

Article

Spatial and Temporal Variability of Parasite Communities: Implications for Fish Stock Identification

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Abstract: The spatial and temporal variability of parasite communities have received little attention when used as biological tags for identifying fish stocks. This study evaluated the potential spatial and temporal variability of the parasite communities affecting three marine fish species collected between 1993 and 2017. To avoid the potential effect of host age in parasite communities, individuals of similar ages were selected: 1123 *Engraulis ringens* (12–24 months old), 1904 *Trachurus murphyi* (24–36 months old), and 630 *Merluccius gayi* (36–48 months old). Most taxa show differences in the prevalence at the spatial and temporal scales, but the prevalence of some larval endoparasites remains constant at the temporal scale. At the spatial scale, an analysis of similarity (ANOSIM) showed differences in the parasite communities of three species; a canonical analysis of principal coordinates (CAP) showed low values of correct allocations (CA; $\approx 50\%$) and values of allocation due to chance (AdC) were lower than the CA. At the temporal scale, an ANOSIM showed differences between the three species. A CAP showed low values of CA ($\approx 50\text{--}60\%$) and the AdC was always lower than CA. Samples at the spatial scale were well allocated to their localities or nearby localities, suggesting a spatial stability. Samples from different years were not well discriminated, suggesting temporal variability. Therefore, in studies regarding parasites as a tool for stock identification, temporal variability must be taken into account.



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1. Introduction

Fishery management units are spatially defined by geographic or fishing grounds, but conventional stock assessment models assume that a fishery resource is a single, homogeneous population [1] with similar population parameters such as growth rate, recruitment, maturity and mortality; in addition, young fish in a group are produced by previous generations of the same group [2]. However, when evidence supports differences in population parameters in different areas (stocks), those must be assessed and subsequently managed as independent units [3]. Consequently, a requisite for the evaluation and posterior management of fish stocks is the correct identification [2]. The complexities of marine ecosystems present many challenges for defining population structures; hence, a variety of tools have been developed for stock identification, including: physical and electronic tags; morphometric and meristic variation; morphology and/or the chemical composition of otoliths; genetic molecular analysis; and parasite burdens [1].

Parasites have been successfully used in stock identification [4]. Nevertheless, since the pioneering studies [5,6] to the present [7–9], the criteria for using parasites as biological tags (BT) for stock identification have changed and evolved [10,11]. The features that parasites

must present to be considered BTs, the procedures and methods for their application and their advantages and limitations have received much attention, including as the subject of many reviews [10,12,13]. Nevertheless, despite the potential spatial and temporal variability in parasite fauna being a key issue in fish stock identification, such variability has received much less attention [14–16].

The importance of spatial variability is clear. Spatial variability in the composition and abundance of parasite assemblages makes the use of parasites as BTs for fish stock identification possible [15–17]. If the loads and species of the parasites of two groups of fish are similar, then they are likely to have had a common history; if their loads are significantly different from another group, then the groups are likely to have had a different life history [18]. However, variability in parasite fauna does not necessarily mean that fish with different parasite loads belong to different stocks [19]. To assess the variability in a community, the magnitude of the study area must be considered [20].

In stock identification using parasites as BTs, caution must be taken when analyzing localities that are 100 km or less apart [21]. That is, to capture local variability, samples in locations separated by less than 100 km should be studied. Temporal variability must also be taken into account to consider a parasite as a good BT—levels of infection should remain relatively constant from year to year [12]. However, differences in infection levels within the same population may be greater over time than the differences between populations [22]. It has been suggested that before making large-scale spatial comparisons, it is necessary to have estimates of small-scale spatial variation [23]. Therefore, to reliably infer a fish stock's structure based on parasitological data, an adequate replication of sampling at various spatial and temporal scales is needed [24]. Comparative studies over long periods require a high degree of temporal repeatability in the composition and structure of parasite communities [15,25]. Likewise, parasite communities vary through the ontogeny of their hosts, as communities in older hosts are more predictable, and ontogenetic variations should also be considered [26].

