

# Biomarker sensitivity to temperature and hypoxia—a seven year field study

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**ABSTRACT:** We examined the response of a set of reproduction biomarkers to natural climate variables such as oxygen concentration, temperature and organic carbon (OC) in sediment. The data used was from field studies of the amphipod *Monoporeia affinis* covering 7 consecutive years from 1994 to 2000. Our aims were to evaluate the sensitivity of the biomarker 'malformed embryos' to oxygen deficiency, temperature increase and OC in sediments and to perform a field validation of some reproduction biomarkers targeted at these factors. The biomarker 'malformed embryos' was related neither to oxygen concentration nor to temperature, while a relationship was found with OC content of the sediment. A negative correlation was found between females carrying a dead brood and the oxygen concentration of the bottom water. Fecundity was positively correlated with the carbon content of the sediment but negatively correlated with the temperature of the bottom water. These results confirm the findings of previous laboratory experiments. By combining a set of biomarkers of different sensitivity to various stressors, we have obtained a powerful tool capable of monitoring the effects of both contaminants and other environmental stressors, and with the ability to discriminate between the effects of different environmental disturbances.

**KEY WORDS:** Biomarker · Monitoring · Oxygen · Temperature

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## INTRODUCTION

Biochemical biomarkers are increasingly used in ecological risk assessments to identify exposure to and biological effects of xenobiotics (Sundelin & Eriksson 1998, Lam & Gray 2003). The most relevant biomarkers affect the Darwinian fitness (Lam & Gray 2003). By using responsive reproduction biomarkers, high sensitivity and relevance is achieved, providing information about effects on the next generation. Biomarkers for environmental monitoring should ideally be affected only by contaminants. In reality, all biomarkers are influenced by variables related to hypoxia, malnutrition and temperature stress. Little effort has been made to distinguish between the natural variability and the effects of contaminants, possibly due to the dominance of laboratory studies and lack of knowledge of biomarker response to factors such as hypoxia in the field (Gray et al. 2002). This knowledge deficit is a hindrance to realistic ecological/environmental risk

assessments (ERA) and limits the possibilities for decision-makers to respond correctly to contaminant problems. The International Council for the Exploration of the Sea has addressed a growing concern in these matters by stating (as a criteria for monitoring biomarkers) that 'a recommended method or combination of methods should be able to differentiate the effects of contaminants from natural background variability' (see [www.ices.dk/reports/MHC/2003/WGBEC03.pdf](http://www.ices.dk/reports/MHC/2003/WGBEC03.pdf)).

Variables linked to the reproduction and embryogenesis of the amphipod *Monoporeia affinis* were evaluated for their sensitivity to contaminants in a series of field studies (Sundelin & Eriksson 1998). The set of biomarkers related to reproduction included sexual maturation, fecundity, and embryo aberrations (e.g. malformed, dead and unfertilised eggs). Of these, 1 biomarker, malformed embryos, proved to be sensitive as a contaminant monitor, while dead eggs and dead broods, showed no correlation with contaminants (Sundelin & Eriksson 1998); 'malformed embryos' has

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been used as a contaminant biomarker in a national monitoring programme in Sweden since 1994 (Bott-niska viken 1994–2000, Östersjö 1994–2000).

A common cause of hypoxia is eutrophication, a major worldwide concern in need of indicator systems (Cognetti 2001). The Baltic Sea experiences frequent events of hypoxia and anoxia. Even in the coastal zone, vast areas are subjected to temporary hypoxia despite efforts to improve sewage treatment and reduce the flow of nutrients. In deeper areas, below 70 to 80 m, hypoxia and even anoxia are widespread (Karlsson et al. 2002). Consequently, large areas of the Baltic lack or have strongly diminished populations of sediment-dwelling fauna (Karlsson et al. 2002). Crustaceans are the invertebrate group most susceptible to hypoxia (Gray et al. 2002), and behavioural responses such as decreased swimming activity and changed predator–prey interactions have been reported (Johansson 1997, Nestlerode & Diaz 1998, Tallqvist 2001). Data on the effects of hypoxia on reproduction is scarce (Wu 2002). In one of the few studies linking hypoxia with reproduction, moderate hypoxia increased the occurrence of dead broods in *Monoporeia affinis* while malformed embryos were unaffected (Eriksson Wiklund & Sundelin 2001).

