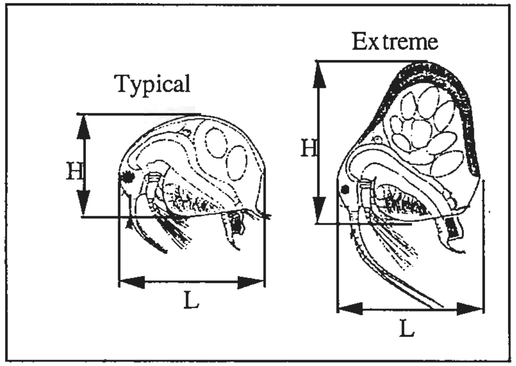

Fig 1. The two forms of Bosmina (E.). Features measured are body length (L) and body height (H).

Methods

Specimens were collected from Lake Östersjön, a eutrophic lake (1,5 km^2; max. depth 32 m) in SW Sweden (57° 93´N, 12° 79´E). The samples were collected in May when both forms are present in the lake. Animals to be used in the experiment were chosen from field samples and kept in aquaria at approximately 22°C, until they were filmed. The animals were filmed in a cubic glass chamber with sides of 2,1 cm, giving a volume of 9,26 cm^3. Illumination was provided by a circular "cold-light"-lamp (Schott) situated approximately 12 cm above the chamber. The water in the chamber was filtered (25 µm) lake water from Lake Östersjön and was held at the same temperature as that in the aquaria. Both forms were held in the same aquarium and were chosen in random order. Each specimen was filmed during a 5 minute period with the three-dimensional technique described by Ramcharan & Sprules (1989) using one camera and four mirrors. The mirror system gives a figure of the aquarium from two directions on the TV-monitor. This makes it possible to see the animals´ movements in three dimensions. The first five sequences, in which the animals swam quite straight, were analysed for a periodof at least 2 sec., the movement was plotted on the screen and the speed was calculated as $(x^2+y^2+z^2)^{1/2}/t$. The average speed was then calculated for each animal. After filming, the body length (L) and height (H) of each individual was measured, and the H/L-value was used as an estimation of the degree of "humpbackness" (see Fig 1).The side profile area (S_{SP}) for 16 randomly chosen individuals of both forms was estimated by weighing pieces of paper of the animal's shape. Thesehad been drawn by using a dissecting microscope with a drawing tube attached to it. The S_{SP} were used in the calculations of drag.

model

There are two kinds of drag, pressure drag (Dp) which depends on inertial forces and friction drag (Dv) which depends on viscous forces. The Reynolds number (*Re*) which is of great importance in fluid mechanics is an estimate of the relative importance of these forces. Since the *Re* for a swimming *Bosmina* range from 0.8 to 5, depending on size and swimming speed, the most important of the two drags will be viscous drag. The other sort of drag, pressure drag, becomes important when *Re*»1 (Vogel 1981; Alexander 1983). Eq. 13,1 in Vogel (1981),

$$\boxed{D=C_{DVS}\cdot\mu\cdot S^{1/2}\cdot U} \qquad (1)$$

D=drag, C_{DVS} is a constant due to the shape of the body, μ is the kinematic viscosity of the fluid, S is the wetted surface area and U is velocity, is trustworthy up to *Re* of ≈1. With that equation as basis, the relative changes in speed (with constant power) and power (with constant speed), due to changes in the H/L-ratio, can be calculated as follows:

A regression line between H/L and the "relative side profile area" (rS_{SP}) was made for the 16 individuals on which the side profile area (S_{SP}) was measured (Fig 2). The parameters were well correlated. rS_{SP} is independent of the body length, without unit but proportional to S_{SP}. If the S_{SP} is assumed to be proportional to the wetted surface area (S), the relative change in S is possible to calculate from different H/L-ratios. The constant C_{DVS} is dependent upon the shape of the body but the difference in shape between the morphs leads to very little change in its value (Vogel 1981), thus, C_{DVS} can be regarde as a constant for all H/L-ratios. The drag (D) then becomes proportional to $S_{SP}^{1/2}\cdot U$. Power(P)=D·U and the relative change in power needed for locomotion keeping the velocity constant can be calculated for different H/L-values as:

$$\boxed{P\mu\ D\mu S_{SP}^{1/2}} \qquad (2)$$

Similarly the relative change in velocity with constant power output is possible to calculate. The result is:

$$\boxed{U\mu 1/S_{SP}^{1/4}} \qquad (3)$$

These equations can give the predictions shown in Fig 3.

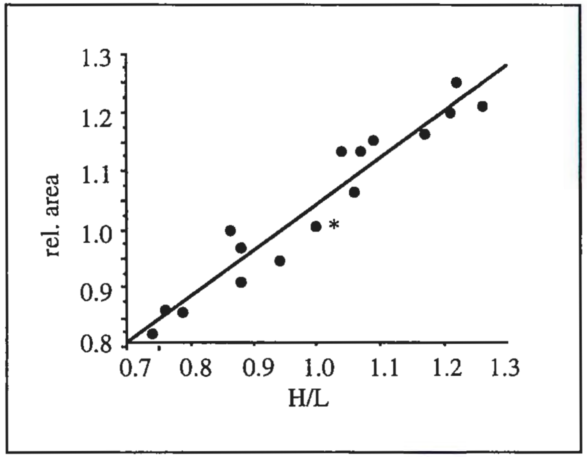

Fig 2. *The regression line (rS_{SP}= 0.805· H/L+ 0.234; r^2 = 0.93) between H/L (see Fig 1) and relative side profile area (rS_{SP}) that was used in the calculations of drag (Fig. 3). Each point represents one animal. The individual marked with * is the starting point whose rS_{SP} was set to 1. The rS_{SP} for remaining individuals can be calculated as: $L_o^2 \cdot A_x / L_x^2 \cdot A_o$. Where: L_o= the length of the individual chosen as starting point; L_x= the length of individual x; A_o= the side profile area of the individual chosen as starting point ; A_x= the side profile area of individual x.*

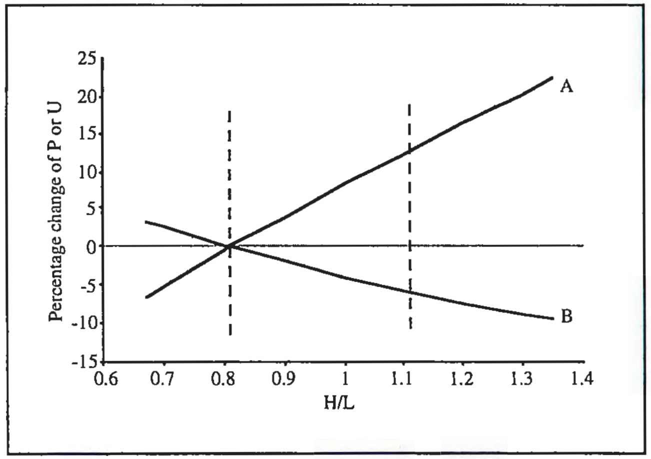

Fig 3. *Curve A demonstrates how much individuals of different H/L (see Fig 1) have to work, to swim at the same speed, in comparison with an individual of the typical form with an H/L-value of the average. Curve B shows how the H/L-value affects the speed if they use the same power for locomotion. The dotted vertical lines represent the mean H/L-values for the typical and extreme form respectively.*

Results

The mean length of the animals used in the experiment did not differ between the two forms (Table 1). The extreme form swam significantly slower than the typical ($p<0.005$ Mann-Whitney U-test, $n=14$). Among the specimens of each form there was no correlation between H/L and speed (F-test, $P>0.05$ in both cases, $n=14$).

Table 1. Mean swimming speed (mm/s), mean body length and mean H/L for typical form (T) and extreme form (E). The sample size was $n=14$ for both forms. The difference in mean speed between forms is significant $p<0.0005$, Mann-Whitney U-test.

	Speed mean ± SD	Body length (mm) mean ± SD	H/L mean ± SD
Forms:			
T	4.08 ± 0.92	0.47 ± 0.07	0.81 ± 0.07
E	2.94 ± 0.56	0.47 ± 0.04	1.11 ± 0.09

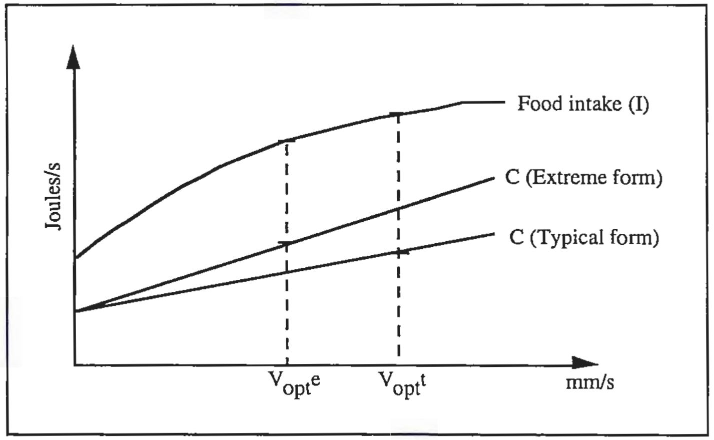

Fig 4. Hypothetical curves of food intake (I) and cost of swimming (C) for feeding Bosmina, of typical and extreme forms, plotted as a function of swimming speed. A simple model that supposes that the animals try to maximise net energy gain per time unit (E/t), E/t=I-C, predicts that the optimal swimming speed, V_{opt}, *is lower for the extreme form then for the typical.* $V_{opt}e$*=optimal swimming speed for the extreme form.* $V_{opt}t$*=optimal swimming speed for typical form.*

Discussion

The results show that the typical form swims almost 40 percent faster than the extreme form. Increased drag seems to be the most likely explanation for the extreme form swimming more slowly than the other form. Our model suggests a relation between the H/L-value and swimming speed due to differences in drag (Fig 3). It is evident that the difference in swimming speed between the mean H/L-ratio of the extreme and the typical form would be only about 6% if they use the same amount of energy when swimming. This figure is considerably less than the observed difference of 40%. The speed of a *Bosmina* varies during a single stroke (Zaret & Kerfoot 1980) with the highest speed being around 10 mm/s in *Bosmina longirostris*. *Eubosmina* swims faster than *Bosmina longirostris* (Stenson 1990) and it may attain a *Re* >10 just after a stroke when the speed is highest. In higher *Re*, where pressure drag becomes the dominating force, the shape of the body becomes more important for the drag (Vogel 1981), thus the difference in drag between the forms is certainly greater than these simplified calculations predict. A relation between H/L and speed (as the model predicts) could not be determined among the specimens of each form. This was probably due to a small variance in H/L and a large variance in speed.

