Ecological effects of the mass occurrence of the Ponto-Caspian invader, Hemimysis anomala G.O. Sars, 1907 (Crustacea: Mysidacea), in a freshwater storage reservoir in the Netherlands, with notes on its autecology and new records

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Abstract

A new Ponto-Caspian invader, the mysid *Hemimysis anomala* G.O. Sars, 1907 (Crustacea: Mysidacea) was recorded for the first time in the Netherlands in 1997. In the summer of 1998 extremely high densities (>6 ind. 1^{-1}) of this neozoon were recorded in one of the Biesbosch reservoirs (Honderd en Dertig). This high abundance can not be explained by a recent invasion. Either *H. anomala* reached the Netherlands via the River Rhine, probably aided by shipping, or through transport with ballast water from the Baltic or Black Sea. The invasion had dramatic effects on the zooplankton composition and abundance: from the end of August onwards hardly any Anomopoda, Ostracoda, Rotifera and invertebrate predators *(Leptodora kindti* and *Bythotrephes longimanus)* were present. Copepod densities, however, were not influenced. Chlorophyll-*a* concentrations were significantly lower compared to previous years, possibly the result of mysids feeding on the algae. Laboratory experiments revealed that *H. anomala* is a voracious predator and also an omnivorous feeder. Life history characteristics and behaviour of *H. anomala* fit into general mysid ecology. The hidden life style of *H. anomala* during the day makes it difficult to assess its present geographical distribution. More attention should therefore be given to appropriate sampling of this mysid. Because of its broad prey-size range *H. anomala* may pose a threat to existing lake restoration programmes (biomanipulation) in the Netherlands. In addition to the records of the Biesbosch reservoirs, *H. anomala* was found in the River Meuse and in reservoir Andijk, in the northern part of the Netherlands.

Introduction

Introductions of exotic aquatic organisms have occurred for centuries, but were accelerated by the global expansion of trade. The transit of large quantities of ballast water from one biogeographical region to another is perhaps one of the most important causes of such unexpected introductions (Jazdzewski, 1980; Carlton, 1985). Two such examples include the invasion of the North American Great Lakes in the 1980's by the bivalve mollusc *Dreissena polymorpha* and the carnivorous onychopod *Bythotrephes cederstroemi* (Mills et al., 1993). *D. polymorpha* dramatically changed the invaded ecosystem and led to large economic losses (Nalepa & Schloesser, 1993). *B. cederstroemi* influenced zooplankton density and

size structure in several lakes (Lehman & Caceres, 1993; Yan & Pawson, 1997). Despite measures taken to prevent unwitting introductions via ballast water, the predatory Ponto-Caspian onychopod *Cercopagis pengoi* recently invaded Lake Ontario (MacIsaac et al., 1998). Also the construction of canals, which connect previously separated biogeographic regions, led to a range extension of many species. During the last two centuries numerous canals have been constructed in Europe, through which species actively migrated or were aided by shipping traffic (Jazdzewski, 1980). The Ponto-Caspian mollusc *Dreissena polymorpha* already spread over Western Europe in the 19th century (Kinzelbach, 1992). Among many other organisms that spread via connecting canals, the Ponto-Caspian amphipod *Corophium curvispinum* is most notable because it dramatically influenced the Rhine ecosystem (Van den Brink et al., 1991, 1993). The Main-Danube Canal, officially opened in 1992, facilitated the invasion of the River Rhine basin by Ponto-Caspian endemics e.g. the amphipod *Dikerogammarus haemobaphes,* the isopod *Jaera istri* and the mysid *Limnomysis benedeni* (Schleuter et al., 1994; Schleuter & Schleuter, 1995; Reinhold & Tittizer, 1998). The transfer of large quantities of crustaceans as an immediate supplementary (live) food for commercial fish species was a common practice in the former Sovjet Union (Jazdzewski, 1980 and references herein). Since the 1950's there has been an increasing interest in the deliberate "inoculation" of crustaceans as food for fish. In Western Europe *Mysis relicta* and *Gammarus tigrinus* have been the objects of such attempts (Fürst, 1967; Jazdzewski, 1980; Langeland, 1981). In Eastern Europe the Ponto-Caspian region has been the favourite source for the "acclimatization" of new species to water bodies, especially new impoundments on large rivers, for the purpose of enriching the fauna and as a food source for fish (Mordukhai-Boltovskoi, 1979; Jazdzewski, 1980). The most frequently used Ponto-Caspian invertebrates have been mysids of the genera *Paramysis, Limnomysis* and *Hemimysis* and the amphipods *Gammarus lacustris, Dikerogammarus haemobaphes* and *Corophium curvispinum* (Gasiunas, 1968; Borodich & Havlena, 1973; Mordukhai-Boltovskoi, 1979; Jazdzewski, 1980). The deliberate introductions sometimes had marked ecological effects: *G. tigrinus* spread throughout the lowlands of Western Europe, out-competed indigenous species (Fries & Tesch, 1965) and is now one of the dominant macro-invertebrate species in many river systems, e.g. the River Meuse (Ketelaars & Frantzen, 1995). Total zooplankton biomass severely decreased and phytoplankton biomass nearly doubled after the establishment of *M. relicta* in Lake Jonsvatn (Norway) (Koksvik et al., 1991). After the deliberate introduction of *M. relicta* in Lakes Selbesjøn and Stugusjøn (Sweden) many cladocerans disappeared from the plankton followed by reduced growth of pelagic arctic char *(Salvelinus alpinus)* in Lake Selbesjøn (Langeland, 1981, 1988; Langeland et al., 1991). Also the introduction of *M. relicta* in North American lakes showed that the introduced populations modified the benthic fauna, phytoplankton, zooplankton and fish communities (Lasenby et al., 1986). From these examples it can be concluded that deliberate or unintentional introductions can cause dramatic changes in the ecosystems involved. The sudden mass occurrence of the mysid *Hemimysis anomala* G.O. Sars, 1907 (Crustacea: Mysidacea) in the Biesbosch storage reservoirs therefore merits attention. In this article we present data on the effects of the invasion, abundances, diel vertical distribution, life history and describe some new records from the Netherlands.

Hemimysis anomala

The order Mysidacea comprises almost 800 known species in about 120 genera, most of which live in marine habitats; only about 25 species of mysids occur frequently in freshwater lakes and rivers (Mauchline, 1980).

Mysidacea can be identified to genus level by Mauchline (1980). The genus *Hemimysis* belongs to the family Mysidae, subfamily Mysinae, and consists of only six species (Mauchline, 1980; Alcaraz et al., 1986). *H. anomala* (Figure 1A) can be distinguished from other species in the genus by the following characteristics (Bacescu, 1954; Komarova, 1991): the 3rd, 4th and 5th male pleopod is well developed, the 4th male pleopod is elongated with a long exopodite and a reduced endopodite, the antennal scale is oblong with long plumose setae on the proximal portion of the outer margin and has no spines (Figure 1B). The telson is broad and uncleft with two long distal spines on both posterior corners and short spines all along the outer margins (Figure 1C).

