





REVIEW

Monogenean *Sparicotyle chrysofhrui*: The major pathogen of the Mediterranean gilthead seabream aquaculture

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Abstract

Following the intensification of the Mediterranean aquaculture over the years, *Sparicotyle* (syn. *Microcotyle*) *chrysofhrui* (van Beneden and Hesse, 1863; Microcotylidae, Monogenea) became one of the most pathogenic ectoparasites for the gilthead seabream (*Sparus aurata* L.) aquaculture. It has demonstrated a remarkable versatility to expand over its known host range, accomplish an efficient transfer between the wild and farmed sparid populations over wide geographic areas, and establish itself as the most detrimental gilthead seabream pathogen. Even though the incidence of *S. chrysofhrui* is closely monitored in marine aquaculture system throughout the Mediterranean, there is a general lack of recent and publicly available data on its epidemiology. Being considered as a parasite whose proliferation is expected to increase under global warming conditions, such lack of data additionally hampers the implementation of integrated pest management strategy at national and Mediterranean level. While EU scientific initiatives greatly contributed to our understanding of *S. chrysofhrui* biology, ecology and sparcotylosis treatment and pathogenesis, there is more ahead to elucidate about the mitigation of the diseases, particularly towards the development of genomic selection breeding programs of parasite-resilient gilthead seabream, omic-based screening of novel drug targets and reversed vaccinology.

KEYWORDS

aquaculture, Mediterranean sea, Monogenea, *Sparicotyle chrysofhrui*, sparids

1 | INTRODUCTION

European Union aquaculture has produced and harvested approximately 552,625 tonnes of farmed fish in 2020, among which, the gilthead seabream (*Sparus aurata*; 93,131 tonnes, data from 2019) and European seabass (*Dicentrarchus labrax*; 81,369 tonnes, data from 2019) represented approximately 32% of the total production.¹ In

2019, Mediterranean aquaculture has reached a total value of 491 and 494 million euros for the two dominant species, respectively.² The production of the gilthead seabream is dominated by Turkey, accounting for 38.5% of world production, followed in descending order by Greece, Egypt, Tunisia, Spain, Italy, Croatia, Cyprus, Israel and lastly Albania.² While the innovation has been driving improvements in the efficiency, competitiveness, and overall

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sustainability of aquaculture, unpredictable effects of threats, such as climate change, increased pathogen resilience and virulence and antimicrobial resistance have exacerbated the challenges faced in the Mediterranean systems. Even though the gilthead seabream is generally considered a more robust and pathogen-resilient species than the temporarily reared European seabass,³ the production still suffers from diseases affecting the productivity and profitability of farms. Except for the main bacterial pathogen, *Tenacibaculum maritimum*, a Gram-negative, gliding, and aerobic rod, the majority of the pathogens affecting the gilthead seabream in aquaculture are parasites, dominated by the microsporidian *Enterosporea nucleophila*, myxozoan *Enteromyxum leei*, dinoflagellate *Amyloodinium ocellatum* and the monogenean *Sparicotyle chrysoiphii*.⁴ Other less frequently isolated and reported species are listed in Table 1. In comparison to other gilthead seabream diseases, such as the winter syndrome (Central Mediterranean), vibriosis (Southern Mediterranean) and nervous necrosis virus (NNV; Western Mediterranean), sparcotylosis ranks as the highest important.³¹ Amongst parasitic taxa, *S. chrysoiphii* is considered the most devastating pathogen by the industry, estimated to contribute to up to 30% of gilthead seabream mortalities, far more of that attributed to *T. maritimum* (14%) in European seabass hatchery and pre-growing stage, or NNV (7.5%) in grow-out phase.³

Sparicotyle chrysoiphii is a gill-attaching, hematophagous monogenean with a direct life cycle and a high infectivity under aquaculture conditions, such as the increased fish density and proximity of different age classes, elevated seawater temperature, lowered seawater circulation due to net's biofouling, and the presence of reservoirs (e.g., wild sparid populations).³² Although mostly present all year round in intensive farming systems, the pathogen causes sparcotylosis as a clinical entity usually at the sudden onset of the seasonal increase in seawater temperature, with few exceptions.³³ The clinical signs are systemic, depending on the extent of disease progression, including lethargy, sluggish movements, body discoloration, more frequent opercular movements or permanently dilated opercula, emaciation, and cachexia, diminished appetite, gill paleness and lastly, generalised anaemia.^{34–37} Since its first record in aquaculture approximately 25 years ago, the parasite has colonised the Mediterranean industry at such a level that it became ubiquitous and endemic, although paradoxically underreported in the scientific literature. Another point to ponder is the cues and causes that enabled this monogenean to emerge as a major issue in aquaculture in contrast to other gill-infecting species, such as monopisthocotyleans *Lamellodiscus echeneis*, *Gyrodactylus* spp. and *Diplectanum aequans* in the

TABLE 1 A recent and non-exhaustive inventory of the pathogens isolated from the gilthead seabream (*Sparus aurata*) farmed in the Mediterranean.

	Disease	Pathogen	Reference
Viral diseases	Viral Nervous Necrosis (VNN)	Betanodaviridae (reassortant RGNNV/SJNNV strain)	5
	Lymphocystis disease (LD)	Lymphocystivirus (Iridoviridae)	6
	Infectious spleen and kidney necrosis (ISKNV)	Infectious spleen and kidney necrosis virus (Iridoviridae)	7
Bacterial diseases	Aeromoniasis	<i>Aeromonas</i> sp.	8
	Pasteurellosis	<i>Photobacterium damsela</i> subsp. <i>piscicida</i>	9
	Tenacibaculosis	<i>Tenacibaculum maritimum</i>	10
	Vibriosis	<i>Vibrio anguillarum</i> , <i>V. harveyi</i>	11,12
Parasitic diseases	Amyloodinosis	<i>Amyloodinium ocellatum</i> (Dinoflagellata)	13
	Cardicolosis	<i>Cardicola aurata</i> , <i>C. mediterraneus</i> (Trematoda)	14
	Ceratomyxosis	<i>Ceratomyxa sparosaurati</i> , <i>C. auratae</i> (Myxozoa)	15,16
	Cryptocariosis	<i>Cryptocarium irritans</i> (Ciliata)	17
	Cryptosporidiosis	<i>Cryptosporidium molnari</i> (Apicomplexa)	18
	Coccidiosis	<i>Eimeria sparis</i> (Apicomplexa)	19
	Enteromyxosis	<i>Enteromyxum leei</i> (Myxozoa)	20
	Enterosporosis	<i>Enterosporea nucleophila</i> (Microsporidia)	21
	Coccidiosis	<i>Gaussia sparis</i> (Apicomplexa)	22
	Gyrodactylosis	<i>Gyrodactylus orrechia</i> , <i>G. longipes</i> (Monogenea)	23,90
	Microsporidiosis	<i>Kabatana</i> sp. (Microsporidia)	24
	Myxosporidiosis	<i>Kudoa iwatai</i> (Myxozoa)	25
	Lamellodiscosis	<i>Lamellodiscus echeneis</i> (Monogenea)	26
	Myxosporidiosis	<i>Leptotheca sparidarum</i> (Myxozoa)	27
	Myxosporidiosis	<i>Ortholinea auratae</i> (Myxozoa)	28
	Myxosporidiosis	<i>Sphaerospora sparis</i> (Myxozoa)	29
Trichodinosis	Trichodinids (unidentified)	30	

European seabass, which favour the same conditions, but fail to establish at the same extent.^{38,39}

To help in answering questions on *S. chrysophrii* biology and ecology, this review compiles the meritorious findings of published reports and researches, critically analyse the existing knowledge and proposes future research directions.

2 | LITERATURE SEARCH STRATEGY

A non-exhaustive search of peer-reviewed literature (including conferences' abstracts) for keywords including aquaculture AND diseases AND parasite, OR gilthead seabream, OR *Diplodus*, OR *Microcotyle*, OR Polyopisthocotylea, OR *Sparicotyle*, OR Sparidae, OR *Sparus*, was conducted through the Google Scholar, Web of Science, PubMed and Scopus databases. Keywords in title, abstract and keywords were last searched on 20 April 2023.

3 | TAXONOMY AND PHYLOGENY

Sparicotyle chrysophrii (van Beneden & Hesse, 1863) belongs to polyopisthocotyleans, a subclass of Monogenea that on the posterior part of the body, called opisthaptor, harbours an array of clamps in addition to the sclerotized haptor. Originally described as *Microcotyle chrysophrii* (Van Beneden & Hesse, 1863), the parasite has been accepted as *S. chrysophrii*,⁴⁰ forming a monospecies genus *Sparicotyle*. The latter is characterised by a genital atrium armoured with two circles of spines, four median spines that are upright and with double hooklets at their ending, seminal vesicle merged with the posterior part of the atrium, and a single vagina.⁴⁰ The genus is representative of the subfamily Atrasterinae,⁴¹ encompassed within the family Microcotylidae,⁴² which includes an enormous diversity of species, that is, more than 160 species distributed in 50 genera of 7 subfamilies, with a cosmopolitan distribution.⁴³

Molecular phylogeny of the Microcotylidae and the groupings at the subfamilial level still shows a poor resolution, although the available molecular data for their representatives have been multiplying over the last decade. Therefore, Microcotylinae remains polyphyletic with only Prostatomicrocotylinae subfamily having a strong branch support, while Atrasterinae resolves in a separated and poorly supported clade.⁴³ This encourages a more extensive sampling effort combined with the application of multilocus genetic analyses and novel-omics approaches that would achieve better accuracy of the phylogenetic reconstruction, especially at the levels of subfamilies, as well as over wider geographic areas.

4 | MORPHOLOGY AND PHYSIOLOGY

The morphology of the adult monogenean is shown in Figure 1a. For a more detailed description of the monogenean, readers should consult the original description.^{40,44}

4.1 | Attaching apparatus

Already the non-clamp-bearing post-larvae (H1, or 'hooked type 1') show posterior hooklets and hamuli similar in size to those in adults, except for the width of the handles of the posterior hooklets that are still not fully transformed.⁴⁵ Through H1 stage growth, posterior hooks are oriented backward from the opisthaptor centre, grouped in a narrow terminal lappet. This lappet narrows down during their development, so that the hooks and hamuli get closer till their final overlapping, while lateral hooks remain anteriorly. Clamps start to appear where the most posterior lateral hooklets are situated, from 7 to 15 days post-infection (dpi) at 20°C. During the growth of clamps, the lateral hooklets move to the lateral edges of the opisthaptor, where they remain till the surrounding tissue remodels, and they become lost. Clamps continue to develop and grow within the anterior end of the opisthaptor, even after the parasite reaches maturity (Figure 1b). Post-larvae move using both the anterior glands and haptor but become more sessile as they grow and anchor by opisthaptor as sedentary adults.⁴⁵

The highest number of clamps (72) was observed at the end of in vitro experiment (51 dpi),⁴⁵ while another study reported 120–150 clamps in adult specimens.⁴⁴ This might help develop monogenean growth models for simulations of sparicotylosis epidemics and planning of treatment regimes, similar to simulations developed for Atlantic salmon sea lice.⁴⁶ Considering that 1.4 clamp pair is added daily,⁴⁵ correcting the formula so that it encompasses different rhythms of parasite sigmoid growth (i.e., slow-fast-slow), and including a set of relevant variables (abiotic factors, such as temperature, salinity, currents; and biotic factors, such as fish size/age, density, health status), it is tempting to suggest the development of algorithms that would predict and simulate monogenean growth, dispersion, and infection rate.

