

**Recent trends in the
prevalence of neoplasia
in the Baltic clam
Macoma balthica (L.)
from the Gulf of Gdańsk
(Baltic Sea)***

OCEANOLOGIA, 47 (1), 2005.
pp. 61–74.

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KEYWORDS

Disseminated neoplasia
Prevalence of the cancer
Baltic clams
Gulf of Gdańsk
Pollutants

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Received 14 June 2004, revised 15 December 2004, accepted 17 January 2005.

Abstract

This study discusses the occurrence of neoplasia in the Baltic clam *Macoma balthica* from the Gulf of Gdańsk in recent years and investigates potential relationships between toxic compounds in the environment and the presence of the cancer. The disease was identified at four sampling stations during 1999–2002. Comparison with previous results highlighted the substantial prevalence of the tumour between 1998 and 2002. The prevalence of the cancer was strongly dependent on the sampling location ($p < 0.001$): it was highest at sampling point H45 (the deepest part of

* This study was supported by funds from the Marie Curie Host Fellowships (No QLK5-CT-2001-60036) provided by the European Commission together with scholarships supported by the French Government and the University of Paris VI.

the gulf) and lowest at station PB30 (central part of the gulf). Monthly studies showed a trend towards an increasing prevalence of neoplasia during the warm months. However, over several sampling months no strong statistical correlation between the prevalence of the disease and the sampling time was found.

The results of the study suggest that several environmental factors may promote the progress of the cancer in *M. balthica* from the Gulf of Gdańsk: it is most probably an indirect effect of pollution, although causality cannot be proven at this stage. Seriously polluted and exhibiting a considerable asymmetry of contamination, the ecosystem of the gulf provides an ideal environment for testing potential cause-effect relationships between pollutants and their biological effects.

1. Introduction

Since the 1970s, considerable changes in the ecosystem of the Gulf of Gdańsk have been observed in parallel to its increasing eutrophication. Among the most important are increasing contamination by nutrients (Nowacki et al. 1993, Andrulewicz 1996), heavy metals, radionuclides (Szefer 2002), organic compounds such as pesticides and cyclic hydrocarbons (Konat & Kowalewska 2001, Albalat et al. 2002), and military waste deposited on the sea bottom after the Second World War (Korzeniewski 1999). This has resulted in an integrated and functional response at the population, community, and ecosystem levels, and includes an elevated incidence of teratological forms in diatoms (Bogaczewicz-Adamczak et al. 2001), poor physiological condition, a higher degree of parasite infection in marine vertebrates and invertebrates (Sokołowski et al. 1999, Chibani et al. 2001), and the appearance of toxic algal blooms (Pliński et al. 1998). One of the most negative phenomena recently observed in bivalves is neoplasia (Elston et al. 1992). In polluted coastal and estuarine systems, some bivalves develop this tumour in their haemolymph and tissues. Most cases of neoplasia reported in bivalves have been described as an impaired growth pattern of haemolymph cells, characterised primarily by excessive cell proliferation. The main features associated with neoplastic cells include nuclear and cellular polymorphism, nuclear hyperchromatism, together with a high rate of proliferation. In bivalves, most identified neoplasias have been described as sarcomas of haematopoietic origin (e.g. Rodriguez et al. 1997) owing to the proliferation of enlarged cells with a large lobate nucleus. Gonad and gill cancer have also been found (e.g. Pekkarinen 1993, Alonso et al. 2001). In bivalve molluscs, these disorders were reported for the first time by Farley (1969). Since then, similar pathological changes have been described in 20 bivalve species from different locations (Elston et al. 1992, Villalba et al. 2001), including *Macoma balthica* (L.) (Pekkarinen 1993). In *M. balthica* from the Gulf of Gdańsk, neoplasia was identified for the first

time by abnormal metaphases in the respiratory system (Thiriot-Quévieux & Wołowicz 1996, 2001, Smolarz et al. 2003).

Although the etiology of bivalve mollusc neoplasia has been reviewed by a number of authors (e.g. Farley et al. 1972, Van Beneden et al. 1993, Roy et al. 1998), it remains essentially undefined. The Gulf of Gdańsk, a seriously polluted area, is characterised by a considerable asymmetry in contamination (Szefer et al. 1996, Hummel et al. 2000). The occurrence of the disease is therefore assumed to be linked to the pollution in the gulf, which presumably promotes tumour growth. This ecosystem provides an ideal environment for testing potential cause-effect relationships between pollutants and their biological effects.