Temperature is another growing global concern. Summer temperatures in northern Europe are predicted to increase by 3 to 5°C and winter temperatures by 5 to 10°C in the next 50 to 100 yr (Climate Change 2001). This would be detrimental to the Baltic Sea since it responds very quickly to climatic change (Stigebrandt & Gustafsson 2003). A major effect of increased temperature is increased metabolism (Lockwood 1968), which results in decreased fecundity in scallops (Martínez & Pérez 2003) and amphipods (Eriksson Wiklund & Sundelin 2001). Laboratory experiments on *Monoporeia affinis* have shown that, in addition to decreased fecundity, elevated temperature causes dead broods, but has no effect on the occurrence of malformed eggs (Eriksson Wiklund & Sundelin 2001).

Using field data covering 7 consecutive years (1994 to 2000), we have examined the response of a set of reproduction biomarkers of *Monoporeia affinis* to oxygen concentration, temperature and sediment carbon content. Our main goals were to evaluate the sensitivity of the biomarker 'malformed embryos' to oxygen deficiency, temperature increase and sediment carbon content and to field-validate some reproduction biomarkers, i.e. dead eggs and dead broods, targeted at these factors.

## MATERIALS AND METHODS

Amphipod data for the years 1994 to 2000 were obtained from an ongoing national monitoring pro-

gramme. *Monoporeia affinis* is a semelparous species, and gravid females are sampled once a year, at the end of January, at 7 coastal stations (see Table 1). Sampling is carried out during late embryogenesis, when the ability to determine embryo aberrations is optimal (Sundelin & Eriksson 1998). At each station, 5 van Veen grabs were taken and the sediment was sieved through 1.0 mm to separate the amphipods. The amphipods were stored in cool water (4 to 6°C, 24 to 48 h) in a natural light regime until analysis of reproduction variables. Live individuals were examined under a stereomicroscope and gravid females were characterised in terms of sexual maturation, unfertilised oocytes, fecundity and dead broods. The eggs and embryos were removed from each female and examined for stage of embryo development and aberrations such as the percentage of malformed embryos, unfertilised/undeveloped eggs, dead eggs and embryos (for details see Sundelin & Eriksson 1998). Participating scientists regularly analysed 50 broods independently in an intercalibration routine to assure that assessments were in agreement.

Bottom-water temperature data were obtained from the SHARK database (Swedish Meteorological and Hydrological Institute, Västra Frölunda, Sweden). Temperature was monitored at a depth of 38 to 40 m, 2 to 4 times a month at a station in the vicinity of the amphipod monitoring stations. Temperature data covered the gonad development and mating period (August to November) in the years immediately preceding (1993 to 1999) the annual sampling of gravid amphipods (1994 to 2000) (see Eriksson Wiklund & Sundelin 2001 for details). A mean temperature for the period was calculated by integrating temperature data between sampling occasions.

Oxygen concentration was monitored yearly from 1997 onwards during the early autumn when oxygen concentrations in the bottom water are usually at a minimum (SHARK database). In mid-September, 3 Kajak-type cores were collected from each station. The oxygen concentration was measured at the sediment surface by a portable oxygen meter (WTW Oxi 323-B, Weilheim) calibrated according to Swedish standards SS-EN 25813 (SIS 1993a) and SS-EN 25814 (SIS 1993b). Mean values of 3 replicates per station and year are presented in Table 1.

The mean organic carbon (OC) content of the top 1 cm of the sediment, measured in January, was calculated for each station from the data available for 3 of the 7 years (see Table 1).

The biomarker data were evaluated using linear mixed-effects models in which station and year were included as random-effect factors. Oxygen concentration, OC and temperature were included as covariates. For all variables except dead broods, oxygen concen-

Table 1. Station locality and natural background data characteristics. Organic carbon ( $n = 3$ ) measured in January 1994–1996, oxygen content of the bottom water calculated as a mean value of 3 replicates per station measured yearly in the middle of September during 1997–1999