In the southeastern Pacific Ocean (SEPO), the most important pelagic commercial species are the Peruvian anchovy (*Engraulis ringens* Jenyns, 1842) and the Chilean jack mackerel (*Trachurus murphyi* Nichols, 1920), and one of the most important demersal species in the SEPO is the South Pacific hake (*Merluccius gayi* Guichenot, 1848) [27]. For these three species, parasitological studies with stock implications have been published [14,16,28–34]. Consequently, parasitological records and stock structures have been proposed for these species in the SEPO. Therefore, these species are good models for the study of the spatial and temporal variability in parasite communities and the stock assessment implications.

The goal of our study was to test for spatial and temporal variability when metazoan parasite communities are used as a tool for stock identification.

2. Materials and Methods

2.1. Ethics Declarations

This study was approved by the Ethical Commission of the Universidad de Antofagasta, Antofagasta, Chile. This study did not consider experiments with live animals. All fishes were obtained from commercial catches and none of the species are subject to conservation measures. Commercial fishermen follow national regulations concerning these fisheries.

2.2. Fish Sample

A total of 7142 individuals belonging to three species—*E. ringens* ($n = 2050$), *T. murphyi* ($n = 4021$) and *M. gayi* ($n = 1071$)—were collected from different localities in the SEPO, including two landing ports in Peru—Paita (Northern Peru, NPE), 5° S; and Callao (Central Peru, CPE), 12° S—and five landing ports along the Chilean coast—Antofagasta (ANT); 23°40' S; Coquimbo (CQM), 30° S; Valparaíso (VAL), 33° S; Talcahuano (THN), 36°40' S; and Puerto Montt (PTM), 41°30' S—covering a spatial scale of more than 4600 km. The

closest landing ports were Coquimbo and Valparaíso, separated by ca. 300 km. (Figure 1). The temporal scale reached a maximum of 24 years, between 1993 and 2017.