This study discusses the occurrence of neoplasia in the Baltic clam *M. balthica* from the Gulf of Gdańsk in recent years, and potential relationships between toxic substances in the environment and the prevalence of this disease. The presence of the cancer was individually identified by the occurrence of abnormal metaphases and neoplastic lesions in all tissues.

2. Material and methods

Baltic clams *M. balthica* (L.) (16 ± 4 mm mean valve length) were collected monthly by dredging at four sampling stations in the Gulf of Gdańsk (Baltic Sea) (Fig. 1) from 1999 to 2002. The number of animals taken ranged from 20 to 38 at each sampling station. The sampling areas were chosen on the basis of previous studies (Thiriot-Quévieux & Wołowicz 1996, 2001) and represent heterogeneous hydrological and environmental conditions in the Gulf of Gdańsk. Stations V40 and V60 are situated off the mouth of the river Wisła (Vistula). V60 is beneath the thermocline and halocline. Station H45 is located in an intermediate layer in the deepest part of the gulf, where conditions are unstable and subject to rapid change. Station PB30 is situated in the central part of the gulf. The numerical part of each station name designates its depth in metres. After sampling, the clams were maintained for a few days under laboratory conditions (salinity 7–8 PSU, $11^\circ\text{C} \pm 2^\circ\text{C}$) and fed daily with *Isochrysis* sp. algae. Prior to analysis, the animals were incubated for 8 h in colchicine (0.005% in sea water, 7 PSU). Used in cytogenetic research, colchicine is an antimetabolic agent which arrests mitotic cell division at the metaphase.

For the histological analysis, the soft body of each individual was placed in Davidson's fixative (1200 cm³ sea water, 1200 cm³ 95% ethanol, 300 cm³ formaldehyde, 400 cm³ glycerol and 30 cm³ glacial acetic acid) for 48 hours. Samples were dehydrated through an ascending ethanol series, cleared in xylene and embedded in paraffin wax. Histological blocks were sectioned

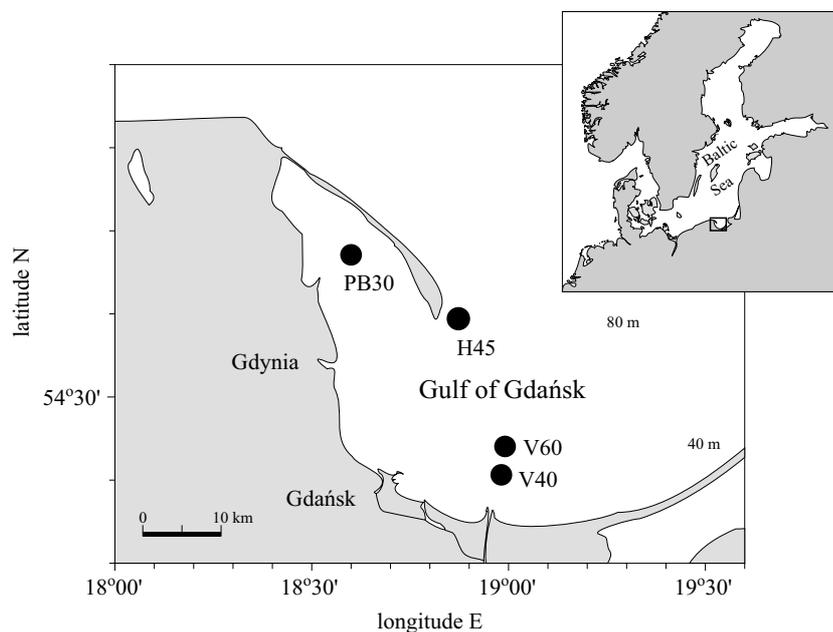


Fig. 1. The sampling area. Note that the station code numbers represent depth in metres

into slices $2\ \mu\text{m}$ thick, which were then stained with hematoxylin and eosin (H&E) and examined under a light microscope to determine the presence of neoplastic cells.

For the cytogenetic analysis, the gills of each individual were dissected out and treated for 45 min in a 0.9% solution of sodium citrate in distilled water, then fixed in a solution containing absolute ethanol and acetic acid (3:1) with four changes of bath: two of 20 min and two of 10 min duration. Slides were prepared from each individual using an air-drying technique (Thiriot-Quiévreux & Ayraud 1982) and stained for 8 min with Giemsa (4%, Sigma) in distilled water (pH 6.8). The counting of mitosis under light microscopy was carried out to confirm whether or not cells in the gill tissue were affected by neoplasia.

