Vol. 175: 155–166, 1998 | MARINE ECOLOGY PROGRESS SERIES Mar Ecol Prog Ser | Published December 17

Ecophysiological differentiation of *Capitella capitata* (Polychaeta). Sibling species from different sulfidic habitats

I. Gamenick[1,4,*], B. Vismann[2], M. K. Grieshaber[3], O. Giere[4]

[1]Department of Life Sciences and Chemistry, Roskilde University, Postbox 260, DK-4000 Roskilde, Denmark
[2]Marine Biological Laboratory, University of Copenhagen, Strandpromenaden 5, DK-3000 Helsingør, Denmark
[3]Institut für Zoophysiologie, Heinrich-Heine-Universität, Universitätsstraße 1/26.12, D-40225 Düsseldorf, Germany
[4]Zoologisches Institut und Zoologisches Museum, Universität Hamburg, Martin-Luther-King Platz 3, D-20146 Hamburg, Germany

ABSTRACT: The ecophysiological differences of 4 sibling species of the *Capitella capitata* species complex from habitats with different sulfide concentrations were studied: *Capitella* sp. S (small) from a North Sea intertidal flat, living in the upper sediment layer containing low sulfide concentrations (up to 20 µmol l^{-1}); *Capitella* sp. L (large), sympatric to *Capitella* sp. S, but living in deeper sediment layers with high sulfide concentrations (up to 350 µmol l^{-1}); *Capitella* sp. M from the Mediterranean Sea from highly sulfidic sediments (up to 710 µmol l^{-1}) close to shallow hydrothermal vents; and *Capitella* sp. I from eutrophicated coastal areas of the North Atlantic. *Capitella* sp. L, *Capitella* sp. M and *Capitella* sp. I are significantly more tolerant to anoxia and 760 µmol l^{-1} sulfide than *Capitella* sp. S from the upper North Sea sediments. Respiration rates showed that only *Capitella* sp. S can be characterized as an oxyconformer. The oxygen consumption of *Capitella* sp. S becomes successively reduced with declining ambient oxygen tensions. The 3 other sibling species are all oxyregulators with different regulation abilities. At moderate oxygen concentrations the aerobic metabolism of *Capitella* sp. S is inhibited at low sulfide levels (30 µmol l^{-1}). Conversely, at moderate oxygen levels the anaerobic metabolism of *Capitella* sp. S is increased at 20 µmol l^{-1} sulfide. In contrast, even at sulfide concentrations of 130 µmol l^{-1}, the aerobic metabolism of *Capitella* sp. L is not affected. The anaerobic metabolism of *Capitella* sp. L is not increased at sulfide concentrations <100 µmol l^{-1}. The anaerobic metabolism of *Capitella* sp. M from the hydrothermal vents is not affected even at higher sulfide concentrations. This study shows that sibling species of the *C. capitata* complex from different habitats can be differentiated by their ecophysiological characteristics.

KEY WORDS: *Capitella capitata* · Sibling species · Sulfide · Anoxia · Tolerance · Oxygen consumption · Anaerobic metabolism

INTRODUCTION

The cosmopolitan endobenthic polychaete *Capitella capitata* (Fabricius) is known as an indicator for organically polluted and disturbed marine environments. *C. capitata* dominates the macrobenthic community in areas which are highly eutrophicated by paper mills (Pearson & Rosenberg 1978), fish farming (Tsutsumi 1987, Tsutsumi et al. 1990), waste discharge (Chang et al. 1992) or oil spills (Grassle & Grassle 1974, Sanders et al. 1980).

The population density, reproduction and growth rate of *Capitella capitata* are strongly correlated with the content of organic material (Tenore 1977, Warren 1977, Chesney & Tenore 1985, Tenore & Chesney 1985, Tsutsumi 1987, Grémare et al. 1988, Qian & Chia 1991, Bridges et al. 1994). The sediments of eutrophicated areas are often anoxic and highly sulfidic due to bacterial degradation of the organic material. Hitherto the dominance of *C. capitata* under such conditions has mainly been explained by its ability to sustain increased mortality rates by continuous reproduction (e.g. Grassle & Grassle 1974, Pearson & Rosenberg 1978, Gray 1984). Recently Levin et al. (1996) proposed

*E-mail: gamenick@virgil.ruc.dk

possible demographic mechanisms (e.g. reduced maturation time) which would explain *C. capitata*'s propensity to dominate organically enriched sediments. Little is known, however, about possible ecophysiological adaptations of *C. capitata* (Gamenick & Giere 1994, Gamenick et al. 1998) to 2 of the key factors in the benthic environment (Vismann 1991, Giere 1992), oxygen depletion and sulfide accumulation. In many other polychaetes ecophysiological adaptations to hypoxic and sulfidic conditions have been described. Their major adaptation strategies are a high capacity for long-term anaerobiosis (Theede et al. 1969, Theede 1973) and/or the ability to oxidize (= detoxify) sulfide to thiosulfate (Vismann 1991, Bagarinao 1992, Grieshaber & Völkel 1998).

Capitella capitata consists of a complex of several sibling species which show minor differences in adult morphology, but differ markedly in their reproduction modes and enzyme patterns (Grassle & Grassle 1976). Moreover, Eckelbarger & Grassle (1983, 1987) described differences in the ultrastructure of eggs and follicle cells, in genital setae, sperm and larval morphology between sympatric sibling species from the North American East Coast. Several *C. capitata* populations, which differ in enzyme patterns (Wu et al. 1991) and reproduction modes (Zhang & Wu 1988, Pearson & Pearson 1991) as well as in tolerance to hypoxia and sulfide and standard respiration rates (Gamenick & Giere 1994), have been described from China, Great Britain and Germany.

In this study, 4 sibling species of *Capitella capitata* from 3 geographic regions with different sulfide regimes are studied. Former studies revealed that they do not cross breed and are distinguishable by total protein pattern analysis (Gamenick et al. 1998). Two sibling species inhabit the intertidal zone of the North Sea near the island of Sylt, Germany: the small *C. capitata* Type S (hereafter termed *Capitella* sp. S) and the large *C. capitata* Type L (hereafter termed *Capitella* sp. L) (Gamenick & Giere 1994). The third sibling species is *Capitella* sp. M from the Mediterranean Sea near the Island of Milos, where it lives in highly sulfidic sediments close to shallow water hydrothermal vents (Thiermann et al. 1997). It is characterized by high survival rates under anoxic and sulfidic conditions (Gamenick et al. 1998). The fourth sibling species is the well-known *Capitella* sp. I from eutrophicated sediments of the US East Coast (Grassle & Grassle 1974, 1976), which dominates sulfidic sediments near sewage outfalls (J. P. Grassle pers. comm.).

The sibling species were *in situ* exposed to different oxic and sulfidic conditions and we addressed the following questions: Do the *Capitella* sibling species have different survival rates under anoxia and sulfide exposure? How do hypoxia, anoxia and sulfide affect their aerobic and anaerobic metabolism? Are there genetically fixed ecophysiological differences between these *Capitella* sibling species?

To answer these questions, tolerance to hypoxia and sulfide and respiration rates were measured in laboratory-cultured worms under different oxygen and sulfide concentrations. In addition succinate (as an indicator for anaerobic metabolism; Grieshaber et al. 1988), sulfide and thiosulfate concentrations in the worms tissue were analyzed after exposure to different oxygen and sulfide levels.

MATERIAL AND METHOD

Field. The depth distribution of the 2 sympatric *Capitella* sp. S and *Capitella* sp. L was measured in sediment cores taken with plexiglass tubes (inner diameter 5 cm, n = 3) and subdivided into 0–3, 3–6 and 6–9 cm horizons. Samples were fixed in buffered formaldehyde (final concentration 5 to 10 vol. %), sieved (250 µm mesh size) and the worms counted. Pore water sulfide concentrations were analyzed photometrically using the colorimetric method of Gilboa-Garber (1971), modified by Howarth et al. (1983). The term sulfide refers here to total dissolved sulfide, i.e. undissociated H_2S, dissociated HS^- and S^{2-}.

