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THE VERTEBRAL ABNORMALITIES IN EELPOUT *ZOARCES VIVIPARUS* (LINNAEUS, 1758) (PISCES, ZOARCIDAE)

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ABSTRACT

The different vertebral abnormalities in eelpout are described for the first time. Population differences in the malformation frequency for eelpout have been found. The high incidence of vertebral abnormalities have been recorded in eelpout from the Wadden Sea (North Sea) as compared to the samples from the Chupa Inlet (White Sea) and Gulf of Finland (Baltic Sea). The degree of malformations in fishes correlates with the levels of various water toxicants in the same areas.

Key words: Baltic Sea, eelpout, North Sea, vertebral abnormalities, *Zoarces viviparus*

РЕЗЮМЕ

В статье впервые описаны позвонковые аномалии у бельдюги. Обнаружены популяционные отличия по частоте встречаемости рыб с такими скелетными нарушениями. Наибольшее число рыб с позвонковыми аномалиями отмечено у бельдюги из юго-восточной части Северного моря по сравнению с выборками из Финского залива (Балтийское море) и губы Чупа (Белое море). Полученные данные обсуждаются в связи с уровнем загрязнения прибрежных морских акваторий.

INTRODUCTION

Biological indices are necessary for assessing the effects of global climatic and man-induced environmental stresses on fish species and other marine organisms. There are different methods which are now being used for these purposes. One of them is the detection and description of abnormalities in certain indicator species which are sensitive to specific stress factors. Eelpout *Zoarces viviparus* (Linnaeus, 1758) is the common fish species in the North, Baltic, Norwegian and White seas. Since eelpout is characterized by ovoviviparous reproduction and relatively non-migrating behaviour, it is a very convenient object to estimate of the influence of environmental stress on the population parameters of fishes from different regions. In 1995 the X-ray analysis of eelpout from the Wadden Sea was performed to count the number of rays of unpaired fins and vertebrae. During these osteological examinations the vertebral abnormalities were found in several specimens from this part of the North Sea. It was of interest to find out whether these defects occur in eelpout from other regions and are there any population differences in proportion of malformed fishes. The aim of this study was to investigate the degree of skeletal abnormalities in eelpout from different parts of distribution area in relation to their ecological conditions.

MATERIAL AND METHODS

The sampling area and positions are shown in Fig. 1. The fishes were caught by beam-trawl (July 1995, East Frisian Wadden Sea, North Sea, Germany), on hook and line (April 1996, eastern part of the Gulf of Finland, Russia) and by traps (July-August 1996, Chupa Inlet, Kandalaksha Bay, White Sea,