4.2 | Digestive system

Paired buccal suckers help with the feeding of the adults (Figure 1c,d), becoming visible in the post-larval stage with three pairs of clamps. In contrast, the pharynx is visible since the early stage and continues to grow, being posteriorly limited by four elliptical dark spots at the gut level that are later associated with the caecum.⁴⁵ The spots scatter after 9–15 dpi, increase in number, and become lighter, remaining always related to the intestine, therefore indicating that these might form vitelline follicles, securing nutrients for the developing embryo. The intestine is firstly sac-like, becoming bifurcated later (17 dpi).

While the feeding nature of *S. chrysophrii* has been simply assumed as hematophagous, only recently a more tangible evidence for this was provided.⁴⁷ Authors intravenously injected fluorescent 1 µm-diameter microspheres in the infected gilthead seabream, withdrawing the blood from fish and isolating feeding monogeneans 18 h post-injection. By counting the microspheres recovered from the host blood and the parasite-digested tissues under a fluorescent microscope, authors calculated that the parasite feeds on average with

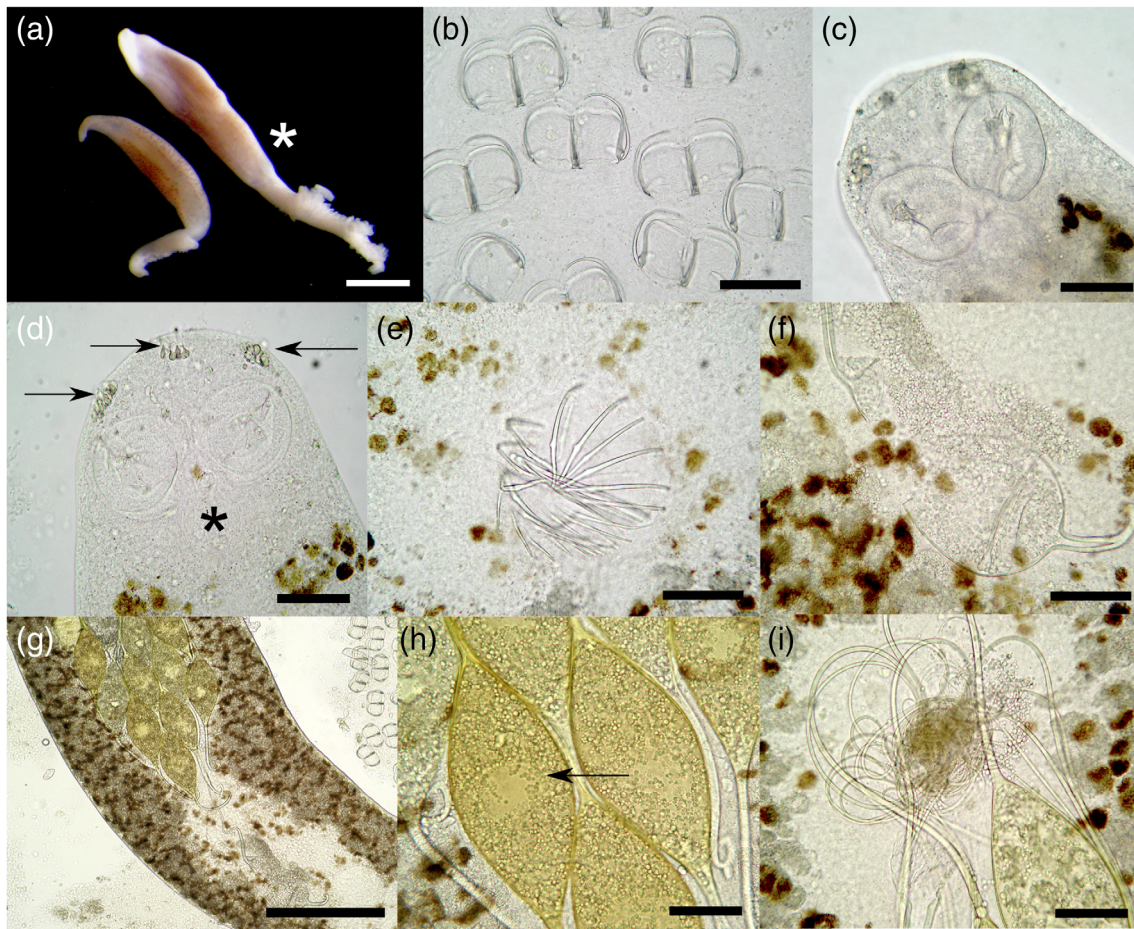


FIGURE 1 Details of *Sparicotyle chrysophrii* (Monogenea, Microcotylidae) body observed under the stereo- (a) and the light microscope (b–i): (a) Two adult monogeneans isolated from farmed gilthead seabream (*Sparus aurata*) gills from Croatia and Italy (white asterisk) showing a considerable difference in size; (b) Clamps in the opisthaptor; (c) Anterior part or prohaptor with paired distinct buccal suckers; (d) A detail of the prohaptor showing three aggregations of dome-shaped papillae (arrows) and a bulbous, muscular pharynx (black asterisk); (e) Sclerotised spines of the genital atrium; (f) Sclerotised prostatic reservoir of the male copulatory organ; (g) Mid body with fertilised eggs within the uterus. Note a part of the opisthaptor (upper right corner); (h) Fertilised eggs undergoing embryonic development. The arrow shows a centrally located early embryo; (i) A detail of the entangled egg's polar filaments in the uterus; Scale bars: 500 μm (a); 100 μm (b, c); 250 μm (d); 25 μm (e, g); 50 μm (i). Credits: I. Mladineo.

4.31 μL of blood per hour, explaining the anaemia, a hallmark of sparicotylosis.⁴⁷

4.3 | Reproductive system

Sparicotyle chrysophrii is protandrous monogenean, and the genital atrium appears at 19 dpi, coinciding with the appearance of testes primordia (Figure 1e,f). On 21–26 dpi, the first germarium is observed carrying developing oocytes. At 26 dpi, the first eggs appear in the uterus (Figure 1g,h), while at 36 dpi, all parasites bear mature eggs.⁴⁵ *S. chrysophrii* adults produce approximately 20 eggs per day, usually released into the environment as egg bundles.⁴⁸ Eggs have an oval shape, 100 \times 27 and 110 \times 30 μm in size, and two long tendril-like filaments at the opposite polar ends (Figure 1i), one of which is armed with small hooklets at the edge. These structures are particularly

relevant for the attachment of eggs to filamentous structures, acting similarly to Velcro® fasteners. They allow the eggs to attach to natural thin filamentous structures present in the fouling, or artificial structures, such as frayed nets and ropes present in the cages or equipment in contact with the fish.⁴⁸

5 | LIFE CYCLE

Sparicotyle chrysophrii life cycle follows the pattern of other monogeneans, being susceptible to abiotic conditions as described further.⁴⁹ The whole life cycle, from egg to the egg of the next generation takes 26–30 days at 20°C.⁴⁵ Repullés-Albelda et al. described parasite's early development in vitro that remains so far the only published study on this topic.⁴⁸ In the egg, the embryo centred between the vitelline material shows a haptor positioned in the direction of the

shorter polar filament (2 days post-deposition; dpd). The haptor sclerites become more prominent (3 dpd); first, the primordium of the hamuli and then the hooklets, same as the adhesive glands on the prohaptor. This is followed by the appearance of the first cilia (3.5 dpd), the eyespots (3–4 dpd), lateral hooklets and pharynx (5 dpd), and a prominent egg operculum. Oncomiracidia hatch by contractile movements within 1–2 min. On average, they survive in vitro for 11.9 h post-hatching (hph; 52 hph maximum), but most (28%) live for 7–12 hph.⁴⁸ Interestingly, this equals half of the life span of *Zeuxapta seriolae* oncomiracidia, another highly pathogenic polyopisthocotylean Monogenea infecting the yellowtail amberjack (*Seriola lalandi*) and farmed greater amberjack (*Seriola dumerilii*) reared in the Mediterranean.^{50–52} Repullés-Albelda et al. observed either vertical or horizontal swimming of larvae, with frequent speed changes (mean 3.97 mm/s).⁴⁸ Oncomiracidia grow slowly while attempting to attach to gill lamellae, but once attached, the growth progress to a fast phase until they reach maturity. Afterward, the growth again slows down till the maximum number of clamps has been incorporated within the opisthaptor. During this sigmoid growth, *S. chrysophrii* adds 1.4 clamp pairs/day or grows 105.5 $\mu\text{m}/\text{day}$, so that eventually the haptor contributes to 2/7 of the total body length.⁴⁵

The growth kinetics in the natural *S. chrysophrii* infection has not been studied yet and it might infer differences from the above in vitro model. In *Z. seriolae*, wild populations that show smaller size at sexual maturity correlated with a higher genetic diversity are considered to have a higher evolutionary and transmission potential to a farmed host.⁵¹ This indicates that *S. chrysophrii* populations might as well be prone to local adaptations that would determine their life strategy and success of infection, however, this remains yet to be elucidated.

6 | EPIDEMIOLOGY

6.1 | Geographic range

The reported geographic range of the parasite, either from the wild or farmed fish is limited to the Mediterranean basins and the Red Sea, except for the Canary Islands, the archipelago off the coast of north-western Africa in the Atlantic Ocean.⁵³ The unexhaustive reports include the Bay of Trieste and Venice in the North Adriatic, the Central and the East coast of the Adriatic (Archipelago of Zadar, Island of Brac, Bay of Boka Kotorska), the Aegean Sea, the Israeli coast, Eilat, the Bardawil area of the Egyptian North Sinai and Alexandria, the Gulf of Tunis, the Spanish Mediterranean coast, the Bay of Lyon, Southern part of the Island of Corsica and the Tyrrhenian and the Ligurian Sea.^{38,44,54,55}

6.2 | Abiotic factors

Environmental factors, such as the seawater temperature, pH,^{56,57} photoperiod⁵⁸ and salinity are crucial parameters for the propagation of monogeneans in general, and particularly during their larval stages.

