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Infection status and microhabitat of polyopisthocotylean Monogenea found on the gills of goldlined seabream, *Rhabdosargus sarba*, from Musairah Island, Oman

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One of the major problems in fish production is that parasite-induced episodes of disease can result in significant impacts to the health of stock, mortalities and, economical losses. Seabreams are economically important fish in both the capture fishery and aquaculture sectors in Oman and represent potential species for aquaculture. The current study set out to investigate infection of polyopisthocotylean monogeneans on the gills of wild goldlined seabream, *Rhabdosargus sarba,* caught from Musairah region in Oman. The prevalence, mean intensity and abundance of polyopisthocotyleans on 145 fish was found to be 52.41%, 5.65 and 3.04, respectively. Three species of microcotylid parasite were isolated, one being *Atrispinum acarne* while the remaining two species require further study to identify them. There was no significant difference in the infection rate between host sex, however, parasite burdens were higher on larger sized fish (p<0.05) and were found to favour the first gill arch (p< 0.05). The study found that the sample of *Rhabdosargus sarba* landed in the waters around Musairah are heavily infected, with a predominantly immature population of microcotylids. Further work is required to better understand the potential risk each species poses to the health of its host before a decision is made on the suitability of new candidate fish species for commercial aquaculture.

Key words: Goldlined seabream, *Rhabdosargus sarba*, polyopisthocotylean monogeneans, *Atrispinum acarne*

Introduction

The world has a high dependency on marine resources for its food security and economy. Seabreams are an important group with significant volumes resulting from both capture fisheries (1,149,589 tonnes) and aquaculture (371,835 tonnes); in 2017 (FishStatJ, 2021). Among the various seabream species, gilthead seabream, (*Sparus aurata*) is the most important aquaculture species (218,099 tonnes in 2017; FishStatJ, 2021), and dominates as key species in the Mediterranean (Sánchez-García et al., 2013). From FAO Fish StatJ (2021), total of 218,099 tonnes were produced in 2017, with the top four producers being Turkey (61,090 tonnes; 28.01% of global production), Greece (55,948 tonnes; 25.65%), Egypt (35,221 tonnes; 16.15%) and Spain (17,005 tonnes; 7.80%). There is also interest to expand seabream aquaculture production through the exploration of new seabream species.

Looking at capture fisheries, most of the seabream species in the Indian Ocean and the Arabian Gulf, are caught using various fishing gear, dhows (= tradi-

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tional sailing vessels) and trolling lines (FAO, 2019). The Arabian seas host a total of 16 species of sparid (Machkewskyi et al., 2013); Omani waters contain many species that serve as potential candidates for aquaculture including the goldlined seabream, *Rhabdosargus sarba* (Yoon et al., 2013). In Oman, the first aquaculture activities began in 1986 with the giant tiger shrimp, *Penaeus monodon* (FAO, 2019). There is now interest to progress fish production toward sustainable fishing and aquaculture, which includes the possible culture of local species such as the goldlined seabream.

When the parasite fauna of these seabreams is considered, then the monogeneans *Furnestinia echeneis* and *Sparicotyle chrysophrii* are among the most significant (Mladineo et al., 2010). Copepods are also commonly encountered on both wild and captive reared fish (Dezfuli et al., 2011), and if not appropriately managed, then heavy infections can result in the direct loss of stock or as a complication of secondary infections (Repullés-Albelda et al., 2013). There is, therefore, an importance in understanding the natural parasite fauna of a species and the potential risks that each parasite species might pose when reared intensively under captive conditions (Sánchez-García et al., 2013; Shawket et al., 2018). Notable parasite-based mortality events in captive-bred seabreams include a 40% mortality of juvenile *Sparus aurata* in Italy 2006, due to an *Ergasilus* spp. infection (Dezfuli et al., 2011), and a 6.7-60% mortality of juvenile black seabream, *Acanthopagrus schlegelii*, in 2015 in a culture facility in South Korea due to an infection of amoebae (Kim et al., 2017).