So how could the difference in swimming ability influence the food intake of these animals? DeMott (1982) writes that "the continuous swimming of *Bosmina* could be a behavioural adaptation to increase the encounter rate with food particles". As algae are slow moving particles, increased speed in the predator implies higher encounter rates (Gerritsen & Strickler 1977). This means that swimming speed would be correlated to the efficiency in food gathering, thus the lower the speed the lesser the food intake by the filter-feeding zooplankton. Accordingly, this could imply a greater cost for the extreme form than for the typical.

A model on optimal foraging of a *Eubosmina*, if food intake is correlated to swimming speed, may look something like Fig 4. Without swimming the movement of the filtering apparatus itself gives some food intake which then rises asymptotically (functional response type 2 (Holling 1966)) with increased swimming speed. The energy consumption rises linearly from a basal metabolism (linearly because a *Bosmina* regulates its swimming speed mostly by varying the time between strokes (Zaret & Kerfoot 1980)) and the slope of the line depends on the drag. The model predicts that the form with increased drag optimises its net food intake if it reduces its swimming speed.

It has not been proven that the food intake depends on the swimming speed but if the concentration of food particles is uneven, optimal foraging would be to swim slowly in high food concentration and more quickly if the concentration is low in order to leave the latter of these two as fast as possible. In a comparison between slow swimming *Ceriodaphnia dubia* and fast swimming *Daphnia pulex*, it was shown that only the faster swimmer was able to locate the good food patches (Cuddington & McCauley 1994). This means that the slower swimming speed may be a cost even if the food intake does not depend on the swimming speed directly, because it takes more time to swim from a poor food patch to a good one.

Another explanation for the extreme form swimming more slowly may be differences in swimming behaviour. Lower speed reduces the risk of encounters with predators (Gerritsen & Strickler 1977). It could be that the extreme form has a behaviourally lower swimming speed regardless of the presence of a predator or not. Contradictory to this hypothesis is the fact that the typical form suffers more from encounters with predators and thus should swim slower than the extreme form.

The relative importance of different ultimate costs remains to be solved in the case of this as well as other species and may be affected by conditions such as the food concentration. At low food concentrations, animals probably suffer more from reduced swimming speed and feeding efficiency because the net energy gain falls faster (with reduced food concentration) for an animal with increased drag (Ware 1975). Interestingly, the extreme form is most often found in eutrophic lakes (Stenson personal observations).

We conclude that drag is probably an important force working against the evolution of certain phenotypical adaptations used as predator defence in zooplankton. Extreme *Eubosmina* forms, which are prevalent under high predation risks, have a morphology that significantly increases drag. When predation risks decline, on the other hand, selection will favour a morphology that is less costly.

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IV

Habitat distribution of two *Eubosmina* species, and *Leptodora* in Lake Östersjön.

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Abstract

We investigated the vertical and temporal distribution of two co-existing species of *Eubosmina*, *Bosmina (E) coregoni* and *Bosmina (E) longispina*, and an important predator, *Leptodora kindtii* in eutrophic Lake Östersjön. Although the lake has extensive populations of fish, *Leptodora* did not exhibit vertical migration.The high water turbidity, leading to reduced visibility for the fish, and the transparency of *Leptodora* may explain this. *B. coregoni* was more abundant near the surface, while *B. longispina* was more evenly distributed in the water column. Interspecific competition, and differences in vulnerability to *Leptodora* predation are proposed as explanations to this. The more extreme body form of *B. coregoni* may be accompanied by costs, which forces *B. coregoni* to spend more time in food rich strata near the surface. Further, *B. coregoni* was more abundant in summer, i.e. at times with high densities of *Leptodora,* and mean size in *B. coregoni* was positively correlated with the abundance of *Leptodora*, which was not the case for *B. longispina.*

Introduction

Predation can have a major impact on zooplankton communities. Large and conspicuous species, which are vulnerable to visually hunting predators like fish will be excluded if predation pressure is sufficiently high. This may favour smaller and competitively inferior species (Brooks and Dodson 1965; Vonder Brink and Vanni 1993). Small zooplankton may on the other hand be more preyed upon by invertebrate predators, who's possibilities to catch and handle the prey often depend on the size and structure of the prey. (Dodson 1974, Lynch 1980, Zaret 1980).

Cladocerans exposed to invertebrate predators have in several cases evolved morphological features such as spines and helmets to reduce mortality. These structures tend to make them more difficult to catch and handle (Havel 1987; Stenson 1987; Dodson 1989; Harvell 1990). Under high predation risk from invertebrate predators, prey with antipredatory structures should be selectively favoured. However, the morphological features are in many cases associated with some kind of cost, for example lower reproductive rate (Kerfoot 1978; Dodson 1984; Black and Dodson 1990), which may explain why such features often occur only when predation pressure is high.

Two species within the subgenus *Eubosmina*, *Bosmina longispina* (Leydig) and *Bosmina coregoni* (Baird) (Fig 1), are found in Lake Östersjön, SW Sweden. The two species succeed each other during the season with highest densities of *B. coregoni* in summer, while *B. longispina* is more common in spring and autumn (Hellsten and Stenson 1995). *B. coregoni* also undergoes a seasonal morphological change with a higher carapace and longer antennule in the summer (Hellsten and Stenson 1995). The succession, and the morphological changes coincide with the hatching of *Leptodora kindtii*, the dominating invertebrate predator in the lake. This cladoceran is an efficient predator on smaller zooplankton (Herzig and Auer 1990). Both *Bosmina* species are quite small (body length 0.1-0.7 mm) and probably vulnerable to invertebrate predators such as *Leptodora* (Lunte and Luecke 1990; Urabe 1990; Branstrator et al. 1991; Lehman 1991), although *Bosmina* species may also be exposed to fish predation (Vonder Brink and Vanni 1993). Under high predation pressure, *B. coregoni* with its more extreme body form may suffer less from a tactile predator than *B. longispina*. The morphological features reducing the predation risk (Hellsten et al. MS), may be accompanied by costs in terms of increased drag, and thereby increased energy expenditure for motion and speed (Lagergren et al. in press). This may in turn lead to reduced fecundity as found in other *Bosmina* populations with morphological defences (Kerfoot 1977). *B. longispina*, with less extreme morphological features, may therefore be competitively superior when predation pressure is low. Leptodora is a large zooplankton (body length ≤ 10 mm), and one problem for this predator is to avoid being discovered by visually dependent fish. This can be achieved in two ways. By vertical migration to greater depths, with reduced light intensity, the possibility of being detected can be decreased. Another possibility is to avoid discovery within the sensory sphere of the fish by being cryptic. *Leptodora* does not contain much pigment, i.e. hemoglobin, carotenoids, resulting in extreme transparency (Zaret 1980).

The purpose of the present work is to study the vertical and temporal distribution of *Leptodora* and the two *Bosmina* species in Lake Östersjön. We predict that *Leptodora* should migrate to greater depths in day time in order to reduce the predation risk from fish.The extreme morphology in *B. coregoni* may lead to higher energy expenditure compared with *B. longispina*, and should therefore stay in regions with high food density, i.e. near the surface. Further, we also analyse the variation in body size of the two *Bosmina* populations in relation to densities of *Leptodora*.

Materials and methods

Vertical and temporal distribution

Samples were taken at three occasions in 1993 and at one occasion in 1995 in the western part of Lake Östersjön (57° 93'N, 12°79'E). Further information about the lake is given elsewhere (Dahlbäck 1991; Hellsten and Stenson 1995). Samples were collected May 19th, July 1th and August 14th in 1993, and July 24th in 1995. At each occasion samples were taken from three different depths at 5 a.m., 12 a.m., 5 p.m., 9 p.m. and 12 p.m. (in August 1993 and July 1995 no samples were taken at 9 p.m.). A plankton net (100 µm mesh) with a closing mechanism was hauled vertically between 2-0, 5-3 and 8-6 meters depth (maximum depth is 8 meter in this part of the lake). From each depth two hauls were taken, pooled together, concentrated by filtration through a 150 µm filter, and preserved in 70% ethanol. At each date water temperature was also measured at 2, 5 and 8 meters depth.

All *Leptodora*, *B.coregoni* and *B. longispina* found in the samples were counted and estimated to density per litre. Mean diel densities of *B. coregoni*, *B. longspina* and *Leptodora* collected at each sample date were calculated, and differences in mean diel densities among dates were tested with Kruskal Wallis One-way Analysis of Variance (Statview). Since the two *Bosmina* species were collected in the same samples we tested for dependency between densities of *B. longispina* and densities of *B. coregoni* with Spearman rank correlation test (Statview) for each date respectively.