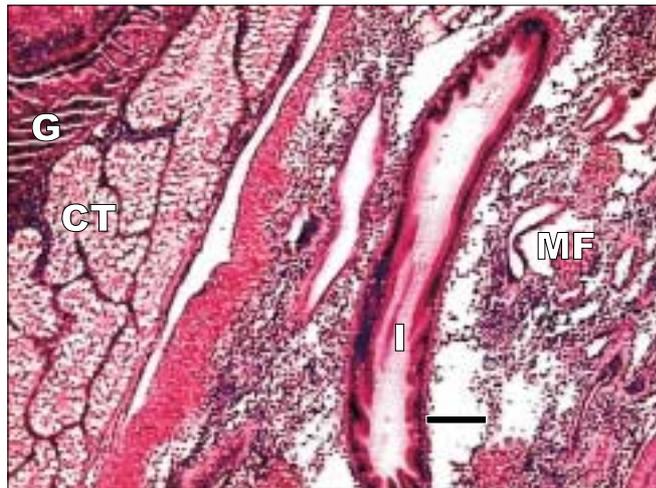
The Kruskal-Wallis test and Friedman's ANOVA, both non-parametric tests, were run to test the relations between sampling season, location, and the occurrence of neoplasia.

3. Results

The prevalence of neoplasia in *M. balthica* from the Gulf of Gdańsk was studied on the basis of histology and cytogenetics. Figs 2a and 2b show histological representations of tissues with cancerous changes. The affected

cells were large, actively proliferating, with pleomorphic nuclei (Fig. 2b). In later stages of the disease, the affected cells often invaded surrounding organs and diffused through the connective tissue into the body wall. Four stages of the disease were identified in clams with neoplasia (Smolarz 2004).

a



b

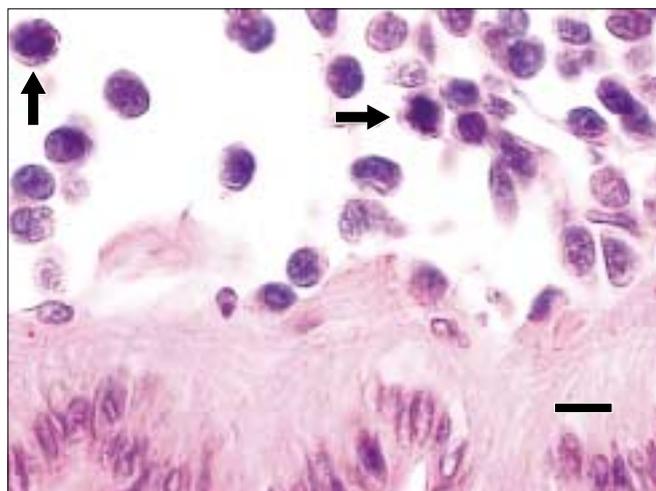


Fig. 2. Histological sections through *Macoma balthica* with neoplasia showing stage IV of the disease. This is manifested by severely damaged gills and uncontrolled proliferation of neoplastic cells; intestine (I), muscle fibres (MF), connective tissue (CT), gills (G), bar 200 μm , H&E (a); large neoplastic cells, actively proliferating with pleomorphic nuclei (arrow pointing upwards) and mitotic figures (arrow pointing right), bar 10 μm , H&E (b)

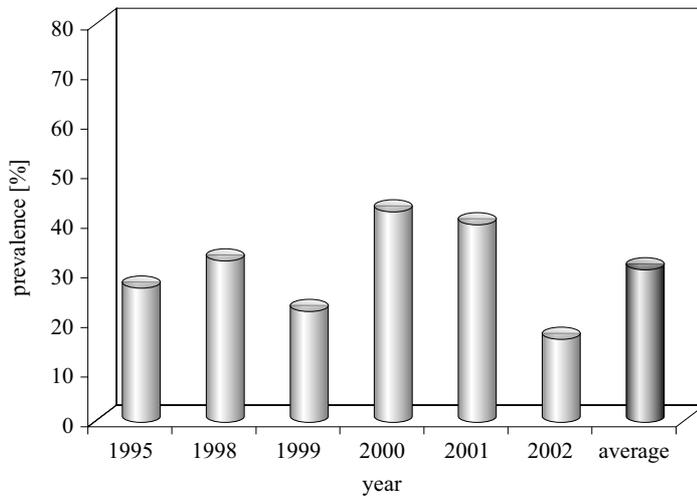


Fig. 3. Long-term changes in the mean prevalence of neoplasia at all sampling stations. Note: the data concerning the years 1995 and 1998 are after Thiriot-Quévieux & Wołowicz (1996, 2001); the data for 1999–02 were collected by Smolarz (2004)

