Impaired reproduction in the amphipods *Monoporeia affinis* and *Pontoporeia femorata* as a result of moderate hypoxia and increased temperature

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ABSTRACT: Laboratory experiments were performed on the amphipods *Monoporeia affinis* and *Pontoporeia femorata* in order to test the hypotheses that specific reproductive variables are affected by moderate hypoxia and/or slightly increased temperature. These reproductive variables include unfertilised/undeveloped eggs, dead eggs and females carrying a dead brood. In a series of experiments, amphipods were exposed to increased temperature during different parts of the reproduction cycle. Exposure prior to mating caused an increased number of dead eggs and a higher percentage of females carrying dead broods. Furthermore, the effects of low oxygen concentrations (2 to 6 mg O_2 l^{-1}), solely and in combination with contaminated sediments, heavy metals and hydrophobic organic contaminants (polycyclic aromatic hydrocarbons and polychlorinated biphenyls), were tested during the reproduction cycle. Exposure to low oxygen concentrations induced a higher frequency of females carrying dead broods, and females exposed to hypoxia or contaminants had a lower fertility rate compared to controls. Our results show that oxygen concentrations and temperature conditions normally occurring in the Baltic Sea and that are not otherwise detrimental to adults could severely affect the gonads and the embryos. Females with dead broods, undeveloped/unfertilised eggs and dead eggs are proposed as tools for use in monitoring non-contaminant stressors.

KEY WORDS: Reproduction · Temperature · Hypoxia · Monitoring · Amphipods · *Monoporeia affinis* · *Pontoporeia femorata*

INTRODUCTION

Monoporeia affinis and *Pontoporeia femorata* (Amphipoda: Crustacea) are both important inhabitants of the Baltic macrofauna community. *M. affinis* is considered to be a key species in structuring benthic communities (Lopez & Elmgren 1989). The species have different origins and geographical distributions: *M. affinis* is a glacial relict, found in lakes below the highest coastline and in the Baltic; *P. femorata* is a marine species, seldom found north of the southern Bothnian Sea due to the salinity gradient in the Baltic. In the Baltic proper, both species co-occur and feed on the top surface sediment layer (Lopez & Elmgren 1989). They differ however in depth distribution, and a larger proportion of *P. femorata* is found deeper in the sediment than *M. affinis*, although more than 80% of both species is found in the top 5 cm (Hill & Elmgren 1987).

The life cycles of the 2 species are similar. In shallow waters (less than 15 to 20 m), *Monoporeia affinis* usually has a 1 yr life cycle although in the Baltic proper at depths where the species is most frequent (20 to 50 m) the life cycle is 2 yr, whereas *Pontoporeia femorata* has a 2 yr life cycle at least down to 45 m (Cederwall 1978). At greater depth, both species have a 3 yr life cycle. The reproduction cycle starts at the beginning of August and is triggered by the reduction in light (Segerstråle 1971). The gonads mature during the

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autumn and mating starts in the beginning of November, after which males die within some weeks (Sundelin & Eriksson 1998). However, deviations from the seasonal reproduction cycle have been observed for both species. Sexually mature adults, both male and female, have been noticed at times other than the normal reproduction season (Eriksson et al. 1999).

Several studies in contaminated areas have demonstrated the usefulness of *Monoporeia affinis* reproduction variables in monitoring effects of xenobiotics in the field (Elmgren et al. 1983, Sundelin & Eriksson 1998). The embryonic development of the amphipod was found to be particularly sensitive to exposure to various contaminants and the frequency of malformed embryos is used as a biomarker in the Swedish National Monitoring Programme for monitoring effects of contaminated sediments. Besides malformed embryos, other deviations from normal development have been observed, i.e. unfertilised/undeveloped and dead eggs and embryos, and females with dead broods in their marsupium (brood chamber) (Sundelin & Eriksson 1998, Andersson et al. 1999). However, these variables were not correlated with pollution gradients in industrial recipients (Sundelin & Eriksson 1998).

The occurrence of dead eggs and females carrying dead broods is more common in the Baltic proper, than in Bothnian Bay (Andersson et al. 2000). Due to higher eutrophication level, hypoxia and hydrogen sulphides occur more frequently in the Baltic proper sediments than in those of the Bothnian Sea (Andersson et al. 2000, Kajrup 2000). Thus, we hypothesise that dead eggs and females carrying a dead brood result mainly from hypoxia and not from contaminants. We also have indications that unfertilised/undeveloped eggs are more common following autumns with higher water temperatures (unpubl. data). In a series of experiments, we examined the effect of natural environmental variables such as temperature and oxygen on different reproductive processes. The aim was to: (1) de-velop tools for monitoring the effects of natural environmental as well as climate variables, and (2) evaluate the possibility of differentiating anomalies caused by stressors such as hypoxia from those caused by contaminants.