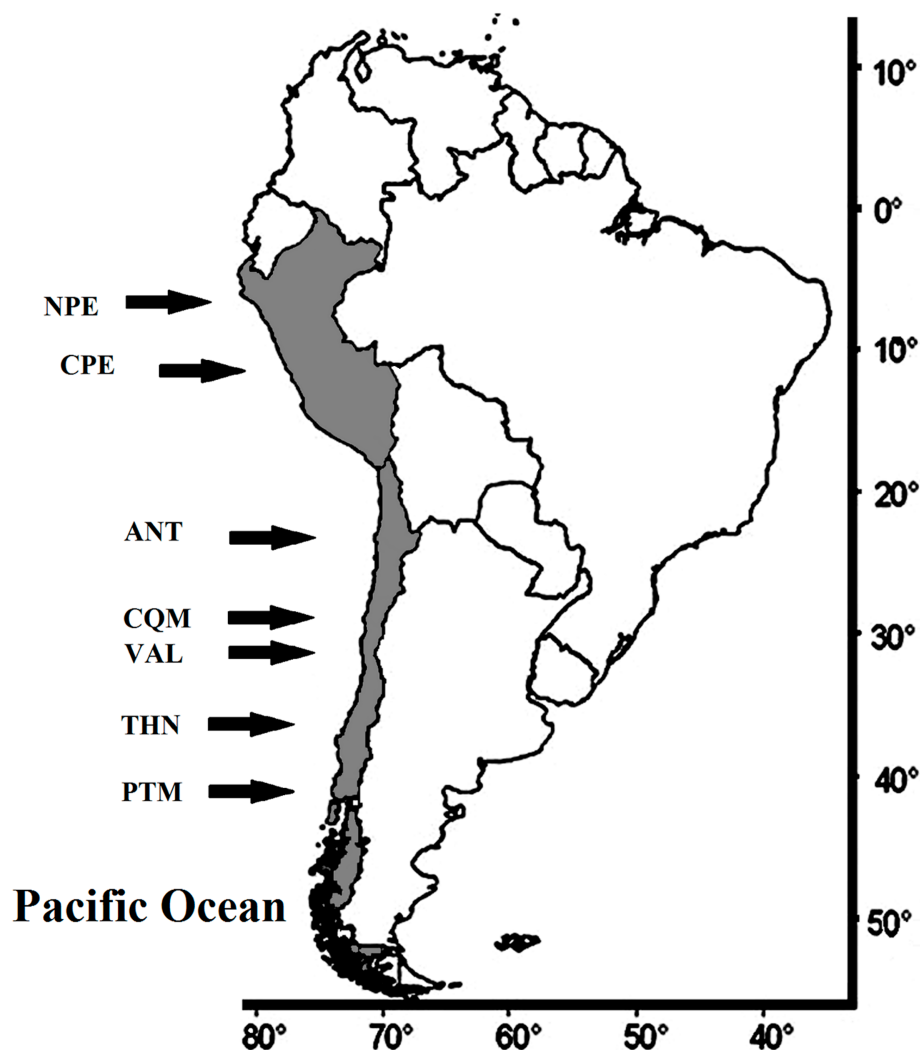


Figure 1. Approximate position of sampling localities. NPE = Paita 5° S (Northern Peru); CPE = Callao 12° S (Central Peru); ANT = Antofagasta 23°40' S (Chile); CQM = Coquimbo 30° S (Chile); VAL = Valparaíso 33° S (Chile); THN = Talcahuano 36°40' S (Chile); PTM = Puerto Montt 41°30' S (Chile).

In the laboratory, the total length (TL; nearest cm) of the *E. ringens* and *M. gayi* and the fork length (FL; nearest cm) of the *T. murphyi* were measured. Then, the specimens were dissected and examined for metazoan parasites that (as reported previously) affect these species [14,28,31,34]. Specifically, after a necropsy, each fish was carefully examined, first for ectoparasites, in the skin, gills and buccal cavity, and then for endoparasites. All organs, including heart and blood vessels, were examined. To count parasites, each visceral organ was separately dissected and washed in running water. All the material retained in a mesh (0.5 mm) was examined under a stereomicroscope (20×).

Because the parasite communities change through the ontogeny of their fish hosts [26], to avoid the potential effect of host age in the parasite communities a subsample of specimens of similar ages was selected for each host species. To estimate fishes' ages, the von Bertalanffy growth function was used; the population parameters for *E. ringens* were $K = 0.91$, $t_0 = -0.01$ and $L_\infty = 18.72$ [35]; for *T. murphyi* they were $K = 0.16$, $t_0 = -0.13$ and $L_\infty = 75.0$ [36]; and for *M. gayi* they were $K = 0.2$, $t_0 = -0.07$, and $L_\infty = 81.9$ [37].

2.3. Bibliographic Search

In order to search for references regarding the potential impact of temporal and spatial variability when parasites are used as biological tags, we searched the Web of Science (www.webofknowledge.com, accessed on 8 June 2021) with the following search term: Parasite * stock; Fish * stock identification; fish * stock * discrimination; Parasite * Biological tag. The search was restricted to the years 2000–2020.

2.4. Univariate Analysis

The prevalence and abundance [38] were calculated for each parasite taxon and for each locality and year for each fish species of the same age. To test the hypothesis of an absence of significant differences in the prevalence of each parasite taxon between host species at a spatial scale, samples from the same year were tested ($\alpha = 0.05$). In a similar way, the hypothesis of an absence of significant differences in the prevalence of each parasite taxon between host species at a temporal scale, samples from the same locality were tested ($\alpha = 0.05$). Significance in differences in prevalence was tested with Fisher's exact test [39], and this analysis was performed with R software [40].

Because parasites with a prevalence <5% can be considered accidental [34], only parasite taxa with a prevalence >5% in at least one locality and year were considered in the multivariate analysis.

2.5. Multivariate Analysis

Nonmetric multidimensional scaling (nMDS) was used to graphically visualize patterns of similarity in parasites' community structures at temporal and spatial scales for each host species. Ordination nMDS plots were constructed from the Bray–Curtis resemblance matrix using abundance values ($\log n + 1$). This analysis was performed on component communities (the mean abundance of each parasite taxon in each fish species) and infra-communities (parasite taxa per individual fish). To evaluate the similarity in parasite communities between localities (spatial) and between years (temporal), we used a permutation-based one-way analysis of similarity (ANOSIM) [41] with the Bray–Curtis index as the similarity measure for abundance data. global R, the statistic for the ANOSIM, ranged from 0 (total similarity) to 1 (total dissimilarity). The statistical significance of the differences between and within sites and years was assessed after 9999 permutations.