Effects of time of day and depth on distribution for the three species were tested with Spearman partial rank correlation by using SAS statistical package (SAS 1988) for each of the four sample dates. Each sample event was used as one sample unit, i.e. five sample occasions per day and at each occasion from three different depths. Since multiple tests adressed a common null hypothesis (no difference in distribution among time of day or depths) we adjusted α-levels by the sequential Bonferroni method to ensure an overall significance level of 5% (Rice 1989; Chandler 1995). Statistical tests are two-tailed.

Prey body size and predator abundance

Animals collected from May to September in 1991-1992, from May to August in 1993 and in July 1995 were used to test for the relationship between prey body size and density of *Leptodora*. In this analysis all samples were collected with a plankton net (100 μm mesh) hauled from bottom to surface at 12 a.m. At each sample occasion temperature was also measured at 2, 5 and 8 meter, and mean temperature was then calculated. Usually 20-60 *B. coregoni* and *B. longispina* were picked out randomly from each sample and measured in lateral view at 100 x magnification using an occular micrometer fitted to an inverted microscope. When less than 20 animals were found, all animals were measured. We measured body length and body height (Fig. 1). A size index was obtained by multiplying length by height. All *Leptodora* found in samples were counted and estimated to density per litre. We then tested for a correlation between mean prey body size and *Leptodora* density (n=14, sample dates with_*B. coregoni*; n=11, sample dates with *B. longispina*. Finally, correlation between density of *Leptodora*, *B. coregoni*, *B. longispina* and temperature was tested.

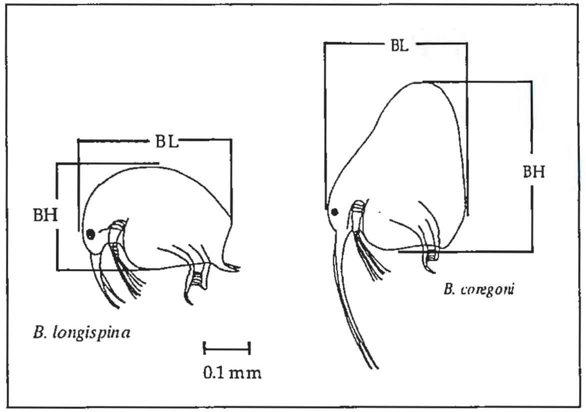

Fig. 1. Lateral view of adult females of B. longispina and B. coregoni. On figure are also indicated features measured: body length (BL) and body height (BH).

Results

Vertical and temporal distribution

The lake was not stratified at any of the dates. Mean temperatures were, 16° C in May, 19° C in July, 19° C in August 1993, and 19° C in July 1995.

Mean diel densities of *B. coregoni*, *B. longispina* and *Leptodora* are presented in Table 1. *B. coregoni* and *Leptodora* were most abundant in July, ($p<0.05$, multiple comparisons, Siegel and Castellan 1988), whereas *B. longispina* did not vary significantly in abundance among the sample dates. Densities of *B. longispina* and *B. coregoni* were not significantly correlated at any sample date ($r_s<0.23$, $p<0.40$, May; n=14, July; n=12, August; n=12, July; n=12).

Densities of *Leptodora* were not significantly correlated (Fig. 2) with time of day when depth was controlled for ($0.02 \le r_s \le 0.52$, $p \ge 0.14$, May; n=14, July; n=12, August; n=12, July; n=12, Spearman partial correlation). Nor did densities of *Leptodora* vary significantly with depths, when time of day was controlled for ($-0.31 \le r_s \le 0.17$, $p>0.38$).
When depths were controlled for, densities of *B. longispina* did not vary significantly (Fig. 3) with time of day ($0.01 \le r_s \le 0.7$, $p>0.05$, May; n=14, July; n=12, August; n=12, July; n=12, Spearman partial correlation). Nor did densities of *B. longispina* vary significantly with depths, when time of day was controlled for ($-0.06 \le r_s \le 0.58$, $p>0.08$).

Densities of *B. coregoni* did not vary significantly with time of day when depths was controlled for. However, when time of day was controlled for, densities were negatively correlated with depth (Fig. 4). Depth was therefore an important factor influencing the distribution of *B. coregoni*.

Table 1. Mean diel density (ind l^{-1}) ± SE of *B.coregoni*, *B.longispina* and *Leptodora* sampled in May, July, and August 1993, and July 1995. Samples were collected from 2-0, 5-3 and 8-6 meters depth at 5 a.m., 12 a.m., 5 p.m., 9 p.m. and 12 p.m. Missing values from 5-3 meter at 12 p.m. in May (n=14), and from all depths at 5 a.m. in July 1993 (n=12). No samples collected at 9 p.m. in August 1993, and July 1995 (n=12 respectively). Differences in mean density between sample dates in 1993 were tested with Kruskal-Wallis One-way Analysis of Variance for the three species respectively.

	B. coregoni	N	*B. longispina*	N	*Leptodora*	N
May 1993	14.8 ± 3.2	14	8.9 ± 1.9	14	0.51 ± 0.18	14
July 1993	46.6 ± 13.3	12	9.2 ± 2.0	12	4.2 ± 0.98	12
August 1993	4.4 ± 0.95	12	9.9 ± 2.4	12	2.7 ± 0.60	12
July 1995	18.0 ± 3.7	12	8.0 ± 2.3	12	0.65 ± 0.17	12
	p=0.0001		p=0.92		p=0.0002	

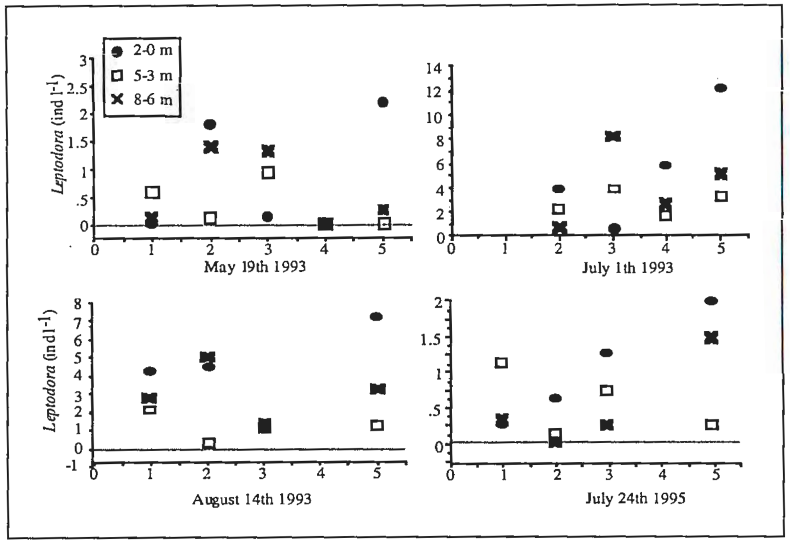

Fig. 2. Relationship between Leptodora densities (ind l^{-1}) and time of day (5 a.m., 12 a.m., 5 p.m., 9 p.m. and 12 p.m.) and depths (2-0, 5-3, and 8-6 m), in May (n=14), July (n=12) and August (n=12) 1993, and July 1995 (n=12). Numbers on x-axis (1, 2, 3, 4, and 5) denote 5 a.m., 12 a.m., 5 p.m., 9 p.m. and 12 p.m. respectively.

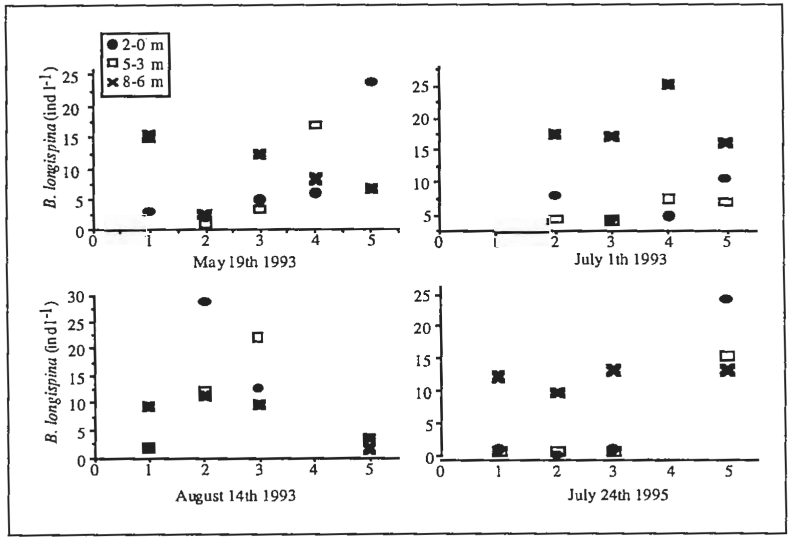

Fig. 3 Relationship between B. longispina densities (ind l^{-1}) and time of day (5 a.m., 12 a.m., 5 p.m., 9 p.m. and 12 p.m.) and depths (2-0, 5-3, and 8-6 m), in May (n=14), July (n=12) and August (n=12) 1993, and July 1995 (n=12). Numbers on x-axis (1, 2, 3, 4, and 5) denote 5 a.m., 12 a.m., 5 p.m., 9 p.m. and 12 p.m. respectively.