MATERIAL AND METHODS

Sampling and experimental set-up. Experiments were performed in the flow-through microcosm system described by Sundelin (1983). Briefly, the microcosm system consists of 2 l Ehrlenmeyer flasks (bottom area 227 cm^2). The water inlet is placed below the outlet to produce a natural flow of water and to minimise gradients. The flasks were wrapped in green plastic to simulate the wavelength *in situ*, and a timer regulated light to natural day-length. The incoming water from 37 m depth (salinity 7‰) was filtered through a 280 µm net to avoid predator larvae, and followed the natural seasonal regime (Fig.1). The amphipods were prevented from surface contact by fine nets (mesh size 0.25 mm) below the water surface. The temperature *in situ* has been yearly monitored since the early 1980s. Variation at this depth is very small between years, and follows the same pattern every year. Between 1992 and 1999 the average temperature in the area at 40 m depth in September has been 5.7 ± SD 1.2°C and in October 5.8 ± SD 1.4°C (SHARK database: Swedish Meteorological and Hydrological Institute, Västra Frölunda).

The sediment and amphipods *Monoporeia affinis* and *Pontoporeia femorata* used in the experiments were collected with a benthic sled (Blomqvist & Lundgren 1996) at a reference site near the Askö Laboratory, a Stockholm University field station in the northern Baltic proper. At this site, background levels of both metals and organic contaminants in sediment and amphipods have been recorded (Sundelin & Eriksson 2001). Amphipods were carefully collected after sieving the sediment through a 1.0 mm net, and immediately transferred to containers with cool water (4°C) for further transport to the laboratory. The amphipods were sorted according to species and year-classes (separated by size). To exclude larvae of amphipod predators the sediment was sieved through a 0.5 mm net.

Biological variables. The adults and embryos were examined in regard to different aberrations (Table 1) from normal development according to Sundelin & Eriksson (1998). Variables studied for females were fertilisation success, fe-
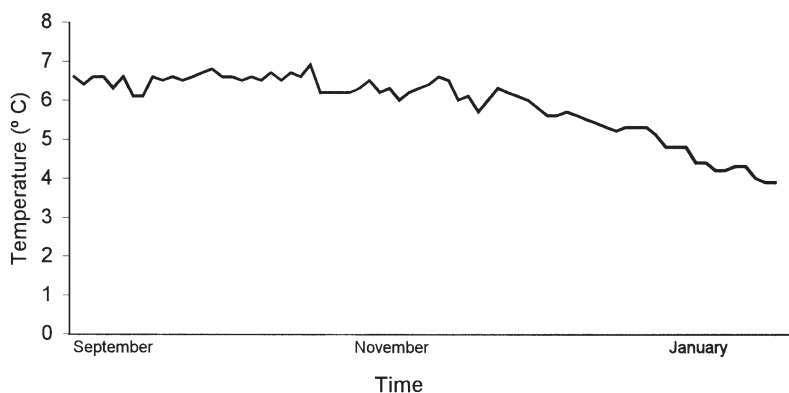

Fig. 1. Temperature of incoming water in flow-through microcosm used in experiments

Table 1. *Monoporeia affinis* and *Pontoporeia femorata*. Female reproductive variables and embryo variables

Variable	Explanation
Fecundity	Eggs per female
Fertilised females	Females carrying fertilised eggs in brood pouch
Females with dead brood	Females, carrying fat residue in brood pouch consisting of decomposing, dead, and unfertilised eggs. Residue is homogenous cell mass, in which single eggs are no longer identifiable
Developmental stage	Embryo development, divided into 9 arbitrary stages, from newly fertilised egg to hatched juvenile
Malformed egg	Fertilised egg showing different types of aberrations
Unfertilised/ undeveloped egg	Egg that is unfertilised or has ceased to develop before gastrulation
Dead egg	Dead egg that has not decayed and is thereby still countable

cundity (eggs/female), and dead brood. Developmental stage and percentage of malformed embryos, unfertilised/undeveloped eggs, dead eggs and dead embryos were recorded (for further details see Sundelin & Eriksson 1998).

Temperature experiments (Expts 1 and 2). Two experiments were carried out to examine the effect of increased temperature on reproduction. To each aquarium we added 400 ml of sediment (water content 70 %) and 50 sub-adult amphipods (1+ yr). A sex ratio of 1:1 was expected (Segerstråle 1937); this has been checked regularly by the authors over several years (1985 to 2000, unpubl. data). To increase the water temperature, test aquaria were supplied with water from tanks equipped with a heater and a thermostat; temperatures in the aquaria and tanks were checked daily. Expt 1 started in early September, during the period of gonad maturation, and was terminated in November, when mating occurs. Expt 2 started at the same time in September, but was terminated in January when embryogenesis has lasted for about 2 mo. Expt 1 was performed on *Monoporeia affinis*, while Expt 2 included both *M. affinis* and *Pontoporeia femorata*.