Laboratory. All experiments were conducted on laboratory-cultured worms. Stock cultures of *Capitella* sp. S, *Capitella* sp. L, *Capitella* sp. M and *Capitella* sp. I were reared in aerated glass tanks with sediment (no detectable sulfide) and 32 to 35‰ artificial seawater at 18°C. Worms were fed once a week with commercial fish food mixed with dried green algae.

Tolerance experiments. *Capitella* sp. S, *Capitella* sp. L and *Capitella* sp. I were exposed to 4 treatments (for further descriptions see Gamenick et al. 1998) in artificial seawater (32 to 35‰ S, for pH stability buffered with 10 mM HEPES, without sediment) at 16°C: (1) normoxia (control), aerated continuously; (2) anoxia, percolated with pure nitrogen prior to inserting the worms; (3) anoxia plus 160 µM sulfide; and (4) anoxia plus 760 µM sulfide, bubbled with nitrogen with subsequent addition of a sulfide stock solution (made from $Na_2S \cdot 9H_2O$ crystals).

Oxygen concentrations were measured with a sulfide-insensitive polarographic oxygen sensor (POS) from Orbisphere (Geneva, Switzerland); pH was measured with an Ingold electrode (Steinbach/Taunus, Germany) and sulfide concentrations were analyzed colorimetrically as described above. Experimental conditions of the sulfide treatments are given in Table 1.

The term 'normoxia' is defined as 100% air saturation (oxygen partial pressure, pO_2 = 21 kPa); the term 'anoxia' is used when oxygen concentration is below the POS detection limit (= 0.08 kPa).

Table 1. Experimental conditions of the tolerance tests. Sulfide and pH values were measured at the beginning and at the end of each experiment (n = 6 to 8)

Capitella	Worms/ treatment	Number of experiments	Anoxia + 160 µM sulfide		Anoxia + 760 µM sulfide	
			Sulfide (µmol l^{-1})	pH value	Sulfide (µmol l^{-1})	pH value
sp. S	6	4	150 ± 30	8.13 ± 0.3	760 ± 46	8.31 ± 0.3
sp. L	4	4	150 ± 30	8.13 ± 0.3	760 ± 46	8.26 ± 0.4
sp. I	6	3	170 ± 15	8.35 ± 0.5	775 ± 36	8.48 ± 0.1

Respiration rate experiments. Oxygen consumption (= respiration rate) of the worms at different oxygen levels and sulfide concentrations was measured in a flow-through respirometer set-up (Fig 1).

Experimental set-up: The incubation medium was pumped with a peristaltic pump from 2 bottles, one containing seawater (buffered with 10 mmol l^{-1} HEPES, pH 7.9), the other a sulfide stock solution (400 to 600 µmol l^{-1}, pH 7.89). Oxygen concentrations were controlled by a gas-mixing pump (Woesthoff, Bochum, Germany). The medium was driven through a mixing chamber (1 ml; flow rate 27 ml h^{-1}) into the flow-through system (made of glass) with 2 POS mounted before and behind a flexible animal chamber and a pH electrode near the outflow. The POS were connected to a 2 channel oxygen meter (Cyclobios, Innsbruck, Austria) equipped with a 2 channel recorder. The flow-through unit was inserted in a 40 l water bath with controlled temperature.

Experimental procedure: Each experimental run was composed of an electrode calibration phase (air = normoxia, nitrogen = anoxia, 10 to 12 h), a respiration measuring phase with worms (3 to 4 h) under normoxic conditions (= standard respiration rate), a blank rate measuring phase without worms (2 h), and subsequently a second respiration measuring phase at different oxygen (and sulfide) conditions. The run was concluded by a second blank rate measuring phase at same conditions (i.e. sulfide).

The worms were sieved out of the culture several hours prior to the experiments and placed in petri dishes without sediment for clearance of their gut contents. For each experimental run 2 to 15 worms (depending on size) were taken and inserted into the animal chamber. After the 2 respiration measuring phases the wet weight of the worms was determined. In experiments with sulfide, the sulfide concentrations were determined at the beginning and at the end of each second respiration measurement phase using the colorimetric method described above. Oxygen consumption rates of all 4 *Capitella* sibling species were measured at oxygen tensions of 21, 19, 17, 15, 13, 11, 8, 6, 4, 2 and 0.4 kPa.

Oxygen consumption of the 2 North Sea species *Capitella* sp. S and *Capitella* sp. L was measured at 13, 11, 10, 9, 8, 7, 6, 5, 4, 3 and 2 kPa with 100 µmol l^{-1} sulfide (100 ± 30 µmol l^{-1}, n = 15), and at 7 and 11 kPa with different sulfide concentrations between 10 and 140 µmol l^{-1}. Oxygen consumption was not measured at normoxia plus sulfide due to technical limitations of the respirometer. During measurements the pO_2 variation was about 0.02 kPa and sulfide decreased usually less than 10%.

Incubation experiments. Worms were exposed to different pO_2 and sulfide concentrations in a closed incubation chamber (Fig. 2). After exposure, tissue succinate, sulfide and thiosulfate concentrations were analyzed.

In the chamber, oxygen was measured with a sulfide-insensitive POS (Radiometer, Copenhagen, Denmark) modified after Revsbech & Ward (1983) and connected to a PHM 73 pH/oxygen meter (Radiometer) using a calomel electrode (Radiometer) as reference. pH was measured with a combination electrode (Radiometer) connected to the PHM 73 and sulfide was determined with an Ag-Ag_2S electrode (Vismann 1996) connected to an Ion analyzer (Ion 85, Radiometer) using a calomel electrode as reference.

Experimental set-up: The incubation chamber (1 l) was filled with artificial seawater (32‰ S, 16°C), and

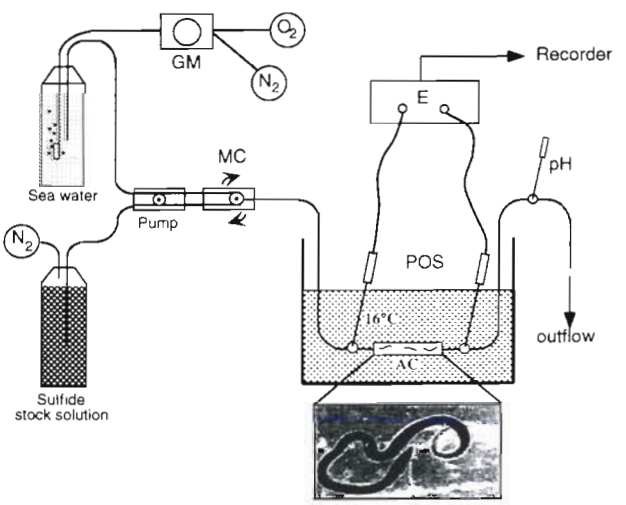

Fig. 1 Schematic view of the flow-through respirometer set-up connected to different medium bottles and mixing chamber (MC) for respiration rate measurements at different pO_2 and sulfide concentrations. AC = animal chamber, E = electronic unit, GM = gas mixing pump, pH = pH electrode, POS = polarographic oxygen sensors

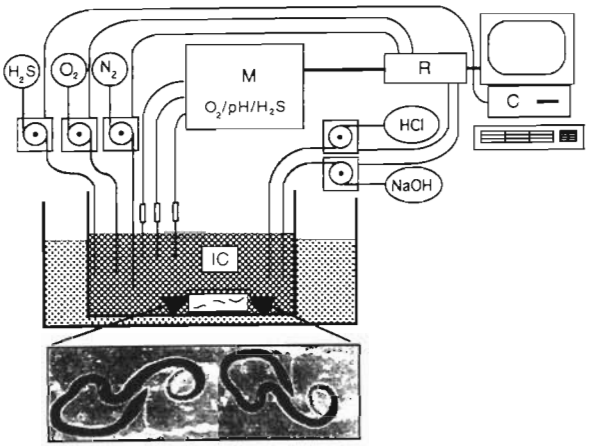

Fig. 2. Schematic view of the computer-controlled incubation set-up. C = calculating section, IC = incubation chamber, M = monitoring section, R = regulating section

pH, oxygen and sulfide concentrations were continuously controlled by a computer (for further details see Vismann 1996).

