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Digenean metacercariae parasites as natural tags of habitat use by 0-group common sole *Solea solea* in nearshore coastal areas: A case study in the embayed system of the Pertuis Charentais (Bay of Biscay, France)

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ABSTRACT

This study focused on the spatio-temporal variation in the host-parasite system, 0-group sole–digenean metacercariae, in nearshore coastal areas at relatively small spatial scale. 0-group soles were sampled using a standard beam trawl in April, May, June, August and October 2005 at nine different sites in the Pertuis Charentais area (Bay of Biscay, France). Sole density, size, Fulton's condition factor *K* and digenean metacercariae communities were analysed. 0-group sole concentrated in shallow and muddy areas where they accumulated digenean metacercariae. Parasite communities displayed strong spatial patterns tightly linked to the distribution of the first intermediate mollusc hosts. These parasitological data suggest that 0-group sole during their first period of growth are mainly sedentary with limited movements between the different parts of the habitat. Size and density data revealed spatial heterogeneity in terms of habitat quality so that a limited zone (Aiguillon Bay) within the study area could be identified as *sensu stricto* nursery habitat for 0-group sole. The use of digenean metacercariae as natural tags appears as a novel powerful tool to evaluate habitat use and movements of juvenile flatfish, which could find applications in fisheries and coastal zone management programs.

1. Introduction

Coastal areas are highly productive ecosystems that are essential habitats for the juveniles of many marine fishes and especially flatfishes. However, these habitats display highly variable natural environmental conditions to which often superimpose anthropogenic disturbances of various origins. As a consequence, on a spatial basis and for a given species, juvenile habitat quantity and quality appear highly variable (Beck et al., 2001; Dahlgren et al., 2006). In order to achieve habitat conservation and sustainable management of fisheries, it is therefore critical to understand fish juvenile spatio-temporal dynamics within these coastal habitats (Rice, 2005).

Different methods exist to study fish spatio-temporal dynamics: i) distributions based on capture data (*e.g.* occurrence, abundance and size structure); ii) use of artificial tags (*e.g.* conventional tags, acoustic tags and archival tags); iii) use of genetic markers (*e.g.* microsatellites, RAPD, mitochondrial DNA); and finally iv) use of natural tags (*e.g.* otolith microchemistry and shape, stable isotopes, parasites). Each of these methods has its own spatio-temporal resolution range with advantages and limitations in terms of sampling representativity, effort, logistics, analysis time, price, etc. However, among them, the use of natural tags appears often as a good compromise and they are especially well suited to study the movements of fish juveniles within and between different coastal habitats (Gillanders et al., 2003).

Parasites are a natural part of all ecosystems and play an important role in their functioning especially in shallow coastal areas where they represent a key component of the biodiversity (Combes, 2001). Among natural tags, parasites have an extensive history of use in fish population studies (MacKenzie, 2005). The basic principle underlying the use of parasites as tags is that fish can become infected with a parasite only when they come within the area suitable for the transmission of that specific parasite (MacKenzie, 2005). Based on these characteristics, parasites have been used mainly at large spatial scale (hundreds of kilometres) to evaluate migratory routes or to discriminate stocks in fish population. Very few studies have used parasites at a smaller spatial scale (hundreds of meters to few kilometres) and that was essentially to study fish connectivity between inshore and offshore habitats (Olson and Pratt, 1973; Sujatha and Madhavi, 1990; Vignon et al., 2008).

On coastal nursery grounds, 0-group flatfish juveniles become infected especially with digenean metacercariae parasites (El-Darsh and Whitfield, 1999; Durieux et al., 2007a). Like many internal parasites, digeneans have a complex life cycle with molluscs as first

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intermediate hosts, invertebrates and small fishes as second intermediate hosts and vertebrate predators (such as fishes, birds or mammals) as definitive hosts. Cercariae are digeneans' larval stage that are produced in quantity in the mollusc first intermediate host; once developed, cercariae are expelled from the molluscs into the water column; then after locating the second intermediate host, they actively penetrate through the skin, encyst as metacercariae in the body and transmission to the definitive host occurs through predation upon the second intermediate host. Based on these biological characteristics, digenean metacercariae appear promising natural tags to study habitat use of juvenile flatfish acting as second intermediate hosts.

The common sole *Solea solea* (L.) is a widely distributed and economically important flatfish of the North East Atlantic and Mediterranean Sea. As most of commercial demersal species in this region it is also reported as over-exploited for most stocks (ICES, 2005). After a planktonic larval stage, 0-group sole juveniles colonize and settle in discrete shallow soft bottom coastal habitats such as estuaries, bays, lagoons or sandy beaches (Amara et al., 2000). During the first growing season 0-group sole are especially sensitive to both natural and anthropogenic stressors exhibited in these areas, which imply variability in their biological performances and survival and *in fine* can influence adult stock recruitment. Therefore a number of studies have focused on 0-group sole habitat suitability (Eastwood et al., 2003; Le Pape et al., 2003b; Le Pape et al., 2007) and quality (Gilliers et al., 2006; Amara et al., 2007; Vinagre et al., 2008b). As results of these studies, the main factors characterising the habitat and influencing its quality have been identified. However there is still a clear need to estimate habitat use and movement capacities of 0-group sole to define the connectivity within the mosaic of habitats that constitutes coastal areas in order to better understand the functionality of each habitat at small spatial scale.