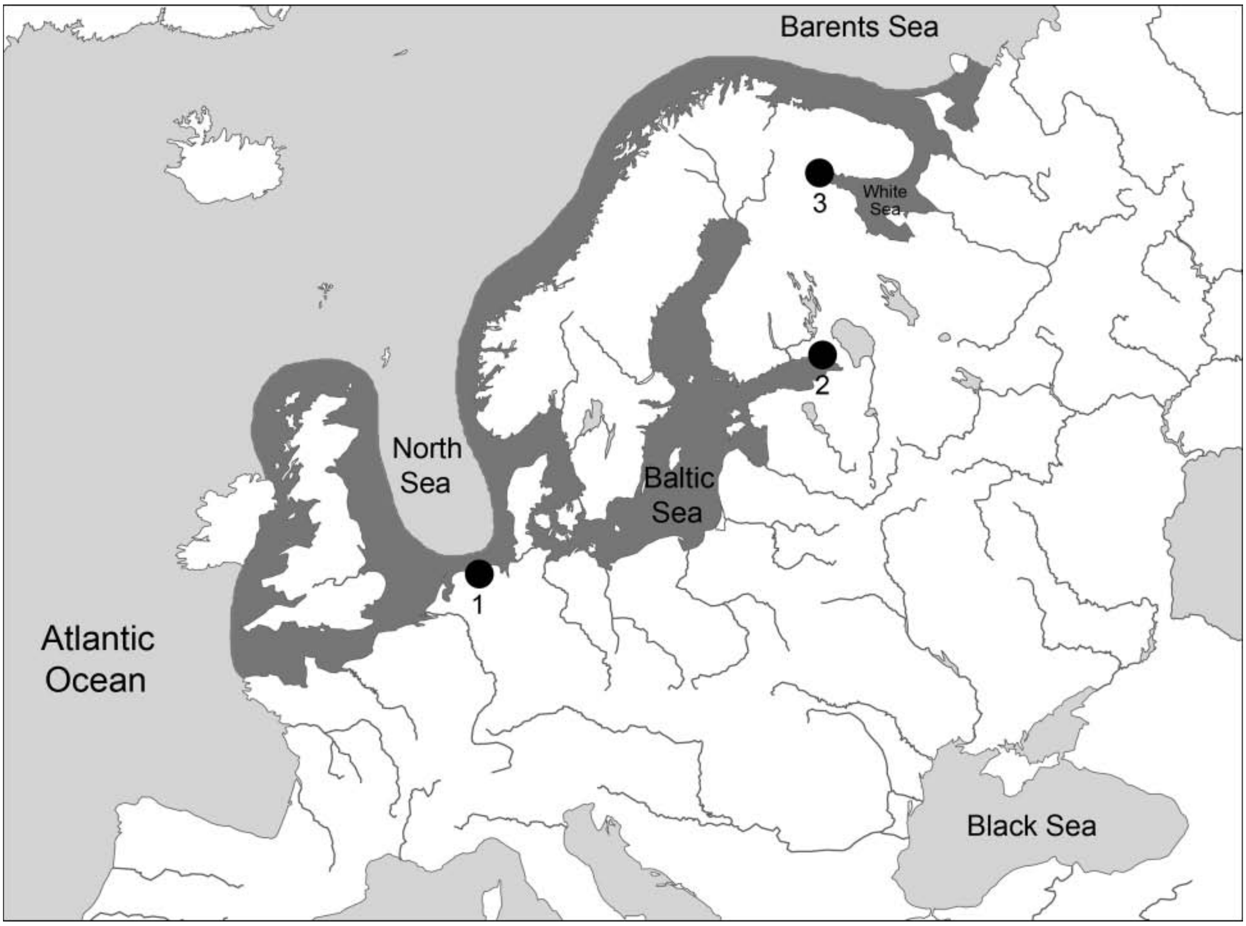

Fig. 1. Distribution range (based on Whitehead et al. 1986) and sampling locations of eelpout: 1 – Wadden Sea/North Sea ; 2 – Gulf of Finland/Baltic Sea; 3 – Kandalaksha Bay/White Sea.

Russia). The fishes of all sizes were fixed in alcohol and then X-rayed. The radiographs were checked for occurrence of defects under binocular. Only distinct abnormalities in the vertebral column were included into consideration. A total of 54 specimens of eelpout (length from 10.2 to 27.0 cm) from the Wadden Sea, 99 (13.5–21.7 cm) – from the Gulf of Finland and 44 (17.4–23.7 cm) – from the White Sea were used in this study. For more information on vertebral abnormalities in fish species from the Wadden Sea, 81 juvenile (0+-old age) specimens of shorthorn sculpin *Myoxocephalus scorpius* (Linnaeus, 1758), encountered in the same beam-trawl (July 1995, East Frisian Wadden Sea, North Sea, Germany), were X-rayed and the pictures were checked for occurrence of defects.

RESULTS

The results show that the vertebral abnormalities occur in eelpout from all localities where the samples were taken. The visible spinal abnormalities resembling scoliosis, lordosis and kyphosis, were not registered. The observed vertebral defects differ morphologically and can be classified into following groups: 1) deformation of vertebrae (compression; distortion); 2) fusion of two or several vertebrae (Fig. 2). Usually one type of the above-noted abnormalities was detected in particular individual. Only two specimens were found with two different types of abnormalities in the vertebral column. It is worth noting that these two fishes were caught in the Wadden Sea. Malformations occurred in both truncal