The significance of differences between samples from different localities (spatial scale) and years (temporal scale) was tested using a permutational multivariate analysis of variance (PERMANOVA). When differences were detected by the PERMANOVA, pair-wise comparisons allowed us to define which sample (samples) differed.

When significant differences among a priori groups in multivariate space, but not observed in an unconstrained ordination (such as in an MDS plot) [42], a canonical analysis of principal coordinates (CAP) was used as a best alternative [42,43]. To test for significant differences between samples, a permutation trace test (the sum of squared canonical eigenvalues) was applied, and P was obtained after 999 permutations. After the CAP analysis, a correct assignment test was performed [21]. All multivariate analyses (nMDS, ANOSIM, PERMANOVA and CAP) were performed with PRIMER v6 software [44].

3. Results

Of the 7142 individuals belonging to the three host species, a subsample of 3687 individuals were selected, including 1123 *E. ringens* (12 to 24 months old; range size 11.3–15.7 cm TL), 1904 *T. murphyi* (24 to 36 months old; range size 21.7–29.5 cm FL) and 630 *M. gayi* (36 to 48 months old; range 37.3–45.6 cm TL). The number of hosts collected in each locality and year are indicated in the supplementary material (the sheet Samples).

A total of 9 parasite taxa were found in *E. ringens*, 15 in *T. murphyi* and 23 in *M. gayi*. The complete list of parasites found in each host species is given in the supplementary material (the sheet Parasites). Prevalences greater than 5% were observed in: 7 taxa (2 at the larval stage and 5 adults) for *E. ringens*; 13 taxa (6 at the larval stage and 7 adults) for

T. murphyi; and 17 taxa (7 larval stage and 10 adults) for *M. gayi*. The remaining taxa were removed from subsequent analyses. The values of prevalence for each parasite species found in each host species (within subsamples of the same age) and locality are shown in the supplementary material (the sheet Prevalence).

3.1. Spatial Analysis

For the three host species, most of the parasite taxa showed differences in prevalence at a spatial scale for the same year. *Bothriocephalus* sp. in *E. ringens* did not show differences at the spatial scale, and *Pseudanthocotyloides heterocotyle* and *Anisakis* sp. showed differences between localities in 2007 and 2016. *Ceratothoa gaudichaudi* in *T. murphyi* did not show differences between localities in 1993, 2008 and 2012. Six parasite taxa—*Corynosoma* sp. and *Caligus bonito* (for 1993); *Rhadinorhynchus trachuri* *Anisakis* sp. and *Lernanthropinus trachuri* (for 2008); and *Hysterothylacium* sp. (for 2011)—did not show differences at the spatial scale. All parasites of *M. gayi* except two (*Corynosoma* sp. and *Anisakidae* gen. sp.) showed differences between localities, and three (*Parabrachiella insidiosa*, *Anthocotyle merluccii* and *Rhadinorhynchus* sp.) showed differences only in one year; see the supplementary material (the sheet Spatial analysis).

The ANOSIM showed significant differences in the parasite communities for different localities among the three species. For *E. ringens* (global $R = 0.054$; $p < 0.001$) and *T. murphyi* (global $R = 0.141$; $p < 0.001$), the pairwise test was significant for all pairs of comparisons. For *M. gayi* (global $R = 0.052$; $p < 0.05$), the pairwise test was significant for all pairs of comparisons except CQM–VAL ($R = -0.023$; $p = 0.870$), CQM–THN ($R = 0.009$; $p = 0.275$) and VAL–THN ($R = 0.007$; $p = 0.168$). PERMANOVA showed significant differences in the parasite communities from different localities in the three host species. For *E. ringens* (pseudo- $F = 39.491$; $p < 0.0001$) for *T. murphyi* (pseudo- $F = 103.45$; $p = 0.001$) and *M. gayi* (pseudo- $F = 18.963$; $p < 0.0001$). Pairwise tests were significant for all pairs of comparisons.