In the present case study, we propose a novel approach using digenean metacercariae as natural tags to estimate habitat use and movements of 0-group sole in nearshore coastal areas at relatively small spatial scale. The aims of this study were to: i) evaluate the spatio-temporal change in 0-group sole density through the first growing season within the Pertuis Charentais (France), one of the main area for sole juveniles in the Bay of Biscay; ii) evaluate the spatio-temporal change in digenean metacercariae infection of 0-group sole; iii) test the use digenean metacercariae as natural tags to determine movements and habitat use of 0-group sole in the study area; and finally iv) estimate habitat quality based on fish size and body condition in the study area.

2. Materials and methods

2.1. Study area

The Pertuis Charentais are located on the French Atlantic coast (Bay of Biscay) north of the Gironde estuary (Fig. 1). This area is an embayment complex constituted by two main straits (Pertuis Breton and Pertuis d'Antioche) with a total surface area of 1300 km^2 comprising 340 km^2 of shallow (0–5 m depth) soft bottom areas essentially located in the inner bays and north of the Pertuis Breton close to the mainland. The substrate of these large shallow soft areas is essentially muddy but the north of the Pertuis Breton, that is constituted of thin sand. The Pertuis Charentais area is considered as the largest suitable habitat for 0-group sole in the Bay of Biscay (Le Pape et al., 2003b). This macrotidal area is largely characterised by a marine water influence. Only small rivers flow into each strait (average annual river-flow of 5, 10, 50 and 10 $m^3 s^{-1}$ for the Le Lay, Sèvre Niortaise, the Charente and Seudre Rivers, respectively). The tidal range is 6.4 m and the average tidal current is 0.5 m s^1 during spring tides, which generates rapid renewal of marine water and well-mixed environment, particularly in the shallowest areas. In addition, most intertidal areas that are suitable for 0-group sole are used for the cultivation of Pacific oyster (Europe's largest production area) and mussel with estimated standing stock of 125,000 and 20,000 tons respectively in 2001 (Goulletquer and Le Moine, 2002).

2.2. Fish sampling and parasite collection

0-group soles were sampled using a standard beam trawl (2 m wide, 0.4 m high, mounted with a 5 mm stretched mesh net in the cod end) at 2.5 knots for 20 min. 0-group sole were sampled in April (21st and 28th), May (23rd and 24th), June (27th and 28th), August (9th and 10th) and October (4th and 5th) 2005 in the Pertuis Charentais on seven sites located in shallow areas (0–5 m) and two sites (one per strait) located in deeper areas (5–10 m) (Fig. 1). All sites were located on muddy bottom but sites PB1 and PB2 were located on sandy bottom. Directly after catch, fish were placed on ice, so that at the laboratory 0-group soles were counted, measured for standard length (SL to nearest mm) and weighed (M_W to the nearest 0.01 g). We used a nested sampling design with up to 120 individuals per month/site randomly selected for SL measurement and, within this sample, up to 30 individuals randomly subsampled for M_W measurement. For parasite analysis, up to 20 individuals measured for SL and M_W were randomly subsampled per month/site and individually stored at −20 °C. Thereafter, the soles were thawed and entirely dissected under a binocular microscope to check for digenean metacercariae parasites (essentially located in muscle tissue), which were identified to the lowest taxa and counted (see Durieux et al., 2007a for details).

2.3. Data analysis

Sole density was calculated as: $D = N^*1000/S$; with D as density (nb ind 1000 m^{-2}), N as number of 0-group sole captured per haul and S the sampled surface (in m^2). Fulton condition factor was calculated as $K = (M_W \times 100)/SL^3$, with M_W in g and SL in cm. Levels of parasitic infection were assessed using classic epidemiological parameters: prevalence, the percentage of infected fish in fish sample; mean intensity, the mean number of parasites per infected fish; and mean abundance (Ab), the mean number of parasites per fish (Bush et al., 1997).

Parasite taxa abundances were compared between months and sites using two-way ANOVAs followed by Tukey post hoc test. SL and K were respectively compared between months and sites using two-way ANOVAs followed by Tukey post hoc test. Discriminant Function Analysis (DFA) was performed for each month/strait in order to reclassify individuals to the different sampling sites using intensity (number of parasites in an individual fish) of the parasite taxa as explanatory variables. As part of the DFA, digenean metacercariae communities were compared by MANOVAs between sites for each month/strait using Pillai's trace test. Prior to the final set of DFAs, a first set of DFAs was performed with all sites together and showed high site fidelity of 0-group soles, thereafter since few connectivity seemed to occur between the two straits (Pertuis Breton and Pertuis d'Antioche), it was decided to perform DFAs on the two different straits separately for both ecological relevance and power of the analysis. A jackknife re-sampling approach was used to reclassify individuals to the different sites using the established DFA functions. Parasite intensities were $\log(n+1)$ transformed to meet normality and homogeneity of variance. Results were considered significant at $\alpha = 5\%$.

3. Results

3.1. Spatio-temporal change in 0-group sole density

A total of 1732 0-group soles were captured. From May onwards individuals were present on all sampled sites with peak densities in most sites located in shallow areas (Fig. 2). Densities varied strongly