Total length of the males is 8-10 mm, females are slightly longer, typically up to 11 mm in summer (Bacescu, 1954; Komarova, 1991), or even 16.5 mm in winter (Bacesco, 1940). The colour varies from deep red (from chromatophores) to ivory-yellowish or even transparent, depending on background colour, light and temperature variations (Bacescu, 1954; Komarova, 1991, Salemaa & Hietalahti, 1993).
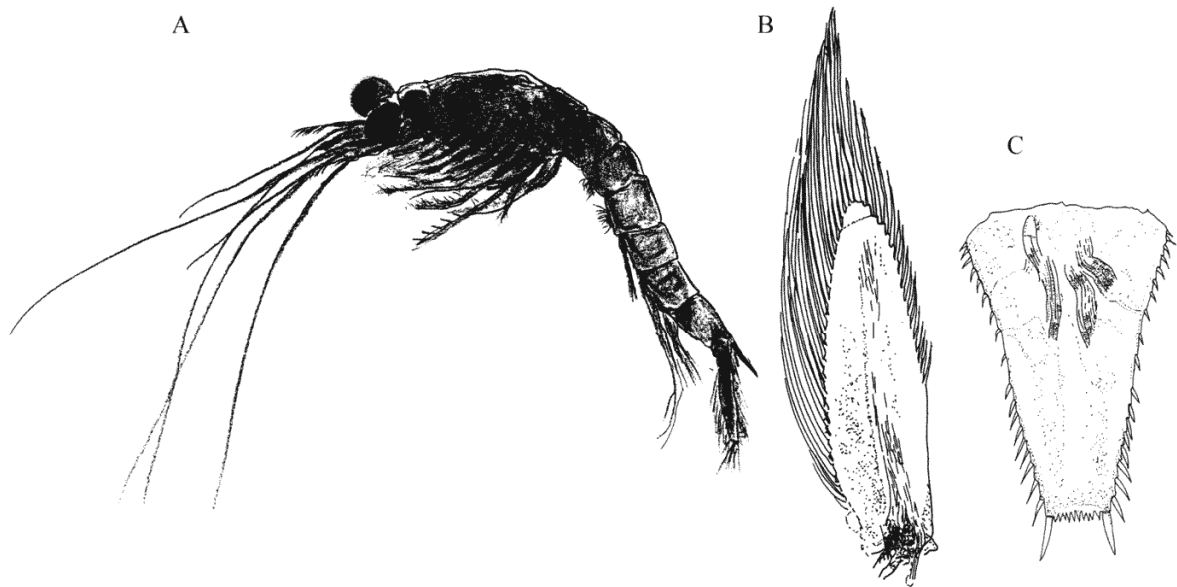
Figure 1. *Hemimysis anomala* G. O. Sars, 1907 adult male from the Biesbosch reservoir Honderd en Dertig (September 1998). A: Habitus (total length = 8 mm); B: Antennal scale (length = 1.26 mm); C: Telson (length = 1.15 mm). Originals.
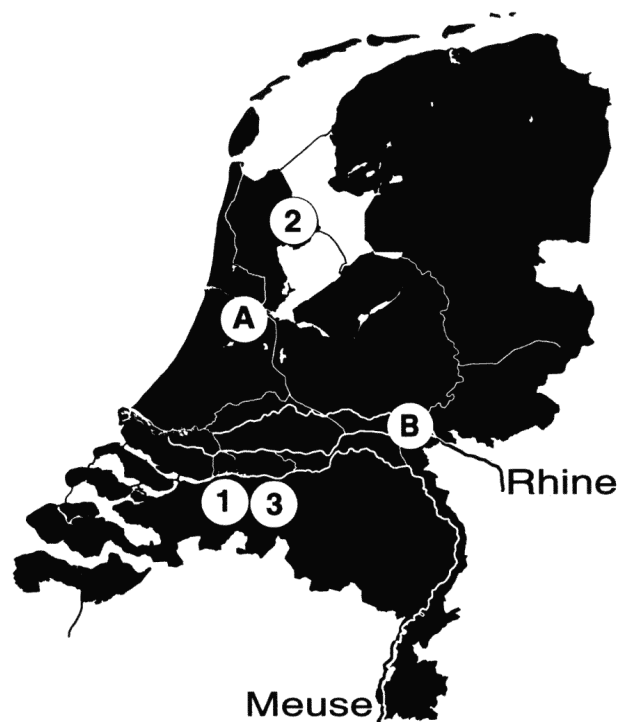

Figure 2. Locations where *Hemimysis anomala* is recorded in the Netherlands (October 1998). 1: Biesbosch reservoirs; 2: Andijk reservoir; 3: River Meuse (all current study); A: Lake Noorder-IJ (Faasse, 1998); B: River Rhine near Nijmegen (in fish guts, Kelleher et al., 1999).

H. anomala is a Ponto-Caspian (Caspian and Black Sea region) species, occurring there in a wide range of salinities: from 18 ‰ to freshwater (Bacescu, 1954; Mordukhai-Boltovskoi, 1979; Komarova, 1991). It has been observed in the coastal regions of the Caspian, Black and Azov Sea, in their lagoons and up to 50 km upstream the rivers Don, Dnestr, Dniepr, Pruth and Danube (Bacescu, 1954, 1966; Dediu, 1966; Komarova, 1991). In the 1950's and 60's *H. anomala* has been intentionally introduced into several impoundments on the River Dniepr (Ukraine), water reservoirs near Chernorechensk and Simferopol on the Crimean peninsula and the Dubossary reservoir (Moldavia) (Zhuravel, 1960; Komarova, 1991). In 1960 *H. anomala* was successfully transferred from the Dniepr hydropower reservoir to reservoirs in Lithuania, e.g. the Kaunas reservoir on the River Nemunas (Gasiunas, 1968; Mordukhai-Boltovskoi, 1979). From there it spread into the Baltic Sea, where it was first recorded in 1992 (Salemaa & Hietalahti, 1993). The first records in the Netherlands date from 1997 and 1998. In June 1997 and November 1998 several swarms were observed in the littoral zone of the brackish Lake Noorder-IJ (surface area 57 ha, max. depth 30 m), close to, but isolated from the Northsea Canal (Figure 2) (Faasse, 1998). Kelleher et al. (1999) found *H. anomala* in stomachs of young percids caught in the River Rhine near Nijmegen (Figure 2) in September 1997. In the German Rhine system several individuals were found in November-December 1997 in the River Neckar near Neckarsteinach (Neckar/km 44.2-44.6) and in the River Rhine near Koblenz (Rhine/kms 589.8 and 576.1) (Schleuter et al., 1998). In July 1998 it was recorded in the River Main (Schleuter & Schleuter, 1998).
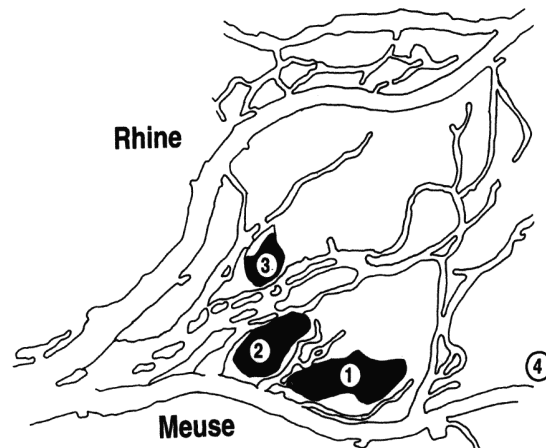

Figure 3. Location of the Biesbosch reservoirs at the confluence of the rivers Rhine and Meuse. 1: De Gijster; 2: Honderd en Dertig; 3: Petrusplaat; 4: Monitoring station Keizersveer.