While knowing the optimal ranges for parasite development and multiplication is essential for planning disease management and treatment strategies in the facility, ideally, seasonal oscillation of abiotic factors should be considered already during the planning of future marine aquaculture sites.

A single comprehensive in vitro analysis of abiotic parameters on the hatching success, swimming performance, and survival of *S. chrysophrii* oncomiracidia was undertaken.⁴⁹ The study projected the outputs within the context of climate changes predictions primarily for the Mediterranean, as well as other geographic areas where the gilthead seabream is reared (e.g., Red Sea, Canary Islands). The temperature (14, 18, 22, 26°C, and extreme values of 10 and 30°C), which is closely associated with the metabolic rate and consequently the development of the parasites and host immunity⁵⁹ unsurprisingly proved to be the most significant variable. Larvae exhibit a considerably wide optimal temperature range (measured as a hatching success being over 88%); from 14°C to 22°C that mainly corresponds to spring and autumn in the Mediterranean, which could partially explain their wide presence in the Mediterranean. Temperatures 22 and 26°C induce faster emergence of the early eye-spot oncomiracidia (96 h), and also shortened the incubation and hatching period. In contrast, the extreme temperature (30°C) appears as the upper thermal margin that limits the hatching and oncomiracidial survival period.

The decrease in pH value on *S. chrysophrii* ontogenesis exerts a marginal effect on the embryonic development and incubation period, but negatively affects the hatching period and success, swimming ratio and the mean/maximum survival periods. The pH tested under the climate change predicted scenario (pH 7.0) in comparison to the Mediterranean average (pH 7.9) resulted in expectedly higher resilience of the early stages protected within the egg.⁴⁹

Darkness influences larval emergence beyond hatching rhythms, so that most of the hatchings (>66%) are synchronised with the night (dark periods), and constant darkness also enables oncomiracidia protracted swimming.⁴⁹ Nocturnal hatching strategy might be an evolutionary adaptation to gilthead seabream night resting, or grouping for foraging at dusk common for sparids. Similar strategies have also been described in other groups of fish parasites, such as in ciliates *Cryptocaryon irritans* and *Ichthyophthyrus multifiliis* where infective theronts hatch from encysted tomites during the night.⁶⁰ While the photoperiod in sea cages is difficult to manipulate, lowering the salinity using freshwater baths can be deployed to slower embryonic development (27 ppt)⁴⁹ or induce detachment of adults. The natural inflow of freshwater or areas of brackish water has been observed to considerably reduce the number of parasites by interfering either with their development or that of their intermediate hosts.⁶¹

6.3 | Hosts

Host range is limited to species from the family Sparidae, both the wild and aquaculture fish populations.^{62,63} The type host is the gilthead seabream, but the monogenean has been also isolated from the sharpnout seabream (*Diplodus puntazzo*),^{62,64,65} and the bogue

(*Boops boops*)⁶⁶ to a lesser extent. A recent study undertaken within an H2020 project ParaFishControl evidenced the capacity of the monogenean to infect another sparid, the blochet pickerel (*Spicara maena*) in the area of intensive aquaculture activities in the Zadar Archipelago, Adriatic Sea.⁶⁷ While above reports reference the authority upon which the monogenean identification has been based, the majority do not contain parasite morphological descriptions or figures (micrographs or camera lucida drawings) that would unbiasedly confirm the monogenean identification. Considering that many microcotylids have been described in wild sparids,⁴³ it is possible that some records in fact represent misidentification. In the particular case of *S. chrysophrii* isolated from the bogue and pickerel, the identification has been confirmed by sequencing of different molecular markers, supporting the observation that the host range is not limited only to a single species. This is an important finding as *S. chrysophrii* has been for a long time considered a host specialist, an adaptive feature of a parasite that primarily establishes itself within a predictable resource (host) with a predictable life history.⁶⁸ Since host specialisation increases the risk of parasite local extinction and lowers its likelihood for successful establishment in new regions,⁶⁹ being the opposite case for *S. chrysophrii*, it is very likely that more robust sampling efforts would reveal more sparid hosts bearing this monogenean. Screening parasitic taxa in reared and wild fish within farming sites (including sparids *B. boops*, *Diplodus sargus* and *Diplodus vulgaris*) in the Mediterranean (Community of Valencia, Spain) and Atlantic (Canary Islands), revealed a significant difference in *S. chrysophrii* prevalence in the farmed gilthead seabream between the regions; 36% versus 9%, respectively. However, the monogenean was absent in wild sparids other than the gilthead seabream.⁵³

Host size and age influence monogenean population patterns in different ways and are dependent on the host origin. In the wild gilthead seabream, susceptibility to the infection within host length cohorts was the same between smaller (<196 mm, e.g., <1 year old) and larger fish (234–302 mm, e.g., <2 years old), except for a cohort over >3 years old that showed a tendency of the parasite aggregation.⁷⁰ In farmed fish, smaller fish are heavier parasitised in terms of mean intensity (<20 cm), while larger sizes class (>30 cm) have a higher prevalence.³³ Unfortunately, in the latter study, the small fish category encompassed fish over +1 year, meaning that the monogenean infection pattern in fingerlings seeded in cages has not been evaluated. It is interesting to observe that the gilthead seabream diplectanid *L. echeneis* shows the same pattern of distribution as *S. chrysophrii* reported earlier³³; the highest prevalence in the oldest age category and the highest mean abundance and intensity in the lower age category.⁷¹ This could be attributed to different circumstances; either to a more developed immune system in older fish that rejects a considerable number of parasites, which nevertheless, aggregate over a longer host life span (3–4 yrs), and/or to parasite ability to inhabit a larger gill surface.^{71,72} Irrespectively of the cause, a well-designed study of *S. chrysophrii* distribution in relation to host size/age category over at least two production cycles should be helpful in the planning of the treatments or other intervention schemes on the farms.

6.4 | Fish-to-fish transfer in the farms

Biological characteristics of *S. chrysophrii*, such as the hermaphroditism that allows all the adult parasites to produce fertilised eggs; high prolificity of the adults; and short generation time that results in multiple infections of fish in the same or neighbouring cages over a single production cycle (around 8–10 months in the cage) allow high incidence of sparicotylosis. In addition, the parasite infectivity ratio in the farms is enhanced by the limited egg dispersal within the cages due to net biofouling, and the high density of fish in the same water volume. The resulting levels of infections and reinfections in the farms have contributed to the establishment of sparicotylosis as an endemic disease in many farms producing the gilthead seabream and in an increasing number of geographic areas.

6.5 | Wild-farmed fish transfer

The transfer of *S. chrysophrii* between wild sparids and cultured gilthead seabream has been previously observed,⁶³ figuring as an important risk factor for the propagation of the monogenean in aquaculture.⁷³ Potentially, an additional load of the pathogen is transmitted by farmed gilthead seabream escapees that once released accidentally from the sea cages due to net damage (mechanical tearing, extreme weather conditions, gilthead seabream grazing on the biofouling), huddle to their original farming site, or join neighbouring farming sites. Arechavala-Lopez et al. evidenced that escapees can survive at least 4 weeks, imposing considerable ecological and epidemiological consequences on nearby cultured and wild gilthead seabream stocks.⁷⁴ Although it was beyond the scope of study to account for *S. chrysophrii* infection in tagged escapees, it is feasible that those fish could represent a particular risk for sparicotylosis dispersion. Except for initiatives surged at national scientific and non-profit stakeholders' levels, there is no common management plan in place in Mediterranean countries to prevent fish escapees. However, there is a body of literature on gilthead seabream escapees' adverse effect on farmed mussels (*Mytilus galloprovincialis*) and oysters (*Crassostrea gigas*)⁷⁵ through predation, and wild gilthead seabream populations through the loss of genetic diversity.⁷⁶ The role of escapees in the potential transfer of ubiquitous parasites such as *S. chrysophrii* seems largely understated, therefore any initiative in that direction could represent a worthwhile effort.

It is difficult to differentiate whether the aquaculture facilities where the monogenean is present at higher intensity, have more impact on the wild fish populations attracted to the cages by the presence of feed pellets and increased biological productivity associated with the presence of organic matter, or the wild fish serve as pathogen reservoir to reared fish. However, molecular tools have solved some of the arising questions. For example, the use of a single mitochondrial marker (cytochrome oxidase II) to infer the intraspecific similarity between monogeneans present on wild sparids and farmed seabream failed to confirm the hypothesis.⁶⁶ In contrast, recent data from the ParaFishControl project (Horizon 2020; <https://www.parafishcontrol.eu>)⁶⁷

where the genetic structure of the monogenean from the wild and cultured fish population in Spain, Italy, Croatia, and Greece was evaluated using restriction site-associated DNA sequencing (RAD-Seq) supported the ‘transfer hypothesis.’ The study was based on 246 generated SNPs (Single Nucleotide Polymorphism) and although the relatedness analysis revealed some structure in *S. chrysophrii*, no great distances among different groups were noted and parasite genotypes from different countries and different origins (wild vs. farmed fish) overlapped to a certain degree. Noteworthy is that while the monogeneans from Greek farmed and wild fish were the most genetically non-differentiated (i.e., most closely related), they showed high similarity to the parasites from Croatian farmed fish. Such an exchange of genotypes between two allopatric populations that have no obvious contact might suggest that it originated a while ago by human intervention, for example through accidental transport of monogenean eggs or even movements of infected fish. To corroborate this, more ample sampling should be undertaken, focusing also on wild and farmed gilthead seabream populations along the coast of the Ionian Sea. The study also showed that while parasites from Croatian wild and farmed fish were weakly differentiated, potentially originating from a small effective size population, Italian assemblages showed more structure and heterogeneity, potentially related to the existence of stronger barriers that impede admixture (e.g., offshore sea-cages with stronger currents, the lower population density of wild sparid at aquaculture sites). Lastly, *Sparicotyle* genotypes from Spanish farms (no wild parasites have been provided in this case) expectedly showed to be slightly different from those from other countries. However, the main conclusion is that overall, all wild monogenean genotypes were found clustering together, suggesting that they might be the sources of any population encountered at the end of the distribution, that is, wild specimens provided a starting point for genotypes that have been established in aquaculture facilities.⁷⁷ Despite the large geographical distance, that is, from Spanish to Greek farms, the monogenean shows the panmictic distribution, a feature that would be unlikely achievable by a specialist parasite. This was confirmed by a recent study, although within a smaller geographic area (spanning from the Spanish Mediterranean coast to Sardinia) that inferred a weak difference among *S. chrysophrii* haplotypes using a mitochondrial and a nuclear marker.⁷⁸

6.6 | Population parameters and epidemiological aspects

6.6.1 | Gilthead seabream as a host

According to the available records, reports on the presence and incidence of the monogenean in the wild gilthead seabream populations started almost a decade earlier than the first sparcotylosis reports in the farmed fish, the latter unsurprisingly coinciding with the intensification of the aquaculture sector Mediterranean-wide. The most important reports are summarised in Table 2 and reported below in a chronological order to better follow the sparcotylosis progress over the time.

