The use of parasites as biological tags in multidisciplinary stock identification studies of small pelagic fish

Ken MacKenzie, Pablo Abaunza and Neil Campbell

School of Biological Sciences (Zoology), The University of Aberdeen, Tillydrone Avenue, Aberdeen AB24 2TZ, UK [tel: +44 1224 272861, fax: +44 1224 272396, emails: k.mackenzie@abdn.ac.uk, neil.campbell@abdn.ac.uk.
Instituto Español de Oceanografía (IEO), PO Box 240, 39080 Santander, Spain [+34 942 291060, fax: +34 942 275072, email: pablo.abaunza@st.ieo.es.

*Contact author.

Abstract

Parasites have been used as biological tags in a number of studies of stock structure in small pelagic fish. Here we review these studies and discuss the particular features of the parasite fauna of small pelagic fish, the advantages and limitations of biological tagging compared with other methods, and the methodology of using parasites as biological tags. While a multidisciplinary study has the major advantage of permitting comparisons to be made between results from different methods of stock identification, compromises usually have to be made with regard to the methods of preservation and processing to suit the requirements of all the methods involved, and these may not be ideal for one or more of the disciplines involved, including the preservation and identification of parasites. Based largely on our experience of the EC-funded HOMSIR and WESTHER projects, we propose guidelines for the use of parasites in multidisciplinary studies of small pelagics.

Keywords: parasite tags, small pelagic fish, stock identification.
Introduction

MacKenzie and Abaunza (2005) described the general principles of using parasites as biological tags in the stock identification of marine fish in general, and proposed guidelines for the use of parasites in such studies. In the present paper we focus on small pelagics and discuss how these principles and guidelines apply in studies of their stock identification. We also review publications on stock identification of small pelagics using parasites as biological tags and compare studies in which parasites were the sole method used with multidisciplinary studies in which parasite tags have been one of a number of methods used.

General principles

The basic principles of using parasites as tags for small pelagics are the same as for any other group of fish. This is that fish can become infected with a particular parasite only when they come within the endemic area of that parasite, the endemic area being the geographic region in which transmission of the parasite can take place. If infected fish are found outside the endemic area, we can infer that these fish had been within that area at some time in its past history.

Advantages and limitations of parasite tagging

In any multidisciplinary study of stock identification, each method will have its own strengths and weaknesses compared to other methods.

- Parasite tagging is recognized as being more appropriate than artificial tagging for studies of small delicate species of fish which have poor survival rates following the stressful processes of capture, handling and tagging. This applies particularly to small pelagic species.
- In parasite tagging each specimen sampled represents a valid observation, whereas with artificial tags each individual must be sampled, tagged and recaptured to obtain a valid observation.
- Parasite tagging is less expensive because samples can be obtained from routine sampling programmes and research vessel surveys.
- The use of parasite tags eliminates doubts concerning the possible abnormal behaviour of artificially tag hosts.
- Parasites can have some advantage over genetic studies in that they can be used to identify host subpopulations distinguished by behavioural differences, but between which there is still a considerable amount of gene flow. For example, a gene flow rate of only 1% will give genetic homogeneity among samples (Ward, 2000).

On the other hand, lack of information on the complex biology and ecology of many marine parasites can limit their value as tags. However, as research adds to our knowledge, the use of parasites in stock identification is becoming more efficient. The specific identification of closely related parasite species may be also difficult. The recent application of molecular biology techniques to parasite taxonomy has resulted in the identification of two or more “sibling” or “cryptic” species in parasite groups which had previously been assumed to be a single species. Conversely, the same techniques have occasionally shown that parasites that had previously been regarded as separate species may in fact be conspecific.
Features of the parasite fauna of small pelagics

Small pelagics are generally infected with fewer parasite species than demersal fish (Polanski, 1966). Within the group, piscivorous species and those with relatively diverse diets have richer parasite faunas than planktivorous species. The parasite fauna of small pelagics tends to be dominated by larval forms, particularly helminths, which mature in the predatory species that prey on them.

Methodology and selection of tag parasites

Two different approaches to the use of parasites as biological tags in general are recognized.

1) A small number of parasite species are selected according to selection criteria that have been described by a number of authors and have changed and evolved over the past 42 years (Kabata, 1963; Sindermann, 1983, MacKenzie, 1983, 1987; Williams et al., 1992). A large number of host individuals are then examined specifically for these parasites. The selection criteria for the most appropriate tag parasites can be summarized as follows.

- They should have significantly different levels of infection in the target host in different parts of the study area.
- They should persist in the target host for more than one year.
- Parasites with single-host life cycles, such as monogenetic trematodes and most parasitic crustaceans, are the simplest to use. However, parasites with complex life cycles involving developmental stages in different hosts can also be used effectively, given sufficient information on their life cycles and ecology.
- The level of infection should remain relatively constant from year to year. The effects of annual variations can, however, be taken into account by following infection levels in single year-classes of the target host over several years.
- They should be easily detected and identified. Examination of the host should involve the minimum of dissection, otherwise time can become a limiting factor.
- Parasites that are serious pathogens should be avoided.