Expt 1 included 10 replicates for each of 3 experimental conditions: (1) control (temperature of incoming water unchanged), and temperature raised by (2) 3.2°C and (3) 5.4°C. After termination of the experiment in November, the number of fertilised and unfertilised females in each microcosm was counted.

Expt 2 comprised 3 series: (1) control, in which incoming water followed the natural temperature regime; (2) temperature of incoming water raised during gonad maturation, before mating; (3) temperature

raised after the mating period, i.e. during embryogenesis. The temperature was increased by 3.2 ± 0.2°C. Each series included 5 replicates.

Oxygen experiment (Expt 3). Decreased oxygen concentrations were tested in combination with contaminated sediments, resulting in 4 series: (1) control, high oxygen concentration; (2) low oxygen concentration; (3) low oxygen concentration in combination with contaminated sediment; (4) high oxygen concentration in combination with contaminated sediment. To each aquarium we added 400 ml of sediment (water content 70 %) and 50 sub-adult amphipods (1+ yr), and 5 replicates of each series were included. The experiment was only performed on *Monoporeia affinis*, due to low abundance of *Pontoporeia femorata* in the field, which caused collecting difficulties. The experiment started in September and ended in January.

Low oxygen concentrations were generated by lowering the incoming water-flow to the microcosms from 50 ml min^{-1} in controls to 8 ml min^{-1} in the experiments. The original oxygen concentration (8 mg O_2 l^{-1}) was gradually reduced over 2 wk by lowering the water flow. The average oxygen concentration in the low oxygen incubation series was 3.6 ± 1.3 (SD) mg O_2 l^{-1}, ranging from 2 to 4.5 mg O_2 l^{-1} (2 to 6 mg O_2 l^{-1} when the first weeks of acclimation are included). Oxygen concentration in the high-oxygen series ranged from 7.5 to 11 mg O_2 l^{-1} with an average of $9.7 \pm$ SD 1.4 mg O_2 l^{-1}. This procedure was an attempt to mimic the natural variation *in situ*. The oxygen concentration was measured daily by a portable oxygen meter (WTW Oxi 323-B, Welheim, Germany) according to SS-EN 25813 (SIS 1993a) and SS-EN 25814 (SIS 1993b). In Series 3 and 4, 150 ml contaminated sediment was added to 250 ml control sediment, resulting in the same sediment volume as in the non-contaminant series. Contaminated sediment formed the surface sediment layer, where amphipods feed. This sediment was collected from Stockholm harbour and was contaminated by trace metals and persistent hydrophobic contaminants, e.g. polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) (for details see Sundelin & Eriksson 2001). Toxicity of the settling sediment material and sediment at sampling site has been shown by Ishaq et al. 1999 and Sundelin & Eriksson 2001).

Statistical evaluation. Data from Expts 1 and 2 were evaluated by a 2-way analysis of variance, with temperature and species as factors. Data from Expt 3 were analysed by analyses of variance followed by a Dunnett post hoc procedure. The analyses were based on mean values per replicate with a logistic transformation, $\log_{10}[x/(1-x)]$, applied to the data prior to the analyses. Since low fertilisation in the oxygen experiment resulted in loss of replicates for the embryo
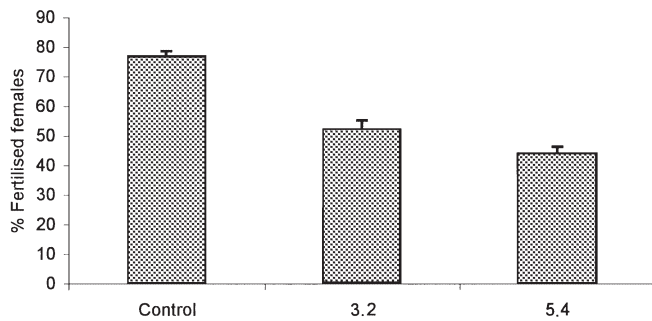
Fig. 2. *Monoporeia affinis.* Expt 1. Percent fertilised females exposed before mating to 3 temperatures: control (temperature unchanged), temperature increased by 3.2°C and temperature increased by 5.4°C. Data are based on mean values of 10 replicates and error bars represent 95% confidence interval

analyses, calculations were based on total number of eggs in each series, and differences between proportions were compared according to Zar (1984, p. 400–404). For multiple comparisons between control and treatments we used a Dunnett-type post hoc procedure with arcsine-transformed proportions (Zar 1984). In all statistical evaluations, the conventional significance level of α = 0.05 was applied.

RESULTS

Temperature experiments (Expts 1 and 2)

In Expt 1, the proportion of fertilised females *Monoporeia affinis* decreased significantly with increasing temperature prior to mating (Fig. 2).

