Three-dimensional internal spatial structure of young-of-the-year pelagic freshwater fish provides evidence for the identification of fish species

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Abstract

Active acoustic detection and characterization in three dimensions (3D) with multibeam sonars is a powerful technique for ecological studies of schooling fish. The alpine Lake Annecy provides ideal conditions for sampling fish with active acoustic methods: it has calm water, low species diversity, and the density of pelagic fish schools is high. We carried out investigations on the internal 3D morphological characteristics of young-of-the-year (Y-O-Y) pelagic fish schools, using high resolution multibeam sonar in the vertical plane. The objective was to discriminate between the two fish species that school in the lake: perch (Perca fluviatilis) and roach (Rutilus rutilus). To discriminate between these Y-O-Y fish species, we used only one characteristic of their internal spatial structure: the total number of vacuoles, defined as empty volumes inside the school, relative to the school volume. Two distinct linear relationships between school volume and the total number of vacuoles were determined. These two types were consistent with the proportions of the percentage occurrence of the two different species as obtained from pelagic trawl sampling in 2004. In 2008, perch was dominant (93%) in the lake, and only one relationship was detected: this relationship corresponded closely to the one in 2004 attributed to perch. Thus, we assume that the schooling behavior is a phenotypic expression that could be used for remote fish species identification.

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More recently, three-dimensional (3D) observations of entire fish schools (Gerlotto et al. 1999; Mayer et al. 2002; Paramo et al. 2010) or Antarctic krill shoals (Brierley and Cox 2010) have been used in ecological and behavioral studies (Gerlotto et al. 2004; Brehmer et al. 2006; Guillard et al. 2010); however, these have been seldom used for species identification. In this article, we report on investigations into the internal 3D morphological characteristics of pelagic fish schools (Partridge 1982), recorded using a high resolution multibeam sonar, to discriminate between two species of freshwater fish. An improvement in fish species discrimination has the potential to greatly improve fisheries management, not only to provide a better estimate of abundance and distribution, but also by helping to decrease by-catches and discards, and improving our knowledge of ecological processes and thus on the ecosystem functioning.

Materials and procedures

Lake Annecy is a deep oligotrophic monomictic lake located at about 600 m above sea level in the French Alps (45°51’24” North, 06°10’20” East) (Perga et al. 2010). It has a maximum depth of 69 m (Fig. 1) and is 14.6 km long, 2 km wide, with a total area of 27.4 km². In autumn, fish populations in Lake Annecy (Guillard et al. 2006b), in common with other lakes in temperate areas, exhibit a vertical distribution that is strongly related to the thermocline (Fig. 2). Under the thermocline, the deeper layers are inhabited by salmonids (Mehner et al. 2010); above it, in the surface layers, juveniles of Cyprinidae (*Perca fluviatilis*) and Percidae (*Rutilus rutilus*) school during the day and disperse at sunset to feed (Masson et al. 2001; Probst et al. 2009).

Daily acoustic surveys, at a mean speed of 6 km h⁻¹, were carried out in the larger part of the lake (Fig. 1) on 14 Sept 2004 and 23 Sept 2008. A Reson Seabat 6012 multibeam sonar operating at a frequency of 455 kHz (Gerlotto et al. 1999; Nøttestad and Axelsen 1999) was pole-mounted on the side of a 6.4 m boat with the transducer submerged to a depth of 1 m (Guillard et al. 2006a). The 60 beams (beam dimensions 1.5° athwartships and 17° along ships) allowed for a 90° athwartship observation plane, oriented perpendicularly to the bottom and parallel to the surface (sonar was orientated at 45° to the vertical perpendicular to the line of travel—see Fig. 3).

The multibeam sonar has been checked several times by the manufacturer. It was set to operate a 20 Log R Time Varied Gain (TVG), with a pulse length of 0.06 ms and a range set at 50 m. The system was not calibrated, thus it was not possible to compare absolute levels of backscatter with other systems; the study was limited to the analysis of the school spatial structure. In the case of a nonlinear system, we will not have an absolute threshold to define the vacuoles, and whatever biases may be produced by the selection of vacuoles in our study will be the same across the range of schools that we studied. Our study was a comparative analysis using the multibeam sonar to identify species, not quantify biomass. In this regard, the calibration required to produce absolute levels of backscatter is not an essential requirement for our analyses and should not invalidate our conclusions. The data storage precision was 8 bits, i.e., 1024 samples per beam at 50 m range, the range resolution of sonar was 4.9 cm. During each survey, more than 400 sonar sequences with schools were recorded. These were then extracted using the SBViewer 5.01 digital video analysis software (Gerlotto et al. 1999). Only

[Fig. 1. Map of Lake Annecy showing the survey design carried out during the two acoustic surveys, and the associated locations of trawl samples (September 2004 and 2008). The black point indicates the position of the temperature profile done during each survey.]

[Fig. 2. Temperature profiles of the Annecy Lake carried out during the two acoustic surveys.]
sequences without noise and bottom interferences were retained. A total of 12 descriptors were then extracted using image analysis techniques (Gerlotto et al. 1999) for each fish school. Among these descriptors was the number of vacuoles per school ‘H’ (Partridge 1982; Vabø and Nøttestad 1997; Misdund et al. 1998). This parameter was redefined by Gerlotto and Paramo (2003), using the same device and software as in our case study, as empty volumes in the 3D fish school structure, which is very heterogeneous (Partridge 1982; Fréon and Misdund 1999). Fish schools with more than 1024 vacuoles were eliminated from the database due to limitations of the image analysis software. Relationships between school volume ‘V’ and the number of vacuoles ‘H’ were analyzed (Guillard et al. 2006a), and we set the ratio between ‘V’ and ‘H’ using an unsupervised classification method, the quantization (Gersho and Gray 1991). This method consists of representing each point of the data space by a unique element of a finite subset of the data space. Specifically, we used the L1 quantization method proposed by Lalœ (2010). Linear regressions for each data set were computed and a Student test was performed using the Gaussian law of the estimators of the slopes to compute a slope comparison test, performed using the R package (Hornik 2010). For each case, the hypothesis of the test was $H_0$: the slopes are identical.