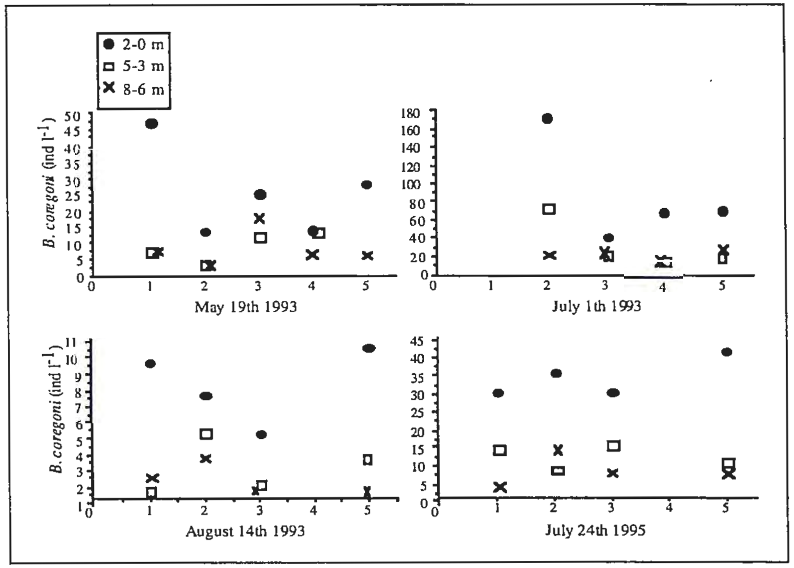

Fig. 4. Relationship between B. coregoni densities (ind l^{-1}*) and time of day (5 a.m., 12 a.m., 5 p.m., 9 p.m. and 12 p.m.) and depths (2-0, 5-3, and 8-6 m), in May (n=14), July (n=12) and August (n=12) 1993, and July 1995 (n=12). When depth was controlled for, densities with time of day did not vary significantly (*$-0.40 \le r_s \le 0.12$*,* $p \ge 0.22$*). When time of day was controlled for, densities of B. coregoni were negatively correlated with depths (*$-0.89 \le r_s \le -0.62$*,* $p<0.05$*, Spearman partial correlation with sequential Bonferroni adjustments). Numbers on x-axis (1, 2, 3, 4, and 5) denote 5 a.m., 12 a.m., 5 p.m., 9 p.m. and 12 p.m. respectively. Missing values in May from 5-3 meter depth at 12 p.m., and in July from all depths at 5 a.m. No samples collected at 9 p.m. in August 1993 and July 1995.*

Prey body size and predator abundance

Mean size of *B. coregoni* was positively correlated with densities of *Leptodora* (Fig. 5). When excluding the outlier, the magnitude of the correlation changed but was nearly significant (r_s =0.55, p=0.06). No significant correlation was found between mean size of *B. longispina* and densitites of *Leptodora* (r_s =0.22, p=0.49, Spearman rank correlation, n=11 sample dates).

Densities of *Leptodora* and temperature were positively correlated (r_s =0.63, p=0.01, n=14). Neither densities of *B. coregoni* nor of *B. longispina* were significantly correlated with temperature (r_s = -0.10, p=0.75, n=14, r_s =0.25, p=0.45, n=11).

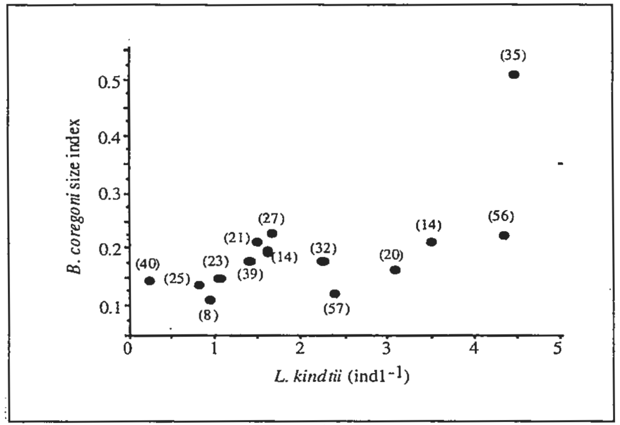

Fig. 5 Relationship between mean size of B. coregoni and density of Leptodora (r_s=0.64, p=0.02, Sperman rank correlation). Pooled data from samples collected in 1991-1993 and 1995 (n=14, sample dates), with number of B. coregoni measured in brackets.

Discussion

B. coregoni was most abundant in July, which is in accordance with our previous field studies. *B. longispina* was found in the July samples, although it was absent during that month in previous years (Hellsten and Stenson 1995), probably reflecting inter-year variation. Density of *Leptodora* was also highest in July, and seem fairly consistent with other studies (Lunte and Luecke 1990; Vijverberg 1991). *Leptodora* densities were strongly correlated with mean temperature. Predation rate of *Leptodora* increases with temperature and prey density (Herzig and Auer 1990), explaining high densities in summer. However, this was not the case for the two *Bosmina* populations, indicating that temperature is of minor importance in influencing their population densities, although filtration rate may increase with increasing temperature (Wetzel 1983).

Leptodora did not exihibit diel vertical migration as shown in other studies (Vijverberg 1991, Ketelaars et al. 1995). Lake Östersjön has extensive populations of planktivorous fish (Dahlbäck 1991), and *Leptodora* could potentially reduce the predation risk by migrating to greater depths in day time. However, Lake Östersjön is eutrophic, with a Secchi depth of about 0.5 meter (Dahlbäck 1991), making *Leptodora* less vulnerable to fish predation. The tendency to migrate may therefore be less pronounced. In addition, *Leptodora* almost lacks pigmentation, making it more or less transparent. The transparency should decrease their susceptibility to visually dependent predators, and thus enabling them to co-exist with fish (Zaret 1980). Although *Leptodora* may feed during the day (Browman et al. 1989; Herzig and Auer 1990) it may also reduce predation risk from fish by feeding mainly at night. We have indications of a more nocturnal feeding mode, shown by higher percentage gut content during night (Hellsten and Stenson unpubl).

Surface waters are usually considered to be more rich in food (especially in eutrophic lakes), and grazing zooplankton are therefore expected, to spend most of the time in this area (Lampert 1989). In this study *B. coregoni* was negatively correlated with depth when time of day was controlled for. Depth was therefore important for the distribution, implying the surface water being more food-rich, and the results are in accordance with our prediction. Given that the surface strata of the water column are more food-rich, and fish predation of minor importance, *B. coregoni* should be most abundant in the upper strata.

The extreme body form has been shown to be correlated with costs, and *B. coregoni* has therefore to put more energy into locomotion than *B. longispina* (Lagergren et al. in press). On the other hand, *B. longispina* did not vary in abundance among depths. Other studies have reported *Bosmina* populations being differently distributed in the water column (Matveev 1985; Hudec 1995), and in one of the studies different temperature optima for the species was proposed as an explanation (Hudec 1995). However, in this study the water column was not stratified at any occasion, why we suggest other explanations. One is that competition may force less competitive species to spend more time in less nutritional parts of the lake as shown by Jakobsen and Johnsen (1987), although in their study the species segregated horizontally. They argued that spatial segregation, aiming to reduce competition, would be advantageous for the inferior competitor. Most cladoceran grazers do not distinguish between low-quality and high-quality food. *Bosmina* is probably an exception having the possibility of actively selecting food particles (DeMott and Kerfoot 1982). Therefore, it is possible that the *Bosmina* populations in Lake Östersjön are partly separated in food preferences.

Another explanation is predation. *Leptodora* is a size limited predator, and can not handle too large prey items (Herzig and Auer 1990). In Lake Östersjön body size in *B. coregoni* and predator abundance was positively correlated (Fig. 5), implying that *B. coregoni* may reduce predation risk from *Leptodora* by growing larger and more extreme. The correlation could also be an effect from size selective predation. As *Leptodora* becomes more abundant, smaller size classes of *B. coregoni* are more exposed to predation, resulting in a higher proportion of large prey. *B. longispina*, with a smaller and less extreme morphology, may have evolved other adaptations to reduce the predation risk. One possibility for *B. longispina* to reduce encounter rate with a tactile predator, is by dispersing in the water, and not aggregating in the surface. *Leptodora* is a cruising predator according to the definition in Gerritsen and Strickler (1977), and with a relatively high swimming speed (13 mm s^{-1}). This relatively high swimming speed leads to a higher encounter rate with prey organisms that move around more slowly. Although the two *Bosmina* species differ in swimming speed (Lagergren et al. in press) the difference is probably not large enough to generate any significant difference in encounter rate between the predator and any of the prey species. A simple calculation using the Gerritsen and Strickler model for encounter rates (1977) gives an encounter difference between the two *Bosmina* species of 1.5% (swimming speeds: *Leptodora*=13 mm s^{-1}; *B.coregoni*= 2.1mm s^{-1}; *B. longispina*= 4.1 mm s^{-1}; conditioned that the *Bosmina* species have the same densities).

To sum up, in this study *Leptodora* did not exhibit vertical migration behaviour. The result could be explained by the predators transparency, and the high water turbidity, possibly making *Leptodora* difficult to detect for visually hunting predators.

B. coregoni was more abundant near the surface at each sample date, as predicted. We propose that this should be the case, since *B. coregoni* have to pay a cost for being large and morphologically extreme, thus forcing it to stay in regions with high food densities. *B. longispina*, on the other hand, was more evenly distributed in the water column. Interspecific competition, and different vulnerability to *Leptodora* predation may contribute to these results.