Table 2. *Monoporeia affinis* and *Pontoporeia femorata.* Expt 2. Statistical parameters of 2-way ANOVAs. Tests were performed for differences between species (factor = species), for an effect of temperature across both species (factor = temperature) and for a difference in the effect of temperature between the 2 species (factor = species × temperature). In addition, tests were designed to compare the effect of temperature specifically for *Monoporeia affinis* and for these comparisons the magnitude of the effect was also estimated (Est.d.) In *Pontoporeia femorata* large inter-replicate variation, due to low fertilisation success, made the corresponding species-specific comparisons uninformative. ns = not significant

Dependent variable	Factor	F	Est.d	p
Fertilised females (%)	Species	83.14		<0.001
	Temperature	6.37		0.004
	Interaction Species × Temperature	0.15		ns
	Control—before		0.55	0.006
	Control—after		−0.12	ns
Fecundity	Species	37.45		<0.001
	Temperature	32.27		<0.001
	Interaction Species × Temperature	6.48		0.006
	Control—before		22.31	<0.001
	Control—after		9.08	0.003
Developmental stage	Species	10.87		0.004
	Temperature	1.47		ns
	Interaction Species × Temperature	0.36		ns
	Control—before		−0.27	ns
	Control—after		−0.62	0.02
Dead broods (%)	Species	26.43		<0.001
	Temperature	4.75		0.02
	Interaction Species × Temperature	4.88		0.02
	Control—before		−0.79	0.02
	Control—after		−0.26	ns
Dead eggs (%)	Species	0.002		ns
	Temperature	2.62		ns
	Interaction Species × Temperature	1.25		ns
	Control—before		−1.22	0.001
	Control—after		−0.43	ns
Unfertilised/undeveloped (%)	Species	4.41		ns
	Temperature	1.18		ns
	Interaction Species × Temperature	0.47		ns
	Control—before		−0.88	0.04
	Control—after		−0.58	ns
Malformed (%)	Species	21.52		<0.001
	Temperature	2.89		ns
	Interaction Species × Temperature	1.97		ns
	Control—before		−0.27	ns
	Control—after		−0.29	ns

In Expt 2, the rate of fertilised females also decreased when exposed to increased temperature prior to mating, but to a lesser extent than in Expt 1 (Table 2, Fig 3A). The effect was similar in both species, although statistically significant only for *Monoporeia affinis*. Examination of the unfertilised female *Pontoporeia femorata* revealed a considerable difference in gonad development between specimens. We found females with both mature degenerated gonads and females with small immature gonads in which development was delayed by about 2 mo; this occurred irrespective of temperature treatment and reduced the number of fertilised *P. femorata* females available for examination.

Increased pre-mating temperature caused a substantial reduction in fecundity in both species, whereas increased post-mating temperature caused but a moderate reduction (Table 2, Fig. 3B). Similar results were

obtained whether females with dead broods were included in or excluded from the fecundity calculations. Exposure to raised post-mating temperature resulted in a significantly higher stage of embryo development in *Monoporeia affinis* (Table 2, Fig. 3C). Pre-mating exposure to increased temperature did not affect embryonic development in either species. Regardless of treatment, fecundity was higher and embryos further developed in *M. affinis* than in *Pontoporeia femorata* (Table 2, Fig. 3C).

The proportion of females carrying a dead brood was higher after exposure to increased temperature than in controls (Table 2, Fig. 3D). The effect was statistically significant for pre-mating exposure in *Monoporeia affinis* (Table 2).