Experimental procedure: Worms were placed in 20 ml vials covered with gauze (150 µm). For each sibling species 15 to 30 specimens were incubated in 3 vials (i.e. 5 to 10 specimens per vial). The worms were exposed for 12 h to different treatments (Table 2) and for 24 h to anoxia.

Succinate determination: At the end of each exposure the worms were removed from the incubation medium, quickly rinsed with demineralized water, blotted on a paper towel, weighed and frozen at −80°C. The frozen samples were homogenized in a 1 ml glass microhomogenizer (Jencons, Bedfordshire, England) on ice with 150 µl of 0.6 N perchloric acid. The homogenate was centrifuged (14000 × *g*) for 15 min at 15°C and the supernatant neutralized in an ice bath with 5 N KOH. The precipitated potassium perchlorate was again centrifuged and the supernatant frozen at −20°C for storage. Succinate was determined photometrically according to the enzymatic method of Beutler (1985). We used succinate and not lactate or opines as an indicator of anaerobiosis since it has been shown to be a sensitive indicator of mitochondrial anaerobic metabolism in other polychaetes, e.g. *Arenicola marina* (Schöttler et al. 1984, Völkel & Grieshaber 1992) and *Hediste* (*Nereis*) *diversicolor* (Schöttler et al. 1984), and in the oligochaete *Tubificoides benedii*, which is of comparable body size (Dubilier et al. 1994).

Table 2. Experimental conditions of the computer-controlled incubations for succinate and thiole (*) determinations. µM = µmol l^{-1} Data acquired every minute (n = 720)

Treatment	pO_2 (kPa)	Sulfide (µmol l^{-1})	pH value
Normoxia	21.5 ± 0.2	–	7.90 ± 0.10
17 kPa	17.1 ± 0.4	–	7.51 ± 0.09
8 kPa	8.2 ± 0.2	–	7.72 ± 0.10
Hypoxia	0.5 ± 0.2	–	7.56 ± 0.08
Anoxia	0 ± 0	–	7.60 ± 0.09
8 kPa + 20 µM sulfide	8.3 ± 0.4	18.6 ± 1.2	7.50 ± 0.01
Hypoxia + 20 µM sulfide	0.5 ± 0.1	20.1 ± 1.2	7.50 ± 0.01
Anoxia + 20 µM sulfide	0.0 ± 0.1	19.7 ± 1.7	7.51 ± 0.01
8 kPa + 100 µM sulfide	7.8 ± 0.2	106.4 ± 15.5	7.51 ± 0.01
Hypoxia + 100 µM sulfide	0.5 ± 0.2	103.8 ± 7.3	7.51 ± 0.01
Anoxia + 100 µM sulfide	0.0 ± 0.1	113.4 ± 15.0	7.51 ± 0.01
*Normoxia	21.5 ± 0.2	–	7.90 ± 0.10
*8 kPa + 100 µM sulfide	7.8 ± 1.1	104.4 ± 13.5	7.51 ± 0.01
*Hypoxia + 100 µM sulfide	0 ± 0	111.1 ± 3.3	7.51 ± 0.01

Determination of reduced sulfur compounds: Sulfide and thiosulfate concentrations in the worms tissue were measured with the HPLC method (modified by Fahey & Newton 1987). Monobromobimane (mBBr) (Calbiochem, Giessen, Germany) forms fluorescent adducts with thiols such as sulfide and thiosulfate, which can be separated by reversed-phase HPLC.

At the end of each exposure the worms were removed from the incubation medium, quickly rinsed, blotted, weighed and homogenized on ice in 75 µl buffer (50 mmol l^{-1} HEPES, 5 mmol l^{-1} EDTA, pH 8.0) with 75 µl acetonitrile and 10 µl mBBr (48 mmol l^{-1}). The samples were incubated in darkness for 15 min at room temperature and derivatization was stopped by adding 100 µl methane sulfonic acid (65 mmol l^{-1}). The homogenate was stored at −80°C.

Chromatography was carried out at room temperature with a Merck/Hitachi HPLC-System (L-6200 Intelligent Pump with LC Organizer; Merck, Darmstadt, Germany/Hitachi, Tokyo, Japan) using a reversed-phase column (LiChroSpher 60, RP-select, Merck). The HPLC system was connected to a C-R1 A chromatopac integrator (Shimadzu, Tokyo), allowing online recordings and peak area calculations.

Statistical analysis. Results are given as mean values (x) with their standard deviation (SD). Statistical analysis was carried out on median survival time, respiration rates, succinate and thiole concentrations using the nonparametric *U*-test of Wilcoxon, Mann and Whitney (Sachs 1984). Data are considered as significantly different when $p \leq 0.05$.

RESULTS

Field

In the North Sea intertidal zone the vertical distribution of *Capitella* sp. S was markedly different from that of the large *Capitella* sp. L (Fig. 3). From 95 to 100% of the

small *Capitella* sp. S occurred in the upper 0 to 3 cm of the sediment where sulfide concentrations never exceeded 20 µmol l^{-1}, whereas 67 to 100 % of *Capitella* sp. L were found in the deeper sediment layers (3 to 9 cm) with anoxia and sulfide concentrations of up to 350 µmol l^{-1}.

Laboratory

For comparison, morphological features (see Grassle & Grassle 1974) and life history in the 4 laboratory-cultured sibling species of *Capitella* are given in Table 3. *Capitella* sp. S from the North Sea was the smallest sibling species, never exceeding a body length of 1.5 cm, whereas adults of *Capitella* sp. L could grow in culture up to 4.5 cm body length. No other differences in morphology among the 4 species were found in the present study. Differences in brood sizes and reproductive modes were, however, remarkable. *Capitella* sp. S was the only species producing benthic juvenile larvae, while the 3 other species had free-swimming trochophore larvae.

Tolerance to anoxia and sulfide

Comparison of the *Capitella* sibling species in survival rates under anoxia and anoxia plus high sulfide reveals species-specific differences (Fig. 4a–c). At anoxia and at anoxia plus sulfide the median survival times of *Capitella* sp. S (LT_{50}, high sulfide: 28 ± 11 h, n = 4) were significantly shorter than median survival times of *Capitella* sp. L (LT_{50}, high sulfide: 47 ± 6 h, n = 4) and *Capitella* sp. I (LT_{50}, high sulfide: 65 ± 6 h, n = 3), which was the most tolerant sibling species.

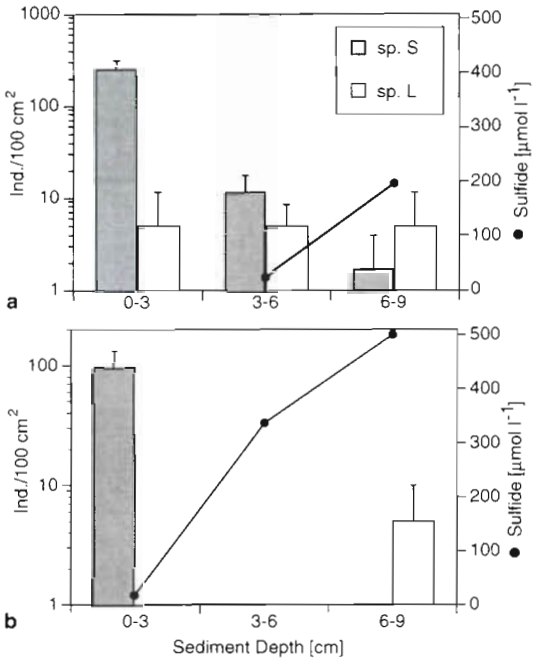

Fig. 3. Mean vertical distribution (n = 3) of *Capitella* sp. S and *Capitella* sp. L compared to pore water sulfide concentrations in (a) June and (b) August 1991 in the intertidal zone of the island of Sylt (North Sea). Note logarithmic scale

Oxygen consumption

The standard respiration rates of the 4 *Capitella* sibling species at normoxia (21.0 kPa) are given in Table 4 and used as the 100 % value in Fig. 5.