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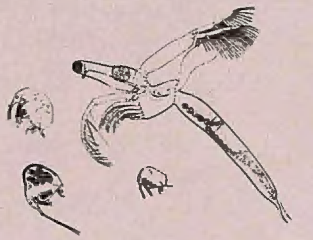

Can extreme morphology in *Eubosmina* reduce predation risk from *Leptodora*? An experimental test

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Abstract

In this study we tested whether extreme morphological traits in *Eubosmina* species can act as a defence against a tactile predator. Many invertebrate predators hunt by mechanoreceptors, and by developing morphological traits (helmets and spines, unwieldy size or shape) the prey can reduce the risk of being attacked and captured by such predators. We tested three different *Eubosmina* forms, each different in morphology from the other, which are found in lakes together with the predator *Leptodora kindtii*. *B. (E) longispina* has a relatively low and elongated body, and short antennule (a more typical *Bosmina* morphology), *B. (E) coregoni gibbera*, and *B. (E) coregoni retro extensa* have a higher and more protruding carapace, and much longer antennule (more extreme *Bosmina* morphology). In one experiment *B. longispina* and *B. gibbera* were exposed for 12 hours to *Leptodora*, in petri dishes. In a second experiment, escape efficiency of *B. longispina*, *B. gibbera* and *B. retro extensa*, and handling time of *Leptodora* were measured in direct observations. In the first case *Leptodora* more often ate *B. longispina*, although both species were eaten. In the second experiment *B. gibbera* and *B. retro extensa* had a higher escape efficiency than *B. longispina*. Predator handling time was correlated to predator body length and antennule length of the prey. The results support the hypothesis that morphological traits in *Eubosmina* can act as antipredator defences.

Introduction

Predation is considered to be one of the most influencial factors for dynamic population properties and community structure (Zaret 1980; Sih et al 1985). By catching, killing and consuming prey animals, the predator can control prey populations, in some cases eliminating them, or influencing absolute and relative densities in the prey community. Besides these direct effects, predation can also act as a selective force influencing the evolution of defence strategies in the prey (Zaret 1980; Jeffries and Lawton 1984; Lima and Dill 1990). Some prey have evolved traits reducing encounter rate with predators, while others have adaptations increasing the possibility to escape after encounter (Sih 1987). Zooplankton may decrease the risk of being detected by visually hunting fish by migrating to greater depths during light hours (Lampert 1989). Reduced amounts of visible body pigmentation may also decrease the predator's possibility to detect the prey (Zaret 1980).

Many invertebrate predators, on the other hand, rely on chemical or hydromechanical cues for localizing prey (Pastorok 1980; Stenson 1987; Herzig and Auer 1990). However, independently of their hunting strategy, or how they detect the prey, they have to hold and manipulate the prey item after catch. When exposed to such predators, other antipredator defences may be more effective. For example, if detected, copepods as well as cladocerans can actively swim away, and thus using speed as an escape response (Gerritsen and Strickler 1977; Larsson and Dodson 1993; Zaret 1980; Svensson in press). Another way to reduce the possibility of being attacked and captured is to develop morphological traits (spines, helmets, unwieldy size or shape), thereby making it difficult for the predator to capture and handle the prey. Several such examples are found in the literature (e. g. Dodson 1988; Havel 1987; Kerfoot 1977; Stemberger and Gilbert 1987).

Leptodora kindtii (Cladocera) is an efficient predator which can severly influence various zooplankton populations during summer (Branstrator and Lehman 1991; Cummins et al 1969; Edmondson and Litt 1987; Herzig and Koste 1989; Lane 1978; Lehman 1991; Lunte and Luecke 1990). *Leptodora* has a tactile mode of hunting with setae on the first thoracic limb acting as mechanoreceptors. Together with thorax and head the other thoracic limbs form a feeding basket in which the prey is pushed (Herzig and Auer 1990; Zaret 1980). The feeding basket is strongly correlated with the predators body length (2-10 mm), and *Leptodora* prefer smaller prey items (< 1.5 mm) (Branstrator 1994; Herzig and Auer 1990; Manca and Comoli 1995). A detailed description of *Leptodora*'s feeding behavior is given in Branstrator et al. (1989). Although Leptodora's efficiency as a predator is well documented, no studies have investigated how different morphological traits in the prey may influence the handling efficiency of the predator.

Bosmina (Cladocera) show a variety of phenotypes, especially within the subgenus *Eubosmina*, where many of the varieties exhibit large differences in body size and shape, and antennule length (Lieder 1983). One possible reason for the variation in morphology, might be that some traits has evolved as antipredator defence against tactile predators. This could also explain why many of the morphological structures in *Eubosmina* varieties occur in a cyclic manner, *i.e.* under periods of high predation pressure. Morphological traits in different *Daphnia* species as a means of reducing the predators handling efficiency is well documented (Dodson 1989). Such relationships are less clear and more contradicting in *Bosmina*. Some studies point to predator defence as an important factor (Black and Hairston 1983, Kerfoot 1978, 1987, De Stasio et al 1990, Sprules et al 1984), while others have failed to document this (Gerritsen 1983, Johnsen och Raddum 1987). Moreover, these studies are mainly made on *B. longirostris*, a species where the morphological traits are less dramatic, comprising differences in antennule and mucro length. However, within the *Eubosmina* species complex few studies, if any (but see Johnsen and Raddum 1987), have been made on the importance of morphological traits in relation to invertebrate predators.

The aim of this study is to test whether *Bosmina* with different morphology are equally vulnerable when exposed to *Leptodora*, or if unwieldy size or shape increase the escape possibility of the prey. In the study we test three different *Bosmina* forms (subgenus *Eubosmina*) (Fig. 1), each different in morphology from the other. All three forms are found in lakes together with *Leptodora*. *B. (E) longispina* (Leydig 1860) has a relatively low and elongated body, and short antennule, *i.e.* a more typical *Bosmina* morphology. *B. (E) coregoni gibbera* (Schoedler 1863) and *B. (E) coregoni retro extensa* (Liljeborg 1901) have a more extreme body shape in comparison with *B. longispina*, and much longer antennule. Further, during summer *B. gibbera* and *B. retro extensa* grow larger and the carapace becomes more protruding in shape, and the antennule also grows much longer, while *B. longispina* during the same period does not change in morphology. In the following we refer to the three *Bosmina* forms as *B. longispina*, *B. gibbera* and *B. retro extensa.*

Methods

Animals were collected from two different lakes in the beginning of July in 1995 and 1996. In Lake Östersjön (57° 93'N, 12° 70' E) *B. gibbera* and *B. longispina* co-exist together with *Leptodora*, and in Lake Södra Färgen (56° 55'N, 12° 20'E) *B. retro extensa* and *B. longispina* co-exist together with *Leptodora*.

In a first experiment we tested survival of *B. gibbera* and *B. longispina* during a 12 hour period (night-experiment) when exposed to *Leptodora*. Two days prior to experiment animals were collected in Lake Östersjön with a plankton net (100 µ) drawn by vertical hauls from near the bottom to the surface. *Leptodora* were picked out, put in tanks (250 ml) containing filtered lakewater (through 100 µm mesh), and starved 24 hours before the experiment. *B. longispina* (body length 0.55 ± 0.09, mm ± SD) and *B. gibbera* (body length 0.56 ± 0.07, mm ± SD) were picked out and put in Petri dishes. Nineteen replicats, each containing 10 *B. gibbera*, 10 *B. longispina*, and one *Leptodora* (body length 3.7 ± 1.2, mm ± SD) were then kept in darkness for 12 hours at 20° C. Eleven petri dishes with 10 *B. gibbera* and 10 *B. longispina* in each, but no predator served as control group. All Petri dishes contained approximately 70 ml of filtered lake water. After 12 hours all alive *Bosmina* were counted and we also noted if the predator was still alive. If not, these Petri dishes were excluded from analysis. As dead prey we defined either those missing or actually seen dead in the dishes.

In the second experiment prey were collected approximately one week in advance, placed in tanks (250 ml) with filtered lake water, and kept at 20° C. *B. gibbera* and *B. longispina* were collected from Lake Östersjön and *B. retro extensa* from Lake Södra Färgen. *Leptodora* were collected from Lake Östersjön, and Lake Södra Färgen a few days before experimentation, and starved for 20-35 hours at 18° C.

The observations were carried out in the following way; approximately 60 individuals from one *Bosmina* population were put in a Petri dish (hunting-dish), containing 70 ml of filtered lake water, together with three or four *Leptodora*. Whenever a *Leptodora* had attacked and captured a *Bosmina* (retaining it in its feeding basket) it was transfered as fast and carefully as possible, to an empty dish (eating dish) with a plastic pasteur pipette having a wide opening. By doing so, we were able to follow each encounter and the handling process from the catch to an eventual escape, or to the final eating of each prey. The observation technique allowed us to register an event within the time span of maximum 10 seconds. *Bosmina* that succeeded to escape, were controlled for a period of 90 minutes. Animals where then preserved in ethanol (70%). Injuries and parts eaten on prey were also noticed. Transfered predators were replaced by new ones in the "hunting-dish". Both Petri dishes were illuminated from 30 cm above with a swan-neck cool point source (Volpi Intralux 150H), giving a light intensity of about 2300-2800 lux in the "hunting dish" and 1200-1800 lux in the "eating dishes". As the Petri dishes were placed on a white background both prey and predator were easily seen watching their shadow image.

The proportion between dead and alive prey and mean survival in the first experiment, and escape efficiency in the second was then estimated. Escape efficiency was defined as the ratio of prey escapes to total number of predator attacks and captures, for each of the three *Bosmina* forms. When captured, handling time was estimated as the time between first contact, ingestion, and finally release of the eaten prey. Further, we measured predator length, and body length, body height and antennule length of the prey (Fig. 1). Since mutiple tests adressed a common null hypothesis (no difference in body measurements among the *Bosmina* forms) we adjusted α-levels using sequential Bonferroni method to ensure an overall significance level of 5% (Rice 1989; Chandler 1995).

Finally, the importance of prey body measurements, and predator body length in relation to handling time was tested with multiple regression analysis.