Study sites

The Biesbosch reservoirs are situated near the confluence of the rivers Rhine and Meuse (see Figures 2 and 3). The Biesbosch reservoir scheme consists of three interconnected man-made reservoirs used for the supply of raw water for drinking water treatment plants in the south-western part of the Netherlands (see Table 1 for some characteristics). About 14% of the Dutch population depend on these reservoirs for their drinking water. Water is pumped from the eutrophic River Meuse and is retained, on average, for five months in these reservoirs. Despite relatively high nutrient levels (annual average total phosphorus concentration 0.10-0.28 mg l^{-1}, annual average total nitrogen concentration 3-5 mg l^{-1}) algal biomass is low (annual average 6-14 μg l^{-1} chlorophyll-*a*), thanks to artificial mixing and zooplankton grazing (mainly *Daphnia galeata* and *D. pulicaria).* The invertebrate predator *Leptodora kindti* has been present since the commissioning of the reservoirs in the 1970's. Another invertebrate predator, *Bythotrephes longimanus,* invaded the reservoirs in the mid-eighties and has established a self-sustained population since then (Ketelaars & van Breemen, 1993). The invasion of this onychopod did not lead to changes in phytoplankton biomass, but it distinctly influenced zooplankton size distribution: smaller size classes were

preferentially removed (unpublished data). Submerged macrophytes are almost absent and the shorelines of the concrete embankments are devoid of emergent vegetation. The lack of suitable spawning substrates and nursery areas restrict successful reproduction to only a few fish species: perch *(Perca fluviatilis),* zander *(Stizostedion lucioperca)* and ruffe *(Gymnocephalus cernuus).* Fish biomass (without 0+ fish) in the three reservoirs was estimated to be 40, 50 and 100 kg ha^{-1} for the reservoirs Petrusplaat, Honderd en Dertig and De Gijster, respectively (Kubecka et al., 1998).

The Andijk reservoir (see Figure 2 and Table 1) is a relatively deep, man-made reservoir used for the production of drinking water for a part of the province of North Holland. From March until October the reservoir is artificially destratified. Andijk reservoir is supplied by Lake IJssel, which is part of the Rhine basin. Being part of this system the chloride concentration is relatively high (100-200 mg 1^{-1}).

Table 1. Characteristics of the Biesbosch reservoirs and Andijk reservoir

Reservoir	De Gijster	Honderd en Dertig	Petrusplaat	Andijk
Surface area (ha)	305	210	100	45
Volume (10^6 m^3)	40	33	13	5
Maximum depth (m)	27	27	15	22
Average depth (m)	13	15	13	11
Retention time (weeks)	11	9	4	8–10
Chloride (mg 1^{-1})	52	55	56	100–200

Materials and methods

Routine vertical plankton hauls (mesh size 250 μm, net opening ø: 25 cm) were taken weekly during daytime from 24 m to the surface at the deepest sampling station in reservoir Honderd en Dertig (max. depth at this site is 27 m) with a hauling speed of circa 0.3 m s^{-1}. The net was lowered to 24 m and after at least two minutes the net was hauled to the surface. From 14 September 1998 onwards additional vertical plankton hauls were taken from bottom to surface at a speed of 0.6-0.9 m s^{-1}. In addition, some hauls from bottom to surface were also taken at a shallower pelagic sampling station (depth = 13 m) during September. Vertical distribution was determined on 21 September during the day (11.00-12.00 a.m.). Hauls were taken from the bottom, 20 m, 15 m and 10 m to the surface. At night (10.00-11.00 p.m.) this was repeated including also the depths 7.5 m, 5 m and 2.5 m.

Routine samples were preserved in 70% ethanol. Initially the extra samples were preserved in a 4% sugar formalin solution (Haney & Hall, 1973), but afterwards in 70% ethanol, because this preservative makes the mysids less brittle and fragile and the chromatophores are preserved better (Tattersall & Tattersall, 1951; Holmquist, 1972). Samples were analysed with a dissecting microscope (Wild M7, Wild M8 and Leica MZ APO at 20-50 x magnification).

Stomachs from 3 adult and 3 juvenile *H. anomala* (collected on 21 September 1998 and preserved in 4% formalin) were dissected out under a dissecting microscope (Wild M8) and teased apart in drops of glycerine, and permanent mounts of the contents were examined with 100 x magnification (Olympus BH2).

Length measurements were made on *H. anomala* juveniles, males and females from the day and night samples of 21 September using a dissecting microscope (Wild M8) with a monochrome video camera (Sony 1CCD) connected to a framegrabber and image analysis software (Quantimet Q500IW, Leica) on a personal computer. Total length (TL) was determined from the anterior margin of the carapace to the distal end of the telson according to Tattersall & Tattersall (1951) and Komarova (1991). The image analyis system allowed us to follow the body's curvature when the animals were bent (Carpentier et al., submitted). Sex determination was as follows: males were recognized by the long 4th pleopod. Females could easily be recognized when they carried offspring in their brood-pouch (marsupium) or, when it was empty, by two lamellae (oostegites, which form the ventral brood-pouch) at the base of the last two thoracic legs. Brood size was determined after removing the eggs, embryos or larvae from the marsupium.

Zooplankton densities in reservoir Honderd en Dertig were determined weekly during the growing season (March-November) and bi-weekly for the remaining period. Samples were taken using a 2 1 Rutt-

ner sampler (Hydrobios, Kiel) at every two meter, from the surface to 1 m above the bottom at two pelagic sampling stations (13 and 27 m deep). The samples were sieved through a net (mesh size 100 μm); the resulting composite sample was preserved in a sugar-formalin solution (Haney & Hall, 1973) with an end-concentration of 4% formalin. Preserved samples were concentrated and the zooplankton was identified to genus level and counted with an inverted microscope (Leica DMIL at 50 or 125 x magnification). When zooplankton density was high, the sample was split with a Folsom Plankton Splitter (Hydrobios, Kiel). The aliquot was identified, counted and numbers were calculated for the entire sample. At least 200 individuals were counted (not including *Dreissena polymorpha* larvae). *Bythotrephes longimanus* and *Leptodora kindti* were always counted in the entire sample.

Chlorophyll-*a* concentrations were determined weekly from samples taken at the outlet of reservoir Honderd en Dertig according to the Dutch Standard Method (warm-ethanol extraction) (NNI, 1981). Heterotrophic plate counts were determined bi-weekly from the same site according to the Dutch Standard Method (3 days incubation at 22 °C on glucose-yeast extract) (NNI, 1982).

Statistical analyses were performed using the statistical software package Statistica for Windows (Stat-soft Inc., 1997). Significance of the product moment correlation coefficient between brood size and length of the female was tested with the critical values mentioned by Sokal & Rohlf (1995). Effects of the invasion were tested with analysis of variance (AN-OVA). Zooplankton densities and heterotrophic plate counts were log-transformed before analysis. ANOVA with planned comparisons was used for comparisons among (groups of) means (1995-1997 vs 1998) (Sokal & Rohlf, 1995; Statsoft Inc., 1997).

Results

Occurrence

H. anomala was first recognised in a routine plankton net haul from 24 m to the surface at the deepest pelagic site in reservoir Honderd en Dertig (max. depth at this site is 27 m) on 13 July 1998. From this date onwards it was consistently found in weekly samples from this reservoir. *H. anomala* was also found in the other two reservoirs (Petrusplaat and De Gijster). The identification of the mysid prompted a re-examination of stored zooplankton samples (taken with a 2 liter Ruttner sampler at different depths of the water column), which revealed that *H. anomala* had already been present in November 1997 in reservoir Petrusplaat (one juvenile specimen). In October 1998 we found one juvenile *H. anomala* in a drift net (250 μm mesh size; ø: 25 cm; 5 minutes at a depth of circa 2 m) sample from the River Meuse at the monitoring station Keizersveer (see Figures 2 and 3).

In two vertical net haul samples from the bottom upwards in October 1998 in reservoir Andijk (Figure 2) we found one juvenile *H. anomala* amidst 70 *Neomysis integer* individuals (19 October) and 16 *H. anomala* individuals (3 adult females, 2 adult males and 11 juveniles) amidst 493 *N. integer* (26 October).