6.6.2 | Wild fish

In the wild gilthead seabream in the Gulf of Lyon (euryhaline Sète area), a very high frequency of parasitisation was reported; the parasite exhibited seasonal variations, with an increase in prevalence (by 25%, but not the abundance) in springtime when the host entered the deeper Mediterranean waters, after the wintering in the brackish Thau lagoon. In general, the monogenean population showed to be stable, with moderate aggregative distribution, moderate numbers at infrapopulation levels, and no interspecific competition with the monopisthocotylean *L. echeneis*.⁷⁰ Interestingly, the observed low intensities contrasted previous reports in wild gilthead seabream from the same, but the wider geographic area.^{79,80} Although Noisy and Maillard sampled the fish from the Thau lagoon,⁷⁹ and Oliver from Salses-Leucate,⁸⁰ a lagoon further SW from the Sète area, Reversat et al. dismissed the possibility that the difference could be due to different abiotic profiles of the sampling sites, arguing that it rather arised from sampling artefacts.⁷⁰

6.6.3 | Farmed fish

In the farmed gilthead seabream populations, the monogenean in general shows proliferation (herein implied as an increase in one or more parasite population indices, for example, intensity, abundance, prevalence) during warmer seasons in most of the Mediterranean aquaculture facilities (Table 2). The prevalence of infection is often higher compared with that of wild fish populations, with the presence of all developmental stages, regardless of the fish size. The exception is Southern Corsica, where *S. chrysophrii* was monitored in the three farms each season over one and a half years, and the highest prevalence and abundance were recorded in the wintertime.³³ Although no mortalities were observed in the farmed fish, authors related higher monogenean population parameters (i.e., total prevalence and mean abundance) to the greater vulnerability of fish during the cold period. Water temperatures, especially in winter, are lower in NW Mediterranean due to geographical conditions and specific hydrographic regimes, and the difference was even more pronounced before the effect of global warming become noticeable. These lower and sometimes fluctuating temperatures had a relevant impact on the metabolism of gilthead seabream,⁸² being associated with pathologies, such as the winter syndrome.^{83,84} In many cases, gilthead seabream affected by winter syndrome exhibited pale gills and high numbers of *S. chrysophrii*, suggesting a synergic effect of comorbidity.

Two studies undertaken in different geographic areas in the Adriatic Sea and with 5 years of difference indicated an increase in the *S. chrysophrii* population in the farmed gilthead seabream.^{63,85} It can only be speculated whether this is related to the stronger ‘establishment’ of the monogenean within the farming environment over the years, and/or it is simply related to the increase of fish production and therefore parasite habitat.

In gilthead seabream farming, the monogenean is mainly found as a parasite and pathogen in cage production systems. In Spain, it was

TABLE 2 Overview of ecological and epidemiological studies, and case reports on *Sparicotyle chrysopharii* (Monogenea, Microcotylidae) searched in bibliographic databases covering scholarly literature.

Host	Origin	Geographic area	Mean prevalence (%)	Mean abundance	Mean intensity	Season/Year	Reference
Gilthead seabream (<i>Sparus aurata</i>)	Wild	Sète area, Hérault, France	85	12.4		Autumn	79
	Wild	Salses-Leucate lagoon, France	34.6	12.9		Autumn	80
	Wild	Sète area, Hérault, France	51.5 (autumn); 75.0 (spring)	1.46 ± 0.28 (autumn); 2.34 ± 0.50 (spring)	2.83 ± 0.43 (autumn); 3.15 ± 0.61 (spring)	September –mid-December, 1984; April –mid-July, 1985	70
	Farmed	Alexandria, Egypt	80 (March); 57.1–60 (summer); 27–35 (autumn); 42.9–48.8 (winter)		30 (March); 6.4 (summer); 4.4–6.2 (autumn); 12.1–21.2 (winter)	January–December 1986	54
	Farmed	Southern Spain				February, 1992	34
	Farmed	The eastern coast of Peloponnese, Western Saronikos Gulf, Greece				November 1990–November 1991	81
	Farmed	Island of Chios (farm I); Eidiavros (farm II), Greece	21 (farm I); 10 (farm II)		++ (farm I); + (farm II)	2000–2003	62
	Farmed	South Tyrrhenian Sea (farm I); Pachino (farm II); Castellamarre (farm III) Italy	50 (farm I); 100 (farm II); 100 (farm III)		++ (farm I); +++ (farm II); + (farm III)	2000–2003	62
	Farmed	Eastern Greece (farm I); Southern Greece (farm II); Northern Greece (lagoon)	61.5 (farm I); 13.3 (farm II); 100 (lagoon)			January–December, 2001 (farm I, farm II); January, June, July 2001 (lagoon)	35a
	Farmed		12.3 (inshore floating cages); 17.4 (submersible cages)			July 2002–March 2005	87
	Farmed	Adriatic Sea, Croatia	17.16 (farm I); 2.83 (farm II); 34.88 (farm III); 36.95 (farm IV)	0.24 (farm I); 0.05 (farm II); 0.57 (farm III); (farm IV)		June 2002 – March 2003	63
	Farmed	Masinova (N 45° 18' E 16° 27'); Lamjana (N 44° 02' E 15° 13'); Adriatic Sea, Croatia	66.7	4.7		Summer, 2008	66
	Farmed	Southern Corsica (3 farms), France	53.2 (total average); 40–80 (farm I); 20–70 (farm II); 40–73.3 (farm III)	0.4–2 (farm I); 0.2–1.3 (farm II); 0.5–1.5 (farm III)		January 2008–May 2009	33

TABLE 2 (Continued)

Host	Origin	Geographic area	Mean prevalence (%)	Mean abundance	Mean intensity	Season/Year	Reference
	Farmed	The central part of the east Adriatic Sea (N 45° 18' E 16° 27'), Croatia	35.86 (28.22–44.12 95% CI)	0.97 (0.71–1.32 95% CI)	2.71 (2.21–3.40 95% CI)	September 2008–September 2009	85
	Farmed	Castellón (3 sea cages, IATS facility); Tarragona (sea cages, intensive earth pond); Valencia (sea cages); Cádiz (earth pond), Spain	20–100 (Castellón sea cages); 0 (IATS facility, earth ponds); 10–50 (Tarragona); 11.1 (Valencia)		1–70.6 (Castellón sea caged); 0 (IATS facility, earth ponds); 1–1.7 (Tarragona); 1 (Valencia)	2 years (1999–2000); occasionally through 2003–2007	37
	Farmed	Off Murcia, Spain	73		4 ± 3		45
	Farmed	Province of Livorno, Italy	50 (farm I); 60 (farm II); 70 (farm III)			July, 2010	88
Sharpsnout bream (<i>Diplodus puntazzo</i>)	Wild	Coast of Santa Pola, Valencian Community, Spain (N 38° 11' 23" W 0° 33' 20")	40 (19.1–63.9, 95% CI)	1.2		2007	65
	Farmed	Central Tyrrhenian Sea, Greece					64
	Farmed	Island of Chios (farm I); Epidavros (farm II), Greece	6 (farm I); 8 (farm II)		++ (farm I); + (farm II)	2000–2003	62
	Farmed	South Tyrrhenian Sea, Italy	11.6 (polyculture); 1.6 (monoculture)		+++ (polyculture) + (monoculture)	2000–2003	62
	Farmed	Adriatic Sea, Croatia	13.45 (farm I); 42.95 (farm II)	0.44 (farm I); 1.36 (farm II)		June 2002–March 2003	38
	Farmed	Adriatic Sea, Croatia	13.45 (farm I); 42.95 (farm II)	0.44 (farm I); 1.36 (farm II)		June 2002–March 2003	63
Bogue (Boops boops)	Wild		22	0.5		Summer, 2008	66

Abbreviations: CI, confidence intervals; IATS, Instituto de Acuicultura de Torre de la Sal.

* Authors presented the cumulative prevalence of Monogenea, calculating together fish infected with *Furmetinia echeneis* and *Sparicotyle chrysophrii* (syn. *Microcotyle chrysophrii*).

not found in the gilthead seabream reared in earth pond extensive or semi-intensive systems (typically based in the south of Spain, and known as 'esteros') or indoor facilities (mostly pre-grow-out facilities), even during successive sampling. Fish seeded in the sea cages become infected after 3 months, regardless of their initial age.³⁷ Taking into account that samplings were performed in farms with a prior history of sparcotylosis, and therefore, the infection was endemic in the farm, the detection of the parasite was most probably associated with reinfections from the surrounding cages of the farm. Knowing the exact time of primo infection is important for planning preventive and therapeutic measures on the farm, but there is still no conclusive data on when naïve fish first become infected by *S. chrysoophrii* and whether this is mainly sourced from wild or reared host population. However, the oncomiracidia of another monogenean, *L. echeensis*, take 10 days to colonise monogenean-free fingerlings originating from a biosecurity hatchery system, and oncomiracidian haplotypes are shared with *Lamellodiscus* haplotypes parasitising adult reared gilthead seabream, inferring that the latter fish represents the source of infection for the naïve fingerlings.⁸⁶ A carefully planned experiment at the seeding time of the gilthead seabream fingerlings over different Mediterranean regions that also takes into the account population genetics of the monogenean should give a better understanding of the trends of primo infection.

The absence of *S. chrysoophrii* in earth ponds was possibly associated with higher water turbidity, lower water quality and a higher diurnal and seasonal oscillation of water temperatures in the shallow ponds.³⁷ In contrast, in an extensive lagoon system in Greece, parasite prevalence reached 100%.³⁵ Such large differences between the two systems might be related to site-specific abiotic and biotic factors shaping the monogenean community, however, any conclusion is speculative without wide-scale comparative studies.

In Italian cages, the first records of the parasite in farms showed a low mean prevalence (6.1%),⁸⁷ while later, the increased load of the parasites was associated with significant mortalities related to comorbidity with the bacterium *Photobacterium damsela* subsp. *damsela*.⁸⁸ The co-infections of *S. chrysoophrii* and other pathogens in gills are frequent, in particular with monopisthocotyleans, such as *L. echeensis*,⁸⁹ *Gyrodactylus longipes* and *Gyrodactylus orechia*.^{90,91} *S. chrysoophrii* can also be detected in gills co-infected by bacteria, such as epitheliocystis⁸⁹ and *Tenacibaculum* sp. (Padros, personal observations). In respect to epitheliocystis, it has been recently shown that the main principal causative agents of the disease are members of the novel Ca. Ichthyocystis genus,⁹² which cause severe proliferative lesions already described by Padros and Crespo.⁸⁹ The epitheliocystis proliferative lesions together with the feeding activity of *S. chrysoophrii* in the co-infection cases cause increased mortalities in the affected farms (Katharios, personal observations). On the other hand, *Tenacibaculum maritimum* is considered as a primary, but also many times as an opportunistic and secondary pathogen. Co-infections are likely due to the attachment of clamps and secretion of excretory/secretory products during the parasite's feeding activity, which causes foci of necrosis and inflammation in the gill tissues. This in consequence easily becomes infected by bacteria, in particular, *Tenacibaculum* sp. due to its specific tropism towards fins, skin and gill epithelium.