In *Capitella* sp. S the oxygen consumption became successively reduced with declining ambient oxygen tensions (Fig. 5). At high oxic levels (19.1 kPa and 16.8 kPa) the standard respiration rate of 11.95 ±

Table 3. Morphological features and life history in 4 *Capitella* sibling species. nm: not measured

Capitella:	sp. S	sp. L	sp. M[a]	sp. I
Geogr. region:	Sylt, Germany	Sylt, Germany	Milos, Greece	Woods Hole, USA
Sulfide (µmol l^{-1}):	20	350	710	High (nm)
Adult morphology (from cultures)				
Body length (mm)	6–15	25–45	15–25	15–20
Wet weight (mg)	0.3–2.5	3.2–12	3.6–9.2	3–12[b]
Sex	Monoecious	Monoecious	Mon-/dioecious	Mon-/dioecious
Prostomium	Broad triangular	Broad triangular	Broad triangular	Broad triangular
Setae (teeth)	Present	Present	Present	Present
(Row and formula)	(1/4)	(1/4)	(1/4)	(1–3/3–5)[b]
Eggs				
Number	30–50	>70	100–300	30–400[b]
Diameter (µm)	240–250	170–180	228 ± 10 (n = 30)	260 × 180[b]
Larvae				
Length (µm)	1047 ± 178 (n = 16)	328 ± 55 (n = 24)	456 ± 8 (n = 30)	210[c]
Larval mode	Benthic	Lecithotrophic	Lecithotrophic	Lecithotrophic
Free-swimming	–	Several hours	Several hours	Several hours

[a]Data from Gamenick et al. (1998). [b]Data from Grassle & Grassle (1976). [c]Fixed for SEM (Eckelbarger & Grassle 1987)

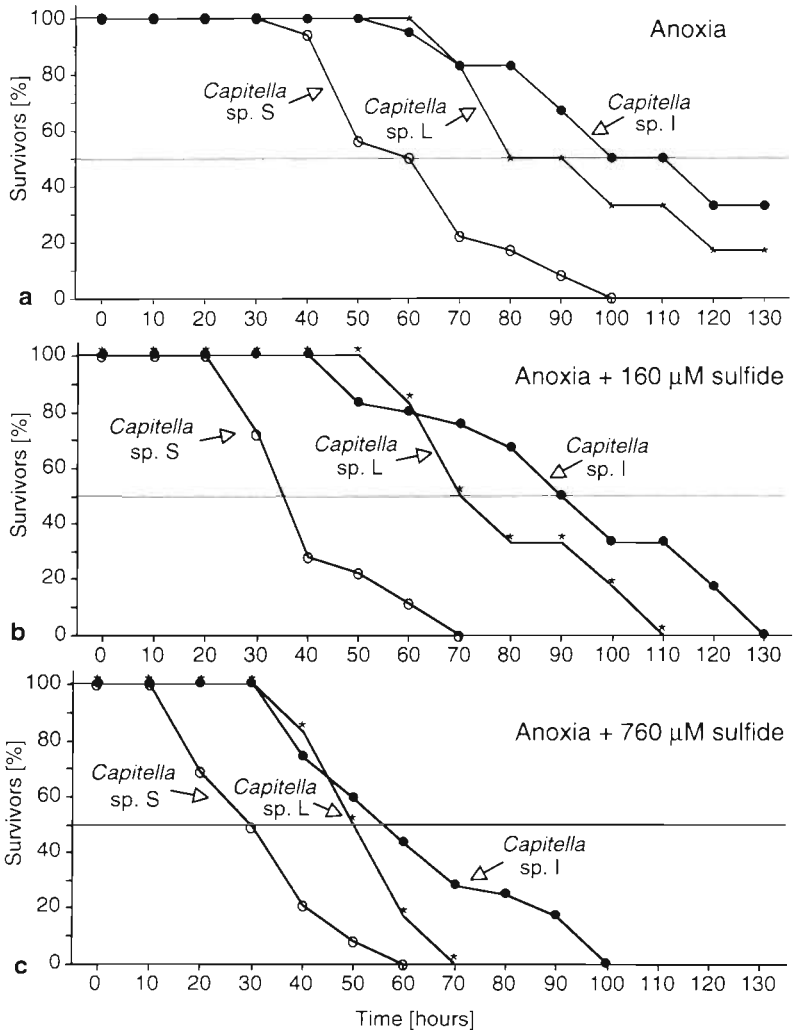

Fig. 4. Average survival rates of *Capitella* sp. S (o), *Capitella* sp. L (*) and *Capitella* sp. I (●) under (a) anoxia, (b) anoxia plus 160 µmol l^{-1} sulfide and (c) anoxia plus 760 µmol l^{-1} sulfide as a function of time. Standard deviations (9 to 29%) are not shown

1.86 µmol O_2 h^{-1} g^{-1} wwt was significantly reduced by 10 ± 4 and 22 ± 6% respectively. At 9.5 kPa, the presence of 100 µmol l^{-1} sulfide caused a significant 78% reduction of oxygen consumption.

In *Capitella* sp. L the oxygen consumption decreased below ambient oxygen tensions of 16.8 kPa. The presence of 100 µmol l^{-1} sulfide led to a slight decrease of respiration. In contrast to *Capitella* sp. S, *Capitella* sp. L was able to maintain its oxygen consumption between 12.6 and 6.3 kPa at a stable level. *Capitella* sp. M maintained a high oxygen consumption (70 ± 12% of the standard respiration rate of 7.74 ± 1.86 µmol O_2 h^{-1} g^{-1} wwt) down to oxygen tensions as low as 2.1 kPa. *Capitella* sp. I maintained its oxygen consumption at a stable level at higher oxygen tensions. A significant decrease was measured below ambient oxygen tensions of 8.42 kPa.

At constant oxygen tensions of 11 kPa the presence of more than 70 µmol l^{-1} sulfide reduced the oxygen consumption of *Capitella* sp. S (Fig. 6). At 7 kPa the oxygen consumption was significantly reduced at 30 µmol l^{-1} sulfide. This contrasts to results for *Capitella* sp. L, where at 11 and 7 kPa sulfide concentrations of up to 130 and 100 mol l^{-1} respectively had no effect on the oxygen consumption.

Succinate content in the tissues

In all *Capitella* sibling species internal succinate concentrations increased with decreasing ambient oxygen tensions and reached maximum values at anoxic conditions (Fig. 7).

In *Capitella* sp. S succinate concentrations increased significantly in the presence of 20 and 100 µmol l^{-1} sulfide at 8.42 kPa. In contrast, in *Capitella* sp. L the presence of 20 µmol l^{-1} sulfide had no effect on succinate levels, but 100 µmol l^{-1} sulfide led to increased succinate content in the worm. In *Capitella* sp. M neither 20 nor 100 µmol l^{-1} sulfide was sufficient to cause an increase in succinate levels. In *Capitella* sp. I at 8.42 kPa the presence of 20 mol l^{-1} sulfide also had no effect, but at hypoxia (0.4 kPa) 100 µmol l^{-1} sulfide led to increased succinate concentrations in this sibling species.