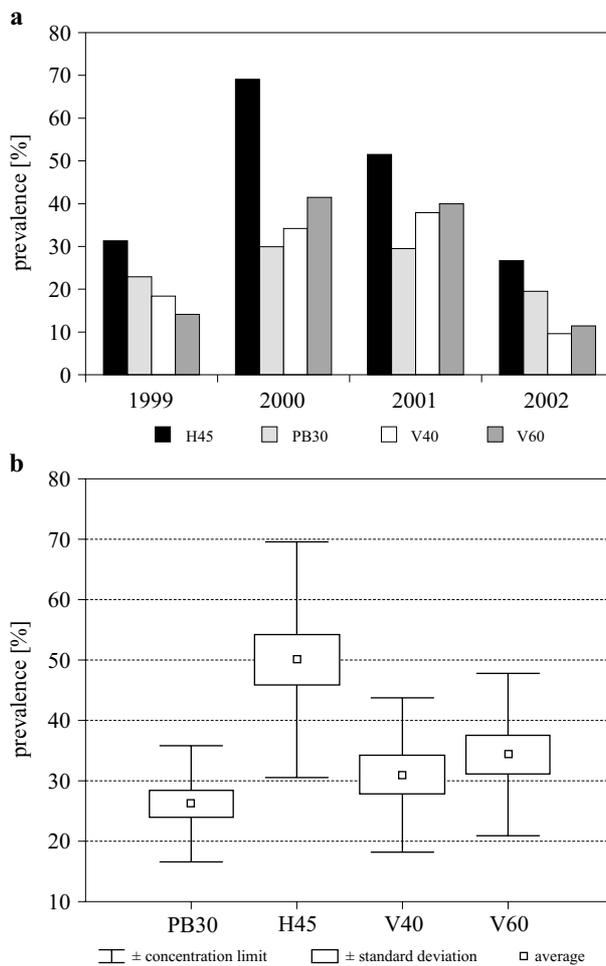


Fig. 4. Long-term changes in the prevalence of neoplasia in different parts of the Gulf of Gdańsk (a), and the mean prevalence of the tumour at the four stations, Friedman's ANOVA, $p < 0.001$ (b)

Previous studies and the present work have shown a relatively high prevalence of the tumour between 1995 and 2002 in the Gulf of Gdańsk (Fig. 3). From 1999 to 2002 a total number of 2320 clams were analysed. Of these, 747 were identified as having neoplasia, i.e. a prevalence of 31.0%. In 1999 and 2002, the average prevalence of the cancer decreased (23.0 and 16.8% respectively), while the highest values were recorded in 2000 and 2001 (40.2% and 42.9%). The prevalence of the disease during the whole sampling period ranged from 6.5% (V40, March 1999) to 73.3% (H45, May and June 2000) and was strictly dependent on the sampling location (Friedman ANOVA, $p < 0.001$) (Figs 4a and 4b). The highest prevalence of the disease occurred at station H45, the lowest at PB30. Monthly studies performed in 2000 and 2001 showed a trend towards seasonal changes in the occurrence of animals with neoplasia. The prevalence of the cancer increased during the warm months as compared to the other seasons (Fig. 5). This trend at two stations – PB30 and V40 – appeared to be much stronger than at the deep stations H45 and V60. However, there was no statistical