The causes of such outbreaks in aquaculture conditions are usually linked to the high density of fish and conditions that facilitate the propagation of monogenean infections (Repullés-Albelda et al., 2012). In the wild, however, monogenean infections may be affected by local water temperatures and dissolved oxygen levels (Shawket et al., 2018), and by salinity (Repullés-Albelda et al., 2011). Warmer water temperatures can accelerate the rates of egg development, hatching and maturation (Repullés-Albelda et al., 2013). Polyopisthocotylean monogeneans, i.e., clamp bearing species that predominantly parasitise the gills of their vertebrate hosts feed on host blood, mucus and epidermis cells (Shawket et al., 2018). As they grow, the volume of blood they can consume and the amount of damage they can inflict through feeding and attachment activity increases (Repullés-Albelda et al., 2011). Consequentially, fish can suffer from anaemia and impairments to respiratory function which can weaken fish, which if left unaddressed can result in mortalities (Repullés-Albelda et al., 2013). Microcotylids are quite large polyopisthocotyleans, possess numerous sets of attachment clamps and so the potential damage they can inflict upon their hosts can be significant (Sánchez-García et al., 2013). In assessing seabream stocks in Omani waters, a number of studies conducted in recent years have identified a number of microcotylids (Yoon et al., 2015), including the description of new species such as *Microcotyle omanea* (Machkewskyi et al., 2013), and a new microcotylid genus *Omanicotyle* (Yoon et al., 2013).

The aims of the present study were therefore to investigate potential polyopisthocotylean infections of goldlined seabream caught in the marine coastal waters of Musairah Island, Oman as a means of gaining a better understanding of the health status of stocks and of the risks that certain species might pose if reared under intensive conditions.

Materials and Methods

Fish collection and parasite examination

A total of 145 specimens of goldlined seabream (av. wt. 241.27 ± 11.13 g (mean ± S.E.; range 69.50-792.5 g); av. length 18.71 ± 0.27 cm (range 12.0 - 29.5 cm); Fig. 1) caught from the waters around Musairah Island, Oman and collected from the central fish market. The specimens were put in ice and transferred directly to the laboratory. The fish were then weighed,

Fig. 1. Specimens of goldlined seabream, *Rhabdosargus sarba* collected from the Musairah region of Oman awaiting dissection.

lengthed and sexed, after which a full parasitological examination was conducted, though the purpose of the current study was to investigate and focus on gill Monogenea. The gills, many of which were notably pale, from each side were excised, separated and placed into labelled Petri dishes. The total number of polyopisthocotylean monogeneans attached to each gill arch were mapped according to the method given by Oliva & Luque (1998). Each parasite was then carefully removed using mounted needles, briefly fixed in Berland's fluid to flatten them and then transferred to 80% ethanol. Parasites were subsequently stained using Mayer's paracarmine and then mounted in Canada balsam and examined under a compound microscope using morphological features of the body and the attachment organs and reproductive armature to facilitate identification.

Data analysis

Data were analysed using one-way analysis of variance (ANOVA) and Tukey's test using Excel 2020. The results are presented as the mean ± standard deviation, unless where otherwise stated, and where a *p*-value below 0.05 is considered significantly different.

Results

Of the 145 fish that were examined and post-mortemed, 51.7% were female, 42.0% were male, while the maturity status of the remaining 6.3% could not be determined. A total of 76 fish were infected and a total of 441 polypisthocotyleans were recovered (Fig. 2a, b); the prevalence, mean intensity and abundance were determined to be 52.41%, 5.65 and 3.04

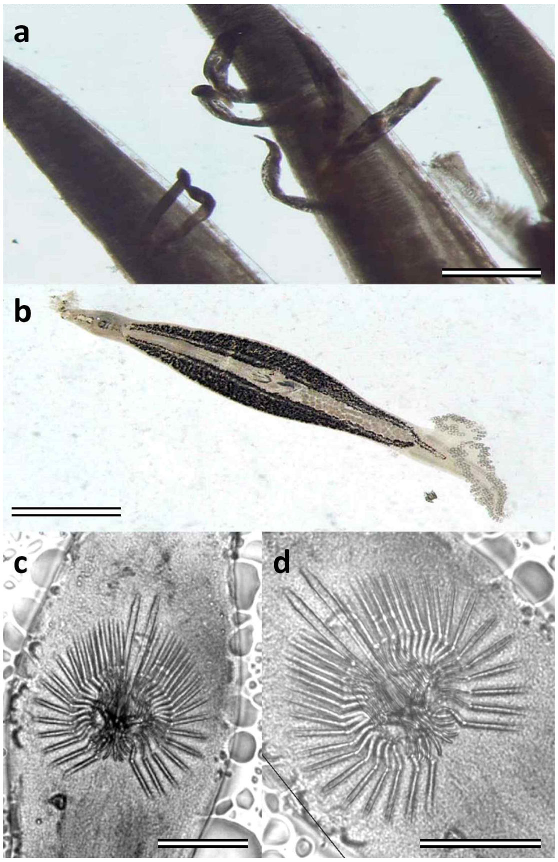

Fig. 2. Polyopisthocotylean monogeneans on the gills of goldlined seabream, (*Rhabdosargus sarba*) collected from the marine waters of Oman. a. monogeneans *in situ* on the gills; b. a processed specimen for identification; c., d. details of the armature of the reproductive organ of species C. Scale bars: a = 500 um; c, d = 250 um.