The percentage of dead and undeveloped/unfertilised eggs in *Monoporeia affinis* was higher when temperature was increased before mating (Table 2,
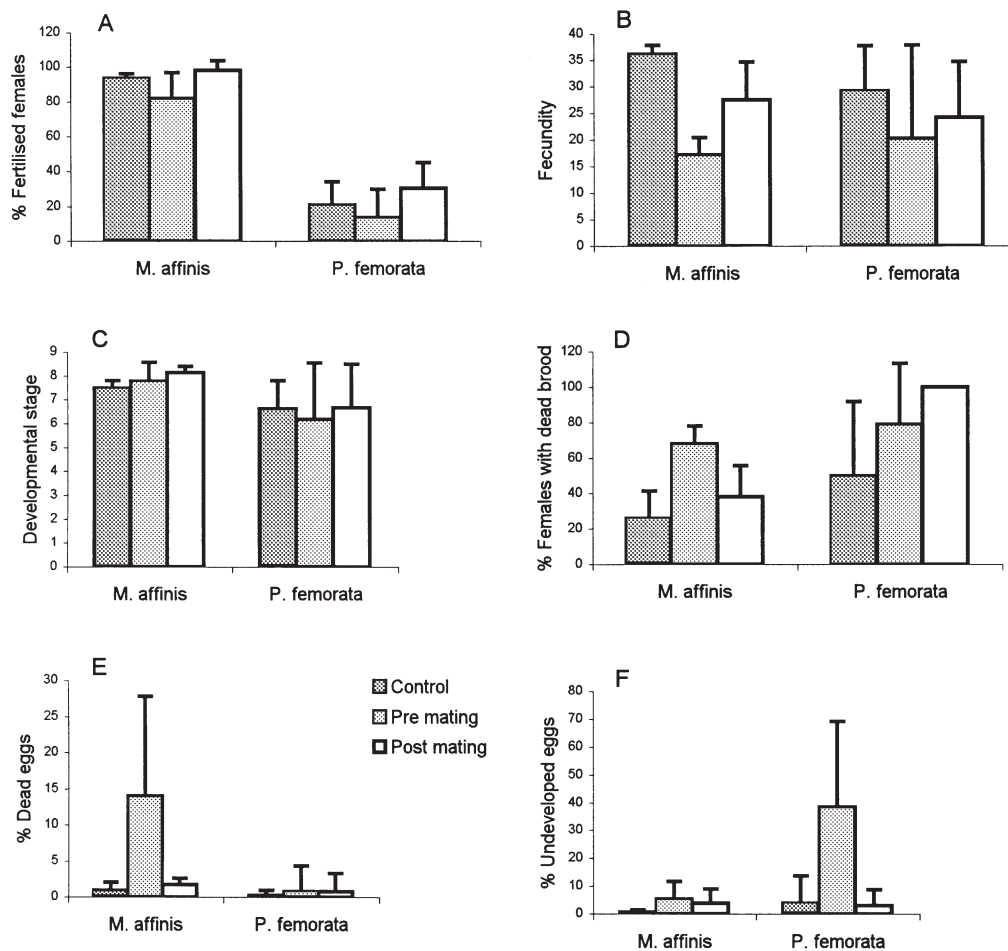

Fig. 3. *Monoporeia affinis* and *Pontoporeia femorata*. Expt 2. Amphipods exposed to increased (by 3.2°C) temperature before or after mating. (A) Fertilised females; (B) fecundity (egg/female); (C) embryo developmental stage; (D) % females with dead brood; (E) % dead eggs; (F) % unfertilised/undeveloped eggs. Data are based on mean values of 5 replicates and error bars represent 95 % confidence interval

Table 3. *Monoporeia affinis:* Expt 3. Statistical parameters of 1-way ANOVAs and Dunnett-type post hoc procedure. low ox.: low oxygen; cont. sed.: contaminated sediment. Other abbreviations as in Table 2. Insufficient numbers of *Pontoporeia femorata* were available for analysis because of low abundance in the field

| Dependent variable	Factor	*F*	Est.d	p
Fertilised females (n)	Treatment	15.49		<0.001
	Control—low oxygen		7.20	<0.001
	Control—contaminated sediment		6.60	<0.001
	Control—low ox. + cont. sed.		5.80	<0.001
Unfertilised/undeveloped	Treatment	6.85		0.007
females (%)	Control—low oxygen		−2.14	0.003
	Control—contaminated sediment		−1.44	ns
	Control—low ox. + cont. sed.		−0.79	ns
Dead brood (%)	Treatment	7.57		0.004
	Control—low oxygen		−1.31	0.007
	Control—contaminated sediment		0.16	ns
	Control—low ox. + cont. sed.		−0.82	0.046
Fecundity	Treatment	6.42		0.013
	Control—low oxygen		2.82	ns
	Control—contaminated sediment		−3.11	ns
	Control—low ox. + cont. sed.		−11.09	0.02
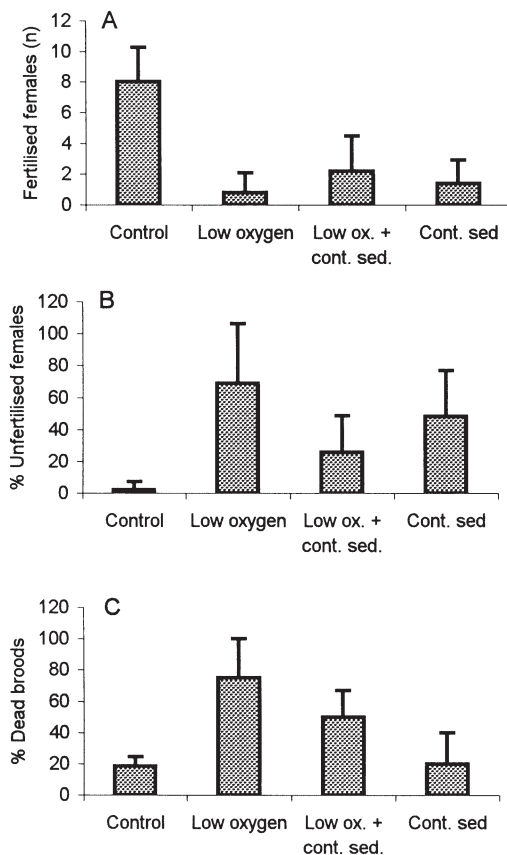
Fig. 4. *Monoporeia affinis.* Expt 3. Amphipods exposed to moderate hypoxia solely and in combination with contaminated sediment during the reproduction cycle (September to January). (A) Number of fertilised females; (B) % unfertilised females; (C) % females with dead brood. Data are based on mean values of 5 replicates and error bars represent SD