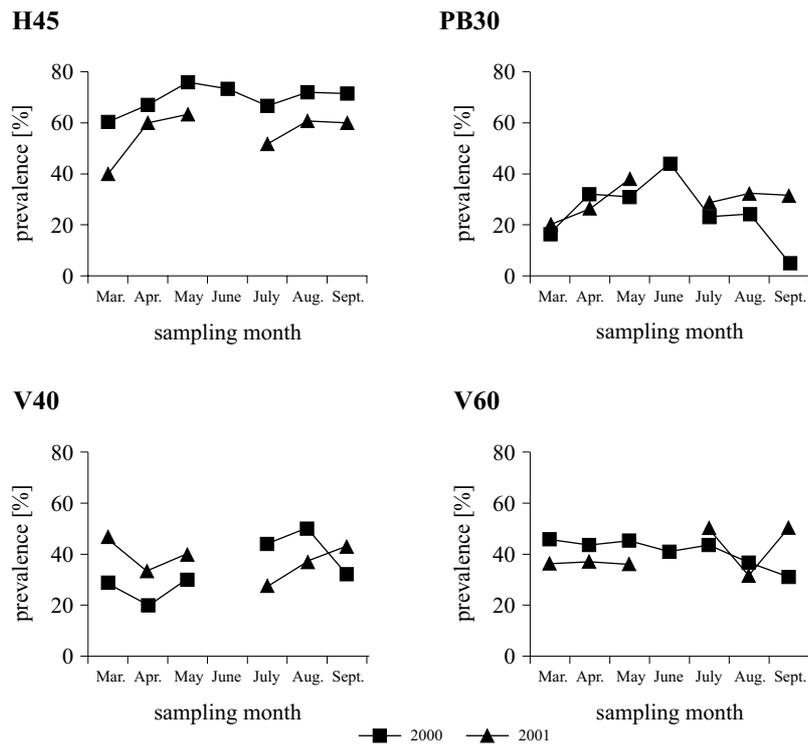


Fig. 5. Seasonal changes in the prevalence of neoplasia at the four sampling stations in 2000 and 2001

correlation between the prevalence of the disease and the sampling season (Kruskal-Wallis test, $p > 0.05$).

Additionally, in the summers of 2000 and 2001, 73 specimens of *Mya arenaria*, 128 of *Mytilus trossulus* and 28 of *Cerastoderma glaucum* from two sampling stations V60 and H45 were analysed cytogenetically. The tumour was observed in the deposit feeder soft-shell clam *M. arenaria* with a mean prevalence of 16.4% (Table 1). In the other bivalve species, *Mytilus edulis* and *C. glaucum*, neoplasia was not recorded.

Table 1. Prevalence of neoplasia in different bivalve species inhabiting the sampling stations

<i>Mytilus trossulus</i>			<i>Mya arenaria</i>			<i>Cerastoderma glaucum</i>	
Number of animals			Sampling station	Number of animals		Number of animals	
studied		with neoplasia		studied	with neoplasia	studied	with neoplasia
2000	55	0	H45	21	4 (19.0%)	15	0
			V60	10	1 (10%)		
2001	73	0	H45	12	2 (16.7%)	13	0
			V60	30	5 (16.7%)		
Total	128	0		73	12 (16.4%)	28	0

4. Discussion

Virtually all bivalve neoplasias have been discovered by histological analysis with a light microscope (e.g. Alonso et al. 2001, Dungan et al. 2002). In our study, the affected clams displayed the same kind of pathological abnormalities as previously described, e.g. nuclear and nucleolar enlargement, hyperchromatism and actively proliferating cells with pleomorphic nuclei (e.g. Elston et al. 1992, Pekkarinen 1993, Smolarz et al. 2003). A high mitotic index and multiple nucleoli were observed in the clams with neoplasia, suggesting a high level of polyploidy and a high proliferation rate.

During the sampling period, the average prevalence of neoplasia in *M. balthica* in the Gulf of Gdańsk was 31%, which allows it to be classified as an epizootic disease (Elston et al. 1992). The disease occurs worldwide,

hence the detailed studies of the cancer's etiology. Initially, viruses, retroviruses and infectious agents were commonly hypothesised as being the main cause of neoplastic changes in marine bivalves (e.g. Farley et al. 1972, Ford et al. 1997). At present, however, environmental carcinogens such as hydrocarbons (Naes et al. 1995), herbicides (Van Beneden et al. 1993) and biotoxins (Roy et al. 1998) have been suggested as risk factors in the development of mutations that could lead to neoplasia. Seriously polluted environments are linked to growing human populations, urbanisation, as well as the industrialisation and intensification of agriculture, and appear to be a reason for the increasing progression of neoplasia in bivalves. As a consequence, the presence of harmful and carcinogenic substances in such ecosystems is more likely and may lead to additional stress for an animal and promotion of the cancer. Studies carried out over many years in the Gulf of Gdańsk have shown that the prevalence of neoplasia in the Baltic clam varied from year to year. Six of 47 clam specimens studied in 1995 exhibited enlarged nuclei and abnormal mitosis, while a further seven displayed moderate abnormalities (Thiriot-Quévieux & Wołowicz 1996). In 1998, Thiriot-Quévieux & Wołowicz (2001) found that 33.0% of the clam populations from the Gulf of Gdańsk were affected by neoplasia. In the present study, an increasing prevalence of the tumour was observed from 1999 to 2001. Station H45 was situated in the deepest part of the gulf close to the Gdańsk Deep. The Gdańsk Deep is known to be an ultimate sink for pollutants and contaminants carried by the Vistula to the estuary. Moreover, in deep regions the clams are subject to adverse conditions, probably as a result of restricted water inflow from the North Sea since 1993. A deficiency of oxygen as well as the presence of hydrogen sulphide have been recorded in the deep parts of the gulf (Janas & Szaniawska 1996). These occurrences may have induced a greater sensitivity to stress. Bacterial outbreaks, which are a part of every anoxic event, probably cause direct bacterial infection of the bivalves (de Zwaan & Babarro 2001). In the autumn of 1997 and 2001 two significant inflows from the North Sea to the Baltic Sea were recorded (Nausch et al. 2003), which probably reduced the prevalence of the disease in 1999 and 2002 to 23.0% and 16.8% of the clam populations respectively. Bivalves inhabiting stations V40 and V60, situated in the mouth of the Vistula, are endangered through exposure to a variety of contaminants, including organic matter, nitrogen and phosphorus compounds, heavy metals (e.g. Cu, Pb, Zn, Cd, Fe, Mn) and organic compounds transported by the river to the estuary (Andrulewicz 1996, Sokołowski et al. 2001). This could explain the high prevalence of neoplasia at both these stations. Furthermore, sediments taken from the four stations were classified as toxic (test *TOXAlert*) according to