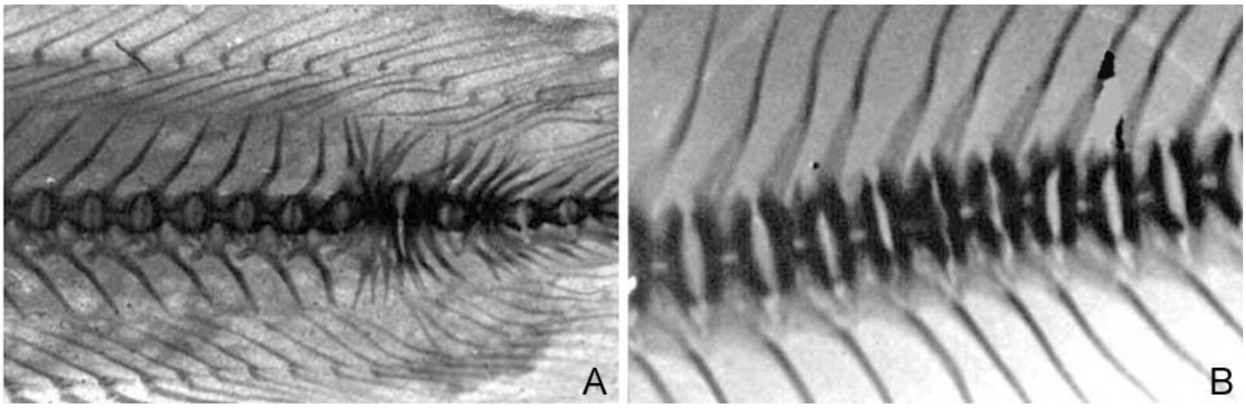

Fig. 2. The vertebral abnormalities in eelpout: A – fusion of several vertebrae; B – compression of vertebrae.

and caudal regions of the vertebral column of fishes. Deformed and normal vertebrae differed in size and shape. The type and degree of vertebral defects varied between fishes. Observed pathological changes in vertebrae usually were associated with deformations of the spines and arches. The significant population differences in the frequency of vertebral abnormalities were recorded between the populations from the southern part of the North Sea and the White Sea (t-test, $p<0.01$). A considerable part of eelpout population from the Wadden Sea had the vertebral abnormalities (24.1% of fish), while in the White Sea malformed fishes took only 4.5%. The analysis of radiographs of fishes from the Gulf of Finland indicated that the malformation frequency had an intermediate value (7.1%), but did not differ significantly from that of the White Sea population. The difference between samples from the Gulf of Finland and southern part of the North Sea was statistically significant ($p<0.05$). In contrast to eelpout shorthorn sculpin had a lower malformation frequency in the vertebral column – 4.9% of the analysed fishes had the defects. All observed abnormalities were deformations of vertebrae, namely distortions. The absence of other defects seems to be connected with the small number of malformed fishes in this species. The significant spinal deformities have not been recorded in this species either.

DISCUSSION

Skeletal deformities, one of the most serious pathological effects in fishes, are well known for many fish species from different areas of the world. Such abnormalities may result from various damage to eggs, embryos, larvae, or to cytotoxic effects on different life stages of fish and authors discussed a possible association between them and environmental stress (Valentine and Bridges 1969; Dahlberg 1970; Dawson 1971; Bengtsson 1975; Valentine 1975; Sindermann 1979; Malabarba et al. 2004; Villeneuve et al. 2005; Boglione et.al. 2006; etc.). Among regions involved in our study, the skeletal anomalies have been recorded for some fish species from the North and the Baltic seas (Ford and Bull 1926; Kandler 1932; Wunder 1971; Kamp 1977; Bengtsson 1980; Dethlefsen 1980; Christensen 1980; Dethlefsen et al. 1996), while no data exist for fishes from the White Sea. The first quantitative data on fish diseases were obtained by Dethlefsen (1980) for southern part of the North Sea (the German Bight). The investigation of 31 species (without eelpout and shorthorn sculpin) showed that skeletal abnormalities, classified as dwarfism, backbone curvatures and bullnoses, occurred in some species from this region. These deformities were found mainly in cod *Gadus morhua* Linnaeus, 1758 (0.8–1.9% abnormal fish) and only in a few number of other species. The cod caught at the inner part of the German Bight, i.e. the estuary of the river Elbe near Cuxhaven, consisted of 10 to 20% of deformed fishes (Wunder 1971). There were deformities of head and spinal abnormalities caused by compressions and synostosis of vertebrae. Different malformations in the North Sea pelagic fish embryos were found during the period 1984–1995 (Dethlefsen et al. 1996). Christensen (1980) found such malformations as shortening and distortion of the spine in fish species from Danish coastal waters.

