Living on the edge: parasite communities of three-spined stickleback fish populations at the southernmost limit of the species' distribution

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Introduction

Parasitism is a common form of life occurring in different ecosystems, and few relationships are as intimate as those between a parasite and its host, leading to strong ecological and evolutionary associations between them [1]. This generates spatially heterogenous parasite communities as their diversity, composition and abundance vary across the geographical range of a host species [2, 3]. To better predict how parasite communities will respond to global climate change, as well as the cascading effect this will have on their hosts, understanding the geographic component of parasite communities is increasingly urgent. Therefore, it is crucial to explore patterns of geographical variation in the diversity and abundance of parasite populations across different environmental gradients, something that has been rarely done [4].

This study represents a first approach to fill this knowledge gap by sampling populations of three-spined stickleback (*Gasterosteus aculeatus* L.), a model aquatic vertebrate species for ecology and evolution studies and aims to characterize the parasite community associated with this species in populations located near its southern limit of distribution [5]. Three-spined stickleback is a widespread fish species that occurs in almost the entire northern hemisphere [6], presenting its southern limit of distribution in the Mediterranean region [7,8]. It is characterized by a complex of phenotypically different populations (including strictly marine or freshwater populations, as well as anadromous ones), resulting from its different adaptations to biotic and abiotic factors imposed by the different types of habitats [9,10]. Such characteristics make this species an excellent model for studying geographic variations in parasite-host interactions, across a wider range of biological and environmental conditions.

Publications on parasites of three-spined stickleback are numerous, but to date have mainly dealt with single species (taxa) of parasites, e.g. [11], or on populations of sticklebacks that inhabit the centre of the species distribution, where sticklebacks are well established [12]. To the best of our knowledge, nothing is known about parasite communities of stickleback at the southern limit of their range. Despite its wide distribution across the Northern hemisphere, stickleback fish are classified as Endangered [EN; 13, 14] species in Southern European countries, including Portugal. A major contributor to this is the degradation of habitat quality and quantity, particularly resulting from ongoing climate change, namely prolonged dry periods in rivers and streams during the summer months, rising water temperatures, and the presence of invasive fish species that, in addition to preying on and competing with sticklebacks, may have introduced new parasites into ecosystems as well [13, 15, 16, 17].

This study involved collecting parasite data from populations inhabiting the most southern end of the species distribution in the North Atlantic (i.e., Portugal), aiming to understand the differences in parasite community associated with the populations that persist at such low latitudes. These data were then compared with similar existing parasite community data from populations of central and northern areas of the species distribution (i.e., Scotland and Iceland). Specifically, we test the hypothesis that stickleback populations at the most southern end of the species distribution in the North Atlantic are exposed to different parasite communities, creating more stressful conditions, than central and northern populations. Differences in parasite community due to changes in the host environment are expected because of the presence of new invasive species such as the mosquitofish (*Gambusia holbrooki,* Girard 1859), a non-indigenous fish nowadays present in all river basins from Portugal and regarded as one of the most dangerous invasive species at a global scale according to the International Union for the Conservation of Nature (IUCN).

Material and Methods

Fish collection

We sampled a total of 31 sites from 22 rivers and five sites from four coastal lagoons, in regions classified as of high probability of three-spined stickleback occurrence throughout mainland Portugal (Table 1; 17). Fish were collected using minnow traps, which were left in each location for a period that lasted between 12 and 24h in each site. Once the traps were collected, individual sticklebacks were counted and a sample in each population was analysed for parasites, as described below. Sticklebacks are an endangered species in Portugal and therefore have a protected status. Collection could only be done under a licence from the Institute for Nature and Forest Conservation (ICNF). Our sampling licence restricted the number of individuals that we could anaesthetise to check for skin parasites to 20 individuals per population and the number we could euthanise to count numbers of eye and gut parasites to 10% of the individuals caught, with a maximum of 10 individuals per location. The fish selected for analyses were haphazardly selected for individuals of all sizes, sex, and breeding condition (Fig. 2).