Interestingly, we found the recently invaded mysid *Limnomysis benedeni* (Czerniavsky 1882) in vertical plankton hauls in a shallow creek surrounding the reservoir Petrusplaat.

Abundance

Although *H. anomala* was found in all three Bies-bosch reservoirs, we only present data from reservoir Honderd en Dertig because the majority of the samples were taken there. From 13 July 1998 onwards *H. anomala* was found with varying densities (0.01-1.43 ind. 1^{-1}). Starting on 14 September 1998 net hauls were taken from bottom to surface at the same sampling station. Densities in these samples varied from 0.11-6.61 ind. 1^{-1}. In this period net hauls from the bottom to the surface were also taken at a shallower pelagic site (depth = 13 m). Densities at that site varied from 3.16-6.31 ind. 1^{-1}.

Effects on the zooplankton community

Two months after we found *H. anomala* in the net hauls, a distinct reduction in anomopod densities, compared to the three previous years, was observed (Figure 4A). The invertebrate predators *Bythotrephes longimanus* and *Leptodora kindti* were not found after 17 and 24 August, respectively; the anomopod *Chydorus sphaericus* was not found after May (data not shown). The copepod densities, however, remained unaffected (Figure 4B). A comparison of zooplankton densities from the period onwards that *H. anomala* was first found in the plankton hauls (13 July-26 October) with those in the same period in

the previous three years (see Table 2), revealed highly significant *(p < 0.0003)* lower densities for *Daphnia* spp. (mainly *D. galeata* and *D. pulicaria), Bosmina* spp. (mainly *B. longirostris),* Ostracoda and Rotifera (mainly *Keratella quadrata, Synchaeta* sp. and *Polyarthra* sp.). Densities of *C. sphaericus* were lower at the 2% level *(p < 0.02).* No significant difference was discern-able for densities of *B. longi-manus, L. kindti,* nauplius larvae, copepodids and adult calanoid and cyclopod copepods. Densities of *Dreissena polymorpha* larvae were significantly higher compared to previous years *(p < 0.02).*
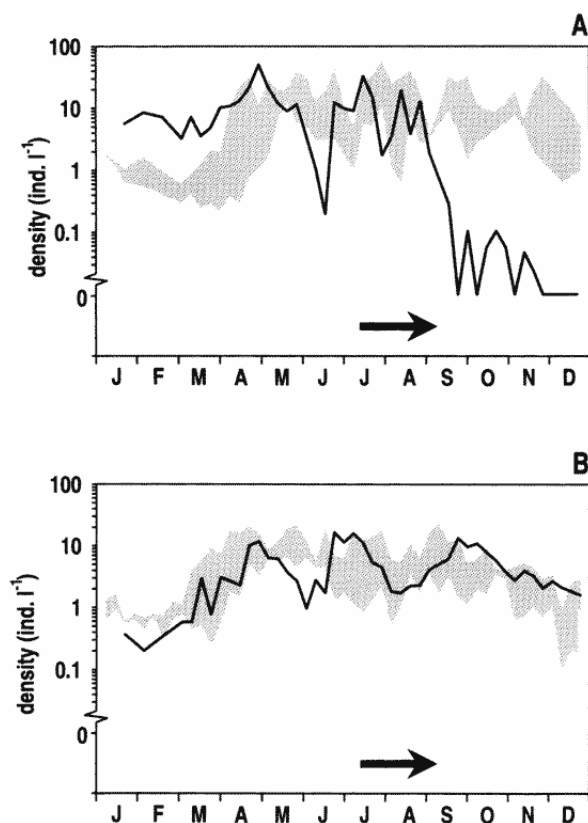

Figure 4. A: Densities of the Anomopoda (*Daphnia* spp. and *Bosmina* spp.) in 1998 in reservoir Honderd en Dertig (solid line) compared to those in the period 1995–1997 (dotted area indicates the range); B: *Idem* for adult Copepoda. Horizontal arrow indicates the start of the period that *Hemimysis anomala* was found in vertical plankton hauls (13 July 1998).

Chlorophyll-*a* was significantly lower, heterotrophic plate counts were unchanged compared to the previous three years (Table 2).

Vertical distribution

Net hauls from different depths to the surface in three consecutive weeks (Table 3) showed that *H. anomala* stayed in the deeper layers during the day and moved upwards at night. Vertical distribution during the day showed large differences: on 14 and 21 September most mysids were found in the bottom layer, whereas on 28 September the largest number was found 7-12 m above the bottom. During the day densities in certain layers were sometimes very high, e.g. on 14 September density in the haul from the bottom upwards was 6.61 ind. l^{-1} and from 20 m upwards 0.51 ind. l^{-1}. Density in the bottom 7 meters therefore was 24 ind. l^{-1}. On 28 September the density in the 10-15 m layer was 8.2 ind. l^{-1}. From the night-sampling data of 21 September (Table 3) can be deduced that the mysids only stayed in the top layer (7.5-0 m) and near the bottom.

During the day adult males and females stayed in the bottom layer and juveniles were found higher up in the water (Figure 5A-C). At night mysids were found higher in the water column. This group consisted almost entirely of males and juveniles. Higher up in the water column smaller juveniles were observed compared to the juveniles from the entire water column. Most females stayed in the layer 2 m above the bottom (Figure 5D-F).

Table 2. Comparison of zooplankton densities, chlorophyll-*a* concentrations and heterotrophic plate counts in reservoir Honderd en Dertig in the period that *Hemimysis anomola* occurred in the plankton samples in 1998 (13 July – 26 October) with the same period in the three previous years. *p*-Level indicates whether the 1998 average is significantly different from the averages of the three previous years or not

	Unit	1995	1996	1997	1998	df*	*p*-level
Rotifera	ind. m^{-3}	11817	3744	4685	1286	1; 51	<0.0001
Leptodora kindti	ind. m^{-3}	117	33	49	21	1; 51	0.2044
Daphnia spp.	ind. m^{-3}	11194	9092	11787	3886	1; 51	<0.0001
Bosmina spp.	ind. m^{-3}	5692	95	1647	13	1; 51	<0.0001
Chydorus sphaericus	ind. m^{-3}	8667	0	2305	0	1; 51	0.0159
Bythotrephes longimanus	ind. m^{-3}	29	40	94	51	1; 51	0.2965
Nauplius larvae	ind. m^{-3}	6234	7934	4933	3901	1; 51	0.0592
Copepodids	ind. m^{-3}	10938	7304	4738	6182	1; 51	0.1159
Adult Calanoida	ind. m^{-3}	2044	930	827	2798	1; 51	0.0857
Adult Cyclopoida	ind. m^{-3}	4015	2974	2912	2403	1; 51	0.9899
Ostracoda	ind. m^{-3}	227	132	290	13	1; 51	0.0002
Dreissena polymorpha larvae	ind. m^{-3}	7963	21837	14997	26698	1; 52	0.0177
Chlorophyll-a	μg l^{-1}	18	12.3	13.3	8.6	1; 59	0.0179
Heterotrophic plate counts	CFU** ml^{-1}	448	211	468	408	1; 28	0.3614

*: df: degrees of freedom; **: CFU: colony-forming units.