The different spinal and vertebral abnormalities were recorded for fourhorn sculpin *Triglopsis quadricornis* (Linnaeus, 1758) from the Baltic Sea (Bengtsson 1980; Bengtsson and Bengtsson 1983). Our data present the first results of X-ray analysis of skeletal anomalies in eelpout and shorthorn sculpin.

Among environmental factors – either natural or man-induced – which may produce such biological effects the water pollution has been considered as most important (Valentine 1975; Sindermann 1979, 1984; Bengtsson 1979). The association between different skeletal malformations in fishes and water pollution has been suggested to be very complicated and dependent on the contaminants, their concentration in the water column, fish ability to excrete or accumulate them and probably other factors. At this point the main idea is that increasing of pollution beyond a threshold level should result in the increasing of such sublethal effects in fish population. Thus, Bengtsson (1980) found that the fraction of fishes with spinal and vertebral defects in fourhorn sculpin collected in the Baltic Sea (Swedish coast) and Gulf of Bothnia ranged from 4 to 38% with higher values in areas heavily polluted by pulp and metal processing industries. Dethlefsen (1980) found the highest average skeletal deformities and infection rates for cod in the near shore polluted areas of the German Bight in comparison with the areas up to 120 miles offshore.

In present study the eelpout from southern part of distribution area (the Wadden Sea) was found to have higher frequency of abnormal fishes as compared to the eelpout from the White Sea and Gulf of Finland. These regions differ greatly in many environmental aspects and observed population differences in malformation frequency might be explained by influence of environmental abiotic factors, habitat-specific natural causes (dietary deficiencies, traumas, parasitic infections, etc.) or pollution. In order to understand what type of stress may produce the high value of malformation frequency in eelpout from the Wadden Sea each of possible factors should be discussed.

The first hypothesis assumes that there is a stress influence of some unknown environmental factor(s) on eelpout in the Wadden Sea, which lead to appearance of fishes with vertebral abnormalities. This influence should be supposed to be strong enough, because the proportion of malformed fishes constitutes 24.1% in the population, i.e. every fifth fish had the vertebral defects. On the other hand, following this hypothesis, the lowest frequency of malformed fishes (4.5%) in the White Sea population could suggest that the hydrologic conditions at the northern part of distribution area are closer to optimal for eelpout. Apparently, it would be logical to ignore such conclusion. Moreover, this low value of the malformation frequency may be regarded as close to the normal level of this parameter ("background noise") in unaffected populations, reflecting the response variability of heterogeneous fish population to the degree of irregularity of abiotic factors (variation in temperature, salinity, currents, etc.). It is interesting that the lowest value of malformation frequency in four horn sculpin from the Baltic Sea also was 4% (Bengtsson 1980). The frequencies of vertebral abnormalities in wild populations of Pacific salmon – sockeye, pink and chum salmon – constituted 2.8%, 3.3% and 2.9% respectively (Gill and Fisk 1966). For another types of skeletal damage the "normal" level of malformations from natural perturbations might be different. Many studies have been conducted on the influence of various natural factors on the morphological characters of fishes at the early life stages. Experiments showed that some environmental stressors (high and low temperatures, low oxygen, low salinity) and their combinations can cause the morphological developmental defects in fish embryos (Stockard 1921; Orska 1957, 1962; Alderdice et al. 1958; Garside 1959; Seymour 1959; Westernhagen 1970; Alderdice and Velsen 1971; Turner and Farley 1971; Pavlov and Moksness 1997; Sfakianakis et al. 2004; Cook et al. 2005; Meeuwig et al. 2005). These embryonic malformations are vertebral deformities and morphological deviations from normal development at the different stages. There are also some evidences from field studies for an involvement of temperature and salinity changes in the occurrence of skeletal anomalies in fish (Hubbs 1959; Orska 1962). For our purposes it is necessary to note that eelpout is ovoviviparus fish and sensitivity of embryos in ovaries to stress factors might be lower as compared to the pelagic fish embryos at the early stages. Probably the impact of environmental changes (for instance, temperature) is operating in the Wadden Sea region and further studies should firstly concentrate on the juvenile eelpout (0+-year old) to test it, however now we have no data supporting the first hypothesis.