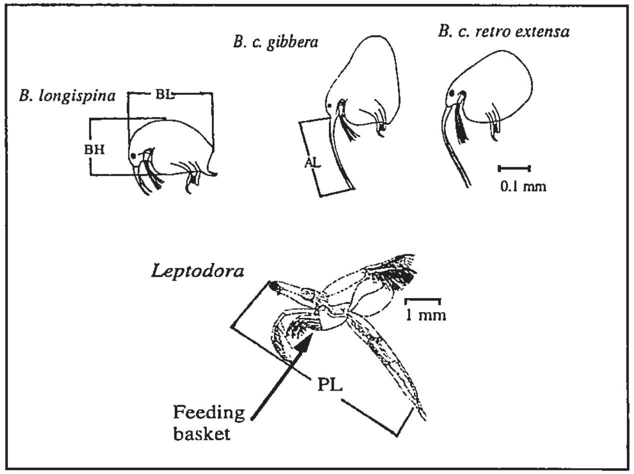

Fig. 1. Lateral view of prey exposed to the predator Leptodora. On figures are indicated features measured. Body length (BL), body height (BH), antennule length (AL), and predator length (PL). On Leptodora also indicated feeding basket. Note that Leptodora is drawn to a different scale than the three Bosmina types.

Results

Mean survival was significantly higher for *B. gibbera* than for *B. longispina* when exposed to the predator in the first experiment (Fig 2.). In the control group both species survived equally well (Fig. 2). *Leptodora* was found in good condition in 16 out of 19 replicats. There was a tendency for *B. gibbera* to get caught more often in the surface tension, why all *Bosmina* trapped in the surface tension were excluded in the analysis. Prey caught in the surface tension were regarded as non accessible prey for *Leptodora.*

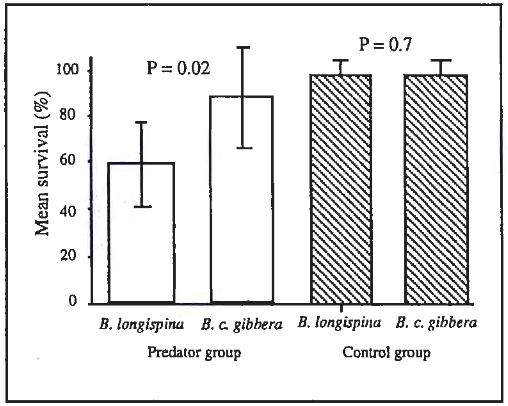

Fig. 2. Proportion of survival (mean ± SD) for B. longispina, and B. c. gibbera exposed to the predator Leptodora during a 12 hour period (night-experiment), and proportion of survival in control group. Differences in survival tested with Wilcoxon signed-rank test. Experiment group n=16, and control group n=11.

In the second experiment *B. gibbera* and *B. retro extensa* escaped *Leptodora* about six times more often than *B. longispina* (Fig 3). The escape efficiency was 67% and 73% respectively of the two extreme forms but only 12% of *B. longispina*. Most *Bosmina* escaped within five minutes (Fig. 4). In some rare cases, individuals escaped unharmed after up to 26 minutes in the feeding basket. The majority of *Bosmina* that escaped were still alive, and with no visible injuries after 90 minutes of observation. Further, *Bosmina* also often showed the "dead-man" response up to 10 seconds after escape, *i.e.* with antennae protectively folded into the lateral sheaths, they passively sink (Kerfoot 1978), and then swim away in a protean way, meaning that the escape behaviour is sufficiently unsystematic to prevent the predator to predict the position in the next moment (Edmunds 1974).

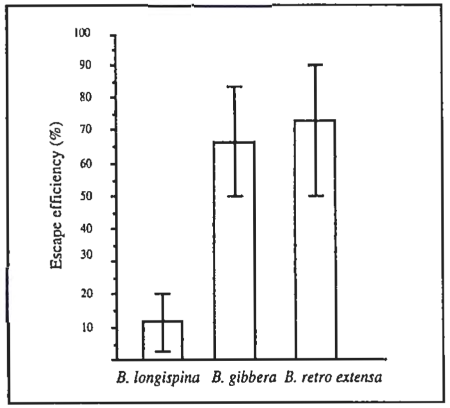

Fig 3*. Escape efficiency (%) with 95% confidence intervals for individuals exposed to Leptodora in the attack experiment. B. longispina (n=43), B. c. gibbera (n=30), B. c. retro extensa (n=20).*

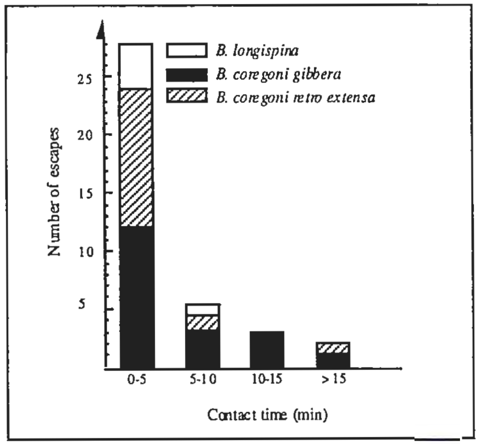

Fig 4*. Number of prey escapes in relation to contact time with the predator.*

Mean antennule length in *B. longispina* was significantly shorter compared with *B. gibbera* and *B. retro extensa*, and mean body height was significantly higher in *B. gibbera* (table 1, $p<0.05$ multiple comparisons, Siegel and Castellan 1988). Predator body length did not vary among groups (table 1).

Body measurements in the prey were strongly correlated (table 2) why we only tested the effect of antennule length, together with predator body length, on handling time. Predator length and prey antennule length explained about 20% of the variation in handling time ($R^2= 0.18$, $p=0.004$, $n= 49$, multiple regression). Predator handling time of ingested prey was negatively correlated with predator body length, and positively correlated with antennule length of the prey (table 3). Finally, there was a tendency towards longer handling time for *B. gibbera* of those prey eaten by *Leptodora* (Table 4.).

Table 1. Mean body measurements (mm ± SD) of prey caught (escaped or handled) by *Leptodora*, and mean body length of *Leptodora* (mm ± SD). Differences in mean body measurements between prey type tested with Kruskal Wallis One-way Analysis of Variance. Asterisks denote $p<0.05$ with sequencial Bonferroni adjustments (probability values from Kruskal Wallis analysis).

	B. longispina (n=43)	*B. c. gibbera* (n=30)	*B. c. retro extensa* (n=20)	P value
Mean length	0.46 ± 0.09	0.47 ± 0.08	0.51 ± 0.08	n.s
Mean height	0.34 ± 0.07	0.49 ± 0.11	0.36 ± 0.06	0.0005*
Mean antennule length	0.19± 0.03	0.53 ± 0.19	0.52 ± 0.08	0.0005*
Mean length of *Leptodora*	3.8 ± 1.0 (n=43)	4.1 ± 0.8 (n=30)	4.2 ± 1.4 (n=20)	n. s

Table 2. Pearson correlation coefficients (R) among body measurements (Fig. 1) of the three *Bosmina* forms in the attack experiment (n= 49).

	log BL	log BH	log AL
log BL	1		
log BH	0.876	1	
log AL	0.474	0.732	1

Table 3. Relationships between predator handling time (dependent variable) and predator body length, and mean prey antennule length in predator-prey encounters with killed and ingested prey. Log PL = predator length, log AL = prey antennule length. N=49 predators. P-value for entire model 0.004, $R^2=0.18$.

Independent variable	Regression coefficient	P value
Log PL	- 0.79	0.02
Log AL	0.30	0.003

Table 4. Mean handling time (± SD) for each of the three *Bosmina* types captured and eaten by *Leptodora*. Differences in handling time tested with Kruskal Wallis One Way-Analysis of variance (p=0.06)

	Mean handling time (min) ± SD	N
B. longispina	29.5 ± 18.6	34
B. c. gibbera	46.8 ± 22.4	9
B. c. retro extensa	22.7 ± 13.8	6

Discussion

The environment in aquatic communities is such that zooplankton have few possibilities to avoid predators by hiding in refugia. To avoid being eaten by visually hunting predators, zooplankton may migrate vertically into the dark depths of lakes. But if they are more exposed to invertebrate predators that hunt with mechanoreceptors, developing morphological defences is a better strategy.

Bosmina is a relatively small zooplankton and can not outrun the large and faster swimming *Leptodora*, but instead have to rely on other defence mechanisms. Therefore, the higher survival of *B. gibbera*, and the higher escape efficiency of *B. gibbera* and *B. retro extensa*, suggest that their morphology can act as a defence against a tactile predator like *Leptodora*. Given the choice, in the first experiment, *Leptodora* preyed more often on *B. longispina*, although *B. gibbera* was also eaten. *B. gibbera* was caught more often in the surface tension, which may have resulted in a higher density of *B. longispina* in relation to *B. gibbera*. However, when animals caught in the surface tension were excluded, *B. gibbera* still had a lower mortality rate. Further, the predator is much more fast swimming than either prey, and due to the limited size of the vials, encounter rate was probably higher than in reality. In nature prey have the possibility to disperse more and other kinds of prey are also available.

The difference in predation on the two *Bosmina* species by *Leptodora*, may contribute to the numeric relations between the species observed in the field. In Lake Östersjön *B. gibbera* is more common during periods with dense *Leptodora* populations, while *B. longispina* some years disappears almost entirely during these periods (Hellsten and Stenson 1995).

Other studies have shown that morphological traits can make the prey less vulnerable to invertebrate predators (Dodson 1988; Havel and Dodson 1984, Kerfoot 1978). One example is *B. longirostris*, which under high predation pressure from copepods, gets a larger body size, and longer antennule and mucro (Kerfoot 1987). Although the morphological traits in this species are less conspicuous compared with the *Bosmina* forms in our experiment, the morphological changes were still large enough to reduce mortality rates.

