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Short communication

Microbiological studies of the round goby (*Neogobius melanostomus*) from the Puck Bay, southern Baltic Sea

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Abstract

In summer 2017 numerous dead round gobies (*Neogobius melanostomus*) and individuals covered with white coating were observed in the Puck Bay. The aim of our research was to determine the microbiological composition of the round goby from the Puck Bay, focusing on the presence of pathogens. Bacteria were identified by biochemical methods and, by sequencing of 16S rRNA. The dominant bacterial species were *Shewanella baltica*, *Pseudomonas* spp. and *Aeromonas* spp. - opportunistic pathogens, commonly present in many fish species, which may become harmful for the organism in unfavorable conditions. It was the first trial to determine the composition of the bacterial flora of *N. melanostomus* from that area.

Key words: round goby, *Neogobius melanostomus*, bacteria, Baltic

Introduction

The round goby (*Neogobius melanostomus*) is one of the so-called 'non-indigenous estuarine and marine organisms' (NEMOs) in the Gulf of Gdańsk. The first individuals were observed in 1990, then in Puck Bay and eastwards to the Vistula tributary by 1997, and westwards to the river Odra estuary. Most individuals in the Gulf of Gdańsk are 8 to 18 cm in length, with a lifespan of three to four years (Sapota 2004). It occurs in shallow waters (from <1 m to about 20 m of depth), where it always occupies the benthic zone independently of the substrate. Its preference is for hard bottoms, but in the Gulf of Gdańsk it also successfully inhabits sandy substrates and even anthropogenic remains. The size of the population is large (Sapota 2004) and the round goby have become the dominant demersal fish species in shallow water in the Gulf of Gdańsk, competing for food items with commercially important benthic fish species like flounder, *Platichthys flesus*.

At the turn of June and July 2017, scientific divers and fishermen observed in part of the Gulf of Gdańsk (the southern Baltic Sea) the round gobies covered with white coating. Dead fish on the sea bottom were also reported. Fish were described as being covered with a white fungal or bacterial coating, therefore the studies were focused on the determination of the bacterial flora of *N. melanostomus* from the southern Baltic Sea, that was suspected as the reason of mortality. The aim of our research was to check whether this phenomenon could be caused by bacterial disease as until now there was lack of general knowledge about bacteria species composition of round goby from the southern Baltic Sea.

Materials and Methods

Towards the end of June 2017, scientific divers observed dead *N. melanostomus* at two sites in the Gulf of Gdańsk: Jama Kuźnicka (4 m depth) and Mechelinki (8.7 m depth). Additionally, at Mechelinki, some specimens with a skin disorder described as a white coating were seen swimming without any other symptoms of disease or abnormality. Immediately after the observations at Mechelinki, water samples were collected at 2 m intervals, from the bottom to the surface, for environmental parameter measurements (oxygen, salinity, pH, temperature). Site photographs were taken for documentation purposes and to obtain the number of dead fish (Fig. 1 a and b). Fish samples were collected *in situ*, however the level of decomposition of the individuals made it impossible to conduct microbiological analysis. Additional sampling of the round goby was organized in that area. Fish were caught as by-catch

by fisherman in Puck Bay at the Mechelinki site. However, fish were not covered with white coating, that might be the result of the sampling method (commercial catch). In total, 35 round goby individuals were analysed. Standard ichthyological analysis was performed and Fulton's body condition factor, K (Fulton 1904), was calculated as follows: $K = (W/L^3) * 100$, where W is the total weight [g] and L is the total length [cm] of the fish.

Samples for microbiological analysis were collected during standard ichthyological analysis. Tissue samples from the fish kidney were taken aseptically, diluted in sterile phosphate-buffered saline (PBS) at a ratio of 1:1 (w/v), homogenized, and then inoculated onto agar supplemented with 5% horse blood (BA) (Biomed), trypticase soy agar (TSA) (bioMérieux), and Cytophaga agar (CA) (Biomed). Agar plates were then incubated at 27°C for 72–96 h (BA, TSA), and at 15°C for 5–7 days (CA). The dominant types of bacteria were reisolated and then pure cultures were examined for morphology and subjected to Gram staining. Biochemical identification and enzymatic properties were performed using the API and VITEK2 systems (bioMérieux) according to the manufacturer's instructions. Each time when biochemical identification was unclear, sequencing of 16S rRNA was carried out as described previously (Pękała et al. 2015, Pękała et al. 2018).