The second possible explanation suggests that natural causes are responsible for vertebral deformities in eelpout. It is well known, that some natural causes (traumas, dietary deficiencies, parasitic infections, microbial diseases) can lead to the morphological malformations in fishes (Kent et al. 2004; Yokoyama et al. 2005; Murcia et al. 2006; Lall and Lewis-McCrea 2007; Murphy et al. 2007). As to possible traumatic origin, the features of vertebral abnormalities in eelpout (fusion, deformation of vertebrae) can not be explained by mechanical impacts. There were no scars on the skin of malformed fishes. So it is unlikely that observed abnormalities are associated with this kind of damage. We have neither data on parasitic press or nutritional deficiencies for eelpout which can be used to support this hypothesis.

The next working hypothesis assumes that high value of malformation frequency in eelpout from the Wadden Sea is an effect of the pollutants. This idea is supported by the data about higher frequency of malformation which was recorded in fishes from more polluted areas as compared to the clean ones. Obviously, the North and the Baltic seas are heavily polluted as compared to the White Sea. Polychlorobiphenyls, polycyclic aromatic hydrocarbons, organochlorine pesticides, heavy metals and other contaminants have been found in water, sediments, benthic invertebrates and fishes from the North and the Baltic seas (ICES 1977; Schmidt 1980; Falandysz and Lorenc–Biala 1984; Larsen and Pheiffer 1986; Cofino et al. 1992; Protasowicki 1991, 1992; Olszewska et al. 1994; Mathieson et al. 1996; Falandysz et al. 1998; Atuma et al. 1998; Vuorinen et al. 1998; Herut et al. 1999; Kress et al. 1999; Schmolke et al. 1999; Ciereszko 2002; Napierska and Podolska 2005; etc.). This ecological situation is closely connected with the promoted industrial development and intense human activities in these regions. Sublethal effects of pollution usually occur in coastal and semi-closed areas of the sea. In the open sea the wave action, currents and chemical alteration rapidly reduce the harmful effects of many contaminants. Similarly, the most polluted areas of the North Sea are the coastal shallow waters, in particular the Wadden Sea (Kersten et al. 1988; Lohse 1990). In 1990 the multidisciplinary field survey was carried out in the German Bight to compare available biological effects monitoring techniques for marine pollution. The chemical analysis demonstrated that the concentrations of total hydrocarbons, polychlorobiphenyls, organochlorines and some trace metals, determined in sediments and liver of dab, *Limanda limanda* (Linnaeus, 1758), steadily declined along the German Bight transect from Elbe/Weser Plume to the eastern part of the Dogger Bank (Cofino et al. 1992; Chapman 1992). The different molecular, biochemical and cellular effects of toxicologically induced degenerative changes in the liver and other tissues were found to exist in dab from most contaminated onshore stations near to Elbe and Weser estuaries (Eggens et al. 1992; Galdani et al. 1992; Moore 1992). The frequency of malformations and abnormalities in dab embryos in the plankton was also elevated in inshore contaminated sites (Cameron and Berg 1992). Various biological effects (immunological, physiological, metabolic, parasitological, cytogenetic) were found in flounder *Platichthys flesus* (Linnaeus, 1758) from the polluted coastal locations in the German Bight (Broeg et al. 1999; Schmidt et al. 2003; Skouras et al. 2003; etc.). Developmental disorders were found in larvae of eelpout from the German and Swedish Baltic coastal waters (Gercken et al. 2006). Malformation levels were significantly lower (range 0–6%) at the Swedish stations in comparison to all German stations (range 50–90%). The different deformations of larvae (spiral or bend shapes of the spinal axis, cranio-facial defects, eye lesions or loss of eyes) were also detected in the broods of eelpout, sampled in some shallow polluted Danish fjords (Strand et al. 2004). The authors suggest that impaired larval development is associated with environmental pollution in coastal waters. Probably similar causality between various sublethal effects and pollutants occur for other fish species from this area. Many experimental data demonstrate the connection between skeletal abnormalities in fish and water pollution at early developmental stages and in fish exposed to different contaminants (Westernhagen et al. 1974; Bengtsson 1975; Mehrle and Mayer 1977; Rosenthal and Alderdice 1976; Couch et al. 1977; Dethlefsen et al. 1996; Jagadeesan 2005; Holm et al. 2005; Muscatello et al. 2006; Yamauchi et al. 2007; etc.). It is neccesary to note that fusion and compression of vertebrae in fishes were recorded among various skeletal deformities induced by toxicants (Kasherwani et al. 2007; Louiz et al. 2007). Taking into account numerous data on pollution-induced stress on dab, flounder and other marine organisms from the southern part of the North Sea, we suppose that prevalence of abnormalities in adult eelpout from the Wadden Sea is associated with environmental contamination of coastal waters in this region.