In the second experiment we showed that the two forms with extreme morphology, *B. gibbera* and *B. retro extensa*, escaped more often than *B. longispina*. This result supports our interpretation that extreme morphology in *Bosmina* can reduce predation risk from tactile predators. The most discriminating characters among the three forms are the shape of the carapace, and antennule length. *B. gibbera* and *B. retro extensa* escaped equally well, which may imply the length of the antennule being important for the escape efficiency. When copepods capture *Bosmina* they often manipulate the prey to expose the soft underparts, and during this manipulation the antennule may interfere with the predator's handling attempts (see Zaret 1980).

The magnitude in escape efficiency between *B. longispina* and the more extreme forms is quite large. Compared with *B. longispina* the other two had almost six times higher chance to escape. In a study by Havel and Dodson (1984) spined *Daphnia* morphs had approximately 1.5 to 2 times higher escape chance than had unspined morphs when exposed to *Chaoborus*. In the experiment we used predators within a limited size range, and it is possible that the escape chance decreases as the predator becomes larger. Predator body length in relation to prey size is important for how successful the predator will be. In *Leptodora* the size of the feeding basket is strongly correlated to body length (Manca and Comoli 1995), and prey availability is thereby correlated to predator length. This was also shown in the regression analysis where handling time decreased with increasing predator length. Although we used prey and predator within a limited size, clearly the prey were not too large. Herzig and Auer (1990) estimated that *Leptodora* with a body length of about four mm would be able to capture prey with a maximum body length of 0.6 mm. The results are in accordance with other studies where prey size and handling time was correlated to the size of *Leptodora* (Branstrator 1994, Browman 1989).

Prey body measurements were strongly correlated (table 2), and we tested the effect of antennule length on handling time in the multiple regression analysis, since antennule length was the character significantly discriminating both *B. gibbera* and *B. retro extensa* from *B. longispina*. Both predator length, and antennule length affected the handling time, but explained only 20% of the variation. Therefore, we suggest that the total form and size of the prey will also influence the handling time. This is supported by the tendency towards longer handling time of *B. gibbera*. The longer antennule and the shape of the body may make it difficult for the predator to turn the prey into position. There are few reports of how *Leptodora* eats the prey. Apparently, it is mainly a fluid feeder, *i.e.* by piercing the carapace of its prey near the head with sharp protuberances it sucks out the juices (Mordukhai-Boltovskaia 1958), but can also tear a hole in the carapace and then suck out the juices (see Browman et al. 1989). It is also possible that the carapace in *B. gibbera* is more robust, making it more difficult for the predator to either pierce or tear a hole in the carapace. This has been suggested for other species (Havel and Dodson 1984, Kerfoot 1978).

To summarize, we have shown that extreme morphological traits in *Bosmina* can be protective against predation from *Leptodora*. We do not know whether the phenotypic varieties found in *B. coregoni* are related to the presence of certain predators. We have shown, however, that the extreme *Bosmina* forms not only had a higher over all survival rate, but also a better escape success when attacked by the predator.

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VI

Is the *Bosmina kessleri*-like morphotype a hybrid between *B. longispina* and *B. coregoni* ? A molecular test

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Abstract

Hybridization has been suggested as an important factor in the diversification of the *Bosmina* species complex, a taxonomically complex genus with a great number of widely varying forms. Previously, putative *Bosmina* hybrids have been identified only through morphological characters. In this study the Random Amplified Polymorphic DNA (RAPD) technique was used to test if morphologically intermediate *B. kessleri*-like specimens are interspecific hybrids between *B. longispina* and *B. coregoni* (subgenus Eubosmina). Eye diameter, sutures on the antennule, and number of spines on the postabdominal claw were also recorded.

The supposed hybrids are morphologically intermediate by having a higher carapace and longer antennule like *B. coregoni*, and a caudal mucro like *B. longispina.*

The RAPD distinguished *B. longispina* and *B. coregoni* from each other, but did not identify the *B. kessleri* -like morphotype as a hybrid between the former two. Rather, the *B. kessleri* -like morphotype seemed genetically more similar to *B. coregoni.* Moreover, the morphological characters examined were not useful for testing the intermediate specimens as hybrids. The results suggest that the *B. kessleri*-like morphotype is not a hybrid, but may instead belong to *B. coregoni*, the difference in morphology possibly being a consequence of phenotypic plasticity.

Introduction

Information on interspecific hybridization among animals have mainly been based on terrestial animals (see Barton and Hewitt 1985, Grant and Grant 1992). However, in the past decade interspecific hybridization in cladocerans have also been described (Hebert 1985, Taylor and Hebert 1992, Spaak 1994). Due to cladocerans capability to reproduce either sexually or asexually via parthenogenesis, hybridization may promote speciation processes because hybrids can circumvent deleterious effects of reduced sexual fertility (see Schwenk 1993).

Although hybrids often have morphological characters being intermediate of those found in their parents (Spaak 1994), these characters alone may not be sufficient to identify intermediate specimens as hybrids, but must also be combined with biochemical methods. Further, in cladocerans large variation in morphological characters is common, making it difficult to distinguish between intra- and interspecific variation in closely related species. Morphological studies have therefore been combined with allozyme electrophoresis to increase taxonomic resolution (Manning et al. 1978, De Melo and Hebert 1994a, 1994b, 1994c), and to identify putative hybrids (Hebert 1985, Hann 1987, Taylor and Hebert 1992).

The cladoceran genus *Bosmina* is regarded as being taxonomically complex, and much of the difficulties are due to the large variation in morphological characters like body size and shape, antennule and mucro length (Nilssen and Larsson 1980, Lieder 1983a, DeMelo and Hebert 1994a). Hybridization as a factor of significance in the diversification of the *Bosmina* species complex has been suggested (Lieder 1983b, 1991). However, in the most recent revision of North American *Bosmina* species, no evidence of interspecific hybridization could be established (De Melo and Hebert 1994b). In Europe, the classification of *Bosmina* species (Nilssen and Larsson 1980, Lieder 1983a), and identification of hybrids (see Lieder 1983b, 1991) have mainly been based on morphological characters.

In Lake Östersjön, SW Sweden (57° 93'N, 12° 79' E) two co-existing species of the subgenus *Eubosmina* are found; *B. longispina* (Leydig) and *B. coregoni* (Baird) (Fig. 1) Additionally, among these an intermediate form occasionally occurs resembling the *B. kessleri* morphotype (Uljanin), by Lieder described as a putative hybrid between *B. longispina* and *B. coregoni* (1983b, 1991). While the body of *B. longispina* is relatively low and elongate with a cuadal mucro, the body of *B. coregoni* is more extreme with a round carapace that grows higher and more pronounced during summer. Further, adult *B. coregoni* have no caudal mucro, and the antennule is much longer than in *B. longispina*. The *B. kessleri*-like morphotype has a carapace form, and length of antennule much like *B. coregoni*, and although shorter, a caudal mucro like *B. longispina.*

I here test whether the Random Amplified Polymorphic DNA technique (Williams et al. 1990, Welsh and McClelland 1990) can be used as a method to identify the *B. kessleri*-like morphotype as being a hybrid between *B. longispina* and *B. coregoni.* The RAPD technique generates DNA markers, consisting of relatively short fragments (about 200-2000 base pair long). The sequence of nucleotides amplified is conventionally called a RAPD locus. The amplification product(s) are separated (by size) electrophoretically on a gel, and the presence/absence of a band of a specific molecular weight is used to assay genetic variation within and between species/populations (Grosberg et al. 1996).

In addition, I also examine some morphological characters, previously used as criteria for hybrid identification (Lieder 1991).

Materials and methods

Samples were collected in Lake Östersjön in mid-June in 1996, and preserved in 70% ethanol. For the DNA extraction specimens of *B. longispina*, *B. coregoni* and the *B. kessleri*-like morphotype were randomly chosen (14 individuals of each). To make certain that only adults were used in the test, I picked out specimens with eggs in the brood chamber, and removed these prior to extraction of DNA. DNA was extracted individually according to the procedures in Winnepenninckx et al. (1993), and measured with a Hoefer 102 flourometer (DNA ranged between 2 and 20 ng/µl). Table 1 lists the protocol I used for PCR amplification; 5 µl loading buffer (50% sucrose, w/v, 0.10% SDS and 0.15% bromophenolblue in 6xTBE) was added to each sample after amplification, and 5 µl of this mixture was loaded onto a 2% agarose gel (MetaphorTM, FMC Bioproducts) stained with ethidium bromide (1 mg^{-1} ml H_2O) and run at 90 V for 1 h and at 80 V for 1/2 h. The gels were electronically captured in UV-light by a videocamera (Ikegami ICD-42E Type F/L) attached to a video copy processor (Mitsubishi P67E).

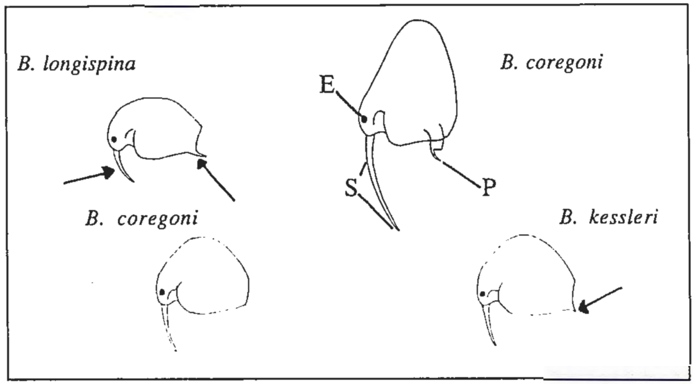

Fig. 1. Morphological characters of adult females of B. longispina, B. coregoni (in different stages of morphological change), and the B. kessleri-like morphotype. Antennule and mucro are indicated with arrows. S, sutures on the distal part of the antennule; P, postabdominal claw; E, eye diameter.