Fig. 3E,F). Due to large inter-replicate variation in series with *Pontoporeia femorata*, the increased percentage of unfertilised/undeveloped eggs was not statistically significant in this species. Finally, increased temperature did not increase the percentage of malformed embryos in either species, although malformations were more frequently in *P. femorata* (3.4 to 9.4%) than in for *M. affinis* (1.9 to 3.8%) (Table 2).

Oxygen experiment (Expt 3)

The number of fertilised female *Monoporeia affinis* was severely reduced in all treatments compared to control (Table 3, Fig. 4A) and the percentage of unfertilised females was higher (Table 3, Fig. 4B). Furthermore, the number of females with dead broods increased when exposed to reduced oxygen levels during the periods of gonad maturation and embryogenesis. Exposure to contaminated sediment did not further increase this aberration (Table 3, Fig. 4C). Exposure to hypoxia did not affect fecundity, while low oxygen concentrations in combination with contaminated sediment decreased fecundity (Table 3).

Due to a low fertilisation rate, there was only a limited amount of data available for analyses of different embryo aberrations. Nevertheless, the proportion of both malformed and unfertilised/undeveloped eggs was shown to increase compared to controls (p < 0.001) (Table 4). The degree of decay among the dead and dying eggs varied considerably, resulting in difficulties in counting the dead eggs. We therefore omitted this variable from the analyses.

Table 4. *Monoporeia affinis.* Expt 3. Results and statistical parameters of multiple comparisons (Dunnett-type post hoc procedure), of the variables malformed and unfertilised/undeveloped eggs. In both cases, main effects resulted in $p < 0.001$ after a comparison between proportions (Zar 1984). –: comparison uniformative because mean proportion lower than control

Parameter	Control	Low O_2 conc.	Low O_2 conc. + contaminated sediment	Contaminated sediment
Malformed eggs (%)	2.8	4.7	8.6	7.8
Transformed proportions	9.72	12.91	17.16	16.33
SE		1.34	0.99	0.98
Critical $q_{0.05,\infty,4}$		2.35	2.35	2.35
Actual q		2.37	7.44	6.76
Unfertilised/undeveloped eggs (%)	1.78	5.69	5.80	0.42
Transformed proportions	7.6	14.17	14.06	4.31
SE		1.34	0.99	0.98
Critical $q_{0.05,\infty,4}$		2.35	2.35	2.35
Actual q		4.90	6.52	–

DISCUSSION

Temperature experiments (Expts 1 and 2)

Monoporeia affinis is a stenotherm, cold-water glacial relict with a previously reported temperature tolerance of 11°C in long-term laboratory experiments (Smith 1972) and 14.5°C in field observations (Ekman 1915). Segerstråle (1937, 1978) reported a much higher temperature tolerance of 22°C in short-term experiments and field observations, while earlier work based on field observations reported that reproduction was already inhibited at 7°C (Samter & Weltner 1904).

Effect of increased temperature on reproduction

In our study, a temperature increase of 3 to 5°C did not inhibit, but clearly affected reproduction in *Monoporeia affinis*. Fertilisation success was affected to a lesser extent in Expt 2, where females were examined in January, than in Expt 1, where females were examined in November. This indicates that mating could be delayed rather than inhibited by increased temperature and that it takes place when the water temperature subsequently falls. Furthermore, there are indications of higher sensitivity during the period of gonad maturation than in the period of embryogenesis. The percentages of dead eggs and females carrying dead broods were higher after pre-mating exposure, while no significant effects were recorded after exposure during embryogenesis.

The correlation between pre-mating exposure and reproductive disorders in terms of unfertilised females and dead broods could be explained by the high temperature-sensitivity of male sperm. In Atlantic salmon *Salmo salar*, sperm mobility (which is considered to reflect fertilisation ability: Aas et al. 1991), was already affected at temperatures above 4°C (Vladic & Järvi 1997). The temperature sensitivity of male sperm is highly relevant for amphipods, since particularly adult males migrate vertically in the water column during gonad development and mating. This period coincides with the maximum water temperatures in late summer and early autumn (Segerstråle 1937, Donner et al. 1987). At the sampling site the temperature at 30 m depth and above shows great variation over the year and as late as October temperatures of 11.8°C have been registered (SHARK database).