Stn	Location	Depth (m)	Bottom type	Organic carbon ( $\text{mg g}^{-1} \pm \text{SE}$ )	Oxygen content ( $\text{mg l}^{-1} \pm \text{SE}$ )
1	58° 46.52' N, 17° 41.50' E	43	Accumulation	2.6 ± 0.2	6.4 ± 0.1
2	58° 44.32' N, 17° 40.85' E	35	Transport	0.9 ± 0.4	7.1 ± 0.3
3	58° 48.67' N, 17° 36.59' E	36	Accumulation	4.8 ± 0.3	5.2 ± 0.1
4	58° 44.68' N, 17° 43.93' E	46	Accumulation	3.3 ± 0.4	6.5 ± 0.3
5	58° 45.54' N, 17° 43.14' E	38	Transport	0.6 ± 0.2	6.9 ± 0.3
6	58° 47.47' N, 17° 43.93' E	37	Accumulation	4.3 ± 0.1	5.7 ± 0.3
7	58° 50.50' N, 17° 47.16' E	32	Accumulation	3.9 ± 0.1	3.0 ± 0.3

tration showed no relation to the response; therefore oxygen concentration was omitted as covariate in these cases. Data on dead broods and embryo aberrations (i.e. malformed, undeveloped and dead eggs) were logit-transformed before analysis. Differences between stations and years were additionally examined using a linear fixed-effects model (ANOVA) with year and station as fixed factors. Pearson correlations were calculated to examine possible relationships between fecundity and embryo variables. Calculations were performed using the MIXED procedure in SAS software, Version 8.2.

## RESULTS

Organic carbon content at the stations showed small yearly variations ( $p = 0.57$ ), but large variations between stations ( $p < 0.001$ ) (Table 1). The average bottom-water temperature during August through November varied from 4.2 to 6.7°C on a yearly basis. Oxygen concentrations showed large variations both with regard to year ( $p < 0.001$ ) and station ( $p < 0.001$ ) (Table 1).

The proportion of malformed embryos showed no yearly variations, although differences between stations were recorded ( $p < 0.001$ ). The mean value for all stations varied between approximately 2.7 and 4% between years. No relationship to bottom-water oxygen concentration or temperature was found, while a significant relationship to OC in sediment was recorded (Table 2). Dead eggs were not correlated to OC, oxygen or temperature. Undifferentiated eggs were positively correlated to OC but negatively correlated to temperature (Table 2).

Results from the regression model showed that the variable 'females carrying a dead brood' was significantly related to the bottom-water oxygen concentration, although not to temperature or OC in the sediment (Table 2, Fig. 1).

The regression model showed that fecundity was positively correlated with the OC of the sediment and negatively correlated with bottom-water temperature (Fig. 2). Correlation calculations showed no correlation between fecundity and the embryo variables (malformed, undifferentiated eggs and dead eggs) ( $p > 0.05$ ).

## DISCUSSION

The occurrence of malformed embryos has been developed as a biomarker for effects of contaminated sediments (Sundelin & Eriksson 1998). In laboratory experiments, malformed embryos showed low sensitivity to elevated temperature and hypoxia (Eriksson Wiklund & Sundelin 2001). This field study confirmed our previous results; i.e. we found no relationship between malformed embryos and low oxygen concentration or increased temperature. A high sensitivity to contaminants (Sundelin & Eriksson 1998) combined with a low sensitivity to other environmental stressors adds weight to the evidence that 'malformed embryos' is an effective and robust biomarker for monitoring contaminants in sediment. Organic contaminants and

Table 2. Statistical parameters of the regression variables. Independent variables were sediment organic content, bottom water temperature and bottom water oxygen content

Dependent variable	Regression coefficient	SE	p
Explanatory variable			
Dead brood			
Constant	2.35	4.28	
Organic carbon	0.048	0.093	0.64
Temperature	-0.38	0.84	0.72
Oxygen	-0.35	0.11	0.020
Fecundity			
Constant	46.5	6.69	
Organic carbon	1.88	0.71	0.045
Temperature	-3.11	1.16	0.044
Malformed			
Constant	-1.82	0.30	
Organic carbon	0.089	0.032	0.041
Temperature	0.0025	0.051	0.96
Undifferentiated			
Constant	-1.66	0.18	
Organic carbon	0.097	0.021	0.0056
Temperature	-0.077	0.031	0.015
Dead			
Constant	-2.90	0.46	
Organic carbon	0.048	0.028	0.15
Temperature	0.150	0.083	0.13