Results and Discussion

Sixteen dead round gobies in an area of approximately 4 m² were counted using photographs taken by divers at the Mechelinki site. The basic seawater parameters were recorded at the site during the same dive: concentration of oxygen 9.3 - 9.6 mg/l, salinity 7.6 - 8.1 PSU, pH 8.0 - 8.1; temperature 17.2 - 17.7°C, conductivity 11.4 - 12.1 mS/cm. Measurements did not reveal any anomalies in the water column, compared with average values for that season and area (Dybowski et al. 2019).

During subsequent, additional sampling, 35 round gobies were collected, most of which were males (91%). The mean length of males (13.4 cm) was smaller than that of females (14.7 cm). Fulton's body condition factor, K , had higher values in the case of males (mean 1.41) compared with females (mean 1.24). Both observation and our sampling took place in the summer time, at the turn of June and July 2017. In the Gulf of Gdańsk round goby spawning takes place from the beginning of May to the end of September (Sapota 2004). The weakened condition of the fish might be explained by fighting and competition during the breeding period. In case of round gobies higher mortality after reproduc-

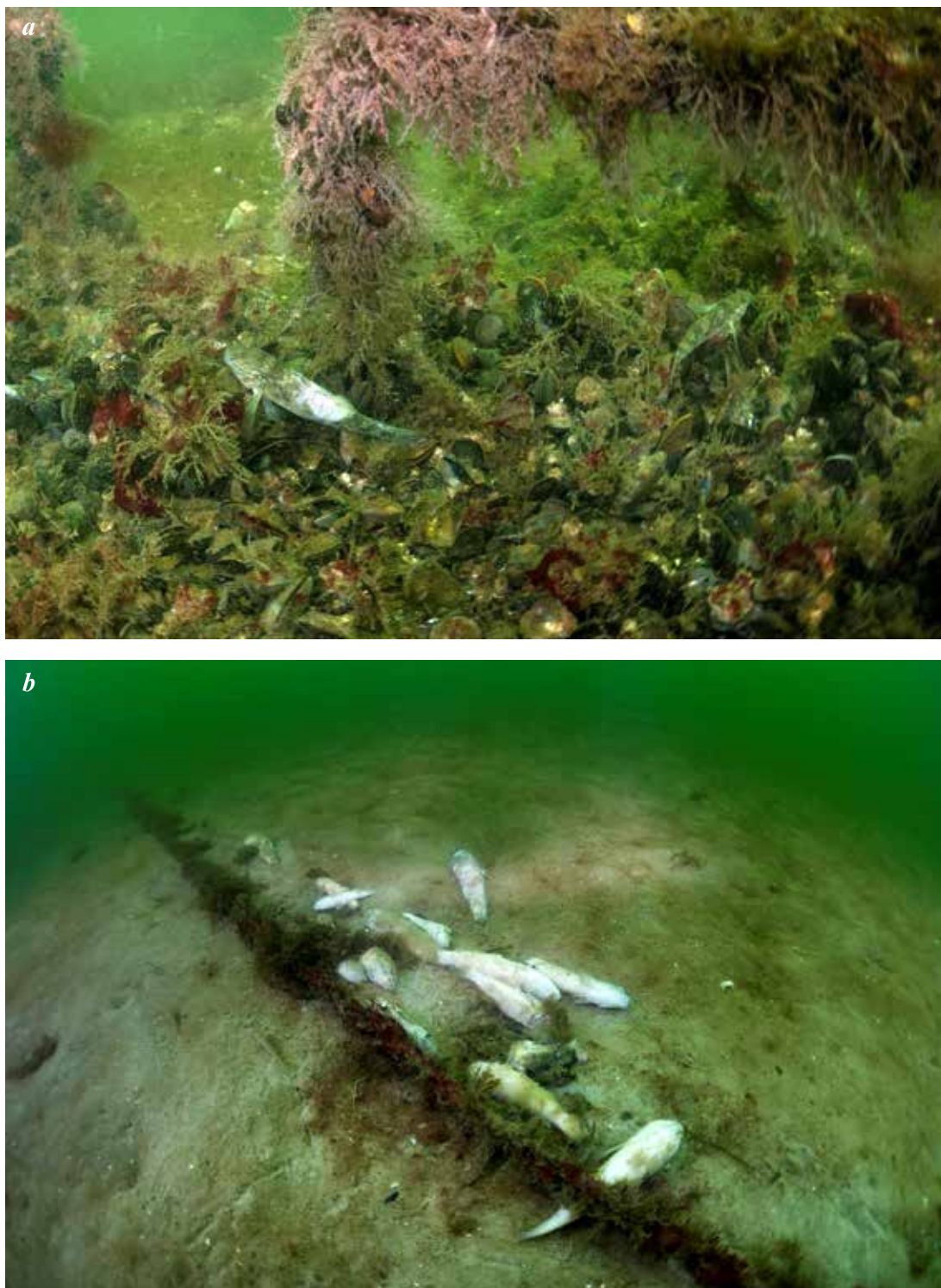


Fig. 1. Examples of the round goby (*Neogobius melanostomus*) from the Gulf of Gdańsk. a) live individual with an abnormal white coating; b) numerous dead fish (photos: P. Balazy)

tion is observed: male round gobies generally survive a single reproductive season. Taking all this into account it is most likely that the dead individuals observed on the bottom are the result of increased natural mortality associated with the spawning season. The stress related with reproductive period, described above, is in line with weakening of the fish organism

and greater susceptibility to pathogens. That explains the observed secondary infection symptoms (white coating).

Microbiological analysis showed the following bacteria strains: the highest prevalence was noted in case of *Pseudomonas* spp. (55%). *Shewanella baltica* was identified in 27% of isolates and *Aeromonas* spp.