Differences between *Monoporeia affinis* and *Pontoporeia femorata*

Dead eggs occurred more frequently in *Monoporeia affinis* than in *Pontoporeia femorata*: the majority of these eggs were undeveloped or unfertilised. *P. femorata* on the other hand had tended towards a higher proportion of undeveloped or unfertilised eggs. We suggest however that both species were affected in a similar way, the difference being that the dead eggs of *M. affinis* were at a later stage in the process of degeneration than the merely unfertilised/undeveloped eggs of *P. femorata*. The developmental stages of *P. femorata* embryos was also found to be lower than those of *M. affinis.*

The percentage of malformed embryos was on a par with Baltic background levels, which are 2 to 4% for *Monoporeia affinis* and 5 to 10% for *Pontoporeia femorata* (Andersson et al. 1999). The reason for the higher malformation rate in *P. femorata* is unclear, but as a marine species living in the Baltic, on the border of its salinity tolerance, its resistance to various stressors may be lowered. Results contradicting our salinity hypothesis were, however, obtained by Johansson

(1997a), who reported that increased salinity did not increase tolerance to hypoxia in *P. femorata*.

The greatest dissimilarity between species was the large variation in gonad maturation observed in *Pontoporeia femorata* regardless of treatment. A large proportion of *P. femorata* specimens deviated from the normal seasonal reproduction cycle. As the reproduction cycle is triggered by the reduction of light after midsummer (Segerstråle 1971), amphipods living at depths where no perception of light is possible sometimes have a more dispersed reproduction period. However, both species in this experiment were collected at the same location at 45 to 50 m depth, a depth that should not influence the normal reproduction period. *P. femorata* in abnormal reproductive stages have also been observed in the field (Sundelin et al. 2000). We have no explanation for the dispersed reproduction observed for *P. femorata*. Normally, embryos are hatched in February to March at the time of the spring bloom; hatching in the 'wrong' season could result in juveniles not receiving sufficient and/or high-quality food.

The observed difference in fecundity between these 2 species is in accordance with data from the monitoring programme in the Baltic, where the fecundity of *Pontoporeia femorata* is lower than that reported for the north-western Atlantic (Steele & Steele 1978). The reason for this fecundity difference is unclear. Dwelling on the border of its salinity tolerance might be the explanation not only of the high malformation rate but also of the lower fertility rate in *P. femorata*.

Oxygen experiment (Expt 3)

Most studies concerning benthos and hypoxia focus on lethal oxygen concentrations (Sprague 1963, Gamble 1970, Johansson 1997a). Fewer studies have dealt with sublethal concentrations (Hagerman & Szaniawska 1986, Breitburg et al. 1994), and even fewer with the sublethal effects of hypoxia on reproduction (Breitburg 1992).

The oxygen concentrations in this experiment were not constant, reflecting the situation *in situ*. Oxygen concentration varied between 2.5 and 6.6 ml O_2 l^{-1} during September and October 1992–1999 (SHARK database). The oxygen measurements were carried out at 40 m depth at a station included in the Swedish National Monitoring Programme and situated close to the sampling site of this experiment.

Effects of moderate hypoxia on broods

The time spent on active brooding increases when oxygen conditions are impaired or when temperature increases. Brooding also increases after gastrulation and decreases at the end of embryogenesis (Dick et al. 1998). In our experiment, low oxygen caused an increased number of dead broods. However, despite increased efforts by the females to oxygenate their broods (i.e. increasing oxygenation by movments of their pleiopods) repeated and/or extended periods of hypoxia may diminish their ability to do this as actively as necessary. This implies that during sensitive parts of the life cycle, temporary hypoxia, which does not affect the adult amphipod, could be lethal do the embryo. Embryonic and larval stages are more sensitive to environmental stressors such as fluctuating hypoxia, temperature and salinity than juveniles (Baker & Mann 1994, Qiu & Qian 1998).

Effects of hypoxia on mating and fertilisation

In the oxygen experiment, the number of fertilised females was lower and the rate of unfertilised females higher in the treatment than in the control group, irrespective of whether solely hypoxia or hypoxia combined with contaminants was tested. Multiple stressors can act either synergistically or antagonistically. The effect of multiple stressors may be other than the sum or product of the individual effect of single factors (Folt et al. 1999). A possible synergistic effect was observed for fecundity in our experiment, as the combination of contaminated sediment and low oxygen reduced fecundity, while none of these factors by itself had any significant effect.