While there are no accessible published records on *S. chrysoophrii* for other important aquaculture actors in the Mediterranean, such as Turkey, Egypt or Tunisia, the reports collected herein (Table 2) show outdated values considering the increase of biomass production and extension of the aquaculture sites in the area over the last years. Country epidemiological data need to be updated, and the monitoring undertaken over subsequent production cycles and geographic areas to manage the disease more efficiently. However, the value of the existing data remains in the possibility to use them as baseline information for building up more robust epidemiological models, climate change predictions in the context of parasitosis, or more structured sampling efforts, offering opportunities for new research directions.

6.7 | Sharpsnout seabream as a host

Sharpsnout seabream is a host to *S. chrysoophrii*, also susceptible to sparcotylosis.⁶⁵ After being previously considered as a promising species for diversification of Mediterranean aquaculture, the farming efforts have been progressively reduced due to fish vulnerability to the myxozoan *E. leei* and other infections, for example, edwardsiellosis.^{93,94} In facilities farming the gilthead and the sharpsnout seabream at the same sites, the monogenean showed a tendency to switch and exacerbate clinical signs in the latter, possibly conditioned by ineffective immunity of the naïve host. Likewise, the differences between infection levels of gilthead seabream and sharpsnout seabream in the Adriatic farm were significant, being higher in the latter.⁶³ *S. chrysoophrii* in farmed sharpsnout seabream induced mass mortality in farms in Greece, either when fish were held in polyculture or monoculture with the gilthead seabream, in contrast to the latter that did not suffer mortality.⁶⁴

In Spanish facilities where sharpsnout seabream was maintained, the risk assessment analysis for sparcotylosis revealed that the possibility of exposure, the pathway to infection, and the probability of infection were extreme, while the consequences and risk were moderate.⁹⁵ Interestingly, it seems that high parasite population parameters were also observed in the wild sharpsnout seabream in Spain, reaching 40% prevalence.⁶⁵ Surprisingly, data from the Adriatic Sea collected during ParaFishControl project suggests that no *S. chrysoophrii* have been isolated from the wild sharpsnout seabream present in sea-cages vicinity, although the finding could have been affected by the sampling effort and study design.⁶⁷

6.8 | Epidemiological considerations

Concerning the impact of infections by *S. chrysoophrii* in the gilthead seabream farming industry, the available information is scarce and unbalanced between geographical areas and countries. Most of the available information is based on clinical case reports or cross-sectional samplings in farms associated with research projects. According to Muniesa et al. which assessed the sustainability of the Mediterranean marine fish-farming sector, mortalities reported in the

on-growing cages associated with sparcotylosis amount to up to 30% of total fish mortalities.³¹ The assessment performed using a multidisciplinary survey, covered the period 2015–2017 and 50 production units, highlighting sparcotylosis as the most frequently reported disease, found in all regions included in the study (Croatia, Cyprus, Egypt, France, Greece, Italy, Portugal, Spain, Tunisia and Turkey).

The published information based on robust epidemiological studies with longitudinal studies with serial sampling of the same batches; several production batches; several farming sites; different geographical areas; and subsequent production cycles (2–3 years) is missing. In contrast, many farms are currently adopting specific strategies for the assessment and control of sparcotylosis, mainly to base their decision about the time for required treatments. Adult monogeneans tend to aggregate at the outmost (fourth) gill arch,⁹⁶ or the anterior hemibranch,⁹⁷ which has been employed by the industry for routine assessments. These comprise regular fish samplings in the cages and examination of the parasitic load in the gills using similar techniques. However, sampling varies from a very simple non-lethal visual inspection and count/scoring technique, to more accurate counts in sacrificed fish and laboratory examination of the gills. Each farm is using own technique and scheme according to possibilities and resources, therefore the obtained data fulfil different levels of accuracy for the downstream analysis. For this reason, consensual and standardised sampling and parasite assessment protocols would be very important for the industry. In contrast to the Norwegian policy for sea lice and amoebic gill disease management,⁹⁸ there are no national platforms for the collection and storage of *S. chrysophrii*-related epidemiological data. While such management of sparcotylosis could be hampered by the diversity of legislative practices in countries where it is present, the development of a similar platform should be encouraged, as the data collected from industry currently remains unused to a greater extent. Information from internal farm assessments is almost always kept confidential by the companies, and this issue has been addressed in the PerformFish project (H2020; <http://performfish.eu>). Within the project, the impact of sparcotylosis in the general disease burden in gilthead seabream farming was preliminary addressed through an internal survey, using aggregated production data managed by companies and national aquaculture associations of Croatia, Greece, Italy, France and Spain. Representing the majority of the EU gilthead seabream producers, the survey highlighted sparcotylosis as the most relevant disease, with the highest burden of disease (scoring 5 on the overall significance scale from 1 to 5).⁹⁹ The disease was also addressed directly through a hybrid DELPHI expert consultation exercise, and indirectly through a series of health-related key performance indicators (KPI). The former, based on a similar exercise in the ParaFishControl project, focused on grading the risks associated with different diseases in aquaculture, including sparcotylosis.^{33,99} The project also provided an impact assessment of parasitosis across marine Mediterranean fish farming sector through specific KPIs identified with the industry and EU major fish farming associations.¹⁰⁰ The KPI “Number of antiparasitic treatments” was associated indirectly with sparcotylosis as the majority of bath treatments undertaken in the gilthead seabream is applied against *S. chrysophrii*,¹⁰⁰ whereas KPI

‘Mortalities–by disease’ was informative for the direct impact of the sparcotylosis. The KPI collection and analytical system is currently available in an innovative web environment developed by ISPRA and five European producers’ associations, running by SAS® Visual Analytics software (ISPRA, 2022 unpublished), and hosted within the National Environmental Information System (SINA) in ISPRA.

7 | INFECTION MODELS

To better understand and study different aspects of the monogenean biology and physiology, especially the traits crucial for pathogenesis in aquaculture, in vivo infection models have been developed to replicate as close as possible the natural infection.^{36,45,101,102} Models rely on the introduction and cohabitation of naturally infected gilthead seabream (donor fish) with naïve, uninfected hosts (receptive fish) for 10 weeks, taking a particular attention to control the level of infection in the naïve fish. Alternatively, the infection can be achieved by direct egg transmission capitalising on their twinning filaments and the positive buoyancy that aids their attachment to various substrates. For that purpose, the eggs are harvested from tanks with donor fish using a 250 µm-mesh held by a PVC ring that floats for 2–3 days in the water upper column. The content is washed with seawater and left to settle in a graded cylinder before being transmitted to receptive fish.³⁶

Under slightly decreasing temperatures from summer to autumn (27–20°C), the transfer of the monogenean to naïve fish is established already 2 weeks from the introduction of donors, showing the presence of all monogenean developmental stages, 100% prevalence and intensity of 4–15 adults in the two external gill arches.¹⁰² In the model that uses eggs to infect recipient fish, the infection is observed only 6 weeks post-challenge, and the prevalence reaches 87%.³⁶ However, when the same authors cohabitated donor and recipient fish, the infection was established a week earlier, and the prevalence reached 100%. This was likely due to the close contact between recipient and donor fish, in addition to a higher temperature, speed of the water flow, type of the net used in the experimental tanks, and the size of the fish, which could have conditioned the success of infection and infection levels in the two models.¹⁰²

The first low mortalities in the model proposed by Rigos et al. started 4 weeks post-cohabitation and lasted until the end of the experiment, although the natural decrease in seawater temperature attenuated the outcome.¹⁰² Fish appeared lethargic with pale gills, and cumulative mortality reached 13%. After 10 weeks of experimental infection, recipient fish significantly lagged in weight compared with the uninfected control fish (almost 26%), further adding to the economic loss attributed to the infection, in addition to direct mortality. An important observation from the latter and in situ study is that a small part of the affected population exhibiting emaciation and anaemic gills may harbour zero or unusually low parasitic load. This is due to the impossibility of the monogeneans to feed on those gill arches and their consequent loss from the host, which can be misleading for the parasitic count.

8 | PATHOLOGY

Pathogenesis initiates at the attachment site when the parasite's clumps tweak and squeeze the gill lamellae and the haptor movements generate damage in the gill epithelium. Bacteria introduced in the wound and the damaged tissues evoke an inflammatory reaction, manifesting with epithelial hyperplasia, capillary congestion, inflammatory infiltrate, development of lamellar synechia and progressive reduction of the interlamellar spaces.¹⁰³ The attachment by the clamps is dynamic, affecting several areas in the gill. Large monogeneans are capable of piercing at least 41 secondary lamellae, sometimes attaching to two neighbouring primary lamellae.³⁶ However, less attention in the pathogenesis has been paid to the parasite's excretory and secretory products (ESP) and extracellular vesicles (EV) that are produced and discharged in the host tissues during *S. chrysophrii* parasitism. While this line of research is conditioned by the annotation quality of monogeneans' genomes, in other parasitic helminths, ESP and EV cargo mostly encompass proteases and protease inhibitors, as well as other factors that engage in tissue dissociation, penetration and migration, digestion, and in particular host immune evasion and interaction with the host-associated microbiome.^{104,105} This corresponds to the secretome inventory of the diplozoid *Eudiplozoon nipponicum*, which is enriched in factors involved in immunomodulation (inhibitors of proteolytic enzymes, CD59-like proteins, fatty acid binding proteins), parasite feeding (proteolytic enzymes cathepsins B, D, L1 and L3), and development (fructose 1,6-bisphosphatase, ferritin and annexin).¹⁰⁶ ESP/EV produced by *S. chrysophrii* have not been scrutinised yet, but they likely play an important role in virulence and pathogenesis.