Sulfide and thiosulfate

Hypoxia (0.4 kPa) plus 100 µmol l^{-1} ambient sulfide concentrations produced an increase in internal sulfide concentrations in all 4 *Capitella* species, compared to non-sulfidic conditions (Table 5). The presence of

Table 4. Average standard respiration rates of 4 *Capitella* sibling species under normoxic conditions. n: number of experimental runs

Capitella	$\dot{M}$ O_2 (µmol h^{-1} g^{-1} wet wt)
sp. S	11.95 ± 1.86 (n = 19)
sp. L	5.78 ± 1.16 (n = 14)
sp. M	7.74 ± 1.86 (n = 18)
sp. I	9.23 ± 1.58 (n = 10)

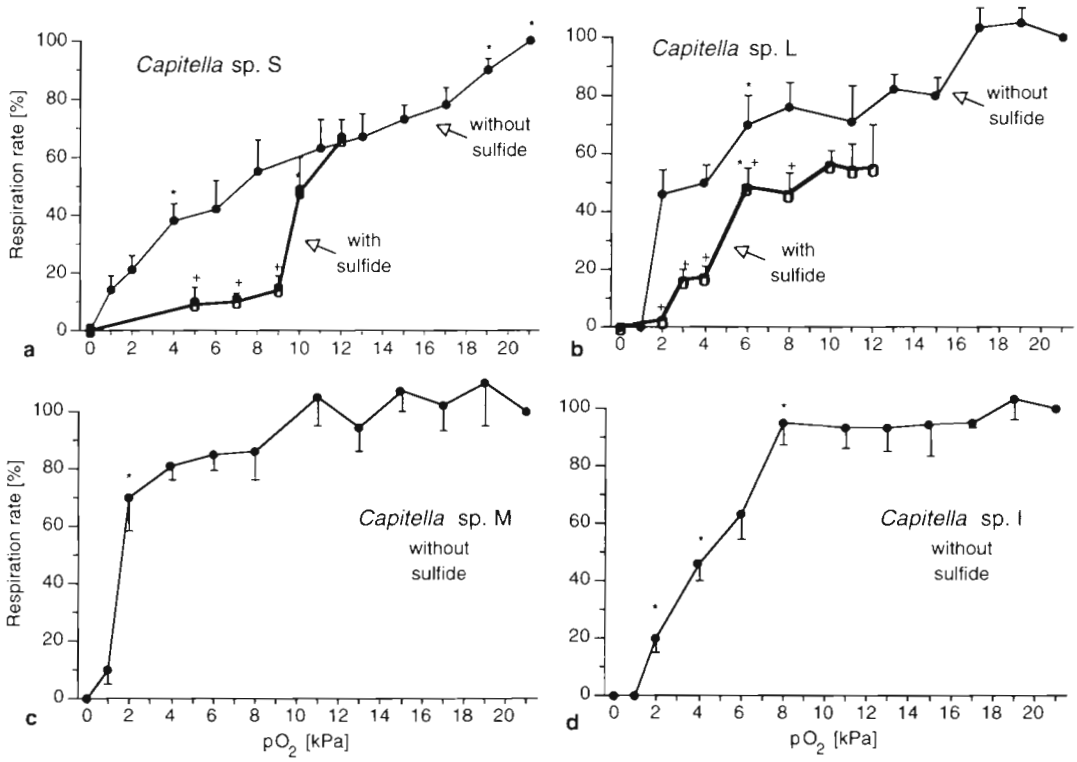

Fig. 5. Standard respiration rate (= 100%, compare Table 4) and mean pO_2-dependent oxygen consumption of (a) *Capitella* sp. S, (b) *Capitella* sp. L, (c) *Capitella* sp. M and (d) *Capitella* sp. I without sulfide (n = 8) and of (a) *Capitella* sp. S and (b) *Capitella* sp. L in the presence of 100 µmol l^{-1} sulfide (n = 6). *Significantly different from former value (lower pO_2). (+) Significantly different from respiration rates without sulfide

8.4 kPa oxygen at sulfidic conditions led to significantly decreased internal sulfide concentrations compared to hypoxia with sulfide.

Thiosulfate concentrations in all 4 *Capitella* species increased when exposed to 100 µmol l^{-1} sulfide and hypoxia, and the presence of oxygen under sulfidic conditions led to a further significant thiosulfate increase (Table 6).

DISCUSSION

Tolerance to anoxia and sulfide

The sibling species of *Capitella capitata* showed significant differences in their tolerance to anoxia and sulfide. *Capitella* sp. S from the upper intertidal sediments had the lowest tolerance to anoxia and sulfide, while the sympatric *Capitella* sp. L from the deeper sulfide-rich sediment layers was more tolerant. A size-dependent tolerance as found in *Arenicola marina* (Groenendaal 1980) does not exist, since the 'medium sized' *Capitella* sp. M from hydrothermal areas of Milos is much more tolerant (Gamenick et al. 1998) than the *Capitella* sibling species from the North Sea and the North Atlantic tested in this study. As described for

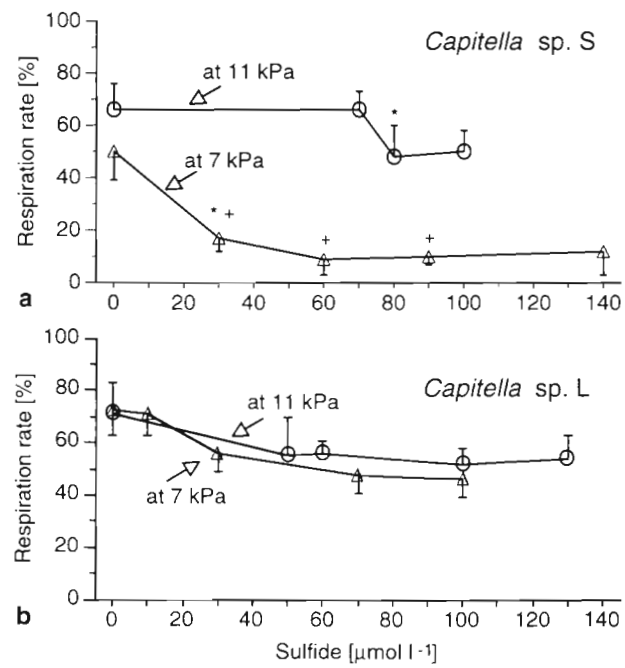

Fig. 6. Mean sulfide-dependent respiration rates of (a) *Capitella* sp. S and (b) *Capitella* sp. L at constant oxygen tensions of 11 and 7 kPa (n = 6). *Significantly different from former value (lower sulfide). (+) Significantly different from respiration rates at 11 kPa

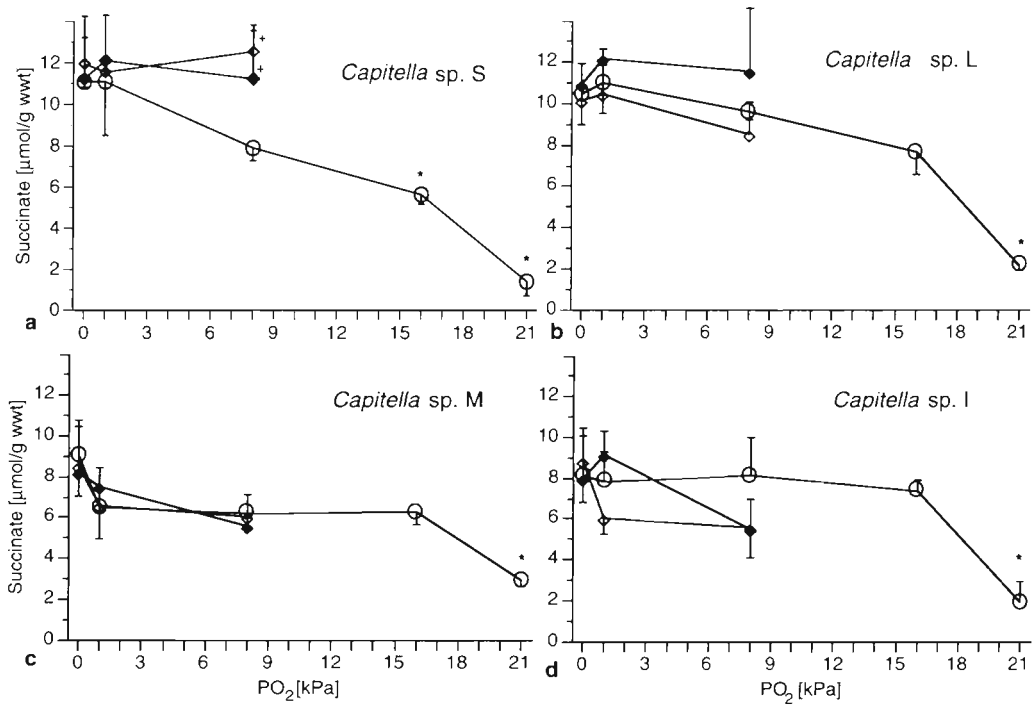

Fig. 7. pO_2-dependent internal succinate concentrations (n = 3) in (a) *Capitella* sp. S, (b) *Capitella* sp. L, (c) *Capitella* sp. M and (d) *Capitella* sp. I after incubations without sulfide (○) and in the presence of 20 µmol l^{-1} (◇) and 100 µmol l^{-1} sulfide (◆). *Significantly different from former value (lower pO_2). (+) Significantly different from value without sulfide