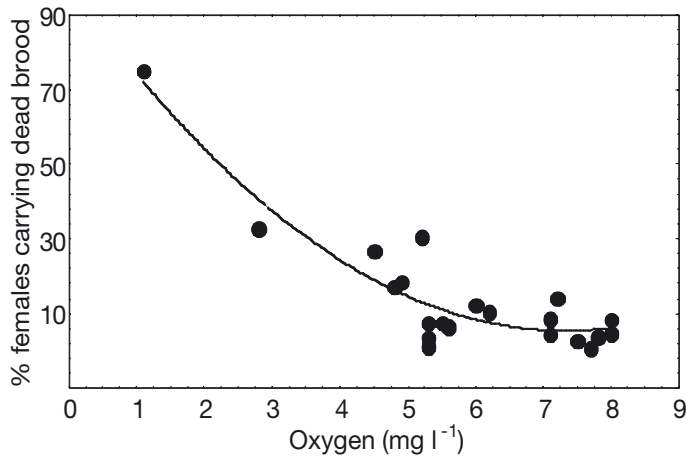


Fig. 1. Percent females carrying a dead brood in relation to oxygen concentration of the bottom water in mid-September. Dead broods are mean values of 5 replicates per station per year, oxygen concentrations are mean values of 3 replicates per station per year

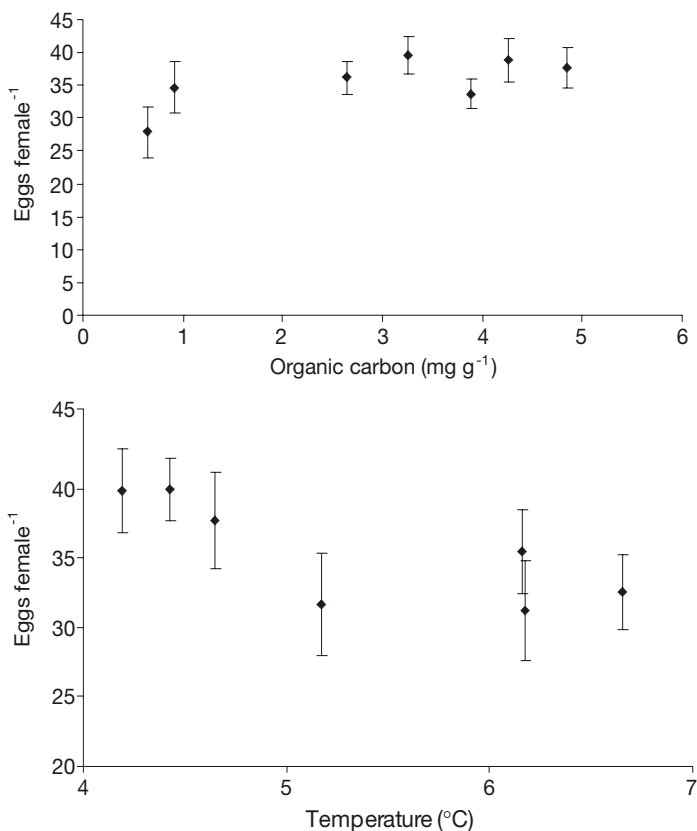


Fig. 2. Annual fecundity (mean  $\pm$  CI of 7 stations sampled in January 1994 to 2000) in relation to sediment organic carbon, (top panel) in relation to mean temperature of bottom water at 38 to 40 m depth from August to November (period of gonad development and mating) during 1993 to 1999 (bottom panel)

OC are often correlated (Sundelin & Eriksson 1998), possibly explaining the relationship between OC and malformed embryos obtained in this study.

Much attention has been paid to the biological effects of contaminants, but factors such as hypoxia may act as stressors, causing damage to populations that is often more dramatic than the effects of contaminants (Wu et al. 2003). Thus, developing new biomarkers for factors like hypoxia is of great importance (Zhou et al. 2000). In laboratory experiments with *Monoporeia affinis* and *Pontoporeia femorata* (Eriksson Wiklund & Sundelin 2001), undifferentiated and dead eggs seemed to be promising biomarkers for effects of oxygen deficiency and elevated temperatures. The present field study did not, however, corroborate these relationships, possibly due to a larger variability in field data for undifferentiated and dead eggs than for malformed embryos. Thus, undifferentiated and dead eggs cannot be considered as optimal monitors of these natural factors.

In laboratory experiments the occurrence of females carrying dead broods was significantly related to moderate hypoxia but not to contaminants (Eriksson Wiklund & Sundelin 2001). Those results were confirmed in the present field study, and females with dead broods showed a significant negative correlation with the oxygen concentration in the bottom water. The low sensitivity to contaminants (Eriksson Wiklund & Sundelin 2001) in combination with the high sensitivity to hypoxia indicates that females carrying dead broods could be used as a biomarker to monitor the effects of hypoxic events.

