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Helminth species richness of introduced and native grey mullets (Teleostei: Mugilidae)

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ABSTRACT

Quantitative complex analyses of parasite communities of invaders across different native and introduced populations are largely lacking. The present study provides a comparative analysis of species richness of helminth parasites in native and invasive populations of grey mullets. The local species richness differed between regions and host species, but did not differ when compared with invasive and native hosts. The size of parasite assemblages of endohelminths was higher in the Mediterranean and Azov–Black Seas, while monogeneans were the most diverse in the Sea of Japan. The helminth diversity was apparently higher in the introduced population of *Liza haematocheilus* than that in their native habitat, but this trend could not be confirmed when the size of geographic range and sampling efforts were controlled for. The parasite species richness at the infracommunity level of the invasive host population is significantly lower compared with that of the native host populations that lends support to the enemy release hypothesis. A distribution pattern of the infracommunity richness of acquired parasites by the invasive host can be characterized as aggregated and it is random in native host populations. Heterogeneity in the host susceptibility and vulnerability to acquired helminth species was assumed to be a reason of the aggregation of species numbers in the population of the invasive host.

1. Introduction

The so-iuy mullet *Liza haematocheilus* (Temminck & Schlegel), native to the Amur River estuary and the Sea of Japan, was deliberately acclimated in the Black and the Azov Seas. After numerous attempts this fish species established a successful reproductive population in the Azov Sea in the early 1980s [1]. *L. haematocheilus* is currently established in the north-eastern Black Sea where it has been subjected to commercial fisheries in Russia and Ukraine since 1991 [2]. The environmental conditions in the Black Sea and the Sea of Azov appear to be favourable to this species whose growth rate exceeds those of the native mullet species [3] Furthermore, Starushenko and Kazanski [2] predicted its expansion towards the Mediterranean Sea, where it is recorded since 1995 [4]. Along shores of Black Sea, its expansion corresponds to a sharp decline of native species of Mugilidae, which it apparently replaces [5]. The other fish species, which parasite diversity was compared here with that of the introduced host is a cosmopolitan species, flathead mullet *Mugil cephalus* L. This fish species occurs in tropical, subtropical and temperate coastal waters in all the world's major oceans [6]. Both species *M. cephalus* and *L. haematocheilus* occur sympatrically in the Sea of Japan and after the introduction of the latter species in the Black and the Azov Seas also in this region, but not in the western Mediterranean.

Helminth fauna of these fish in the northeast Atlantic and in the Sea of Japan is well known [7–20]. Based on an extensive search of the literature and the Host–Parasite Database [19] Kostadinova [20] compiled the checklist of the metazoan parasites of *L. haematocheilus* throughout its distributional range that comprised 69 nominal species of helminth and ectoparasitic crustacean parasites on that time. The comparative analysis of metazoan parasite assemblages provided by this author suggests that a large number of parasite species was lost in the new distributional range whereas an even greater number was gained. Kostadinova [20] supposed that the larger number of parasite species revealed in the invasive population of *L. haematocheilus* than in the native one was biased by the intensity of studies, which were at three fold higher in the Azov–Black Seas than in the NW Pacific. The great parasite diversity discovered in the introduced population of the so-iuy mullet in previous studies [20–22] in general contradicts with the enemy release hypothesis (ERH). The fundamental prediction of the ERH is that the introduced populations lack natural enemies compared to populations within their original range [23]. In other words, introduction of hosts into areas outside their natural distributional range results in a reduction of the number of their parasite species. Enemy release can occur when the invasive species lose its natural enemies from the native range, or/and when the invasive species show increased resistance or tolerance to natural enemies in the invaded localities [23]. Accurate assessment of the enemy loss requires performing a comparative analysis to match: i) the same species across native and introduced populations and ii) populations of an introduced species with populations of one or

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more native species coexisting in sympatry [23]. The present study provides such quantitative comparative analysis of the parasite species richness of the invasive fish and the native cosmopolitan fish, and considers parasites as natural enemies. While, previous works were based on the comparison of the parasite records from the invasive population of *L. haematocheilus* obtained in field studies with those from the native population of this host gathered from literary data [21] or only on literary data about parasite fauna of this host in both the native and introduced ranges [20,22]. The present study provides an opportunity to carry out the direct comparison of parasite diversity of native and invasive populations of *L. haematocheilus* on both the infracommunity and component community levels. The difference between the number of parasite species in the populations of hosts in the native and introduced ranges is the most simplistic measure of parasite release. The present paper introduces a quantitative comparative analysis of parasite communities of invasive and native hosts in the series of papers devoted to two grey mullets, *L. haematocheilus* and *M. cephalus*. For this, estimation of the helminth species richness for each host species and region was done using species richness curves and nonparametric estimators of species richness [24]. The working hypothesis of the present study is that parasite species richness of the invasive host population in the new area should be lower compared to population within their original range and populations of the native host.

2. Material and methods

2.1. Study area, fish sampling and parasite collection

This study is based on 1255 dissected grey mullets from 14 marine areas in the Mediterranean, the Azov–Black Seas and the Sea of Japan during 1998–2013 (Table 1). Collections of fish species differed between sites and seasons both in number and range due to collecting opportunity and differences in local fish fauna. The number of fish typically reached 30 specimens per sample. Only fish at the age of two years old and older within the size range of 17.1–68 cm (total length) were used in the analyses. In total, 695 specimens of *L. haematocheilus* from nine localities were sampled: six in the Black and the Azov Seas and three in the Sea of Japan; and 560 specimens of *M. cephalus* from seven localities: three in the Mediterranean, three in the Black and the Azov Seas and one in the Sea of Japan. Totally 42 samples from two hosts and across all localities, years and seasons are studied here (Table 1).