Gut and stomach analyses

Gut analyses were performed on fish caught in reservoir Honderd en Dertig from 27-30 August 1998. Four out of five perch (standard length (SL) - 16.5-25.5 cm) had ingested *H. anomala,* three were completely stuffed with *H. anomala.* Two out of three ruffe (SL = 10.5 and 13 cm) contained *Hemimysis.* No *H. anomala* was found in 2 bream *(Abramis brama)* (SL = 42.5 and 45 cm), 1 zander (SL = 58 cm) and two roach *(Rutilus rutilus)* (SL = 12 and 13.5 cm). Three stomach analyses of adult *H. anomala* (TL = 8-8.5 mm) showed that cladocerans *(D. galeata* and *Daphnia* sp.) were the predominant prey, though large phytoplankton *(Staurastrum* sp.), rotifers *(K. quadrata, Polyarthra* sp.) and calanoid and cyclopoid copepods were also present. Remains of one *H. anomala* were found in one investigated stomach. Three stomach analyses of juvenile *H. anomala* showed the smallest ones (TL = 3.5 and 4 mm) had ingested predominantly algae *(Staurastrum* sp., cf. *Coelastrum* and *Closterium* sp.). In addition, unidentifiable fine organic material and remains of rotifers, a few small cladocerans and one calanoid copepod were present. The stomach from the largest juvenile examined (TL = 5 mm) was dominated by cladoceran remains. In addition, *Pediastrum* sp., *Staurastrum* sp. and *Keratella* sp. were found.

Life history

Juvenile total length ranged from 1.4 to 9.9 mm *(n* = 1252), males were on average 8.1 mm long (min = 5.5 mm; max = 10.7 mm;SD= 1.05; *n* = 469) and females had an average length of 8.4 mm (min = 6.5 mm; max = 12.5 mm; SD = 0.95; *n* - 184). 74% of these females carried offspring. The mean brood size was 13.3 (min = 1; max = 27; SD = 5.2; *n* = 126). Only 7% of females with a marsupium carried fully developed off-spring (larvae with stalked eyes); 10% carried embryos with discernable eyes, 53% carried embryos without eyes and 30% carried eggs.

Brood size was significantly correlated to female length $(r = 0.666;\ n = 126;\ p < 0.001)$ (Figure 6). Females outnumbered males until the middle of August. Hereafter males outnumbered females (Table 4).
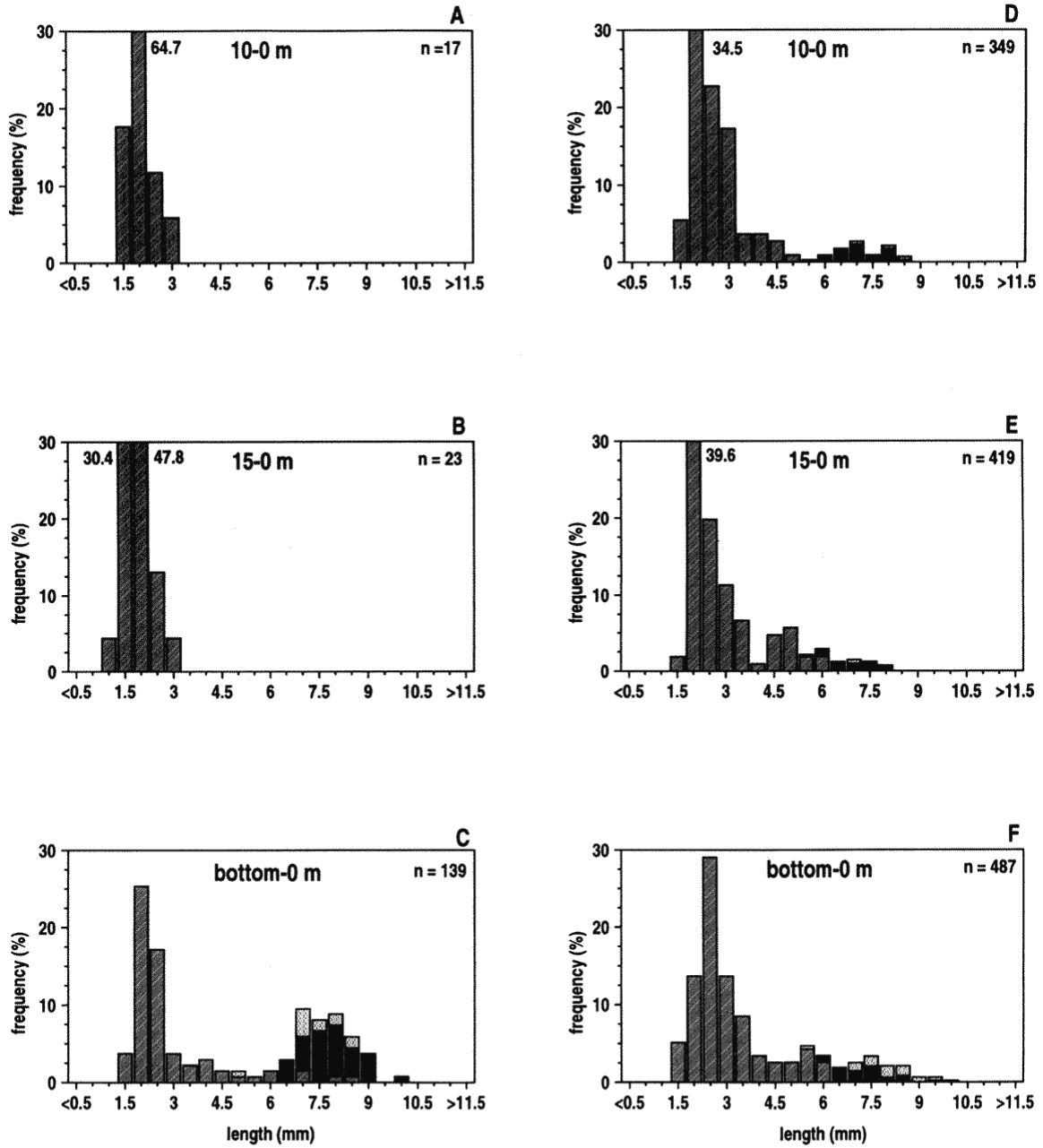

Figure 5. Length–frequency distributions of *Hemimysis anomala* collected in reservoir Honderd en Dertig with vertical plankton hauls from different depths (indicated in the upper centre of the graphs) on 21 September 1998. A–C: During the day (10.00 a.m.); D–F: During the night (10.00 p.m.). Hatched bars: juveniles; solid bars: males; dotted bars: females.

Table 3. Numbers of *Hemimysis anomala* females, males and juveniles in vertical plankton hauls from different depths to the surface in reservoir Honderd en Dertig on three occasions during the day and on one occasion during the night in September 1998

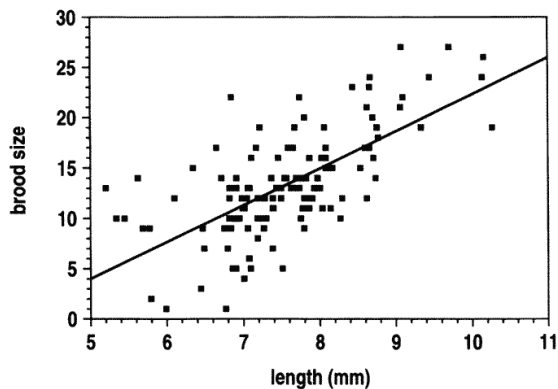| Date and time	Depth	2.5 m	5 m	7.5 m	10 m	15 m	20 m	25 m	Bottom
14–09, 10.00 a.m.	females	–*	–	–	0	3	4	–	260
	males	–	–	–	0	16	152	–	1190
	juveniles	–	–	–	0	375	348	–	7300
21–09, 10.00 a.m.	females	–	–	–	0	0	1	–	12
	males	–	–	–	0	0	1	–	40
	juveniles	–	–	–	17	23	35	–	87
21–09, 10.00 p.m.	females	1	4	2	4	2	2	1	34
	males	3	27	40	26	22	31	42	37
	juveniles	119	190	279	319	395	302	242	416
28–09, 10.00 a.m.	females	–	–	–	0	0	20	–	85
	males	–	–	–	0	0	90	–	140
	juveniles	–	–	–	19	2040	3560	–	1265

*: not determined.