Remarkable species-specific differences in the occurrence of malformation frequencies between eelpout and shorthorn sculpin from the Wadden Sea could be interpreted in relation to ecology of these two species. These species belong to different families and differ greatly in their life histories. Eelpout is a resident ovoviviparus fish species of the Wadden Sea, which is found almost exclusively in the inshore waters between islands and mainland, so it appears to be non-migratory species. Shorthorn sculpin is a common fish species in the southern part of the North Sea, which seems to have small-scale migration. The juvenile fish (0+-year old) inhabit the Wadden Sea area from June–July up to October–November, while the fish older than 1 year live usually offshore islands and in the deep waters of the North Sea. However, they could migrate to shallow coastal waters for feeding and probably for spawning. The longevity for shorthorn sculpin is 3–4 years in this region, the fishes 4+-5+-years old are very rare. Besides, shorthorn sculpin is a species having planktonic larvae stage in life cycle. Taking into account this information, we can suppose that eelpout has higher probability to accumulate the pollutants from water (or food organisms) and to be affected by them because it resides in the Wadden Sea during the whole life cycle as compared to the shorthorn sculpin, which feeds in this area for some months per year only. In other words, the duration of exposure under contaminants will be longer for eelpout. It could be also suggested, that eelpout undergoes sustained stress, e.g. that caused by continuous pollution. The argument that factor maintaining the low malformation frequency in shorthorn sculpin is the elimination of malformed fishes from population via selection seems to be not serious. The observations on the fourhorn sculpin in the Baltic Sea indicated high proportion of fishes with spinal and vertebral abnormalities (up to 54%) in polluted areas (Hansson et al. 1984). It was also shown that these abnormalities had no essential ecological significance on the diet and stomach content of fishes (Hansson et al. 1984). The possible ecological significance of these sublethal effects is not well understood, but it seems they have no obvious effects on survival potential of malformed fishes. Because shorthorn sculpin and fourhorn sculpin belong to the same family Cottidae, similar biological effects for shorthorn sculpin might be expected.

In summary we can conclude that while local environmental impacts on eelpout in the coastal waters of the Wadden Sea are apparent, but the causal stressors remain unknown. Taking into consideration literature data on the effects of environmental contaminants on fish in the North Sea and other coastal marine waters, we suppose that high incidence of vertebral anomalies in eelpout is associated with the increased local pollution impact in this region. Although there is reasonable evidence for localized pollution effect on eelpout, observed anomalies may also be due to other factors, because the effects of pollution-induced stress cannot be separated clearly from the effects of natural environmental changes, like temperature and salinity. Clear and confirmed association of skeletal abnormalities in eelpout from the Wadden Sea with contaminants still require additional research. It is also necessary to investigate the possible connection of these effects with environmental variables.

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