The main clinical signs are therefore induced by parasite attachment of the clamps into the gill epithelium and subsequent blood feeding, exacerbated by the colonisation of secondary bacterial infections, particularly *T. maritimum*.^{35,88,103} By scrutinising the ontogeny of early stages, it was suggested that the first lesions in the gill epithelium are inflicted by post-larval hooks that are present until 21 dpi, before the falling of the terminal lappet.⁴⁵ While these lesions are perforative, as soon as the first clamps appear (7 dpi), lesions also attain a compressive character. After losing the hooks, there is no evident cause of anaemia, until adults start to feed. Accordingly, a strong negative correlation was found between the haemoglobin (Hb) concentration in infected fish and the parasite load, as well as Hb concentration between control and infected fish.^{36,107} Consequently, the main clinical signs include lethargy and progressive anaemia, accompanied in the later phase by progressive emaciation and mortality.^{36,108}

Histopathology findings have been described as focal inflammatory lesions around the monogenean clamps in mild infections, or generalised hyperplasia, congestion of primary and secondary lamella, increased number of mucous cells and thickening of the filament edges in highly infected fish.³⁵ Hypertrophy of the chloride cells, lamellar synechia and clubbing with disruption of the epithelium and microbleeding are present at the attachment site, while the proliferating adjacent epithelium induces secondary lamellae fusion. Similar

findings supported by ultrastructural were observed in greater amberjack infected by *Z. seriolae*.^{50,109} The lesions at the attachment site were characterised by lamellar synechia and subsequent fusion, with mild to moderate epitheliocystis co-infection. Although no severe proliferative cell response was observed, clamp sclerites induced lamellar clubbing, occasionally leading to the rupture of distal epithelial and marginal vascular vessels.

A histopathological study of *S. chrysophrii* conducted within the ParaFishControl consortium corroborated the presence of a cellular infiltrate that surrounded the efferent vessels of the gill arches and consisted of a few leukocytes and many mast-like cells (Figure 2a,b), of which some degranulated. Immunohistochemical staining of the in situ monogenean attached to the gills allowed the identification of targeted elements of the host's innate and adaptive immune response. Phagocytic (Figure 2c) as well as antibody-producing cells (Figure 2d) observed near the lesions confirmed the activation of gill-associated lymphoid tissue (GIALT) components in *S. chrysophrii* infection and the mounting of the adaptive innate response (Figure 2). Although the innate immune elements against *S. chrysophrii* have not been fully characterised (i.e., no confirmation by electron microscopy or target-specific immunolabelling), it is plausible that the same conserved innate elements observed against other helminths are exhibited in this case. Concerning IgM-specific labelling, it cannot be postulated that IgM⁺ cells were produced specifically against the monogenean, and not against any other secondary antigen, especially because the damage of epithelium by clamps introduces bacteria from the gill surface. IgM⁺ cells, such as plasma cells and Ig-bearing macrophage-like cells were mainly located deeper at the base of the secondary lamellae, while eosinophilic granulocytes infiltrate the tissues at the monogenean attachment site. Lastly, the abundant proliferation in the epithelium is supported by the expression of the proliferative cell nuclear antigen (PCNA; Figure 2e), a 36 kd protein involved in DNA replication that confirms an increased cell turnover. In humans, it is mainly related to the onset of neoplasia, but it is expressed also during the persistence of many irritating stimuli, such as toxins and parasites. While light microscopy did not enable the exact characterisation of PCNA⁺ cell types in this case, previous studies attributed PCNA expression to mast cells and to lesser extent fibroblasts in the proximity of parasites.¹¹⁰ Authors related its expression to the enhanced proliferation of mast cells necessary for the repair and remodelling of damage caused by the acanthocephalan *Dentitruncus truttae* infecting intestine of the brown trout (*Salmo trutta trutta*) and nematode *Eustrongylides* sp. encapsulated in the muscles of the European perch (*Perca fluviatilis*). However, the authors did not confirm PCNA expression by double immunolabelling using specific markers for mast cells and fibroblasts, therefore the proliferating cell type still needs to be unbiasedly characterised.

The inducible nitric oxide synthase (iNOS) enzyme catalyses the production of nitric oxide, the latter possessing both signalling and bactericidal functions. Being used as an indicator of the innate and/or inflammatory response of fish, iNOS has shown a moderate expression in *S. chrysophrii* infected gills (Figure 2f). Many cell types express iNOS, including macrophages, neutrophils and fibroblasts after an

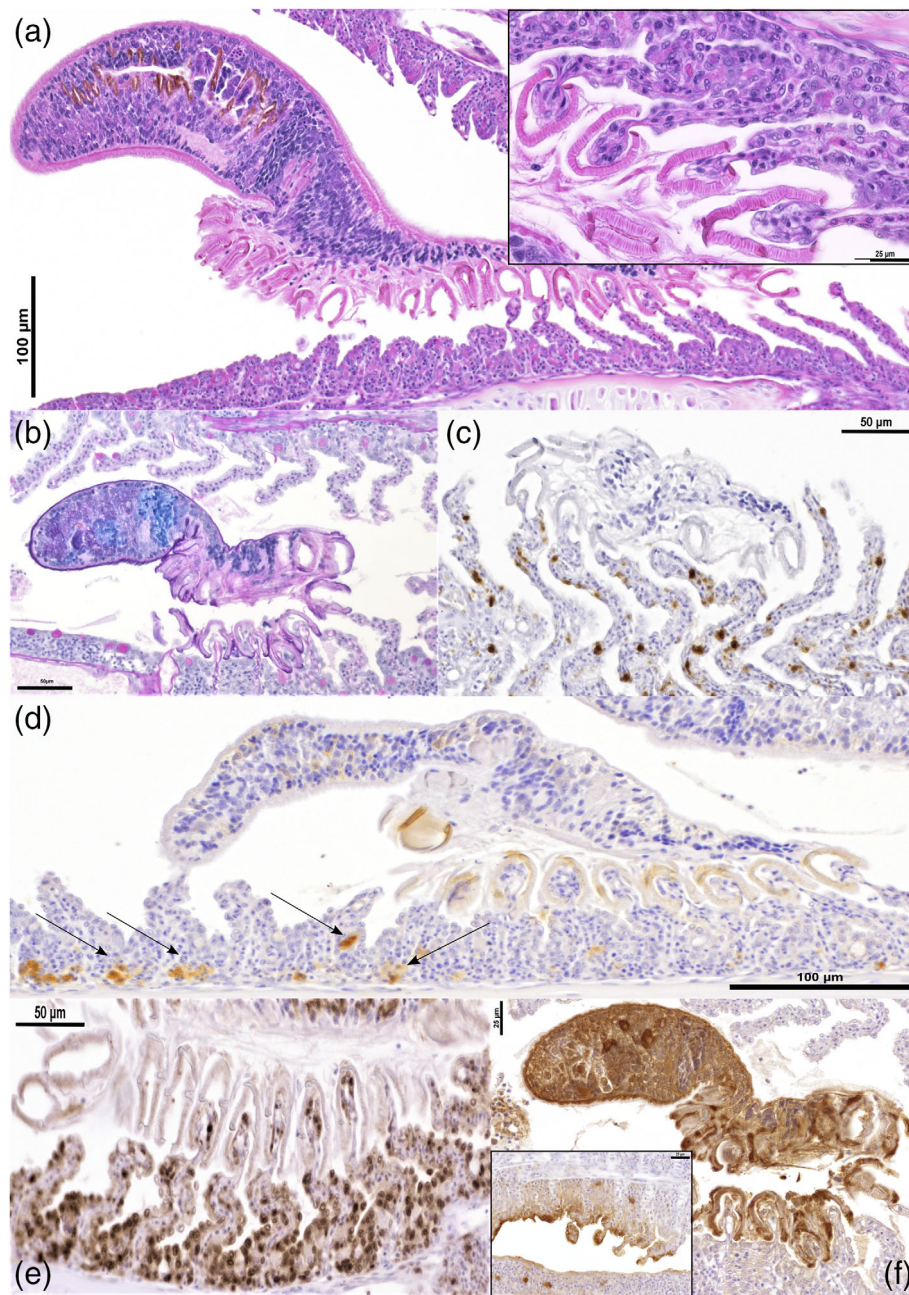


FIGURE 2 In situ histological and immune- and histochemical sections (5 µm thickness) of *Sparicotyle chrysophrii* (Monogenea, Microcotylidae) attaching at the gill filaments of the farmed gilthead seabream (*Sparus aurata*): (a) Haematoxylin and eosin staining (H&E). Inset: detail of sclerotized clamps (light purple pink) constricting the epithelium of the secondary lamella (H&E); (b) Moderate proliferation of mucous cells in the gill epithelium close to the attachment site and the neighbouring gill lamellae (PAS-Alcian blue); (c) Positive staining of eosinophilic granulocytes in the gill epithelium (brown) (mouse monoclonal anti-seabream eosinophilic granulocytes antibody, G7 was developed and kindly given by Dr. Mulero, University of Murcia); (d) Positive staining of IgM⁺ cells (plasma cells and Ig-bearing macrophage-like cells; arrows) mainly located at the base of the secondary gill lamellae. The polyclonal antibody against seabream IgM (University of Trieste) was employed; (e) Positive staining of cells expressing proliferative cell nuclear antigen (PCNA) (Thermo Scientific); (f) Positive staining of the cell expressing inducible nitric oxide synthase (iNOS) enzyme in the monogenean, as well as in the tissues at the attachment site (inset). Credits: P. Beraldo and D. Volpatti.

array of immunological stimuli orchestrated via cytokines and lipopolysaccharide. Its dual role as a critical agent of host defence in infection, as well as a central mediator of pathogenesis¹¹¹ have been observed in different host-parasite systems.¹¹² Cytoplasm of the epithelial cells damaged by the parasite that are consequently detached stained iNOS⁺, but

interestingly, iNOS was also produced abundantly within the parasite's cell. Such antibody recognition of a highly conserved iNOS epitope within the parasite tissues is potentially related to a high metabolic rate of cells during active parasitism, as observed in dinoflagellate *A. ocellatum* infecting the European seabass gills and oropharyngeal cavity.¹¹³

Lastly, systemic reaction to sparcotylosis can be also reflected in the splenic melanomacrophage centres (SMMCs) that increase in size and number in the infected fish. While this is typical for many chronic fish diseases, in this case, lipofuscin and hemosiderin harboured within SMMC might have resulted from the catabolism of damaged erythrocytes during sparcotylosis.¹¹⁴

9 | HOST RESPONSE

Host haematological parameters show significantly low levels of Hb, mean corpuscular haemoglobin content and mean corpuscular haemoglobin concentration, and an increase of the erythroblasts, total leukocytes, neutrophils, eosinophils and plasma cells already 8 weeks post-infection.³⁶

Humoral components of innate immunity are activated by monogenean antigens; infected fish respond with a high production of reactive oxygen species (ROS), but it seems that the monogenean stimulates the respiratory burst, rather than tipping the ROS production in the direction that would damage host tissues and overwhelm its immune system.¹⁰⁷ Namely, oxidative stress represents a misbalance in the production of free radicals and oxygen metabolites (or, ROS) with respect to their elimination rate by antioxidants. This misbalance would consequently lead to damage of cell organelles and their biomolecules in host tissues,¹¹⁵ which has not been observed at least under *S. chrysoophrii* infection models. Fish exhibit an increase in the activity of myeloperoxidase which is used to reduce a load of hydrogen peroxide developed under the respiratory burst. This enzyme is positively correlated with the parasite load, Hb, serum antibodies titre, nitric oxide, and fish weight and negatively with lysozyme. Despite such a response after a 10-week infection, monogenean prevalence remains high, suggesting that *S. chrysoophrii* employs distinct immunity evasion mechanisms, as it has been proven for other helminths.¹¹⁶