In general, across the three host species, the CAP analysis showed significant differences between parasite communities at the spatial scale in the same year. Differences were observed in all cases for *E. ringens*; samples for 1995, 2005 and 2007 showed lower values of correct assignation (CA), at close to 50%, but in 2006 and 2016 the values of CA were higher than 65%. In all cases, values of assignation due to chance (AdC) were lower than those of CA. For *T. murphyi*, differences were evident for three of the five samples; the values of CA were lower, close to 50% (except in 2012 = 96.8%), and the values of AdC in all cases were lower than those of CA except in 1993. For *M. gayi*, significant differences were observed in both samples, the values of CA were lower (<55%) and the values of AdC were lower than those of CA.

The values of the CAP analysis and percentages of CA and AdC for the spatial analysis are shown in the supplementary material (the sheet Correct allocation).

3.2. Temporal Analysis

The prevalence of *Anisakis* sp. (ANT and CQM), *P. heterocotyle* (CQM), *Bothriocephalus* sp. (THN) and *Caligus* sp. (ANT) in *E. ringens* did not show significant differences at the temporal scale. The prevalence of *Scolex pleuronectis* (ANT), *Nybelinia surmenicola*, *Corynosoma australe*, *C. bonito*, *Ceratothoa gaudichaudi* (CQM) and *Pseudoterranova* sp. (THN) in *T. murphyi* did not show significant differences at the temporal scale. The prevalence of *Corynosoma* sp. (CQM, VAL and THN); *P. insidiosa* *Anisakidae* gen sp. (VAL, THN and PTM) and *Clestopothrium crassiceps* (VAL and PTM); *Hepatoxylon trichiuri* (THN); *R. trachuri* (CQM); and *Chondracanthus australis* (CQM) in *M. gayi* did not show significant differences at the temporal scale. The values of Fisher's exact test for each parasite taxon between years are shown in the supplementary material (the sheet Temporal analysis).

For the three host species, the ANOSIM showed significant differences in the parasite communities at the temporal scale. For *E. ringens* (global $R = 0.124$; $p < 0.001$), the pairwise test was significant for all pairs of years except 1995/2007 ($R = -0.088$; $p > 0.05$) and 2005/2007 ($R = -0.041$; $p > 0.05$). For *T. murphyi* (global $R = 0.126$; $p < 0.001$), the pairwise

test was significant for all pairs of years except 2005/1993 ($R = -0.047$; $p > 0.05$), 2006/1993 ($R = 0.006$; $p > 0.05$) and 2008/2011 ($R = -0.002$; $p > 0.05$). Finally, for *M. gayi* (global $R = 0.166$; $p < 0.001$), the pairwise test was significant for all pairs of years except 1995/2017 ($R = 0.00$; $p = 0.465$), 2005/2017 ($R = 0.032$; $p = 0.353$) and 2006/2017 ($R = -0.094$; $p = 0.781$). The PERMANOVA showed significant differences in the parasite communities for different years: for *E. ringens*, pseudo- $F = 65.609$ ($p < 0.0001$); for *T. murphyi*, pseudo- $F = 83.054$; and for *M. gayi*, pseudo- $F = 15.861$ ($p \leq 0.0001$). Pairwise tests showed significant differences for all pairs of comparison across the three host species.

For the three host species, the CAP analysis showed significant differences between parasite communities at the temporal scale for the same locality. For *E. ringens*, the values of CA were lower, close to 50%, and the values of AdC were lower than those of CA in all cases. In *T. murphyi*, the values of CA were lower, close to 50%, except for THN (98%), and the values of AdC were lower than those of CA in three of the five comparisons. *M. gayi* showed significant differences in all cases: the values of CA were high (>69%) and the values of AdC were lower than those of CA in all cases. The values of the CAP and the percentages of CA and AdC in the temporal analysis are shown in the supplementary material (the sheet Correct allocation).