Primers were obtained from Operon Techniques (Alameda, Calfornia).Twelve primers that yielded scorable bands, and showed polymorphism were chosen with sequences presented in Table 2.

Eight individuals from each of the three populations were tested. Bands were scored as present or absent at a given site, using only the positions where bands could be unambiguously scored over all 12 primers respectively. I tested for stringency by replicating the RAPD analysis on two individuals from each *Bosmina* population with primers A-04 and A-07. These primers were selected because they provided distinct bands that were easy to score.

Pairwise similarities between specimens were calculated based on presence and absence of RAPD bands using the Jaccard coefficient (S) (Rohlf 1993): $S=a/(a+b+c)$ where $\underline{a}$ is the presence of a fragment in both individuals, $\underline{b}$ the presence in one but not the other, and $\underline{c}$ the opposite compared to $\underline{b}$. To reveal possible groupings of the specimens (corresponding to genetic units), non-metrical multidimensional scaling (Kruskal, 1964) was used for ordination of the similarity matrix into two dimensions. This is an ordination technique (like principal coordinate analysis, PCA) for representing similarities between objects in a two- or three dimensional space, were similar objects should be placed closer together and dissimilar objects further apart (Rohlf 1993). Similarity calculation and ordination was done with NTSYS.pc ver. 1.80 (Rohlf, 1993).

Sufficient genetic differences between *B. longispina* and *B. coregoni* should show up as a grouping of the individual specimens in agreement with the two species. Given that the *B. kessleri* -like morphotype is an interspecific hybrid of *B. longispina* and *B. coregoni*, individual specimens should show up among the others, and not consitute a group of its own (genetically different from the other two).

To test for grouping, I compared my original results with 10 randomized populations (using MacClade), each with the same frequencies of absences and presences as in the original sample. Each of the randomized populations was analysed in the same way as the original data set. I presented the original ordination, together with one ordination based on the randomized data, to 10 colleagues, asking them which of the two ordinations (if any) showed a concordant grouping. Each colleague was presented a new randomized ordination together with the original. Assuming no difference in degree of grouping between the original and the randomized populations, the null hypothesis was that both populations had the same probability ($p=0.5$) of being scored as most clearly grouped. I then tested the outcome of the ten comparisons by sign test (Siegel and Castellan 1988).

In addition, I picked out individuals from the three forms respectively and measured eye diameter, recorded the number of sutures on the distal part of the antennule, and number of spines in the pecten of the postabdominal claw. Specimens of the *B. kessleri*-like morphotype were rare (approximately 1 to 200) why only six individuals of each were examined.

Table 1. The PCR-protocol for the RAPD analysis of *B. longispina*, *B. coregoni*, and the *B. kessleri* -like morphotype in Lake Östersjön

MgCl2 (25 mM) (Perkin-Elmer)	4 µl
10x Stoffel Buffer (Perkin-Elmer)	2,5 µl
Primer[1]	5 µl
dNTP (2,5 mM each)	10,4 µl
Ampli Taq®DNA Polymerase Stoffel fragment (Perkin -Elmer)	0,2 µl (0,1 U)
BSA (2,5 $\mu g\ \mu l^{-1}$)	0,9 µl
Template (2-20 ng μl^{-1})	2,0 µl

[1] Primers (from Operon Technologies, Alameda, California), OPA-03 (5.2 mM), OPA-04 (5.1 mM), OPA-07 (4.6 mM), OPA-09 (5.2 mM), OPB-07 (5.0 mM), OPB-08 (5.4 mM), OPC-02 (5.3 mM), OPC-05 (5.4 mM), OPC-11 (4.8 mM), OPC-14 (5.8 mM), OPC-15 (4.8 mM), OPC-20 (5.8 mM). Temperature profiles. For all primers: 94°C, 3 min, followed by 46 cycles of 94°C, 20 sec: 42°C, 30 sec, 72°C, 1 min: extension at 72°C for 10 min. Amplification products were kept at 4°C until loaded onto a gel.

Table 2. Sequences of primers used in the RAPD analysis. Sequences read from 5' end.

A-03=AGTCAGCCAC	A-04=AATCGGGCTG
A-07=GAAACGGGTG	A-09=GGGTAACGCC
B-07=GGTGACGCAG	B-08=GTCCACACGG
C-02=GTGAGGCGTC	C-05=GATGACCGCC
C-11=AAAGCTGCGG	C-14=TGCGTGCTTG
C-15=GACGGATCAG	C-20=ACTTCGCCAC

Results and discussion

B. longispina had a mean of 4.6 ± 2.1 (SD) bands per individual, *B. coregoni* and *B. kessleri* a mean of 4.5 ± 2.2 (SD) and 4.3 ± 2.1 (SD) respectively. No primer was monomorphic, that is no primer produced exactly the same amount or position of bands in all individuals. The average reproducibility, measured as the same bands appearing in replicates and test on the gel, was 82% for A-04 and 70% for A-07. No differences in position of bands between replicates and tests were found. In some cases a band appeared in tests but not in replicates and vice versa.

The ordination analysis based on the similarity matrix separated *B. longispina* and *B. coregoni* (Fig. 2), although less clearly than in a previous study (Hellsten and Sundberg MS). However, the analysis did not place the individuals of the *B. kessleri* morphotype among the other two, nor as a group of its own. Rather, these specimens seemed genetically more similar to *B. coregoni*. Moreover, the morphological characters examined were not useful for testing whether the *B. kessleri*-like morphotype is a hyrid (table 3). Eye diameter and number of spines in the pecten of the postabdominal claw did not vary among the three *Bosmina* populations. Eye size may vary due to fish predation (Nilssen and Larsson 1980), and therefore is probably not a good morphological character for identifying hybrids. Number of sutures on the antennule were significantly fewer in *B. longispina* (table 1, $p<0.05$, multiple comparisons, Siegel and Castellan 1988), probably because this *Bosmina* has much shorter antennule.

When testing the combined outcome of original data and randomized data, the null-hypothesis was rejected ($p<0.0005$).

The advantage with RAPD analysis is that it does not require any prior knowledge of the target genome, and also requires such small amounts of DNA that even a tiny single specimens can be used for a number of analyses. On the other hand, in RAPD there is a tendency to get dominant markers (Burke 1994), *i.e.* no distinction between homozygotes and heterozygotes, which may be a problem when identifying hybrids. The RAPD technique may therefore not be ideal for this particular question. However, in a study by Heun and Helentjaris (1994) on F_1-hybrids of corn, 90% of the RAPD markers appeared to be inherited as expected in the F_1 generation. Further, the technique has also been used to identify nuclear markers in *Daphnia* hybrid complexes (Ender and Schierwater 1993).

Table 3. Mean eye diameter, mean number of sutures on the distal part of the antennule, and mean number of spines in the pecten of the postabdominal claw on *B. longispina*, *B. coregoni* and the *B. kessleri* -like morphotype. Differences in number of sutures on the antennule tested with Kruskal-Wallis One-way Analysis of Variance.

	B. longispina (n=6)	B. coregoni (n=6)	B. kessleri (n=6)	P value
Eye (µm ± SD)	36 ± 5	36 ± 6	36 ± 4	
Sutures (± SD)	11.5 ± 1.4	15.7 ± 0.9	17.5 ± 4.4	0.003*
Spines (± SD)	5.2 ± 0.8	6.0 ± 0.6	6.2 ± 1.0	

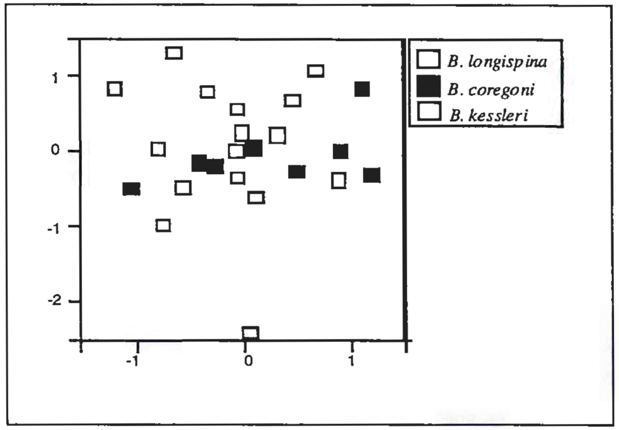

Fig. 2. Ordination of the specimens in two dimensions based on the Jaccard similarity coefficient between individuals.

Phenotypically variable forms within the subgenus *Eubosmina* are well documented (Nilssen and Larsson 1980, Lieder 1983a, 1983b, 1991, Hann 1987). Since many of these forms also change in morphology over the season, distinguishing between intra- and interspecific variation might be difficult. In Lake Östersjön *B. coregoni* undergoes a morphological change from spring to autumn with a higher and more pronounced carapace and longer antennule (Hellsten and Stenson 1995). Further, juveniles of *B. coregoni* have a caudal mucro, which disappears as they become adults (Hellsten and Sundberg MS). Besides having a mucro like *B. longispina*, specimens of the *B. kessleri*-like morphotype resemble adult B. *coregoni* which have not yet developed a very high carapace (Fig. 1). They may therefore not be interspecific hybrids, but instead phenotypic variable forms of *B. coregoni*. Another explanation may be unidirectional hybridization, as suggested in a study on *D. galeata* x *D. hyalina* hybrids. Here the restriction pattern (from amplified cytochrome b segments) were identical to the species-specific pattern of one of the parental species (Schwenk 1993).

Further studies based on genetic analyses are needed to investigate the extent of intraspecific variation and hybridization in *Eubosmina*.

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