Collected fishes were measured and surveyed for parasites within the day of capture or after freezing. The skin, gills, oesophagus, stomach, pyloric caeca, intestine and internal organs were carefully examined under a stereomicroscope for parasites. All helminth parasites were identified and counted. Following the preliminary identification of helminths by the stereomicroscope, selected specimens of: i) microcotylid monogeneans, digeneans and acanthocephalans were fixed in 70% alcohol with subsequent staining in iron acetocarmine [25] or Delafield's haematoxylin [26], dehydrating through an ethanol series (from 70 to 100%), clearing in dimethyl phthalate and mounting as whole mounts in Canada balsam (applied to); ii) nematodes were fixed in 70% alcohol with subsequent clearing in glycerol and examination as temporary mounts; and iii) dactylogyrid and gyrodactylid monogeneans were mounted unstained directly in glycerin jelly [27].

Taxonomic identification was attempted to the lowest possible level. In the case of digeneans, identification keys were proposed by Blasco-Costa et al. [11–15,28]. When specimens of *Dicrogaster* spp. were slightly despoiled, identification was only possible to the generic level. In such cases, an estimate of the number of specimens of each species was assessed by a ratio of 1:1 (following Míguez-Lozano et al. [29]). Identification of some metacercariae was done to genus or even family level (see Appendix A) due to the paucity of morphological features useful for the identification of larval stages and specific requirements for mounting and examination of specimens [20,30]. Identification of monogeneans followed Euzet and Combes [31] and Sarabeev et al. [17], acanthocephalans followed Tkach et al. [18] and nematodes followed Belous [32] and Orecchia and Paggi [33].

2.2. Data analysis

We used two measures to characterize size and diversity of helminth assemblages for each host sample: i) parasite species richness (PSR) that is a total number of helminth species that occurred in the host sample; the PSR can be local and total, which defines the PSR of a sample and a region/sea, respectively; and ii) individual parasite species richness (IPSR) that is the sum of helminth species per individual fish (or infracommunity species richness) and its mean (MIPSR), including uninfected individuals, in the sample. The rarest species, which specimens occurred once per host/region and which prevalence was lower than 1% in samples per region, were not included in the analysis. Predominantly the cut out was applied to casual records of the oioxenous parasites, for example *Ligophorus* species, on nonspecific host. The data analysis was carried out at both the component community and the infracommunity levels to study patterns of the helminth assemblages in grey mullets. The data-set was split into 6 groups based on taxonomy, developmental stage or localization in a host. Those are monogeneans, digeneans (both adults and metacercariae), acanthocephalans, the whole helminth community and endohelminths (the whole community except monogeneans). Parasitic nematodes, as the separate taxonomic group, were not kept for the further comparative analysis because those are rarely occurred in fish and are met at distinct stages of the life cycle in different seas (Appendix A).

Newly obtained data on PSR, IPSR and MIPSR were tested for the normality of distribution using the Kolmogorov–Smirnov test for one sample to ensure the correct processing of further analysis. This test showed that values of PSR and MIPSR (presented in Table 1) fitted normal distribution ($p > 0.05$), while IPSR did not. Moreover, to assess the patterns of the IPSR distribution in host populations the test on fit of both the Poisson distribution and the negative binomial distribution (NBD) was executed. Wherever in the text values of mean are represented as mean $\pm$ standard deviation.

A single field sample of parasite individuals from their host or a pooled data-set of samples represents one point on the species accumulation curve, but we have no way of directly determining where on the curve this point lies. To compare the richness of two different samples it should be standardized to a common number of individuals [34,35]. Rarefaction represents an interpolation of a biodiversity sample to a smaller number of individuals for purposes of comparison among samples. In the present study parasite samples from different hosts and geographic areas were rarefied to sample with 30 host individuals, as well as to the smallest sample represented by 59 individuals from *M. cephalus* in the Sea of Japan to determine if species richness differs for a common number of individuals. This was done using rarefaction curves. Sample-based rarefaction curves (using each fish individual as a sample), species richness estimates and corresponding standard deviations (SD) and 95% confidence intervals were obtained using the analytical programme EstiMateS (v. 9.1.0, available from Colwell [36] at http://viceroy.eeb.uconn.edu/estimates/).

In theory, PSR of the total species richness can be determined for any community because it is limited, but the sampling effort needed to make a full count of species richness is excessive [37]. According to Walther and Morand [37], a non-parametric jackknife estimator is one of the most suitable applications for analysing parasite data. The non-parametric richness estimator Jackknife (first order) was calculated to deduce the total species richness in each component community of host–parasite data sets studied here following [37]. The jack 1 was counted with EstiMateS.

The Kolmogorov–Smirnov test (K–S) for one sample on the normality of distribution and the Poisson distribution was carried out with free statistics software PSPPIRE for Windows (http://pspp.awardspace.com). A web-based tool provided by Reiczigel et al. [38] was used to