different *Nereis* species, the present study confirms the presence of a close correlation between habitat sulfide exposure and survival capacity (Theede et al. 1973, Vismann 1990). Moreover, in the Baltic clam *Macoma balthica* different degrees of sulfide tolerance occur even at population levels (Jahn & Theede 1997). In the present study, the mean tolerance to anoxia varied between 2.2 (*Capitella* sp. S) and 4.2 d (*Capitella* sp. I), confirming that an unusually high resistance to low oxygen tensions does not exist for *C. capitata* (e.g. Warren 1984, Forbes & Lopez 1990). Other polychaetes, e.g. *Nereis* (*Hediste*) *diversicolor*, *Nereis* (*Neanthes*) *virens* and the lugworm *Arenicola marina*, all survive anoxia for 5 d or longer (Theede et al. 1969, Schöttler & Grieshaber 1988, Gamenick et al. 1996). Sulfide tolerance (anoxia plus 760 µmol l^{-1} sulfide) of the *Capitella* species, ranging from 1.2 (*Capitella* sp. S) to 4.4 d (*Capitella* sp. M), is also low compared to other polychaete species. For *Owenia fusiformis* Groenendaal (1980) demonstrated a maximal survival time of 5 d under hypoxia plus 10 $mmol^{-1}$ sulfide.

Effects of oxygen depletion and sulfide on the aerobic metabolism

The patterns of pO_2-dependent respiration rates in response to changes in ambient oxygen tensions differ markedly among the 4 *Capitella* sibling species. Only the oxygen consumption of *Capitella* sp. S becomes successively reduced with declining oxygen tensions. Due to this linear oxygen dependence of its respiration

Table 5. Mean (n = 3) internal sulfide concentrations (nmol g^{-1} wwt) of 4 *Capitella* sibling species at normoxia, at 8.4 kPa pO_2 with 100 µmol l^{-1} sulfide and at 0.4 kPa pO_2 with 100 µmol l^{-1} sulfide

Treatment	sp. S	sp. L	sp. M	sp. I
Normoxia	22 ± 3	24 ± 3	24 ± 5	23 ± 4
8 kPa + sulfide	43 ± 8	39 ± 3	16 ± 9	55 ± 10
Hypoxia + sulfide	68 ± 11	65 ± 20	72 ± 25	60 ± 15

Table 6. Mean (n = 3) internal thiosulfate concentrations (nmol g^{-1} wwt) of 4 *Capitella* sibling species at normoxia, at 8.4 kPa pO_2 with 100 µmol l^{-1} sulfide and at 0.4 kPa pO_2 with 100 µmol l^{-1} sulfide

Treatment	sp. S	sp. L	sp. M	sp. I
Normoxia	16 ± 4	15 ± 4	24 ± 15	18 ± 5
8 kPa + sulfide	174 ± 16	188 ± 17	163 ± 32	199 ± 10
Hypoxia + sulfide	54 ± 11	77 ± 18	74 ± 22	79 ± 1

rate, *Capitella* sp. S can be characterized as an typical oxyconformer (Prosser 1973). A similar oxyconforming pattern was reported by Warren (1984) for a non specified *C. capitata* population.

In contrast to *Capitella* sp. S, the 3 other *Capitella* sibling species can be characterized as oxyregulators (Prosser 1973). They keep oxygen consumption pO_2-independent through increased ventilation over a range of ambient oxygen tensions and switch to oxyconformity below a critical oxygen tension (P_{cr}) (e.g. Herreid 1980, Grieshaber et al. 1994). To be a regulator and thus to keep an energetically more efficient aerobic ATP production as long as possible is of advantage for an organism (e.g. Hochachka & Somero 1984). However, species-specific differences in the regulation ability of the *Capitella* sibling species were found. According to Herreid (1980), *Capitella* sp. L can be termed as a bad regulator because of its high P_{cr} (16.8 kPa). *Capitella* sp. I, as a moderate regulator, is able to keep its oxygen consumption pO_2-independent above a P_{cr} of 7 kPa. In contrast, *Capitella* sp. M has an extremely low P_{cr} of 2 kPa; it can be termed as a good regulator, well adapted to fluctuating pO_2. *Capitella* sp. M maintains the energetically more efficient aerobic metabolism even at low oxygen tensions, which is advantageous for the polychaete in the rigid habitat near the hydrothermal vents. The present study underlines once more that the dichotomous distinction between conformers and regulators is oversimplified; many exceptions and several transitions between 'perfect conformity' and 'perfect regulation' are known (Pörtner & Grieshaber 1993, Grieshaber et al. 1994).

Addition of sulfide leads to a decrease in respiration rate in both investigated North Sea species, due to inhibition of the key enzyme of aerobic respiration, cytochrome *c* oxidase (Somero et al. 1989, Bagarinao & Vetter 1990, Vismann 1991). However, differences were found between these 2 sympatric species. At declining ambient oxygen tensions the presence of 100 µmol l^{-1} sulfide led to a significant reduction in respiration rate in the sensitive *Capitella* sp. S, whereas the tolerant *Capitella* sp. L maintained its oxygen uptake in these conditions. At lower oxygen levels, sulfide concentrations as low as 30 µmol l^{-1} inhibited aerobic pathways of *Capitella* sp. S. In contrast, the respiration rate of *Capitella* sp. L was not affected even at higher sulfide concentrations, underlining the adaptation of this sibling species to hypoxia and sulfidic conditions of its environment, the deeper sediments of the Wadden Sea.

Effects of oxygen depletion and sulfide on the anaerobic metabolism

With declining ambient oxygen tensions, internal succinate concentrations increased in the *Capitella* sibling species, indicating that decreased respiration rates were related to a successive onset of an anaerobic metabolism, not to reduced activity or ventilation rates. This phenomenon has also been described for other marine invertebrates, e.g. *Sipunculus nudus* (Pörtner 1982), *Arenicola marina* (Völkel & Grieshaber 1992), *Hediste diversicolor* (Schöttler et al. 1984), *Halicryptus spinulosus* (Oeschger 1990) and recently also for the small ostracod *Cyprideis torosa* (Jahn et al. 1996) from shallow sulfidic sediments of the Baltic Sea.

A sulfide-induced onset of anaerobic metabolism could be shown not only by respiration data but also by increased succinate levels in all *Capitella* sibling species. Differences among the sibling species were found with the North Sea *Capitella* sp. S being the most sensitive to sulfide. In this species, low sulfide concentrations of 20 µmol l^{-1} led to total anaerobiosis in the presence of moderate ambient oxygen tensions. In the more tolerant *Capitella* sp. L this could only be shown at sulfide concentrations of 100 µmol l^{-1}. In contrast, the same conditions did not elicit increased anaerobiosis in *Capitella* sp. M and in *Capitella* sp. I. In these 2 tolerant species the presence of sulfide only caused an increase in anaerobiosis under hypoxic conditions.

The oxygen-dependent effect of sulfide on the metabolism (inhibition of aerobic pathways and onset of anaerobiosis) shown in this paper for the 4 *Capitella* species has also been described for other marine invertebrates, e.g. *Sipunculus nudus* (Völkel & Grieshaber 1992), *Arenicola marina* (Völkel & Grieshaber 1992, Hauschild 1996), the oligochaete *Tubificoides benedii* (Dubilier et al. 1994) and thalassinidean crustacea (Johns et al. 1997). Sulfide induced anaerobiosis in these species, even at normoxic conditions, and at lower ambient oxygen tensions much less sulfide was needed to give the same effect. Sulfide-dependent anaerobiosis has been discussed as being an adaptation to sulfidic habitats (Grieshaber et al. 1992, Oeschger & Vetter 1992). Comparing different marine invertebrates (exposed to the same oxygen conditions), very different sulfide concentrations inhibit aerobic metabolism (e.g. Oeschger & Vetter 1992, Dubilier et al. 1994, Völkel & Grieshaber 1994). As shown in the 4 *Capitella* species the defense systems against sulfide toxicity which prevent inhibition of aerobic metabolism are species-specific. Indeed, several comparative studies on marine invertebrates have shown marked differences in the ability to detoxify sulfide (Vetter et al. 1987, Bagarinao & Vetter 1990, Vismann 1990, Levitt & Arp 1991, Völkel & Grieshaber 1992).