Microscopy and parasite data collection

To characterize the parasite communities in each fish population, the number of skin parasites (e.g., *Gyrodactylus* spp.*)*, body cavity parasites (e.g., *Schistocephalus solidus)* and eye parasites (e.g., *Diplostomum* spp.) per fish were counted when fish were processed in the following way:

- *Skin parasites:* skin parasites quickly detach themselves once a fish is dead. Therefore, for each individual fish, the number of individual

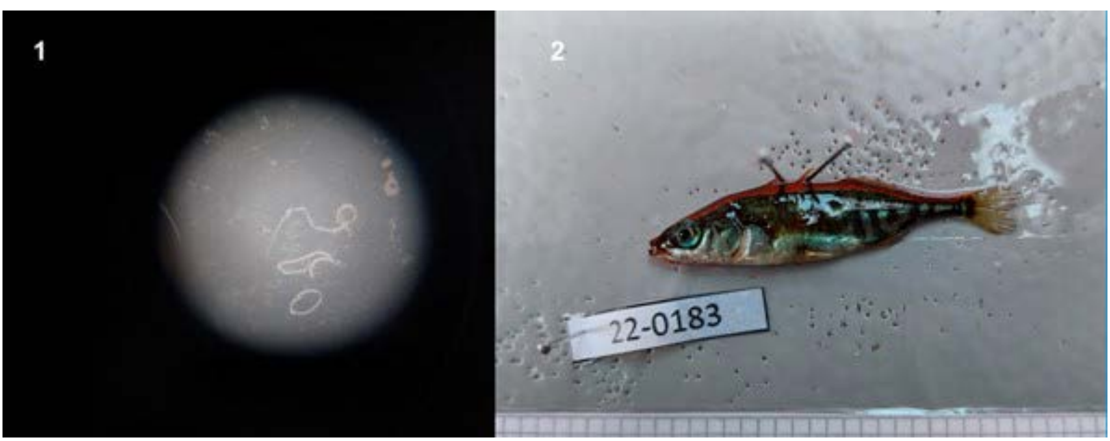

Fig. 1. Pictures by André Moreira of examples of parasites observed in analysed fish (1) and pictures of a specimen of the target species collected during the work in Portugal in site 2 of Âncora River (2).

Gyrodactylus spp. was counted while the fish was mildly sedated. This was done by using an Olympus SZ61 dissecting microscope. Counting of number of skin parasites was done in a standardized way, starting from tail to head (including fins) with the fish turned with the left side up, and then repeating the process with the right side up. Identification of *Gyrodactylus* spp. (and body cavity and eye parasites, see below) to species level was