the Helsinki Commission's Recommendations (HELCOM). The Wilcoxon test ($p < 0.001$) highlighted a strong correlation between sediment toxicity and the prevalence of neoplasia at all stations. The highest toxicity, like the highest prevalence of the cancer, occurred at stations H45 and V60, while the lowest occurred at PB30. There was no evident cause-effect relationship between heavy metal content and the prevalence of neoplasia (Smolarz 2004), neither were elevated levels of polychlorinated biphenyls (PCBs) measured in the sediments at any of the four sampling stations. In the Gulf of Gdańsk, the highest concentration of PCBs was noted in the neighbourhood of two stations – H45 and V60 (Konat & Kowalewska 2001). Moreover, Falandysz et al. (1998) noted the presence of polychlorinated naphthalenes (PCNs) in different fish species and other organisms collected from the south-western part of the gulf.

A trend towards seasonal changes in the prevalence of the tumour was observed, a larger proportion of individuals being affected in the warm months. This may be attributed to a temperature- and spawning-induced increase in metabolic activity of the bivalves at this time of year. Thus, the degree of exposure and the rate of uptake of toxic compounds from the environment is probably stimulated by the plentiful food supplies available and more intensive physiological activities (feeding, respiration, digesting). From March to September (stations H45 and V60), clams with the highest prevalence of neoplasia co-occurred with those in late stages of gametogenesis. Moreover, it has been demonstrated that low water-temperatures inhibit the development of neoplasia (Appeldoorn & Oprandy 1980) and lead to a reduction in mortality rates (Brown et al. 1977). Off the coast of Spain, the highest prevalence of gonadoblastoma in *Mytilus galloprovincialis* was recorded between April and June (Alonso et al. 2001). Double neoplasia peaks with the second maximum in the warm months have been reported (Cooper & Chang 1982, McLaughlin et al. 1996). Also, very high levels of neoplasia have been noted between October and March (Rasmussen 1986, Brousseau 1987) and very low ones between April and August (Leavitt et al. 1990). One can conclude that, on a global level, there is no general rule linking the prevalence of neoplasia and the sampling season.

Wołowicz et al. (2003) noted a new behavioural aspect in neoplasia etiology, associated with the presence of neoplasia in the facultative feeders *M. balthica* and *M. arenaria*. Moreover, neoplasia may indicate a decrease in the immunological resistance of sedentary benthic invertebrates to genotoxins. The disease has a negative impact on reproduction as manifested by a decrease in reproduction (Barber et al. 2002), and on potential genetic heredity (Depledge 1998). It can also lead to mortality

rates of up to 80% (Elston et al. 1992) and as such presents a growing danger to benthic populations. As the disease appears to be most likely attributed to indirect effects of pollution, the ecosystem of the Gulf of Gdańsk provides an ideal environment for testing cause-effect relationships between pollutants and their biological effects. Further studies may help to elucidate the environmental significance of the tumour and to assess its biological risk at the population and ecosystem levels.

Acknowledgements

Our sincere gratitude is extended to Dr. T. Renault from the IFREMER station in La Tremblade (France) for his help in performing the histology examination of clams, and also to A. Mitwer (Northstream, Sweden) for correcting the English text.

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