Table 1. The prevalence, mean intensity and abundance of the polyopisthocotylean monogenean infections when found as either the only species infecting the gills and when co-occurring alongside the crustacean copepod *Lernanthropus theodori*

	Polyopisthocotyleans	*L. theodori*
Prevalence (%)	52.41	11.72
Mean intensity	5.65	3.29
Abundance	3.04	0.39

respectively. From the parasitology evaluations, the fish could be categorized into one of three types of gill infection: 1) infected only with polyopisthocotyleans; 2) infected with polyopisthocotyleans and the crustacean copepod *Lernanthropus theodori*; and, 3) hosts with heavy infections of monopisthocotylean monogeneans (i.e., species that use hooks as their principal means of attachment to their hosts) and no other gill parasites. When a single infection of polyopisthocotyleans were found, they were found randomly distributed across the gills filaments; when polyopisthocotyleans were found alongside the copepod *L. theodori*, then they were found in small aggregations A summary of these infections is provided in Table 1.

Table 2 provides a breakdown of infection across the different host sexes; there were no significant differences in the prevalence, mean intensity and the abundance between the host sexes. When host size though is considered (Table 3), then a significant difference (p<0.05) between the size classes was found. The medium sized fish classified here as those falling between 17-23 cm in length were found to have the highest level of infection and number of parasites. There was no difference (p=0.472) in the distribution of polyopisthocotyleans on the right (234 parasites) and left (207 parasites) sides.

Of the monogenean parasites that were recovered, the monopisthocotylean parasites were dactylogyrids and will not be dealt with further here. The polyopisthocotylean monogeneans were represented by three different species. One species was identified as *Atrispinum acarne,* and identified from images presented in Akmirza (2013), representing 4% of the specimens recovered, the remaining two species B (18% of those recovered) and species C (78% of specimens; Fig. 2c, d), which measured between 10.7-12 mm in total length are currently undergoing identification. There were, however, significant differences (p<0.05) in the distribution of parasites across the different gill arches, with a preference for the first gill arches rather than the second and third arches (Fig. 3). The medial position on each gill arch was preferred occupied by 47% of the total parasite number. There was also a statistical difference (p=

Table 2. The prevalence, mean intensity and abundance of the polyopisthocotylean monogeneans on the different host sexes

Fish	Infected fish	Total parasite nos.	Prevalence	Mean intensity	Abundance
Female	74	231	50	3.122	1.59
Male	61	209	60.7	3.426	1.44
Unidentified	1	1	10	1	0.0069

Table 3. The average number, prevalence, mean intensity and the abundance of polyopisthocotylean monogeneans on different size of goldlined seabream, *Rhabdosargus sarba*

Host size	No. fish	Total no. of parasites	Prevalence	Mean intensity	Abundance
Small (<16 cm)	49	17	28.6	0.63	0.12
Medium (17-23 cm)	77	221	53.3	2.26	1.52
Large (24-31 cm)	19	203	100	10.15	1.4

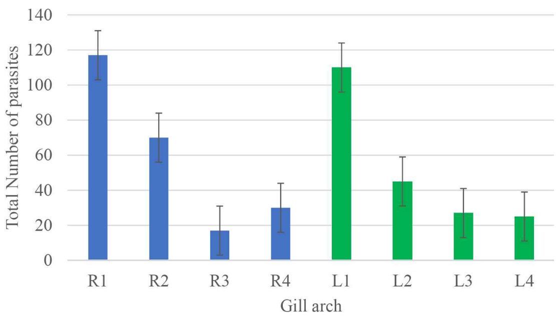

Fig. 3. The total count of polyopisthocotylean monogeneans found on each gill arch from the left and right sides of their host goldlined seabream, *Rhabdosargus sarba*.