The onset of anaerobiosis in *Capitella* sp. L at oxygen levels of 7 kPa (Fig. 7b), while oxygen consumption is not reduced (Fig. 6b), could indicate that oxygen is used for sulfide oxidation (e.g. Grieshaber & Völkel 1998) as shown in increased internal thiosulfate levels

(see below). Thus, in contrast to *Capitella* sp. S, aerobic metabolism is not inhibited under these conditions. Recently Völkel & Grieshaber (1996) showed that the sulfide-tolerant lugworm *Arenicola marina* is able to oxidize sulfide even under high sulfidic conditions via an alternative sulfide-insensitive terminal oxidase. One could discuss that *Capitella* sp. L might also have such an alternative oxidase to survive sulfidic conditions.

The 4 *Capitella* species were unable to prevent the diffusion of sulfide into the body but were able to oxidize it to thiosulfate if oxygen was available. This oxidative step has been interpreted as an adaptation in several animals from sulfidic habitats (for reviews see Somero et al. 1989, Vismann 1991, Bagarinao 1992, Grieshaber & Völkel 1998). The occurrence of internal succinate, sulfide and thiosulfate in all *Capitella* species in the control at normoxia is probably due to the fact that the worms were kept in culture tanks with sediment. Here, they were exposed to hypoxia and sulfide in deeper sediment layers despite aeration of the tank. To remove the worms from sediment prior to experiments for a longer time period was not possible because of starvation symptoms.

The differentiation of the 4 *Capitella* sibling species is much more pronounced on an ecophysiological level than on the morphological level, reflecting the degree of 'sulfide stress' experienced in the natural habitat. This is in addition to different general protein patterns (Gamenick et al. 1998) and reproduction modes (see Table 3).

This study demonstrates that genetically fixed ecophysiological differences occur within the *Capitella capitata* complex. Our results are consistent with the conception that abiotic stress—for example hypoxia and/or sulfide—can be a driving force of genetic differentiation, resulting in physiological divergence. Hence, *C. capitata* is an ecophysiologically much more diverse species complex than has previously been recognized. Thus, the unreflected and summative use of *C. capitata* as an indicator species for disturbed and polluted environments must be questioned.

Acknowledgements. We thank Susanne Völkel for her introduction into succinate analysis and HPLC measurements and for valuable discussions. We thank Lars Hagermann and 4 anonymous referees for their constructive criticism on the manuscript. This work was supported by the German Bundesministerium für Bildung, Wissenschaft und Forschung (BMBF) under the project DYSMON (03F0123D) to O.G. and by the Danish Science Research Council No. 11-8391/0088 to B.V.

LITERATURE CITED

Bagarinao T (1992) Sulfide as an environmental factor and toxicant: tolerance and adaptations in aquatic organisms. Aquat Toxicol 24:21–62

Bagarinao T, Vetter RD (1990) Oxidative detoxification of sulfide by mitochondria of the California killifish *Fundulus parvipinnis* and the speckled sanddab *Citharichtys stigmaeus*. J Comp Phys [B] 169:519–527

Beutler HO (1985) Succinat. In: Bergmeyer HU (ed) Methods of enzymatic analysis. Verlag Chemie, Weinheim, p 25–33

Bridges TS, Levin LA, Cabrera D, Plaia G (1994) Effects of sediment amended with sewage, algae, or hydrocarbons on growth and reproduction in two opportunistic polychaetes. J Exp Mar Biol Ecol 177:99–119

Chang S, Steimle FW, Reid RN, Fromm SA, Zdanowicz VS, Pikanowski RA (1992) Association of benthic macrofauna with habitat types and quality in the New York Bight. Mar Ecol Prog Ser 89:251–253

Chesney EJ Jr, Tenore KR (1985) Oscillations of laboratory populations of the polychaete *Capitella capitata* (Type I): their cause and implications for natural populations. Mar Ecol Prog Ser 20:289–296

Dubilier N, Giere O, Grieshaber MK (1994) Concomitant effect of sulfide and hypoxia on the metabolism of the marine oligochaete *Tubificoides benedii*. J Exp Zool 269: 287–297

Eckelbarger KJ, Grassle JP (1983) Ultrastructural differences in the eggs and ovarian follicle cells of *Capitella* (Polychaeta) sibling species. Biol Bull 165:379–393

Eckelbarger KJ, Grassle JP (1987) Interspecific variation in genital spine, sperm, and larval morphology in six sibling species of *Capitella*. Bull Biol Soc Wash 7:62–76

Fahey RC, Newton GL (1987) Determination of low-molecular weight thiols using monobromobimane fluorescent labeling and high performance liquid chromatography. Meth Enzymol 143:85–96

Forbes TL, Lopez GR (1990) The effect of food concentration, body size, and environmental oxygen tension on the growth of the deposit-feeding polychaete, *Capitella* species I. Limnol Oceanogr 35(7):1535–1544

Gamenick I, Giere O (1994) Population dynamics and ecophysiology of *Capitella capitata* from North Sea intertidal flats. Evidence for two sibling species. Polychaete Res 16: 44–47

Gamenick I, Jahn A, Vopel K, Giere O (1996) Hypoxia and sulfide as structuring factors in a macrozoobenthic community on the Baltic shore: colonization studies and tolerance experiments. Mar Ecol Prog Ser 144:73–85

Gamenick I, Abbiati M, Giere O (1998) Field distribution, tolerance and general protein patterns of *Capitella capitata* (Annelida: Polychaeta) around shallow water hydrothermal vents off Milos (Aegean Sea). Mar Biol 130(3): 447–453

Giere O (1992) Benthic life in sulfidic zones of the sea (ecological and structural adaptations to a toxic environment. Ver Dtsch Zool Ges 85.2:77–93

Gilboa-Garber N (1971) Direct spectrophotometric determination of inorganic sulfide in biological materials and in other complex mixtures. Analyt Biochem 43:129–133

Grassle JF, Grassle JP (1974) Opportunistic life histories and genetic systems in marine benthic polychaetes. J Mar Res 32:253–284

Grassle JF, Grassle JP (1976) Sibling species in the marine pollution indicator *Capitella* (Polychaeta). Science 192: 567–569

Gray JS (1984) Ökologie mariner Sedimente. Eine Einführung. Springer, Berlin

Grémare A, Marsh AG, Tenore KR (1988) Short-term reproductive responses of *Capitella* sp. I (Annelida, Polychaeta) fed on different diets. J Exp Mar Biol Ecol 123:147–162

Grieshaber MK, Völkel S (1998) Animal adaptations for tolerance and exploitation of poisonous sulfide. Annu Rev Physiol 60:33–53

Grieshaber MK, Hardewig I, Kreutzer U, Schneider A, Völkel S (1992) Hypoxia and sulfide tolerance in some marine invertebrates. Verh Dtsch Zool Ges 85.2:55–76

Grieshaber MK, Hardewig I, Kreutzer U, Pörtner HO (1994) Physiological and metabolic responses to hypoxia in invertebrates. Rev Physiol Biochem Pharmakol 125:44–147

Grieshaber MK, Kreutzer U, Pörtner HO (1988) Critical PO_2 of euryoxic animals. In: Acker H (ed) Oxygen sensing in tissues. Springer, Berlin, p 37–48

Groenendaal M (1980) Tolerance of the lugworm (*Arenicola marina*) to sulfide. Neth J Sea Res 14:200–207