Figure 6. *Hemimysis anomala* brood size plotted against female length ($r = 0.666$; $n = 126$; $p < 0.001$).

Table 4. Sex ratios of *Hemimysis anomala* in reservoir Honderd en Dertig. Only samples with more than 10 individuals are included. From 13 July–7 August animals were collected with plankton hauls from 24 m to surface and from 14 August–2 November from bottom (27 m) to surface

Date	13–07	20–07	27–07	10–08	17–08	24–08	31–08
Sex ratio (F:M*)	>13	2	3.5	1.3	2.2	0.5	0.2
Number of ind.	13	24	76	44	41	51	125

Date	07–09	14–09	21–09**	28–09	19–10	24–10	02–11
Sex ratio (F:M)	0.1	0.2	0.6	0.6	0.18	0.73	0.5
Number of ind.	64	1450	123	225	26	45	12

* F: female, M: male; ** day and night samples combined.

Discussion

Invasion

The known distribution of *H. anomala* does not give a clue as to how it reached the Biesbosch reservoirs. It could either have been transported downstream the River Rhine or have been transported with ballast water from the Baltic or Black Sea and released in Dutch ports. Transport via the River Rhine seems possible because of the records in the German (Schleuter et al., 1998; Schleuter & Schleuter, 1998) and Dutch part of the Rhine drainage area (Kelleher et al., 1999). It is remarkable, however, that so few other records in the Rhine drainage area exist. This can be due to the hidden life style of *H. anomala* during the day, when most samples are taken, or to the use of inadequate sampling gear. Mysids have been observed escaping from samplers used in the littoral region (Mauchline, 1980; pers. observ.). In addition, samples are often not taken near the bottom. For example *H. anomala* occurrence in Andijk reservoir was only established after deliberately taking net hauls from the bottom to surface. On the other hand, the record from the brackish waters in the Netherlands indicate transport by ballast water from the Baltic Sea (Faasse, 1998) or the Black Sea. The Biesbosch reservoirs are situated near some large ports (Antwerp and Rotterdam), but are located upstream. Mysids are unable to swim upstream actively (Holmquist, 1978; Mordukhai-Boltovskoi, 1979), but could have been aided by ships. The range extension along the Danube of the Ponto-Caspian mysid, *Limnomysis benedeni,* may have been facilitated by navigation. Both Wittmann (1995) and Reinhold & Tittizer (1998) collected *L. benedeni* in ballast water and on the

outer hull of ships. *H. anomala* may not easily be transported attached to a ship's hull, because it is typically a swimming mysid, in contrast to *L. benedeni,* which clings to surfaces most of the time (pers. laboratory observations). Even transoceanic transport has been described for mysids (Carlton, 1985). A point of interest is that mysids, in contrast to e.g. parthenogenetically reproducing cladocerans, only reproduce sexually (Mauchline, 1980), which means that a female with fertilized eggs or both males and females must have been introduced.

H. anomala either built up a population in the River Meuse, from which water is abstracted for the reservoirs, or in its adjoining backwaters, or the river has just served as a transport medium for several or one (gravid female) individual(s). The time to reach the reproductive age depends on lake trophic status and hypolimnetic temperatures. In Lake Michigan *Mysis relicta* females produced their first brood at one year of age. Males died at this time, but females were found to produce a second brood approximately four months later (Morgan & Beeton, 1978). In oligotrophic Lake Tahoe, however, it took four years for *M. relicta* to reach the reproductive age (Morgan, 1980). Species with more than one generation per year usually occur in latitudinal bands 40°-60° N or are shallow-living neritic and littoral species (Mauchline, 1980). Because of artificial destratification no temperature gradient exists in the Biesbosch reservoirs. This may stimulate fast growth and early sexual maturity for *H. anomala.* Depending on temperature one female can produce several broods. Estimates of rates of production within mysid populations show that a single breeding pair of the mysid *Praunus* spp. produced 588-2450 young per year (Mauchline, 1980). Brood size of these species varies from 40-80 eggs (Mauchline, 1980), whereas *H. anomala* in reservoir Honderd en Dertig only had 13.3 eggs on average. Considering a density of circa 1 ind. 1^{-1} in reservoir Honderd en Dertig, which means in total 33 x 10^9 individuals, it must have taken years for *H. anomala* to establish such a population, even if the number of initial colonists has been high (e.g. 1000).

Experiences with deliberate introductions of a large number of individuals have shown that it takes a long time before a dense population exists. In Lake Selbesj0n (Norway) *M. relicta* had established a dense population six years after the introduction of 100,000 individuals in the lake. It also took six years before *M. relicta* appeared in fish stomachs (Langeland et al., 1991). Schumacher (1966) reported that 200-1000 *M. relicta* were transplanted for four consecutive years in Grindstone Lake (USA), but only a few individuals could be caught after five years. Two years after the introduction of 1.65 million *M. relicta* individuals in Lake Blasjon (Sweden) it had formed a self-sustaining population (Fürst, 1967). The time of first appearance of *M. relicta* in fish stomachs ranged from four years in Lake Mesvattnet (Sweden) to as long as 16 y in Kootenay Lake (Canada) (Lasenby et al., 1986). In the current study perch stomach content analyses showed that *H. anomala* was the preferred prey in the same year as the mysid was first detected in vertical plankton hauls. In gut and stomach analyses *(n* = 113) of different fish species (fry and adults of perch, ruffe, zander, roach, bream, smelt *(Osmerus eperlanus)* and ide *(Leuciscus idus))* from the Biesbosch reservoirs in 1996, no mysids were found at all (unpublished data).

We can only guess when *H. anomala* initially invaded the Biesbosch reservoirs. Considering the high densities recorded in 1998 and the relatively low reproduction rate, the invasion must have occurred at least several years ago. The presence must have gone unnoticed because of the hidden life style near the bottom and the use of inappropiate sampling methods for mysids.

The possibility of a mass inoculation during the last few years does not seem likely. In 1996 Meuse water has extensively been sampled at the reservoir intake from June-August during the day and night with a 60 cm diameter ichthyoplankton net (mesh size 4-6 mm), but no mysids were collected, whereas smaller zooplankton like *Leptodora kindti,* was collected (Ketelaars et al., 1998).