The bi-dimensional graphic representation (nMDS) of the parasitic component communities for each host species at the same locality but in different years is shown in Figure 2. The graphic for *E. ringens* suggests that closer localities (exceptions: THN-05, THN-16) showed higher similarities than farther localities. For *T. murphyi*, three major groups showed similarities at 50% but without a clear spatial or temporal pattern. A different scenario occurred for *M. gayi*, with high values of similarity between all samples except for CQM-05 and CQM-17. The same graphic analysis for infra-communities showed similarities over 50% for all spatiotemporal samples of *E. ringens*, but *T. murphyi* and *M. gayi* did not show evident temporal/spatial patterns. For *M. gayi*, most of the data overlap and a subset (black square) was performed for better visualization (Figure 3).

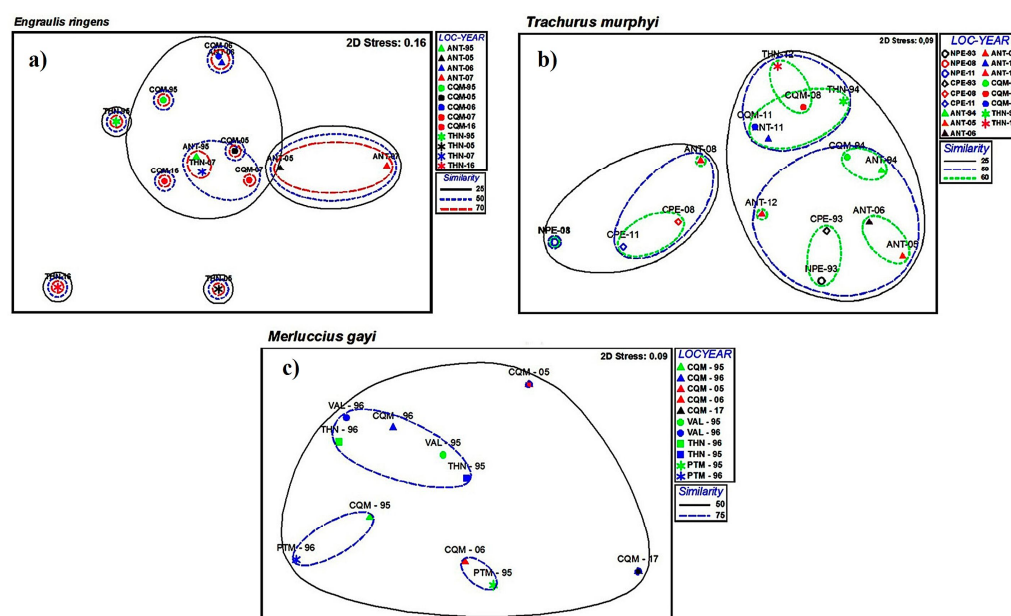


Figure 2. Non-metric multidimensional scaling (nMDS) of parasite communities in three fish species in the southeastern Pacific Ocean: (a) *E. ringens*; (b), *T. murphyi*; (c), *Merluccius gayi*. Circles represent similarity levels between samples of locality-year.