Location	code	Coordinates	Sampling date	No. fish trapped	No. fish checked for skin parasites	No. Gyro	No. fish checked for eye and gut parasites	No. eye parasites	No. gut parasites
Capelinha River	CAPE	37 38 45.2N, 8 34	4/11/22	27	27	0	2	0	0
Torgal River	TORG	37 37 26N, 8 40 1(	4/11/22	0	-	-	0	-	-
Moinho da Asneira – Mira River	ASNE	37 43 47.5N, 8 45	4/11/22	0	-	-	0	-	-
Santo Andre Lagoon	ANDR	38 06 17.8N, 8 47	4/12/22	0	-	-	0	-	-
Melides Lagoon	MELI	38 07 49.4N, 8 47	4/12/22	0	-	-	0	-	-
Grândola River	GRAN	38 09 56.6N, 8 33	4/13/22	3	3	0	0	-	-
Marateca River 1	RIMA	38 35 38.9N, 8 38	4/13/22	122	20	0	10	0	0
Marateca River 2	MARA	38 34 10.8N, 8 44	4/13/22	0	-	-	0	-	-
Sorraia River	SORR	38 55 24.4N, 8 53	4/14/22	0	-	-	0	-	-
Almansor River 1	ALMA	38 51 20.9N, 8 44	4/14/22	0	-	-	0	-	-
Almansor River 2	CANH	38 42 28.4N, 8 23	4/15/22	6	6	2	0	0	0
Ulme River	ULME	39 09 04.6N, 8 41	4/15/22	0	-	-	0	-	-
Almonda River 1 (below dam)	ALM1	39 30 17.0N, 8 36	4/18/22	25	10	0	2	0	0
Almonda River 2 (above dam)	ALM2	39 30 17.0N, 8 36	4/18/22	55	10	0	5	0	0
Alcoa River	ALCO	39 34 41.9N, 9 4 3	4/18/22	0	-	-	0	-	-
Obidos Lagoon 1	OBI1	39 23 10.8N, 9 13	4/18/22	0	-	-	0	-	-
Obidos Lagoon 2	OBI2	39 24 02.4N 9 13	4/18/22	0	-	-	0	-	-
Ançã River	ANCA	39 58 44.2N, 8 34	4/19/22	14	14	8	1	0	0
Anços River	ANSO	39 58 43.5N, 8 34	4/19/22	82	20	6	8	0	0
Corujeira River	CORU	40 22 17.3N, 8 42	4/20/22	23	20	2	0	0	0
Mira Lagoon	MIRA	40 27 03.3N, 8 47	4/20/22	0	-	-	0	-	-
Vouga River 1	AVES	40 33 33.9N, 8 45	4/20/22	0	-	-	0	-	-
Vouga River 2	AVEN	40 42 43.8N, 8 33	4/21/22	13	10	0	1	0	0
Caster River	CAST	40 50 52.6N, 8 38	4/21/22	6	-	-	0	-	-
Seixo River	SEIX	40 50 12.0N, 8 37	4/21/22	5	5	0	0	0	0
Milhazes River	MILH	41 29 57.5N, 8 42	4/22/22	0	-	-	0	-	-
Cavado River 1	CAV1	41 30 52.3N, 8 44	4/22/22	0	-	-	0	-	-
Cavado River 2	CAV2	41 31 21.8N, 8 47	4/22/22	0	-	-	0	-	-
Lima River 1	LIM1	41 41 41.6N, 8 46	4/23/22	1	-	-	0	-	-
Lima River 2	LIM2	41 44 32.8N, 8 38	4/23/22	2	2	0	0	-	-
Lima River 3	LIM3	41 44 44.1N, 8 38	4/23/22	15	15	10	1	0	0
Estorãos River	ESTO	41 45 17.4N, 8 38	4/23/22	0	-	-	0	-	-
Âncora River 1	ANC1	41 48 36.7N, 8 51	4/24/22	6	6	0	1	0	0
Âncora River 2	ANC2	41 48 26.4N, 8 51	4/24/22	27	15	11	2	0	0
Coura River 1	COU1	41 53 15.1N, 8 47	4/24/22	7	7	44	0	-	-
Coura River 2	COU2	41 52 17.9N, 8 48	4/24/22	2	2	0	0	-	-

Table 1. Summary of the sampling (from left to right): Names of sites sampled, abbreviations of their names (code), latitude and longitude coordinates, sampling date, number of fish trapped per site, number of fish checked for skin parasites, number of total *Gyrodactylus spp.* ("Gyro") counted per population, number of fish euthanised and checked for body cavity and eye parasites and numbers of eye and gut parasites counted.

carried out by inspection of microscopic features (e.g., haptors hooks and trophic morphology) using an Olympus CK2 inverted microscope;

- *Body cavity parasites:* each fish was cut from the cloaca to the ventral fins and the body cavity was then checked for parasites such as the tapeworm *Schistocephalus solidus*. Tapeworms parasitising three-spined sticklebacks can range from a few centimetres long to less than a millimetre, so the body cavity of the fish was inspected under an Olympus SZ61 microscope to check for small parasites present within the body cavity;
- *Eye parasites:* eye parasites such as *Diplostomum* spp. (Fig. 1) were identified and counted on preserved fish in the lab at Nottingham University. This was done by using an Olympus SZ61 microscope.

Data analyses and visualisation

Total numbers of fish collected, anesthetized, and euthanized, and total numbers of parasites counted per sampling site can be found in Table 1. Sites where no sticklebacks were found were excluded from further analyses. We calculated average numbers of each type of parasites per site as the total number of parasites found in a given site, divided by the number of fish on that site checked for that type of parasites. Averages and standard deviations of parasites per site were calculated and visualised using the software R. To compare average numbers of parasites in Portuguese sites with those from sites in Iceland and Scotland we plotted average numbers of parasites across 7 sites in Portugal (the ones where we it possible to catch fish from the target species with parasites in the 2022 sampling campaign), 30 in Iceland and 27 in Scotland. Sites with no parasites were excluded from this analysis.