This first approach is the one that has been used most frequently in stock identification studies of small pelagics.

2) In the second approach, entire parasite assemblages are analyzed using multivariate statistical techniques such as discriminant analysis or multiple logistic regression. This approach is more appropriate for large valuable host species with rich and varied parasite faunas. It use for small pelagics is limited by the relative paucity of the parasite faunas of most small pelagic species, but there are exceptions to this general rule (e.g., George-Nascimento, 2000; Timi, 2003).

For both approaches, summary statistics of levels of infection in terms of prevalence, mean intensity and/or abundance should be expressed by the mean value plus and minus the standard deviation, and the range. The biological interpretations of the statistical parameters should be clearly described and understood to avoid misleading interpretations concerning the true nature of the infection (Rózsa et al., 2000). For full descriptions of the statistical methods appropriate for application to parasite data in stock identification studies, see MacKenzie and Abaunza (2005).
Review of publications on parasites as tags in the stock identification of small pelagics

Tables 1, 2 and 3 list the publications in which parasites have been used as biological tags in stock identification studies of small pelagics. They show that the most frequently used tag parasites have been larval nematodes, particularly those of the genus *Anisakis*, while digenean metacercariae, cestode plerocercoids (Figure 1), juvenile acanthocephalans and parasitic crustaceans have also been used to good effect. All of the publications listed used parasite tags as the only method of stock identification, with the exception of those by George-Nascimento and Arancibia (1992), who used host morphometrics in addition to parasites, and Campbell *et al.* (2002), which was based on the multidisciplinary HOMSIR project. Our own experience of the EC-funded HOMSIR and WESTHER projects has shown that the most useful parasite tags for Atlantic horse mackerel *Trachurus trachurus* and Atlantic herring *Clupea harengus* have been digenean metacercariae (Figure 2) anisakid nematode larvae (Figure 3), monogeneans, and juvenile acanthocephalans (Campbell *et al.*, 2002 and unpublished results). Horse mackerel have a much richer parasite fauna than herring, reflecting the more diverse diet of the former.

While multidisciplinary studies have the major advantage of permitting comparisons to be made between results from different methods, planning the sampling and examination protocols must be done with great care so that the requirements of all the disciplines involved are taken fully into account. Compromises will inevitably have to be made and, in the case of the parasite studies, this can mean that the methods of preservation of the parasite material may not be ideal, with obvious implications for precise identification.
Table 1. Studies using parasites as biological tags for stock identification of small clupeoid species.

<table>
<thead>
<tr>
<th>Host species</th>
<th>Study area</th>
<th>Parasites used</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlantic herring <em>Clupea harengus harengus</em></td>
<td>Northwest Atlantic</td>
<td>Anisakid nematode larvae, cestode plerocercoids, myxosporean</td>
<td>Sindermann (1957)</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Anisakis</em> sp. larvae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Northeast Atlantic Baltic Sea</td>
<td><em>Anisakis</em> sp. larvae, cestode plerocercoid, adult digeneans</td>
<td>Parsons &amp; Hodder (1971)</td>
</tr>
<tr>
<td></td>
<td>Baltic Sea</td>
<td><em>Anisakis</em> sp. larvae</td>
<td>Grabda (1974)</td>
</tr>
<tr>
<td></td>
<td>Northeast Atlantic</td>
<td>Digenean larvae, cestode plerocercoid</td>
<td>Gaevskaya &amp; Shapiro (1981)</td>
</tr>
<tr>
<td></td>
<td>Northwest Atlantic</td>
<td><em>Anisakis</em> sp. larvae</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atlantic</td>
<td><em>Anisakis</em> sp. larvae</td>
<td></td>
</tr>
<tr>
<td>Pacific herring <em>Clupea harengus pallasi</em></td>
<td>British Columbia White Sea</td>
<td><em>Anisakis</em> sp. larvae (Nematoda) Gyrodactylidae (Monogenea)</td>
<td>Bishop &amp; Margolis (1955)</td>
</tr>
<tr>
<td>Sprat <em>Sprattus sprattus</em></td>
<td>North Sea</td>
<td>Monogenea</td>
<td>Reimer (1978)</td>
</tr>
<tr>
<td>Sardine <em>Sardina pilchardus</em></td>
<td>Northeast Atlantic</td>
<td>General parasite community</td>
<td>Shukhgalter (1998)</td>
</tr>
<tr>
<td>Anchovy <em>Engraulis anchoita</em></td>
<td>Argentina</td>
<td>General parasite community</td>
<td>Timi (2003)</td>
</tr>
</tbody>
</table>
Table 2. Studies using parasites as biological tags for stock identification of small carangid species.