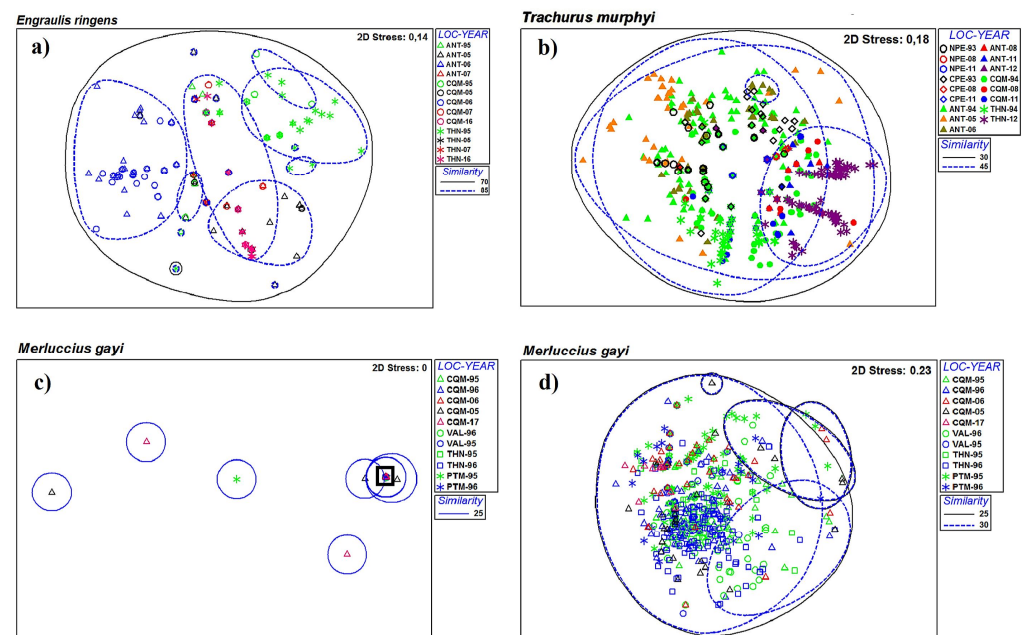


Figure 3. Non-metric multidimensional scaling (nMDS) of parasite infra-communities in three fish species in the southeastern Pacific Ocean: (a), *Engraulis ringens*; (b), *Trachurus murphyi*; (c), *Merluccius gayi*; (d), subset (of the black square) of *Merluccius gayi* in (c). Circles represent similarity levels between samples of locality–year.

4. Discussion

One way to mitigate uncertainty about the sustainability of global fisheries is to achieve precise assessments of the abundance of resources in space and time and collect information on stock structures as a first step [4]. For almost a century, parasites have been successfully used in fish stock identification [5,6]. Currently, parasites are considered an appropriate tool in fisheries management, specifically as BTs for stock identification [4]. When using parasites as BTs for fish stock identification, it is desirable to know the age of host individuals [12]. Although, for some species, an age determination has not been validated [12], most studies have used the lengths of fishes to compare groups of fishes from different localities [45,46]. Of 102 published articles using fish parasites as BTs in the last 20 years, for 66 the goal was stock identification, but host age (or ontogeny stage) was considered in only 14. Fishes of similar length could include individuals of different ages that therefore would have been exposed to parasite infections for different periods, generating a confounding factor in the interpretation of results, specifically for parasites at the larval stage, because these parasites accumulate over time [26].

The utility of parasites as BTs for stock identification is determined to an extent by the temporal stability of the parasite communities, particularly when different localities have been sampled across different seasons or years [47]. However, in this kind of study, spatiotemporal variability in parasite communities has been poorly evaluated.

Of the 102 studies published from 2000 to 2020, 38 included samples from 2 or more years and 12 compared samples from different years; however, the samples were pooled, because the temporal variability in each sample locality was not measured. The variability at the temporal and/or spatial scales was analyzed in only nine studies. Samples of *Clupea harengus* collected between 2002 and 2005 and compared with samples obtained in 1973 and 1982 suggested that parasite fauna are spatially variable but remain temporally stable over the short and long term [48]. Samples of *Mullus surmuletus* in three localities and two seasons in different years, showed that due to the high small-scale variability (across samples from the same localities), it was not possible to identify different stocks [24]. For *Pinguipes brasilianus* collected in different seasons between 2006 and 2007 [25], the spatial effects exceeded seasonal effects. Similarly, an analysis of parasite communities

in *Percophis brasiliensis*, conducted across seasons over one year, showed that parasite communities in seasonal samples had a high degree of homogeneity in infection levels within and between samples throughout the year [15]; therefore, parasites can be used for fish stock identification independent of the season of capture, at least on an annual scale. Parasite communities of *Polydactylus macrochir* from different periods between 2007 and 2009 showed stability over the temporal scale explored [49]. Temporal variability but marked spatial differences in the infection of metacercaria supported the hypothesis regarding two stocks of *Sardinops sagax* [50]. Among studies that evaluated possible spatial and/or temporal variability in parasite communities by comparing samples from temporal scales in the same locality, Moore [51] analyzed the parasites of *Scyliorhinus canicula*, including samples from three localities; however, because samples taken over different periods from the same locality were small, samples from each locality were grouped and analyzed as a single sample. The local variability in the parasites of *E. ringens* studied through five subsamples ($n = 60$ c/u) from two localities less than 60 km apart and collected within two days of separation to avoid potential temporal noise showed that variability could be well represented with a single sample [14]; but in the same species, the magnitude of local variation was higher than regional variability; therefore, the effect of high local variability must be considered [16].