Results

In the sampling campaign conducted in Portugal, stickleback numbers per site were generally low, with no fish from the target species being

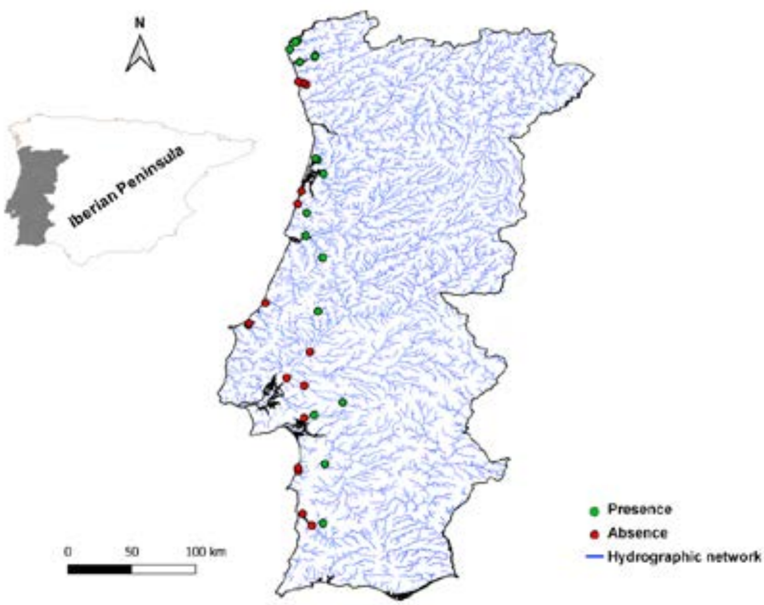

Fig. 2. Location of the sites sampled in Portugal, in the spring of 2022, for three-spined stickleback, with indication of respective presences and absences.

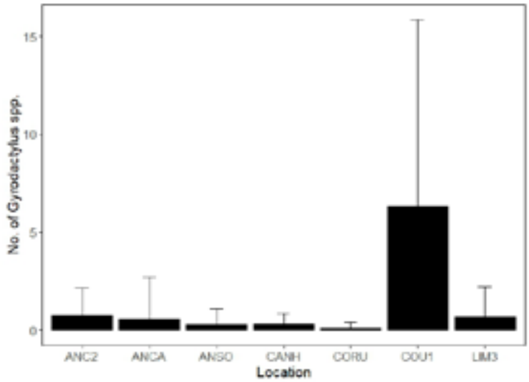

Fig. 3. Average number of *Gyrodactylus* spp. per site, of the seven sites where the parasite species was found. Black bars represent averages and lines the standard deviations.

collected in 17 out of the 36 sites sampled (Table 1; Fig. 1). In the remaining 19 sites, numbers of stickleback captured ranged between 1 and 122, with an average of 23 fish per site. Parasite numbers were also generally low with eye and body cavity parasites being absent from all individuals analysed. Skin parasites, specifically *Gyrodactylus* spp. were present in seven sites. In these southern European populations, the number of *Gyrodactylus* spp. counted in a single fish ranged between 0 and 22. Totals per site ranged between 2 and 44, with average numbers per site and fish between 0.1 (Corujeira River) and 6.3 (Coura River 1) (Fig. 3). When comparing between countries, Iceland populations had, on average, the highest numbers of *Gyrodactylus* spp., followed by Scotland and then Portugal (Fig. 4).

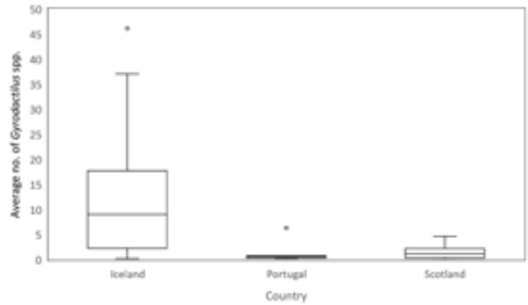

Fig. 4. Box-plots based on average numbers of Gyrodactylus spp. in 30 locations in Iceland, seven in Portugal and 27 in Scotland. Box-plots show the interquartile range (IQR) from 25th to 75th percentile with horizontal lines indicating medians. The range bars encompass all data within 1.5 IQR above and below the upper and lower IQRs, respectively. Dots show individuals outside of that range.