The proportion of malformed eggs was higher in the contaminated sediment series, which agrees with results of Sundelin & Eriksson (1998). The proportion of unfertilised/undeveloped eggs was higher in the series exposed to moderate hypoxia. The lower number of fertilised females, and possibly also the higher proportion of unfertilised eggs could partly be due to low survival rates of males and possibly also to death of fertilised females. A higher sensitivity to hypoxia of males and egg-bearing females has been observed (Vopel et al. 1998). Moreover, the swimming activity of *Monopreia affinis* and *Pontoporeia femorata*, which is strongly connected to the mating process, has been reported to be significantly affected at 3.9 mg O_2 l^{-1} (Johansson 1997b). Non-reproduction has also been observed to be correlated with hypoxia (Trippel & Harvey 1988).

The variable oxygen situation at coastal stations in the Baltic where hypoxia frequently occurs (Elmgren 1989, Andersson et al. 2000) is stressful for the benthic species. The sensitivity to low oxygen concentrations of *Monoporeia affinis* is subject to debate; this species has been classified as moderately tolerant to hypoxia

by Sandberg-Kilpi et al. (1999), while Modig & Òlafsson (1998) consider it very sensitive. *Pontoporeia femorata* was reported by Johansson (1997a) to be less tolerant to hypoxia than *M. affinis*. However, when the 2 species co-occur, *P. femorata* is found deeper in the sediment (Hill & Elmgren 1987), where the oxygen concentration is generally lower. Lower respiration rates have also been reported for *P. femorata* (Cederwall 1979), and might be the explanation of the deeper burrowing of this species. It is therefore difficult to make any statement about the sensitivity to hypoxia of *P. femorata* reproduction.

Bioenergetic considerations

Temperature

Monoporeia affinis (Q_{10} = 1.7 to 2.0; Lehtonen & Andersin 1998) subjected to increased temperature prior to mating could be at a bioenergetic disadvantage. A rise in temperature of 3.2°C would increase the oxygen consumption of this amphipod by approximately 20 to 25% (Schmidt-Nielsen 1997, p. 218–221), and likewise increase its metabolism. The amphipods used in our experiments store energy in the form of lipids from spring to early autumn. The lipid content of Baltic adult amphipods mainly range from 20 to 40%, depending on season and location (Hill et al. 1992, Lehtonen 1995, 1996). A sufficient amount of stored lipids in relation to size is one of the signals triggering the onset of reproduction (Lehtonen 1996). During autumn and winter, lipids will be relocated to gonads and consumed during reproduction. A rise in temperature increases energy cost and a smaller amount of lipids are available for gonad maturation. Lipid storage in *Mysis relicta*, for instance, occurs only in colder water (Chess & Stanford 1999). We argue, therefore, that the reduction in fecundity for females exposed to increased pre-mating temperature in this study is likely to have been a result of increased lipid consumption during gonad development and a subsequent impaired capability to produce eggs.

Hypoxia

Hypoxia could also increase energetic costs due to a higher respiration rate (Wannamaker & Rice 2000). In our study the low oxygen concentrations did not cause any considerable effect on fecundity, although hypoxia in combination with contaminants decreased fecundity. Fecundity is also affected by other reproduction disorders. The effects on fecundity caused by dead broods could for instance be difficult to discriminate from possible effects of increased energetic cost on gonad development due to higher lipid consumption.

Field and monitoring implications

Malformed embryos have proved to be a sensitive biomarker in toxicity tests as well as in field studies of industrial recipients, while females carrying dead broods and dead or unfertilised/undeveloped eggs have shown no correlation with contaminant exposure (Sundelin & Eriksson 1998). The results of the current experiments indicate that the anomalies observed in field-collected amphipods could be explained by hypoxia or increased temperature during critical periods of the reproduction cycle. In the Baltic, gonad maturation occurs during early autumn, a period when moderate hypoxia frequently occurs and elevated temperature occasionally. This could explain our findings.

Our results demonstrate the possibility of separating anomalies caused by contaminants from other types of interference in the field. Most biomarkers for effect-monitoring of contaminants also respond to abiotic variables that vary naturally in the Baltic (e.g. hypoxia and elevated water temperatures). Little effort has been made in studies to examine the sensitivity of biomarkers to these physico-chemical variables, and to separate the effects of contaminants from the effects of other types of stresses. Optimal biomarkers for monitoring contaminants in the field should respond to toxicants but not (or at least only to a lesser extent) to other types of stress. Davis (1993) underlined the urgent need for sensitive early-warning biomarkers with low response to natural climate variables in effect-monitoring of the benthic ecosystem.

In conclusion, for amphipods in the Baltic Sea the most suitable biomarker for effects of increased water temperatures and hypoxia in bottom water and sediments seems to be females carrying dead broods. The proportions of dead or unfertilised/undeveloped eggs are also potential variables in non-contaminant monitoring, although further studies are needed to corroborate these findings.

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