in 12% of isolates. These species are commonly present in many fish species and are known as opportunistic pathogens, which may become harmful for the organism in unfavorable conditions. Bacteria from *Shewanella* group are the etiological agent of shewanellosis, disease with various clinical signs involving skin lesions accompanied by fish mortality (Pękala et al. 2015). Other bacteria strains constituted only 6% of all analysed isolates and were represented by: *Vibrio anguillarum*, *Carnobacterium* sp., *Staphylococcus epidermidis*, *Alcaligenes faecalis*, *Rhodococcus erythropolis*, *Acidovorax* sp. and *Acinetobacter* sp. *V. anguillarum* inhabits the estuarine and marine coastal ecosystems worldwide, it causes a hemorrhagic septicemia, leading to high mortalities and economic losses in aquaculture (Toranzo et al. 2017). The genus *Carnobacterium* contains nine species: e.g. *C. maltaromaticum* can be a fish pathogen, although carnobacteria are also suggested as probiotic cultures for use in aquaculture (Leisner et al. 2007). *S. epidermidis* has been reported as a pathogen in some marine and freshwater fish, but the disease is induced by a sudden rise in water temperatures or other stress factors in the aquatic environment (Kubilay and Uluköy 2004). *A. faecalis* was isolated from a coastal, marine fish-rearing unit, nowadays used as probiotics in aquaculture (Wang et al. 2020). *R. erythropolis* may cause pathological changes of fish in aquaculture (Olsen et al. 2006). Strains belonging to *Acidovorax* sp. are rarely present as the dominant species and no pathogenicity was reported in case of aquatic animals (Chun et al. 2017). The role of *Acinetobacter* in fish pathology is not well understood. However, two species were identified as a factor causing the damage of the tissue of fish species cultured in Poland (Kościńska et al. 2014). The present results complement the lack of the general knowledge about the bacteria species composition of the round goby from the southern Baltic Sea. Nevertheless, no bacterial disease (specific sickness) was confirmed during our analysis. However, it should always be remembered that environmental factors as well as the immune status of the fish are of key importance in the pathogenesis of bacterial fish diseases. Therefore, microorganisms are very often responsible for the secondary infection of organisms in poor condition.

It is worth to emphasize, that our microbiological analysis is the first attempt to describe the composition of the bacterial flora present in the *N. melanostomus* from the southern Baltic Sea. The present results complement the lack of the basic knowledge about the presence of bacteria in marine organisms in that geographic area and constitute a reference material for the future more thorough research.

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