Discussion

Parasitism is one of nature's most intricate relationships, fostering profound connections between parasites and their hosts. This interdependence fuels evolutionary and ecological bonds, driving geographical variations in parasite communities across diverse landscapes [1,2,3]. Knowledge on the diversity in parasite community composition and abundance across a host species' range is pivotal to understanding host populations' health and subsequently predicting how these communities might respond to global climate changes [4]. However, investigating patterns in parasite diversity and abundance across geographic and environmental gradients has remained an understudied topic. This study addressed this significant knowledge gap by analysing the ectoparasite community associated with three-spined stickleback, a model organism renowned for adaptation and evolution with a diverse habitat range across the northern hemisphere that reaches its southernmost edge in the Mediterranean region [5]. This species' phenotypic diversity, and the diversity of habitats it inhabits, spanning from marine to freshwater and anadromous populations, offers a unique lens to scrutinize geographic variations in parasite-host interactions across diverse environmental conditions.

The sampling conducted in Portugal unveiled a strikingly low abundance of stickleback across sites, with 17 out of 37 sampled sites yielding no specimens of the target species, In sites where sticklebacks were found, their numbers were low, averaging 23 per site. Such information confirms the poor conservation status of this species in these southernmost habitats, classified as Endangered by the last revision of the Fish Rede List in Portugal [13]. The fact that stickleback were absent from some populations where they had been recorded in the past [18] and the lower densities in some populations, strongly suggests that abundance is still decreasing. This is likely due to increased habitat degradation exacerbated by climate change, elevated water temperatures and prolonged dry spells in water bodies [15, 16, 17].

While low fish abundance was partly expected, poor environmental conditions and the incursion of invasive fish species like the mosquitofish that potentially introduce novel parasites into ecosystems lead us to hypothesise that populations in this region would be more susceptible to parasitism when compared to central and northern populations (Scotland and Iceland). However, our results revealed a rather unexpected scenario. Parasite numbers mirrored the trend of host scarcity, with eye and body cavity parasites notably absent in all examined individuals. Skin parasites, specifically *Gyrodactylus* spp., were detected in only seven sites.

Strikingly, comparisons among countries showcased a gradient from north to south with Icelandic populations exhibiting the highest average numbers of *Gyrodactylus* spp., trailed by Scotland and then Portugal. This result challenges the anticipated hypothesis of southern populations enduring more severe parasite pressures due to environmental alterations, including the introduction of invasive species. Two factors may contribute to the lower parasite numbers found in Portuguese populations. The first is that Portugal has no natural lakes, so we sampled stickleback in rivers, streams and lagoons, while in Iceland and Scotland we primarily sampled lakes. *Gyrodactylus spp.* is known to cause changes in their host, like erratic swimming behaviour [19], or even cause the hosts' fins to become contracted and the fin rays to fuse together making their swimming in fast flowing water extremely difficult or impossible. Fish highly infected with Gyrodactlylus *spp.* would be unlikely to survive in the streams or rivers but could still live in lakes. The second factor is that low fish density might contribute to maintaining the density of parasites low due to less proximity and contact between individual fish.

In conclusion, while our initial hypothesis posited greater stress on southern stickleback populations due to distinct parasite communities, our findings unveil a complex and unexpected disparity between the central (Iceland and Scotland) and southern regions. These results highlight the importance of demography and environmental factors shaping parasite-host interactions across diverse habitats. Such insights are pivotal in formulating conservation strategies for endangered species like the three-spined stickleback, navigating the tumultuous waters of climate change and invasive species encroachments.

References

1. Combes, Claude. *Parasitism: the ecology and evolution of intimate interactions*. University of Chicago Press, 2001.

2. Johnson, P.T., Wood, C.L., Joseph, M.B., Preston, D.L., Haas, S.E. and Springer, Y.P., 2016. Habitat heterogeneity drives the host-diversity-begets-parasite-diversity relationship: evidence from experimental and field studies. *Ecology Letters*, *19*(7), pp.752-761.

3. Fecchio, A., Pinheiro, R., Felix, G., Faria, I.P., Pinho, J.B., Lacorte, G.A., Braga, E.M., Farias,

I.P., Aleixo, A., Tkach, V.V. and Collins, M.D., 2018. Host community similarity and geography shape the diversity and distribution of haemosporidian parasites in Amazonian birds. *Ecography*, *41*(3), pp.505-515.