Hauschild K (1996) Die sauerstoffabhängige Entgiftung von Sulfid zu Thiosulfat und dessen Ausscheidung beim Wattwurm *Arenicola marina* L. PhD dissertation, Heinrich-Heine-Universität, Düsseldorf

Herreid CF II (1980) Hypoxia in invertebrates. Comp Biochem Physiol 67A:311–320

Hochachka PW, Somero GN (1984) Biochemical adaptation. Princeton University Press, Princeton

Howarth RW, Giblin A, Gale J, Petersen BJ, Luther GW III (1983) Reduced sulfur compounds in the pore waters of a New England salt marsh. Ecol Bull 35:135–152

Jahn A, Theede H (1997) Different degrees of tolerance to hydrogen sulfide in populations of *Macoma balthica* (Bivalvia, Tellinidae). Mar Ecol Prog Ser 154:185–196

Jahn A, Gamenick I, Theede H (1996) Physiological adaptations of *Cyprideis torosa* Jones 1850 (Crustacea, Ostracoda) to hydrogen sulfide. Mar Ecol Prog Ser 142:215–223

Johns AR, Taylor AC, Atkinson RJA, Grieshaber MK (1997) Sulphide metabolism in thalassinidean crustacea. J Mar Biol Assoc UK 77:127–144

Levin LA, Caswell H, Bridges T, DiBacco C, Cabrera D, Plaia G (1996) Demographic responses of estuarine Polychaetes to pollutants: life table response experiments. Ecol Appl 6(4):1295–1313

Levitt JM, Arp AJ (1991) The effects of sulfide on the anaerobic metabolism of two congeneric species of mudflat clams. Comp Biochem Physiol 98B:339–347

Oeschger R (1990) Long-term anaerobiosis in sublittoral marine invertebrates from the Western Baltic Sea: *Halicryptus spinulosus* (Priapulida), *Astarte borealis* and *Arctica islandica* (Bivalvia). Mar Ecol Prog Ser 59: 133–143

Oeschger R, Vetter RD (1992) Sulfide detoxification and tolerance in *Halicryptus spinulosus* (Priapulida): a multiple strategy. Mar Ecol Prog Ser 86:167–179

Pearson M, Pearson TH (1991) Variations in populations of *Capitella capitata* (Fabricius, 1780) (Polychaeta) from the West Coast of Scotland. Ophelia Suppl 5:363–370

Pearson TH, Rosenberg R (1978) Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanogr Mar Biol Annu Rev 16:229–311

Pörtner HO (1982) Biochemische und physiologische Anpassungen an das Leben im marinen Sediment: Untersuchungen am Spritzwurm *Sipunculus nudus* L. PhD dissertation, Heinrich-Heine-Universität, Düsseldorf

Pörtner HO, Grieshaber MK (1993) Critical PO_2 (s) in oxyconforming and oxyregulating animals: gas exchange, metabolic rate and the mode of energy production. In: Eduardo J, Bicudo PW (eds) The vertebrate gas transport cascade. Adaptations to environment and mode of life. CRC Press, Boca Raton, p 330–357

Prosser CL (1973) Comparative animal physiology, 2nd edn. Saunders, Philadelphia

Qian PY, Chia FS (1991) Effects of food concentration on larval growth and development of two polychaete worms, *Capitella capitata* (Fabr.) and *Polydora ligni* (Webster). Bull Mar Sci 48:478–485

Revsbech NP, Ward DM (1983) Oxygen microelectrode that is insensitive to medium chemical composition: use in acid microbial mat dominated by *Cyandium caldarium*. Appl Environ Microbiol 45:755–759

Sachs L (1984) Angewandte Statistik. Springer, Berlin

Sanders HL, Grassle JF, Hampson GR, Morse LS, Garner-Price S, Jones CC (1980) Anatomy of an oil spill: long term effects of the grounding of the barge Florida off West Falmouth, Massachusetts. J Mar Res 38:265–380

Schöttler U, Grieshaber MK (1988) Adaptation of the polychaete worm *Scoloplos armiger* to hypoxic conditions. Mar Biol 99:215–222

Schöttler U, Surholt B, Zebe E (1984) Anaerobic metabolism in *Arenicola marina* and *Nereis diversicolor* during low tide. Mar Biol 81:69–73

Somero GN, Childress JJ, Anderson AE (1989) Transport, metabolism and detoxification of hydrogen sulfide in animals from sulfide-rich marine environments. Rev Aquat Sci 1:591–614

Tenore KR (1977) Growth of *Capitella capitata* cultured on various levels of detritus derived from different sources. Limnol Oceanogr 22(5):936–941

Tenore KR, Chesney EJ Jr (1985) The effects of interaction of rate of food supply and population density on the bioenergetics of the opportunistic polychaete, *Capitella capitata* (type I). Limnol Oceanogr 30:1188–1195

Theede H (1973) Comparative studies on the influence of oxygen deficiency and hydrogen sulfide on marine bottom invertebrates. Neth J Sea Res 7:244–252

Theede H, Ponat A, Hiroki K, Schlieper C (1969) Studies on the resistence of marine bottom invertebrates to oxygen-deficiency and hydrogen sulfide. Mar Biol 2:325–337

Theede H, Schaudinn J, Saff F (1973) Ecophysiological studies on four *Nereis* species of the Kiel Bay. Oikos Suppl 15: 246–252

Thiermann F, Akoumianaki J, Hughes A, Giere O (1997) Benthic fauna of a shallow water gaseohydrothermal vent area in the Aegean sea (Milos/Greece). Mar Biol 128(1): 149–159

Tsutsumi H (1987) Population dynamics of *Capitella capitata* (Polychaeta, Capitellidae) in an organic polluted grove. Mar Ecol Prog Ser 36:139–149

Tsutsumi H, Fukunaga S, Fujita N, Sumida M (1990) Relationship between growth of *Capitella* sp. and organic enrichment of the sediment. Mar Ecol Prog Ser 63: 157–162

Vetter RD, Wells ME, Kurtsman AL, Somero GN (1987) Sulfide detoxification by the hydrothermal vent crab *Bythograea thermydron* and other decapod crustaceans. Physiol Zool 60:121–137

Vismann B (1990) Sulfide detoxification and tolerance in *Nereis* (*Hediste*) *diversicolor* and *Nereis* (*Neanthes*) *virens* (Annelidae: Polychaeta). Mar Ecol Prog Ser 59: 229–238

Vismann B (1991) Sulfide tolerance: physiological mechanisms and ecological implications. Ophelia 34:1–27

Vismann B (1996) Sulfide exposure experiments: the sulfide electrode and a set-up automatically controlling sulfide, oxygen and pH. J Exp Mar Biol Ecol 204:131–140

Völkel S, Grieshaber MK (1992) Mechanisms of sulfide tolerance in the peanut worm *Sipunculus nudus* (Sipunculida) and in the lugworm *Arenicola marina* (Polychaeta). J Comp Physiol 162B:469–477

Völkel S, Grieshaber MK (1994) Oxygen dependent sulfide detoxification in the lugworm *Arenicola marina*. Mar Biol 118:137–147

Völkel S, Grieshaber MK (1996) Mitochondrial sulfide oxidation in *Arenicola marina*. Evidence for alternative electron pathways. Eur J Biochem 235:231–237

Warren LM (1977) The ecology of *Capitella capitata* in British waters. J Mar Biol Assoc UK 57:151–159

Warren LM (1984) How intertidal polychaetes survive at low tide. In: Hutchings P (ed) Proc 1st Int Polych Conf, Linnean Soc of New South Wales, Sydney, p 238–253

Wu BL, Qian PY, Zhang SL (1991) Morphology, reproduction, ecology and allozyme electrophoresis of three *Capitella* sibling species in Qingdao (Polychaeta: Capitellidae). Ophelia Suppl 5:391–400

Zhang SL, Wu BL (1988) Taxonomy of the *Capitella capitata*-complex (Polychaeta). Mar Sci Bull 1:187–192

Editorial responsibility: Otto Kinne (Editor), Oldendorf/Luhe, Germany

Submitted: May 4, 1998; Accepted: August 21, 1998
Proofs received from author(s): December 7, 1998