Host lysozyme and complement activity increase and correlate positively with Hb, similar to the serum anti-protease activity and serum nitric oxide. Ceruloplasmin, a biomarker for inflammatory response does not significantly increase after a 10-week infection, further suggesting monogenean immunomodulating potential. Similarly, the total antibody titre stays at the same level, either attributed to the lack of a marker for a *Sparicotyle*-specific antibody within a total titre, or a delayed start of antibody production in the naïve fish. Interestingly, the higher respiratory burst and lower nitric oxide concentration, and antibacterial and antitrypsin activity in infected fish are in fact suggested to act as incentives for the cellular innate immunity, while suppressing the humoral antibacterial and anti-parasitic components.¹⁰⁷ However, this in turn would make the fish more susceptible to secondary infections, which is indeed observed in natural infections.⁸⁸

Using RNA-sequencing, 2581 differentially expressed genes were detected in infected gill tissues, of which more transcripts related to the immune response and apoptosis were found in sites not directly in contact with the parasite; another cue that suggests a local

silencing effect of the attached monogenean.¹¹⁷ The authors also observed a strong systematic immune response characterised by spleen transcriptomic profile, which surprisingly was mainly downregulated. While gill tissues were enriched in transcripts involved in apoptosis, inflammation, and cell proliferation, spleen tissues expressed genes participating in the inhibition of apoptosis, autophagy, platelet activation, signalling and aggregation and the inflammasome. The splenic downregulation was explained by a potential hypometabolic response that strives to counteract the hypoxia resulting from the gills damage and consequently directs the energy towards defence and repair responses. Alternatively, it can be a result of parasite immunomodulation, as suggested earlier.¹⁰⁸ Although transcriptomic profiles during the infection should be interpreted at the enrichment level of different pathways (e.g., apoptosis) rather than individual perturbed transcripts (e.g., caspase 3), the latter data is valuable for follow-up studies of vaccine and drug targets.

Sparicotylosis clinical progress, observed through the increase in infection levels reflects in the host serology. In total 22 proteins belonging to apolipoproteins, globins, and complement c3 were found significantly correlated with the infection intensity in plasma samples from fish undergoing low (1–50 monogeneans/fish), medium (51–100), and high (>100) infection.¹¹⁸ The proteomics of host plasma showed perturbation of haemostasis, immunity, and lipid metabolism, and transport pathways, supporting the systemic response to the infection. However, authors found that in high infection levels, fish are almost able to restore some level of homeostasis, suggesting that the damage is still reversible, probably orchestrated by common parasite–host evolution history. The mortality that eventually is observed in the farming conditions might be confounded by other secondary infections and/or intense farming pressure exerted on the fish. Such decoupling of the effect of *S. chrysoophrii* and other confounding factors is essential to understand its pathogenesis but yet needs to be studied.

Restriction site-associated DNA sequencing (2b-RAD) was used to explore the genetics of seabream resistance to *S. chrysoophrii* after infecting the gilthead seabream progeny from a controlled parental crossing and detecting SNPs.¹¹⁹ Authors evidenced that the monogenean resistance trait was heritable, observing a decreasing trend of parasite load in heavier and fast-growing families. This moderately favourable genetic correlation between production traits such as body weight and specific growth rate, and the parasite count, suggests the feasibility of gilthead seabream indirect selection for the resistance. Although the genome-wide association analysis (GWAS) revealed no major quantitative trait loci (QTL) regions over the genome-wide significance threshold for the log-transformed parasite count, a signal for a suggestive QTL with one SNP was detected in a linkage group. The low minor allele frequency criteria (a filtering threshold for SNPs) for the particular SNP indicated that the substitution of allele should have a parasite-reducing effect. Considering the other SNPs from other linkage groups, the authors suggested a polygenic nature of the trait with a chance to have few loci with relatively large, and many loci with potentially small effects. Aligning the tag sequence of the highest significantly associated SNP to the gilthead seabream genome,

authors mapped the 15 chromosome and 5 candidate genes; RNA exonuclease 2 (*REXO2*, alias *RFN122*), exportin1 (*XPO1*), nuclear transcription factor of kappa-light-chain-enhancer of activated B cells (*NFKB*), serum response factor and FHF Complex Subunit HOOK Interacting Protein 2A (*FHIP2A*, alias *FAM160B1*).¹¹⁹ Except for the latter and the exportin-1, all other genes have a role in immune processes, such as suppression of antiviral type I interferon, transport of specific mRNAs, transcription in important cellular signalling pathways and neutrophil migration. Application of versatile and high-throughput NGS tools should help to search for cues of *S. chrysophrii* resistance and the development of advanced selection methods to build up seabream lineages resilient to the monogenean.

10 | DISEASE MANAGEMENT

10.1 | Zootechnics

The control of the monogenean presence and proliferation within a farm is paramount in general farm husbandry. It is performed via an integrated control system encompassing different strategies: surveillance of the parasite's presence using diagnostic techniques and the scoring system, periodic cleaning of the most relevant substrates (i.e., nets or ropes where the eggs of the parasite can be attached), cleaning, disinfection and change of equipment for work with fingerlings and older size classes, biomass control in the cages, fast removal of the daily mortalities, distribution of the cages in the farming site following size/batch grouping, and in particular distancing of cages with newly introduced juveniles from those with older fish categories (e.g., keeping the former upstream), implementation of fallowing strategies when possible, improvement in the water filtration in recirculation aquaculture systems or flow-through systems using $\leq 30 \mu\text{m}$ mesh size filters, controlled placement of filamentous shaggy material (e.g., cotton or synthetic fibres) to collect and remove as much of eggs as possible.^{4,109} The farming site itself can be relevant for the risks associated with the disease, as stronger water currents and higher depths, as well as the distance between cages and farms, can favour the natural dispersal of eggs and oncomiracidia, reducing the risk of horizontal transmission.¹²⁰

A frequent assessment of the fouling biomass and periodical net changing is considered a recommended strategy to minimise the presence of parasite eggs in cages. Biofouling in marine aquaculture is one of the main barriers to efficient and sustainable production, accounting for 5%–10% of production costs. The main impact on the industry is effectuated via increased weight of cages, physical damage, modified hydrodynamics, retention and accumulation of pathogens and non-indigenous species, reduction of fish fitness, and consequently farm productivity.¹²¹ Therefore, changing and cleaning the net is essential not only because it reduces the substrate for the entanglement of monogenean eggs, but it also greatly reduces the water flow and oxygenation, additionally limited for fish with respiratory impairment due to the damaged gill epithelium. Moreover, synchronisation of the net cleaning and bath treatments against the monogenean with

the introduction of new fish stock is highly recommended.³⁷ While the number of net changes might incur high labour costs, especially of small-mesh size nets in eutrophic sites with high seawater temperatures, it should not be regarded as the last, but first resort in combating the monogenean. Farmers should also consider the combined application of innovative net materials that reduce the production of microfilaments, and biocide-containing coatings to avoid the attachment of organisms or to repel them. Although available coatings do not provide full biofouling protection for aquaculture nets during the whole production cycle, and novel net materials (e.g., copper alloy nets) might be costly for small family facilities, they are more sustainable in the long term.¹²² Remotely operated vehicle net cleaners have been recently introduced with success in Mediterranean aquaculture. As an attempt to replace copper-based antifoulings, these robots offer revolutionary technology for efficient and gentle cleaning of nets, combining a large volume of water with low pressure.

However, there is no published data on the presence and quantity of *S. chrysophrii* eggs and developmental stages in net biofouling, while contrasting observations have been reported for sea lice. Namely, the presence of biofouling in open-sea Atlantic salmon cages showed no influence on the average density and proportion of samples with planktonic stages of sea lice, nor the planktonic sea lice inhabited biofouling.¹²³ While the Atlantic salmon and gilthead seabream farming technologies, as well as ecological and biological traits of the two pathogens, do not allow for direct comparison and drawing of generalised conclusions, the former study could serve as a robust background for similar research in the Mediterranean.

These strategies have been recently integrated into the fish farmers' guide to combating parasitic infections in the Mediterranean aquaculture,⁴ and addressed in the PerformFish project within the prophylactic practices for Mediterranean farmed fish and risks associated with *S. chrysophrii* infection.^{32,99}

10.2 | Treatments against sparicotylosis

Treatments can be performed in cages, tanks and ponds, mainly as bath and sporadically as oral treatments, and combined with other preventive strategies to improve their efficacy (e.g., net changing, specific feeds to improve fish resilience).³⁷

Concerning chemical treatments, formalin still represents the first choice for most Mediterranean farmers,¹²⁴ even though its ban in Italy will be likely followed up in other EU countries. A recently conducted questionnaire and consensual opinion of the Mediterranean farmers undertaken within the ParaFishControl project showed that a farmer uses approximately 300 ppm (mostly 150–200 ppm) formalin in 60 min baths for fish of 20–200 g, up to two times per week in summer, synchronising the treatment with net changing.¹²⁰ This could easily exceed 10 tons of formalin applied in a commercial cycle of gilthead seabream in the sea cages, for a production of 100,000 fish. Regardless of the current trend to avoid copper-based antifoulings in the management of net changing, nets freshly painted with these chemicals seem to have a synergistic effect with formalin, perhaps due to

the antiparasitic copper properties. Indeed *S. chrysophrii*-challenged gilthead seabream, harboured significantly lower parasitic load when kept in copper-painted compared with unpainted nets in land-based facilities (Rigos, unpublished).