<table>
<thead>
<tr>
<th>Host species</th>
<th>Study area</th>
<th>Parasites used</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black Sea horse mackerel</td>
<td>Black Sea</td>
<td>Helminth fauna</td>
<td>Kovaleva (1965)</td>
</tr>
<tr>
<td><em>Trachurus mediterraneus ponticus</em></td>
<td>North &amp; Northwest Spain</td>
<td><em>Anisakis</em> sp. larvae</td>
<td>Abaunza <em>et al.</em> (1995)</td>
</tr>
<tr>
<td></td>
<td>Northeast Atlantic and Mediterranean Sea</td>
<td>General parasite community</td>
<td>Campbell <em>et al.</em> (2002)</td>
</tr>
<tr>
<td></td>
<td>Eastern Atlantic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atlantic horse mackerel</td>
<td>Chile</td>
<td>Anisakid nematode larvae, juvenile acanthocephalan,</td>
<td>Gaevskaya &amp; Kovaleva (1980)</td>
</tr>
<tr>
<td><em>Trachurus trachurus</em></td>
<td>Pacific Ocean</td>
<td>parasitic isopod</td>
<td>George-Nascimento &amp; Arancibia (1992)</td>
</tr>
<tr>
<td></td>
<td>Chile</td>
<td>Parasitic isopods</td>
<td>Avdeev (1992)</td>
</tr>
<tr>
<td></td>
<td>Chile, Peru</td>
<td>General parasite community</td>
<td>Aldana <em>et al.</em> (1995)</td>
</tr>
<tr>
<td>Horse mackerels</td>
<td>Java Sea</td>
<td><em>Anisakis</em> sp. larvae</td>
<td>George-Nascimento (2000)</td>
</tr>
<tr>
<td><em>Trachurus</em> spp.</td>
<td></td>
<td></td>
<td>Burhanuddin &amp; Djamali (1978)</td>
</tr>
<tr>
<td>Jack mackerel <em>Trachurus symmetricus murphyi</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Round scad <em>Decapterus russelli</em></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3. Studies using parasites as biological tags for stock identification of other small pelagics.

<table>
<thead>
<tr>
<th>Host species</th>
<th>Study area</th>
<th>Parasites used</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atlantic mackerel <em>Scomber scombrus</em></td>
<td>North &amp; Northwest Spain, Northeast Atlantic</td>
<td><em>Anisakis</em> sp. larvae, cestode plerocercoids, general parasite community</td>
<td>Abaunza <em>et al.</em> (1995)</td>
</tr>
<tr>
<td>Pacific saury <em>Cololabis saira</em></td>
<td>Pacific Ocean</td>
<td>Parasitic copepods</td>
<td>Sokolovsky (1969)</td>
</tr>
<tr>
<td>Garfish <em>Belone belone</em></td>
<td>Baltic Sea</td>
<td><em>Anisakis</em> sp. larvae, cestode plerocercoids</td>
<td>Grabda (1981)</td>
</tr>
<tr>
<td>Blue whiting <em>Micromesistius poutassou</em></td>
<td>Celtic Sea and adjacent waters</td>
<td>Myxosporean</td>
<td>Karasev (1988)</td>
</tr>
</tbody>
</table>
Figure 1. *Anisakis* larvae in the visceral cavity of a herring

Figure 2. Cestode plerocercoid.

Figure 3. Digenean metacercariae.
Guidelines for the use of parasites as biological tags for small pelagics

The selection of tag parasites should initially follow the criteria listed above, but we further offer the following guidelines for the use of parasites in multidisciplinary studies of the stock identification of small pelagics, based largely on our own experience of the HOMSIR and WESTHER projects.

- Those doing the parasite study must receive the material to be examined in a form that allows for accurate identification of any parasites found. If whole fish are to be deep-frozen, as was the case in HOMSIR, they should be frozen as soon after capture as possible. Many parasites begin to deteriorate very quickly after death, especially in warm temperatures. Where material is preserved in alcohol, as was the case in WESTHER, the strength and volume of the alcohol used must be sufficient for adequate preservation and should take account of any dilution due to liquids present in the material itself.
- If whole deep-frozen fish are supplied, they should be frozen individually and preferably in individual plastic bags or other suitable containers. Defrosting large blocks of fish can lead to loss of, and damage to, parasites.
- Deep-frozen fish must be labelled in such a way that the labels are not easily lost and do not damage fish tissues or organs. For example, plastic ties around the gills can result in bleeding and damage which can make examination difficult and may result in loss of, or damage to, ectoparasites.
- If the material to be examined is liquid-preserved, the type of container must be selected with great care so that it will not crack or leak and so that numbers of them can be easily packed for transport.
- The labelling system for identifying individual samples should be as simple and concise as possible. The brain most readily processes information that is either the first or last in a string of numbers, so the most important information, such as the sampling position and individual sample number, should be at the beginning and end of the label. The system used in HOMSIR had the sampling position at the beginning and the individual fish number at the end, with the sampling year in the middle – for example 09-01-001 refers to fish no. 1 of sampling position 09 in year 2001.
- If the requirements of other disciplines mean that it is not possible to obtain whole fish for parasitological examinations, those organs and tissues that are the sites of infection of the most potentially useful tag parasites should be identified. For example, in WESTHER we received only the visceral organs of herring preserved in alcohol, because we had previously identified the most likely tag parasites as helminths infecting the visceral cavity and associated organs.

References