Even the shallower regions of our study area regularly showed low oxygen concentrations in the bottom water, at some stations as low as  $0.8 \text{ mg O}_2 \text{ l}^{-1}$  during the early autumn. Concentrations lower than  $4 \text{ mg O}_2 \text{ l}^{-1}$  seriously affect the behaviour of *Monoporeia affinis*, as shown by reduced swimming activity (Lindström & Fortelius 2001). Several physiological responses to hypoxia have been reported, e.g. ventilatory and circulatory adaptations, adjusted metabolism, and increased induction of the stress protein Heat Shock Protein 70 (HSP70) in barnacles (Cheng et al. 2003 and references therein). Cheng et al. (2003) showed that oxygen concentrations sublethal to adults affect embryogenesis, resulting in a decreased recruitment of offspring. Recent results have shown that hypoxia reduces the gamete quality of carp *Cyprinus carpio*, leading to low hatching success and high larval mortality (Wu et al. 2003). Both male and female carp subjected to hypoxia had a low gonadosomatic index (GSI) and low levels of testosterone, estradiol and triiodothyronine. We cannot exclude the possibility that oxygen deficiency could affect vitellogenesis and oogenesis in *M. affinis* resulting in dead broods, since hypoxia reduces scope for growth, indicating that lipid resources might be insufficient for oogenesis (Zhou et al. 2000).

Fecundity is positively related to size of the gammaridean female (Persson 1999). *Monoporeia affinis* is a deposit-feeder; thus higher sediment OC results in larger females and subsequently higher fecundity. Despite large variation in OC between stations, the differences in fecundity were relatively small, indicating differences in carbon quality between the stations or that other factors than food could be of importance for fecundity. Our results show that in a cold-water species like *M. affinis*, fecundity is negatively affected by even a slight temperature increase. *M. affinis* stores lipids from the spring phytoplankton bloom as triacylglycerol, reaching maximum lipid contents in August (Eriksson Wiklund et al. 2003). A temperature increase during the mating season (August to November) increases the metabolism, whereby the reproducing generation consumes stored lipids intended for gonad production. These amphipods feed intermittently, and gut fullness is primarily observed after the spring phytoplankton bloom (Dermott & Corning 1988, Quigley 1988). Recent studies on *Diporeia* spp. have shown that gut fullness declines after the spring bloom but increases later in the autumn (Guiguer & Barton 2002). Between phytoplankton blooms the amphipods can survive without food or subsist on low-quality food by using stored lipids because of their low energy requirement in their low-temperature habitat. The low ammonia excretion rate of *M. affinis* prior to the spring bloom (Lehtonen 1994) is an indication that stored lipids are the dominant energy source during periods of starvation. This indicates that high temperatures during a period of non-feeding could have serious consequences for this intermittently feeding species. In a wider perspective, a large-scale temperature increase would affect stenothermal species more than other coexisting species, reducing the competitiveness of the cold-water species.

Investigating the reproduction of brood-carrying amphipods has been a successful method of contaminant-monitoring in the Baltic Sea for several years. In this paper, we have shown that by combining a set of reproduction variables differing in sensitivity to various stressors we have obtained a powerful tool capable of monitoring the effects of both contaminants and natural climate factors and with the ability to discriminate between the effects of different environmental disturbances.