H. anomala is probably present at more locations in the Rhine and Meuse basin than presently known, but unless the right samples are taken it will not be noticed. Until 1997 *Neomysis integer* was the only mysid occurring in freshwater lakes in the Netherlands (Koomen et al., 1995). In addition to *H. anomala, Limnomysis benedeni* has recently been found on many locations along several branches of the River Rhine (Kelleher et al., 1999). These recent discoveries are not yet widely known. Because of the unawareness of the existence of other freshwater mysids in the Netherlands, any mysid found is perhaps likely to be "identified" as *N. integer.*

Why is *H. anomala* successful in the Biesbosch reservoirs? One reason may be that some feature(s) of these reservoirs are conducive to the development of large neozoon populations. Self-sustained populations of *Bythotrephes longimanus* only developed in artificial reservoirs or newly formed lakes in the lowlands of Western Europe in the late 1980's (Ketelaars & Gille, 1994). Some common characteristics of these lakes and reservoirs are their relatively large depth (> 10 m), the almost complete absence of

macrophytes, low productivity and a fish assemblage dominated by percids. The occurrence of large-sized *Daphnia galeata* and *D. pulicaria* (> 2mm) and the conspicuous *B. longimanus* indicate that fish predation pressure is low in the Biesbosch reservoirs (Ketelaars et al., 1998). Low fish predation pressure could also play a role in the success of *H. anomala* in these reservoirs, because mysids are preferred prey for many fish (Mauchline, 1980; Toda et al., 1982). The euryhalinity of the Ponto-Caspian biota (Dumont, 1998) makes them ideally pre-adapted to invade new environments, as pointed out for the Ponto-Caspian Onychopoda by Dumont (in prep.). After their introduction it took 16-18 years before the mysids *Paramysis lacustris, L. benedeni* and *H. anomala* were fully adapted to the reservoirs in Lithuania (Lazauskiene et al., 1993). The successful "acclimatization" of Ponto-Caspian mysids in reservoirs in Russia, Lithuania and Ukraine proves that these mysids are able to establish populations in "pure" freshwater habitats. The successful invasion of the Biesbosch reservoirs confirms that *H. anomala* is not dispersal limited. It is most likely only a matter of time before they will invade and change more ecosystems in Western Europe. The recent invasions of the Great Lakes basin in North America by the Ponto-Caspian amphipod *Echinogammarus ischnus* (Witt et al., 1997) and onychopod *Cercopagis pengoi* (MacIsaac et al., 1998) is a reminder that *H. anomala* and other mysids may soon invade North America too, as has also been predicted by Ricciardi & Rasmussen (1998).

Abundance

Grabe & Hatch (1982) recorded a maximum abundance of *Mysis mixta,* with a similar size as *H. anomala,* on the bottom of New Hampshire coastal waters of 0.041 ind. 1^{-1}. Maximum densities of *Mesopodopsis slabbed* and *Neomysis integer* (both about the same size as *H. anomala)* in the Baltic Sea were 1.1 ind. 1^{-1} and 0.2 ind. 1^{-1} respectively (Moffat & Jones, 1992). Toda et al. (1982) found a maximum density of *N. intermedia,* a slightly larger mysid than *H. anomala,* in Lake Kasumigaura (Japan) of 2.5 ind. 1^{-1}, and concluded that Lake Kasumigaura supports a large population of mysids compared to published data of abundances of mysids of similar size elsewhere. Fulton (1982) mentions maximum densities of the marine mysids *Mysidopsis bigelowi* (adults > 6 mm) and *Neomysis americana* (adults > 12 mm) in Beaufort, North Carolina of 0.007 ind. 1^{-1} and 0.011 ind. 1^{-1}, respectively. *Mysis relicta,* about twice the size of *H. anomala,* reached maximum densities in aggregations in the thermocline in Lake Michigan of 0.001-0.01 ind. 1^{-1} (Lehman et al., 1990). Morgan (1981) found maximum densities of *M. relicta* in two Californian lakes of 0.002-0.005 ind. 1^{-1}. The latter densities are three to four orders of magnitude lower than those in reservoir Honderd en Dertig. All these comparisons show that in our case reservoir Honderd en Dertig supports an extremely large population, certainly if we take into consideration that our sampling method was not optimal for catching mysids. We used plankton nets with a 25 cm diameter, whereas the use of large diameter nets is the best technique for quantitative studies of mysids (Langeland, 1988; Salemaa et al., 1990).

Effects

Several lines of evidence point toward *H. anomala* as the cause for declines in other zooplankton and phytoplankton biomass. Truly dramatic changes in zooplankton were observed. *Daphnia* spp., *Bosmina* spp., Rotifera and Ostracoda practically disappeared from the zooplankton, a unique phenomenon in the Biesbosch reservoirs. The disappearance of the carnivorous *B. longimanus* and *Leptodora kindti* from the plankton after mid-August could be the result of competition for food or predation by *H. anomala.* The latter is the most likely because *B. longimanus* has been found in the stomachs of *M. relicta* (Fürst et al., 1978, in Langeland, 1981) and both predatory cladocerans were consumed by *H. anomala* in our laboratory feeding experiments (see below). In comparison with previous years, however, no significant difference was found. High densities of *B. longimanus* in July 1998 account for not significantly different densities compared to previous years. No significant difference for *L. kindti* can be explained by very variable densities in the three previous years. *Chydorus sphaericus* was also absent from the plankton, but this is known to occur from time to time, mainly depending on phytoplankton composition. The higher densities of *Dreissena polymorpha* larvae in 1998 are probably not a consequence of the invasion; densities are very variable at this time of year.

   Little is known of the food preferences of *H. anomala.* Bacescu (1954) mentions that detritus and copepods are ingested. In a limited number of feeding experiments in the current study (125 ml poly-

carbonate containers at a temperature of 18.4°C in complete darkness), *H. anomala* voraciously fed on large and small *Daphnia, B. longimanus* and *L. kindti*. After 24 h up to 23 daphnids were eaten and within half an hour after releasing the prey *H. anomala* consumed up to 3 *Daphnia* sp. and 1 *B. longimanus*. Stomach analyses of three adult individuals and one large-sized juvenile, collected in reservoir Honderd en Dertig on 21 September, confirmed a food preference for cladocerans. In feeding experiments *Hemimysis,* however, only occasionally fed on planktonic copepods. It seemed that the dashing movements of the copepods made it difficult for *H. anomala* to get hold of them. This may explain why no changes were found in reservoir copepod densities in 1998 compared to previous years (Figure 4B). The copepods mentioned by Bacescu (1954) as food for *H. anomala* may be related to benthic (harpacticoid) copepods, which do not swim very fast. The disappearance of some major zooplankton groups can either be a direct effect (predation), an indirect effect (competition for algae, detritus or bacteria), or both. Considering the preference of *H. anomala* for animal food it seems likely that *H. anomala* first preyed upon the zooplankton, and after its elimination shifted to phytoplankton. Bacteria seemingly did not play a role. Data on detritus are not available.

It has been shown that mysids consume prey of a broader size-range than most other invertebrate predators (Murtaugh, 1981a). *Mysis relicta* in deep Ontario lakes consumed large (1.3-3 mm) cladocerans, but also smaller cladocerans than those consumed by planktivorous fish (Almond et al., 1996). Different sizes of *Neomysis mercedis,* a freshwater mysid of similar size as *H. anomala,* preyed upon *Daphnia* in a size selective way: small mysids selected the smallest prey available, while adult mysids consumed *Daphnia* of a broad size range (Murtaugh, 1981a). Large daphnids (>2.5 mm) were, however, less efficiently preyed upon by *N. mercedis,* which resulted in more injured large *Daphnia* (Murtaugh, 1981b). In our laboratory experiments large *Daphnia magna* (>3 mm) were also not eaten by *H. anomala*. Maximum length of the Biesbosch daphnids is about 2.5 mm. Increasing body size could be a defence against mysid predation. This would, however, increase vulnerability to visual planktivores. It remains to be seen how the predation pressure of the invaded mysid will influence size structure of the *Daphnia* population and fish composition and biomass.