Other commercial synthetic and natural compounds, such as distilled water, limoseptic[®], hydrogen peroxide, chlorine, and praziquantel (PZQ) were tested against sparcotylosis as bath treatments, resulting in different efficiency.¹⁰⁹

Praziquantel has a remarkable efficacy against a wide range of helminth fish parasites¹²⁵ and it has been regarded as the most promising dietary anthelmintic in sparcotylosis also due to its high absorption in the gilthead seabream.¹²⁶ In past, PZQ showed no major effect when tested in vivo through oral intubation of low (preventive dose of 200 mg/kg) or high doses (curative dose of 400 mg/kg) over 6 consecutive days or orally once per 4 weeks, except for a reduction in the parasite abundance.¹⁰⁹ A considerable unpalatability of the PZQ-medicated diet observed in the later study and stressed elsewhere,¹²⁷ necessitated the inclusion of masking agents in the diet and alterations in the feed management. In particular, the inclusion of krill meal aids considerably the acceptance of PZQ-diets (150 mg PZQ for 3 days) delivered against *Z. seriolae* in the greater amberjack raised in cages.¹²⁸ While gilthead seabream seems more sensitive to PZQ-medicated diets than the latter, the added dietary krill meal coupled with an alternative feeding regime, accomplished a full acceptance of the medicated diet (150 mg PZQ for 3 days) in field trials (Kogiannou, in preparation). In both aforementioned works, the efficacy of PZQ against *S. chrysophrii* exceeded 80%, which is very promising for the value of dietary PZQ as a future replacement of formalin baths (or combination plans) in gilthead seabream. Although formalin baths usually induce 100% parasitic reduction, their associated disadvantages (e.g., cancerogenic for operators, laborious application, costs, weather dependency, environmental unsustainability) have forced aquaculture medicine to search for other, less impacting and polluting antimicrobial measures, such as the dietary PZQ. Importantly, cytotoxicity and ecotoxicity tests of PZQ revealed minor to no toxicity effects on non-target marine organisms.¹²⁹

Fenbendazole (FBZ; 75 mg/kg/day), a benzimidazole drug used against nematodes and cestodes, and PZQ (50 mg/kg/day) were tested in vivo trial side by side administered in feed over a month.¹³⁰ Although the low palatability of PZQ affected the feed consumption and consequently the effective administered dose in one experimental unit, in the other units where the feed has been consumed, PZQ showed to be effective already after the first treatment. Overall, FBZ showed to be the most efficient, while tested commercial feed supplemented with caprylic acid (2%) and garlic (2%), showed no satisfactory improvements over the infection.¹³⁰ However, the oscillating efficiency of tested compounds that authors observed during weekly sampling of the fish, might be also due to the study design that used a low number of fish per trial ($N = 25$) and no replicates, as well as very low PZQ dose.

Among 14 in vitro tested synthetic and natural compounds (bithionate sodium, bitoscanate, camphor, cedrol, (+)-trans-chrysanthemic acid, coumarin, curcumin, diallyl sulphide, *N,N*-diethyl-*M*-toluamid, eucalyptol, garlycin 80%, 4-hexylresorcinol, monocrotaline, pyrethrins 50%), bithionate sodium showed to be the most potent against the monogenean,

with no host cytotoxicity (tested in gilthead seabream SAF-1 cell line), and an additional medium and high potency against two common bacterial pathogens (*Vibrio harveyi*, *V. anguillarum*).¹³¹ Expectedly, natural compounds showed to be approximately 20 (cedrol) or up to 154 times (camphor) less toxic compared with bithionate sodium, but authors suggested their potential use in combined application with synthetic drugs administered through the functional feed.

Based on in vitro studies,⁴⁵ a two-dose treatment of sparcotylosis separated by 2 weeks (eventually three, in case of lower seawater temperature) was suggested to eliminate mature worms and then the hatched post-larvae. The first treatment should eliminate larval, juvenile, and adult monogeneans, and the second, which should be carefully scheduled depending on the seawater temperature, should eliminate the second generation of monogeneans emerging from the resistant eggs. To estimate the time for the second treatment, a farmer needs to consider the maximum incubation length of the eggs according to the site temperature.⁴⁹ However, there might still be a bias between observations attained through in vitro studies and the hatching in the natural environment. The authors highlighted the possibility of unsynchronised infection and the effect of abiotic traits at the facility sites that could affect the length of hatching of the novel generation. Therefore, the farms' routine of periodic monitoring starting with the increase of seawater temperature before every treatment represents a useful measure.

Additional strategies against sparcotylosis rely on the preventive effect of certain compounds administered in feeds as supplements; iron to mitigate anaemia, and immunostimulants to boost the innate host response, such as vitamin E, selenium, mannan oligosaccharides, β -glucans, nucleotides, different plant extracts, fatty acids and essential oils.^{96,132,133}

Caprylic acid was one of the first and still one of the most used feed supplements to combat sparcotylosis, even with its doubtful efficiency.¹³⁰ While its therapeutic application (200 mg/kg) over 2 months can halve the mean intensity of *S. chrysophrii* in seabream with the low initial load of the parasite (1.1–1.5), in cases of a high parasitic load, it is inefficient.¹³³ Therefore, it was suggested to apply the supplement in advance of expected outbreaks and monitor continuously the progress of the disease. Caprylic acid has been further admixed with organic iron (0.2% of diet) to counteract anaemia, and mannan oligosaccharide (0.4% of diet) to stimulate the innate immunity of infected fish.⁹⁶ While the prevalence was not affected by a 10-week treatment, the mean intensity of adults and larvae was again halved, and the body weight of infected fish remained the same as that of the control. The latter is particularly important, as infected fish may increase their food conversion ratio by 0.2–0.4 at high parasite abundance, which reflects in large economic loss generated by an additional administered food and reduced growth (Rigos, unpublished).

A commercially available microencapsulated feed additive composed of garlic, carvacrol, and thymol essential oils (AROTEC-G[®]) administered over 65 days was tested by challenging the naïve fish in cohabitation trial with infected donors.¹³² The feed proved efficient to a greater extent, reducing monogenean abundance by 78% and decreasing the prevalence of most parasitic developmental stages

(not affecting post-larvae). It also proved to be beneficial for gill health, since the authors also scrutinised the transcriptomic profile of gilthead seabream-fed AROTEC-G[®]. They observed several biological processes associated with immunity, biogenesis and metabolic processes, such as the upregulated peptide biosynthesis, and protein and lipid metabolism, indicating the restoration of damaged gill tissues. Additionally, biogenesis pathways likely induced the activation of processes of secretory protein translocation by vesicles, supported also by observed exocytosis. However, expressed cytokines milieu suggested an anti-inflammatory reaction (expression of interleukins *il7*, *il6r*, *il20ra* and *il21r*), which is not surprising given that the transcriptomic profiling of gills was conducted at the end of the nutritional trial in fish, but before the cohabitation infection.

Since the administration of essential oils in fish feed might induce off flavours in the final product, testing of the sensory and physicochemical parameters in fillets of the gilthead seabream (fed AROTEC-G[®] for 4 weeks) showed that organoleptic traits were not changed and that fillets did not exhibit aromatic or anomalous odour, nor aromatic or anomalous flavour. Physicochemical parameters were slightly altered; AROTEC-G[®]-fed fish were darker in colour, and the fillet textures showed increased hardness, adhesiveness and gumminess.¹³⁴

Lastly, propyl-propane-thiosulfinate and propyl-propane-thiosulfonate, two organosulfur compounds from onion (*Allium cepa*), already known for their antioxidant, antimicrobial, antifungal and antiparasitic properties have been tested in vitro and in feed of *Sparicotyle*-infected gilthead seabream.¹³⁵ While the number of parasites significantly decreased in both trials, authors remarked that the degree of efficiency of *Allium* compounds will depend on the composition of the active ingredients and the dose of inclusion in the feed.

Accounting for all published studies on the subject, Mediterranean aquaculture is lacking a registered prophylactic and therapeutic formulation that is safe, effective, practical, weather-independent, environmentally friendly and economically affordable against sparcotylosis. Consequently, efforts to register promising dietary anthelmintics such as PZQ should be a priority for European pharmaceutical companies. Under current conditions, the best prophylactic strategy should therefore integrate functional feeding, routine gill inspection in seasons of expected parasite pressure (e.g., increased temperatures), constant net cleaning with ROVs to avoid the detrimental effects of copper-based antifoulings,¹³⁶ and a consequent treatment with an anti-parasitic compound, based on specific predetermined infection indexes [these levels may vary among Greek farms from 8 to 15 parasites/per fish (total counting of both adults and larvae; Rigos unpublished)]

11 | FURTHER DIRECTIONS FOR STAKEHOLDERS IN THE SECTOR

11.1 | Epidemiologic studies and a national monitoring database

Although farmers are monitoring the incidence of *S. chrysophrii* with the first seasonal increase of the seawater temperature, the data is

not publicly available, and the published scientific studies and reports are obsolete, lacking a robust epidemiological design in terms of replicates, such as fish size categories, cages, and production units at different locations, seasons and subsequent years, even the standardised method for counting of the monogenean. Integrated pest management plans should be drawn relying on the sea lice experience from the salmon aquaculture¹³⁷ and adapted to fit the Mediterranean sector's profile based on national epidemiological surveys. Monogenean surveillance on the farms should be normalised across the countries in the region, and data should be stored and shared through curated national repositories, allowing the data analyses and follow-up of IMP efficiency.

11.2 | The resilience of *S. chrysophrii* to climate changes

The monogenean has been listed as a potential parasite whose proliferation is expected to increase under global warming conditions.¹³⁸ According to the authors, a temperature increase of up to 2.5°C is predicted by 2100 for the western Mediterranean region, which overlaps with the thermal tolerance range both of the gilthead seabream and the monogenean.⁴⁹ Similarly, the annual decrease of 0.004 pH units for the western Mediterranean underwater acidification conditions, predicting approximately a 7.4 pH by 2100, would not greatly affect the monogenean early survival.⁴⁹ Therefore, studies relying on long-term epidemiological data series and carefully designed in vitro experiments testing monogenean resilience to abiotic factors should strive to bring together more understanding of its physiology, helping to enrich our knowledge on *Sparicotyle* drug and vaccine targets.

11.3 | Breeding programs

Implementation of genomic selection in breeding programs could be an efficient methodology to genetically improve the host resistance to *S. chrysophrii*. Indeed, a recent study reveals that the resistance of gilthead seabream against the parasite is significantly heritable,¹¹⁹ therefore, further research on how to genetically improve the host should be encouraged to deliver a valuable tool for the producers to reduce the parasitic prevalence in farmed gilthead seabream.

11.4 | Vaccines and treatments

The ParaFishControl project generated the first genome and transcriptomes of the monogenean (unpublished). This valuable tool should be fully exploited by the scientific community to identify in silico drug-target candidates and test the novel drugs under relevant conditions to accelerate their bringing to the market. Simultaneously, the reversed vaccinology relying on the identification of potential vaccine candidates through screening of the monogenean proteome using computational analyses should be applied to generate the first

anti-*Sparicotyle* vaccine and later into developing vaccination programs. The efforts should be paid off because a glimpse at the last 25 years of research indicates that *Sparicotyle* is bound to stay. The recent promising field observations of dietary PZQ used as anthelmintic against *S. chrysophrii*, coupled with the drug's low ecotoxicity impact and the fact that its central registration is a current target of pharmaceutical companies, render this drug an ideal substitute for formalin, which soon might become banned in EU aquaculture medicine.

AUTHOR CONTRIBUTIONS

Ivona Mladineo: Conceptualization; investigation; funding acquisition; writing – original draft; methodology; visualization; writing – review and editing; formal analysis; project administration; resources.
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Paola Beraldo: Data curation; methodology; visualization; writing – review and editing; investigation; writing – original draft.
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CONFLICT OF INTEREST STATEMENT

The authors declare there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in Google Scholar at <https://scholar.google.com>.

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