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## LITERATURE CITED

- Bottniska viken (1994–2000) Yearly reports from the Marine Research Center of Umeå University, Umeå (in Swedish, with English summary)
- Cheng SH, So CH, Chan PK, Cheng CW, Wu RSS (2003) Cloning of the HSP70 gene in barnacle larvae and its expression under hypoxic conditions. *Mar Pollut Bull* 46: 659–676
- Climate Change (2001) The scientific basis. Third assessment report of the inter governmental panel on climate change IPCC (WG I). Cambridge University Press, Cambridge
- Cognetti G (2001) Marine eutrophication: the need for a new indicator system. *Mar Pollut Bull* 42:163–164
- Dermott RM, Corning K (1988) Seasonal ingestion rates of *Pontoporeia hoyi* (Amphipoda) populations in Lake Ontario. *Can J Fish Aquat Sci* 45:1886–1895
- Eriksson Wiklund AK, Sundelin B (2001) Impaired reproduction in the amphipods *Monoporeia affinis* and *Pontoporeia femorata* as a result of moderate hypoxia and increased temperature. *Mar Ecol Prog Ser* 222:131–141
- Eriksson Wiklund AK, Wiklund SJ, Sundelin B, Axelman J (2003) Dynamics of lipids and polychlorinated biphenyls in *Monoporeia affinis*—a field study. *Environ Toxicol Chem* 22:2499–2507
- Gray JS, Wu RSS, Or YY (2002) Effects of hypoxia and organic enrichment on the coastal marine environment. *Mar Ecol Prog Ser* 238:249–279
- Guiguer KRRR, Barton DR (2002) The trophic role of *Diporeia* (Amphipoda) in Colpoys Bay (Georgian Bay) benthic food web: a stable isotope approach. *J Gt Lakes Res* 28:228–239
- Johansson B (1997) Tolerance of the deposit-feeding Baltic amphipods *Monoporeia affinis* and *Pontoporeia femorata* to oxygen deficiency. *Mar Ecol Prog Ser* 154:175–183
- Karlsson K, Rosenberg R, Bonsdorff E (2002) Temporal and spatial large-scale effects of eutrophication and oxygen deficiency on benthic fauna in Scandinavian waters—a review. *Oceanogr Mar Biol Annu Rev* 40:427–489
- Lam PKS, Gray JS (2003) The use of biomarkers in environmental monitoring programmes. *Mar Pollut Bull* 46: 182–186
- Lehtonen KK (1994) Metabolic effects of short-term starvation on the benthic amphipod *Pontoporeia affinis* (Lindström) from the northern Baltic Sea. *J Exp Mar Biol Ecol* 176: 269–283
- Lindström M, Fortelius W (2001) Swimming behaviour in *Monoporeia affinis* (Crustacea: Amphipoda)—dependence on temperature and population density. *J Exp Mar Biol Ecol* 256:73–83
- Lockwood APM (1968) Aspects of the physiology of Crustacea. Oliver & Boyd, Edinburgh, p 135–140
- Martínez G, Pérez H (2003) Effect of different temperature regimes on reproductive conditioning in the scallop *Argopecten purpuratus*. *Aquaculture* 228:153–167
- Nestlerode JA, Diaz RJ (1998) Effects of periodic environmental hypoxia on predation of a tethered polychaete, *Glycera americana*: implications for trophic dynamics. *Mar Ecol Prog Ser* 172:185–195
- Östersjö (1994–2000) Yearly reports from the Marine Research Center of Stockholm University, Stockholm (in Swedish, English summary)
- Persson LE (1999) Growth and reproduction in two brackish water populations of *Orchestia gammarellus* (Amphipoda: Talitridae) in the Baltic Sea. *J Crustac Biol* 19:53–59
- Quigley MA (1988) Gut fullness of deposit-feeding amphipod, *Pontoporeia hoyi* in southeastern Lake Michigan. *J Gt Lakes Res* 14:178–187

- SIS (Swedish Institute for Standards) (1993a) Water quality—determination of dissolved oxygen—iodometric method. SIS, Stockholm
- SIS (Swedish Institute for Standards) (1993b) Water quality—determination of dissolved oxygen—electrochemical probe method. SIS, Stockholm
- Stigebrandt A, Gustafsson BG (2003) Response of the Baltic Sea to climate change—theory and observations. *J Sea Res* 49:243–256
- Sundelin B, Eriksson AK (1998) Malformations in embryos of the deposit-feeding amphipod *Monoporeia affinis* in the Baltic Sea. *Mar Ecol Prog Ser* 171:165–180
- Tallqvist M (2001) Burrowing behaviour of the Baltic clam *Macoma balthica*: effects of sediment type, hypoxia and predator presence. *Mar Ecol Prog Ser* 212:183–191
- Wu RSS (2002) Hypoxia: from molecular responses to ecosystem responses. *Mar Pollut Bull* 45:35–45
- Wu RSS, Zhuo BS, Randall DJ, Woo NYS, Lam PKS (2003) Aquatic hypoxia is an endocrine disruptor and impairs fish reproduction. *Environ Sci Technol* 37:1137–1141
- Zhou BS, Wu RSS, Randall DJ, Lam PKS, Ip YK, Chew SF (2000) Metabolic adjustments in the common carp during prolonged hypoxia. *J Fish Biol* 57:1160–1171

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