Mysids exhibit two distinct feeding methods: large food items are picked up by the endopods of the thoracic limbs, whereas suspended fine particles are filtered from the incoming currents of water produced by the exopod movements of the thoracic limbs (Tattersall & Tattersall, 1951; Mauchline, 1980). Both feeding methods can be exhibited by the same species (Mauchline, 1980). A wide variety of organic materials is used by mysids: detritus, remains of macrophytes, algae, Annelida, Rotifera, Anomopoda, Ostracoda, Copepoda, Amphipoda, Chironomidae, mollusc larvae and flesh from mussels, crayfish and fish (Blegvad, 1922; Kinne, 1955; Mauchline, 1980). We noticed that *H. anomala,* under laboratory conditions, also fed on aquarium fish food (TetraMin®). This omnivory, combined with the ability to maintain high feeding rates at low prey densities (Fulton, 1982), may explain (in part, see above) the success of *H. anomala* in the Biesbosch reservoirs: almost all organic food resources, including dead animals and conspecifics, are available. Cannibalism was observed for *H. anomala* in our feeding experiments as well. In addition, we saw *Hemimysis* feed on dead conspecifics. Limited stomach analyses from animals caught in the reservoir indicate this occurs in the reservoirs as well. Because only one *H. anomala* was found in these stomachs, the laboratory observations do not necessarily mean that *H. anomala* is severely cannibalistic or a scavenger in the reservoirs. The feeding behaviour observed in the laboratory may be an artefact due to low food or high predator densities.

The deliberate transfer of mysids into lakes in Norway, Sweden and North America, to serve as supplementary food for fish, often led to dramatic changes in the ecosystems involved, including negative effects on fish production (Lasenby et al., 1986). Mysids often had the same effect as overpopulations of planktivorous fish and therefore acted as a eutrophication agent (Langeland et al., 1991). In the Biesbosch reservoirs, however, the almost complete absence of herbivores from mid-September onwards did not result in elevated chlorophyll-a concentrations. On the contrary, chlorophyll-a concentrations were significantly lower than in previous years, possibly due to small-sized juvenile mysids feeding on phytoplankton, as indicated by stomach analyses. In the laboratory we noticed that *H. anomala* fed on *Scenedesmus acuminatus* and large (1-5 mm) colonies of the cyanobacterium *Aphanizomenon flosaquae,* an often dominant phytoplankton species in the reservoirs in September-October. Algal biomass is an important aspect with respect to drinking water production from the Biesbosch reservoirs.Whether the invasion has negative or beneficial effects on water quality may become obvious during the next growing season.

Vertical distribution

Sampling during the day and at night in September revealed that *H. anomala* stayed in the deepest parts of the reservoir during the day. Komarova (1991), citing Russian literature, and Bacescu (1954) describe the same diel vertical migration pattern for *H. anomala.* In general most mysids live just off or on the bottom during the day and migrate upwards during the night (Tattersall & Tattersall, 1951; Mauchline, 1980 and references therein). In addition to diel vertical migration, *Neomysis integer* also exhibited diel horizontal migration: during the day it stayed in swarms near the shore and migrated from the shore into the pelagic zone of the Baltic Sea at night (Debus et al., 1992). Salemaa and Hietalahti (1993) found *H. anomala* swarms avoiding direct light by hiding in crevices or under stones, whereas during the night the animals dispersed. We also observed the small-sized mysids during the day in swarms in shaded littoral areas. SCUBA-divers observed *H. anomala* swarms in the littoral of the reservoir Honderd en Dertig at a depth of 6 m. These swarms only occurred near large stones and not near sandy substrates. Komarova (1991) described the same preference for stony habitats, which makes the large rivers in the Netherlands (with many groynes and stone-covered banks) a suitable habitat for *H. anomala.*

Migration of mysids may be related to sexual activity (Mauchline, 1980). Reviews by Tattersall & Tattersall (1951) and Mauchline (1980) indicate that no clear pattern is discernable in either marine or freshwater mysids. In reservoir Honderd en Dertig females stayed near the bottom during the day and night. A similar pattern was found for the mysid *Gastrosaccus msangii* by Bacescu (1975, in Mauchline, 1980).

During the day juveniles are usually found higher up in the water than adults, though the reverse is also found (Tattersall & Tattersall, 1951). We observed that the "former is also the case in reservoir Honderd en Dertig. *N. integer* in the Western Baltic Sea showed the same behaviour in March, but the reversed was found in October (Valipakka, 1992). The separation from the adults may help to prevent cannibalism, especially at the high densities in the Biesbosch reservoir. The occurrence higher up in the water can also be due to the fact that the small, more transparent juveniles are not as prone to fish predation as the larger adults.

Life history

We found that males are slightly smaller than females, which is a common feature for most mysids (Tattersall & Tattersall, 1951; Mauchline, 1980). This was confirmed by Komarova (1991) and Salemaa & Hietalahti (1993) for *H. anomala,* and the size range we determined is in accordance with their results. Bacescu (1969, in Komarova, 1991) mentioned that length in freshwater populations does not exceed 6.7-8.5 mm. No such limit was found in our case. Schleuter et al. (1998) only mention a few measurements of *H. anomala* from a freshwater habitat (7 juveniles varied from 4.1—4.3 mm and one female measured 8.4 mm). This is well within the range we found.

Literature on brood size is sparse. Bacescu (1954) mentioned that females carry 10-15 eggs (maximum 31), Reznichenko (1959) mentioned an average of 18 (maximum 31) and Derjavin (1929, in Komarova, 1991) mentioned just 6-10 eggs per female. Brood size we found (average 13.3, max. 27) fits in the literature range.

The significant correlation between female length and brood size is a common feature for mysids (Mauchline, 1980; Morgan, 1980; Toda et al., 1982), but can be highly variable and sometimes this relation is not found at all (Grabe & Hatch, 1982).

Changing sex ratio's during the season may be attributed to differential mortality between males and females. Mauchline (1980) e.g. mentioned mortality of females after breeding, which led to a dominance of males. According to Komarova (1991) *H. anomala* breeds from April to October. The change in sex ratio in reservoir Honderd en Dertig indicates that the breeding season ended earlier. In contrast to what is commonly found (Kinne, 1954; Mauchline, 1980; Grabe & Hatch, 1982; Toda et al., 1982; Moffat & Jones, 1992) males outnumbered females more frequently. Investigations need to be carried out the whole year round on a larger number of animals, however, to know the real pattern.

Further elucidation of life history characteristics and behaviour of *H. anomala* will not be a problem, because mysids are easy to maintain and culture in the laboratory (Mauchline, 1980; Léger & Sorgeloos, 1982; pers. observ.). This is in sharp contrast with the onychopod *B. longimanus,* another invertebrate predator that invaded the Biesbosch reservoirs 10 y ago, which could not be maintained in the laboratory longer than a few weeks (Ketelaars et al., 1995).

Concluding remarks

The present distribution of *H. anomala* in Western Europe looks very scattered, and raises the suspicion that not the right samples are taken at the right time (night). Water managers should therefore pay more atttention to appropriate sampling as is also pointed out by Kelleher et al., (1999).

The establishment of this new invader in the Biesbosch reservoirs may have been facilitated by the fact that no resident population of mysids (e.g. *Neomysis integer)* was present. Without competition and with a relative low fish predation pressure it succeeded to build up a dense population in a relatively short time. In the Andijk reservoir, however, a resident population of *N. integer* is present. Comparison of the development of *H. anomala* populations in the reservoirs at both locations may provide clues on the key factor(s) that determine colonization success of *H. anomala.*

Because of the much broader prey-size range of mysids compared to other invertebrate predators, further spread of *H. anomala* in the Netherlands might severely interfere with lake restoration programmes (biomanipulation) in which planktivorous fish are removed in order to increase herbivorous zooplankton densities. A complicating factor is that, in contrast to planktivorous fish, no method is available to remove mysids once they have established populations. If *H. anomala* also exploits the phytoplankton biomass, the overall water quality may look the same, but by reducing the number of trophic levels it will make the ecosystems less stable and more vulnerable to (small) disruptions.