4.1. Spatial Variability

The spatial variability of parasite communities allows the use of parasites as biological tags for fish stock identification [15], because a parasite species is found only in those areas where conditions are suitable for its transmission [12]. A parasite must have specific features to be considered as a BT, parasite taxa must present significant differences in prevalence or load across the study area [12]. For the three host species, most of the parasite taxa showed differences in prevalence at the spatial scale for a given time. In *E. ringens*, only *Botriocephalus* sp. showed similar prevalences in all localities, and the remaining parasite taxa showed differences between localities at least on time. In *T. murphyi* and *M. gayi*, all parasite taxa showed differences at the spatial scale, but with two exceptions in *M. gayi* (*Corynosoma* sp. and Anisakidae gen. sp.). The ANOSIM showed differences in parasite communities between localities in *E. ringens* and *T. murphyi* but not *M. gayi*; nevertheless, the PERMANOVA and CAP showed that significant differences between communities were detected in the three species.

The CAP analysis showed significant differences between communities (with exceptions) and the values of CA were low. The low values of CA could be explained by samples of one locality being assigned to a nearby locality. For example, in *E. ringens* 1995, total CA = 55.8% (ANT = 96.6%; CQM = 0%; THN = 50%), but 56 of 68 samples from CQM were allocated to ANT. In *T. murphyi* 2011, total CA = 55.6% (ANT = 34.0%; CQM = 50.9%; NPE = 0; CEP = 97.5), but 67 of 69 samples from NPE were allocated to CPE. In *M. gayi*, the highest values of CA were allocated to the most geographically distant localities (CQM and PTM). Therefore, although the spatial variability was lower, the communities were well assigned to their hosts' localities of origin or to the nearest locality. These results could be due to specimens belonging to the same stock sharing similar parasite communities but landing in different but closer ports or due to each locality representing a landing port but fishing areas of closer ports overlapping [34].

4.2. Temporal Variability

Comparative studies over long periods require a high degree of temporal repetition in the composition and structure of parasite communities [25], the only way to avoid the potential impact of temporal variability on the structure of parasite communities. Although studies that have analyzed the variability in parasite communities at different temporal scales exist, long-term comparisons are rare [48]. Analyzing long-term samples, most parasite taxa (at the same locality) present different prevalence rates between years, although some taxa (*Anisakis* sp. from *E. ringens*; *N. surmenicola*, *Pseudoterranova* sp. from *T. murphyi*;

and *P. insidiosa*, *C. australis*, *C. crassiceps* H. trichiuri, *Rhadinorhynchus* sp., *Corynosoma* sp. and Anisakidae gen. sp. from *M. gayi*) remained constant throughout different years. Many of these taxa (six) were in the larval stage, and it has been stated that these long-lived parasites confer temporal stability to community parasites at least at the seasonal or annual scale [52]. Our results support desirable long-term temporal stability as a prerequisite for the use of parasites as BTs [12] but for fishes of the same age or the same cohort.

5. Conclusions

In conclusion, for comparative studies over long periods, a high degree of temporal repeatability is required, as suggested previously [15], but in a scenario with temporal variability, caution must be taken when using samples from different years from a given locality. Nevertheless, in a scenario with temporal stability samples from the same locality but different years can be pooled. On the other hand, as suggested recently [26], older hosts harbors relatively more stable parasite communities; consequently, older age classes present in a given sample are preferable when using parasites as BTs.

Supplementary Materials: The following supplementary material is available online at <https://www.mdpi.com/article/10.3390/fishes6040071/s1>, Espinola-Novelo and Oliva.xlsx.

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Data Availability Statement: Data is available in the Supplementary Material.

Conflicts of Interest: The authors declare no conflict of interest.

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