<0.05) in the distribution of polyopisthocotyleans across the dorsal (23% of parasites), median (47% of parasites), and ventral (30% of parasites) parts of each gill arch.

Discussion

Goldlined seabream usually spawn at the end of summer, which in Oman is at the end of August; this coincided with the collection of fish explaining why most of the fish processed had mature gonads. On processing the fish, many of the gills from infected fish were notably pale – this was considered to be a parasite induced anaemia, i.e., the parasites feeding on host blood, rather than a post-mortem change to the condition of the gills. Of the fish that were infected with parasites, only 12% of hosts harboured a dual polyopisthocotylean monogenean and crustacean copepod infection. The abundance and infection of the polyopisthocotyleans was larger in the community aggregations that were found than the collective number of polyopisthocotyleans that were found on gills when they were the only parasite occupying the gills. This suggests that parasites distribute themselves more freely when not limited by available resources, i.e., space and food. Salgado-Maldonado et al. (2019) commented that species may aggregate when other species are present, as this may help secure a food source for each species. Aggregating may therefore reduce competition between species. A further explanation may be linked to the direct life-cycle of monogeneans; the eggs that are produced frequently get tangled within gill filaments and hatch close to the point where they were laid, this facilitates subsequent infection success, which may then lead to aggregations of parasites developing.

The parasite micro-community can, therefore, be high species richness but low density, or can be poor species richness but high density (as seen from the results presented here), to avoid any possible competition. From the fish that were found to have heavy monopisthocotylean infections (n=3), these were all found to have very few or no polyopisthocotyleans, which somewhat contradicts that indicated by Salgado-Maldonado et al. (2019) in that the high density of one species does not limit the occurrence of another species as much as the number of species present. The observation here regarding the presence of "monopisthocotyleans" does, however, require further investigation to determine whether the specimens found represent a single or multiple species.

The fish sample included a higher number of females than males which was suggested by Amir et al. (2018) to be linked to spawning activity at the time of fish collection. Fish measuring between 17-23 cm (Table 2) were found to have the highest parasite burdens; polyopisthocotyleans appear to prefer larger hosts, with larger gill surface areas for feeding (Mladineo et al., 2010). In this study, the polyopisthocotyleans appeared to favour hosts that were under 24 cm in total length; the observed number of parasites may reflect parasite burdens accumulated over time (Shawket et al., 2018). For this latter comment to be acknowledged, the behavior of the parasite should be taken in consideration, as parasites may leave a host if subject to unfavourable abiotic conditions, for example Sánchez-García et al. (2013) observed that the number of parasites on wild caught hosts dropped significantly after the fish had been moved into a culture facility.

The composition of the polyopisthocotylean community on the gills was dominated by species C (78 %), then by species B (18%) and then by 4% *Atrispinum acarne.* The latter species is recorded from the gills of the axillary seabream, *Pagellus acerna,* by Akmirza (2013). Species B and C, while they are not named here had unusual armed genital pores (Fig. 2c, d), and these species are currently in the process of identification.

There was no significant difference in parasite prevalence between the left and right gill sets and this is a finding that has been observed by others (e.g., Soylu et al., 2013). While many monogeneans appear to favour the second and third gill arches, which have the highest water flow and somewhat protected from debris carried by the inhalant current, this was not the case in the current study where the highest number of polyopisthocotyleans was found on the first gill arch (Fig. 3). Most of the parasites recovered from the first gill arch were found to be immature, or with few to no eggs. This might suggest that the first gill arch serves as an initial colonization site by infective oncomiracidia, with the developing parasites then migrating to the second and third gill arches as they grow and mature. In support of this, parasites on the first gill arch were more abundant in the medial found in the median, and most exposed, part of the gill arch. As the parasite matures, it appears to favour the more secluded dorsal part of each gill arch, an observation which is consistent with that of Soylu et al. (2013).

Conclusion

Of the 145 goldlined seabream collected from the marine waters around Masirah Island, Oman, 76 fish were found to be infected with polyopisthocotylean monogeneans, the prevalence, mean intensity and abundance of which were 54.41%, 5.65 and 3.04, indicating that a large proportion of the wild population are infected. The polyopisthocotyleans that were collected were found to be represented by three different species of microcotylid: *Atrispinum acarne* and other two species which are currently under further investigation. The significance of these infections and potential health impacts on the host needs further investigation and will need to be carefully considered before a decision is made on the suitability of this species for commercial aquaculture.

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