4. Pérez-Rodríguez, A., Fernández-González, S., de la Hera, I. and Pérez-Tris, J., 2013. Finding the appropriate variables to model the distribution of vector-borne parasites with different environmental preferences: climate is not enough. *Global change biology*, *19*(11), pp.3245-3253.

5. Barber, I., 2013. Sticklebacks as model hosts in ecological and evolutionary parasitology. *Trends in parasitology*, *29*(11), pp.556-566.

6. Berna, T. M. (2001). *Freshwater Fish Distribution*. United Kigdom: Academic Press.

7. Crivelli, A. J., & Britton, R. H. (1987). Life history adaptations of *Gasterosteus aculeatus* in a Mediterranean wetland. *Environmental Biology of Fishes*, 18(2): 109-125.

8. Rind, K., Rodriguez-Barucg, Q., Nicolas, D., Cucchi, P., & Lignot, J. (2020). Morphological and physiological traits of Mediterranean sticklebacks living in the Camargue wetland (Rhone river delta). *Journal of Fish Biology,* 97(1): 1-13. DOI:10.1111/jfb.14323

9. Bell, M. A., & Foster, S. A. (1994). *The evolutionary biology of the threespine stickleback*. Oxford: Oxford University Press.

10. Foster, S. A., Baker, J. A., & Bell, B. A. (2003). The case for conserving threespine stickleback populations: protecting an adaptive radiation. *Fisheries*, 28(5): 10-18.

11. Weber, J.N., Kalbe, M., Shim, K.C., Erin, N.I., Steinel, N.C., Ma, L. and Bolnick, D.I., 2017. Resist globally, infect locally: a transcontinental test of adaptation by stickleback and their tapeworm parasite. *The American Naturalist*, *189*(1), pp.43-57.

12. Magalhaes, I.S., D'Agostino, D., Hohenlohe, P.A. and MacColl, A.D., 2016. The ecology of an adaptive radiation of three-spined stickleback from North Uist, Scotland. *Molecular Ecology*, *25*(17), pp.4319-4336.

13. Magalhães MF, Amaral SD, Sousa M, Alexandre CM, Almeida PR, Alves MJ, Cortes R, Farrobo A, Filipe AF, Franco A, Jesus J, Oliveira JM, Pereira J, Pires D, Reis M, Ribeiro F, Robalo JI, Sá F, Santos CS, Teixeira A, Domingos I. 2023. Livro Vermelho dos Peixes Dulciaquícolas e Diádromos de Portugal Continental. FCiências.ID & ICNF, I.P. Lisboa.

14. Doadrio I. (2002) *Atlas y libro rojo de los peces continentales de España*. Ministerio de Medio Ambiente, Madrid, Spain.

15. Alexandre, C.M. and Almeida, P.R., 2010. The impact of small physical obstacles on the structure of freshwater fish assemblages. *River Research and Applications*, *26*(8), pp.977-994.

16. Collares-Pereira, M.J., Alves, M.J., Ribeiro, F., Domingos, I., Almeida, P.R., Costa, L., Gante, H., Filipe, A.F., Aboim, M.A., Rodrigues, P.M. and Magalhães, M.F., 2021. Guia dos peixes de água doce e migradores de Portugal Continental

17. Moreira, A., Boavida-Portugal, J., Almeida, P.R., Silva, S. and Alexandre, C.M., 2022. Macro-Habitat Suitability for Threespine Stickleback (Gasterosteus aculeatus L.) Near

the Southern Limit of Its Global Distribution: Implications for Species Management and Conservation. *Fishes*, *7*(5), p.271.

18. Fang, B., Merilä, J., Ribeiro, F., Alexandre, C.M. and Momigliano, P., 2018. Worldwide phylogeny of three-spined sticklebacks. *Molecular Phylogenetics and Evolution*, *127*, pp.613-625.

19. Cable J, Scott ECG, Tinsley RC& Harris PD. 2002 Behavior favoring transmission in the viviparous monogenean *Gyrodactylus turnbulli*. *J. Parasitol.* **88**, 183–18

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