Fish samples were collected for species identification at night, using a pelagic frame trawl (Guillard and Gerdeaux 1993) in the same areas where the schools were previously recorded during daytime. In 2004, 5 trawls were carried out; and in 2008, this increased to 19 trawl operations (Fig. 1). Comparisons of total fish length and mean school volumes per year were made using a Kolmogorov-Smirnov test (Zar 1984).

**Assessment**

As expected only young-of-the-year (Y-O-Y) fish were caught in the trawls carried out above the thermocline (Fig. 2). In 2004, 808 fish were caught, 23% of these were *R. rutilus* and 77% were *P. fluviatilis* (Table 1). In 2008, 909 fish were caught, and more than 93% of these were *P. fluviatilis* (less than 7% *R. rutilus*). The mean size of the two species was significantly different ($P < 0.05$), for both surveys (Table 1). The mean sizes of the same species were also significantly different between each year ($P < 0.05$).

In 2004, 277 schools were analyzed and two different relationships were distinguished (Fig. 4). The L1 quantification methods discriminated 2 groups: the first one, comprising 58.8% of the schools (named Fish Group 1, FG1) corresponded to the upper relationship showed in Fig. 4, and the second one (named Fish Group 2, FG2), comprising 41.2% corresponded to the lower relationship in Fig. 4.

The relationships between the numbers of vacuoles and school volume were analyzed for the two sets of data, i.e., FG1, FG2. These two significant relationships (Fisher test, $P < 0.001$), were defined by Eq.1 for FG1 [$V = 0.796 H + 12.89$; ($r^2 = 0.85, n = 163$)] and by Eq. 2 for FG2 [$V = 0.143 H + 2.55$; ($r^2 = 0.63, n = 114$)]. The two slopes for FG1 and FG2 were significantly different (Student test, $P > 0.99$). The proportions of each group (FG1 and FG2) were similar to the proportions of the species sampled by fishing during our 2004 survey (77% of perch and 23% of roach). The FG1 relationship was, therefore, attributed to perch schools and the FG2 relationship to roach, due to the similarity between the occurrence of the species in the lake and the acoustics data.

In 2008, 379 schools were analyzed using the same protocol and methods but only one Fish Group (FG3) was observed.

**Table 1.** Descriptive statistics of the total number of fish caught during the two acoustics surveys using a pelagic trawl (total fish length expressed in mm). The occurrences show a dominance of one species (*Perca fluviatilis*) in 2008.

<table>
<thead>
<tr>
<th></th>
<th>2004</th>
<th></th>
<th>2008</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Perch</td>
<td>Roach</td>
<td>Perch</td>
</tr>
<tr>
<td>Number caught (n)</td>
<td>626</td>
<td>182</td>
<td>848</td>
</tr>
<tr>
<td>Mean size</td>
<td>61.9</td>
<td>65.4</td>
<td>55.4</td>
</tr>
<tr>
<td>Maximum size</td>
<td>78.0</td>
<td>151.0</td>
<td>68.1</td>
</tr>
<tr>
<td>Minimum size</td>
<td>49.0</td>
<td>48.0</td>
<td>45.3</td>
</tr>
<tr>
<td>Standard deviation of mean size</td>
<td>4.7</td>
<td>8.6</td>
<td>3.8</td>
</tr>
<tr>
<td>Occurrence (%)</td>
<td>77.5</td>
<td>22.5</td>
<td>93.3</td>
</tr>
</tbody>
</table>
The relationship between the numbers of vacuoles and school volume for FG3 was defined by the equation $V = 0.997 H + 50.45; (r^2 = 0.49, n = 379)$. The FG3 slope was similar to the slope of FG1 (Fig. 5), but the linear relationship for the FG3 was statistically significantly different both from FG1, and obviously FG2 (Student test, $P > 0.99$). Mean school volume of FG3 ($222.51 \text{ m}^3$) was significantly different from the FG1 mean volume in 2004 ($160.56 \text{ m}^3$) ($\text{Kolmogorov-Smirnov } P < 0.001$).

In 2008, 4% of the school volumes were extreme values (Tukey 1977), and these were removed from the analysis to form a modified dataset named FG3*: these had a mean volume ($191.41 \text{ m}^3$) that was not significantly different to the FG1 mean volume ($\text{Kolmogorov-Smirnov } P < 0.05$). The slope of the relationship for the modified data from 2008 FG3* [$V = 0.785 H + 64.15; (r^2 = 0.53, n = 370)$] was then not significantly different to the slope of FG1 ($P = 0.13$) indicating that the two relationships FG1 and FG3* were similar.

**Discussion**

The vertical structure of the fish assemblage in Lake Annecy is strongly related to the thermodynamic conditions of the water (Appenzeller 1995; Masson et al. 2001; Mehner et al. 2010). At the end of summer, the thermocline is well established, and the only fish occurring in schools above the thermocline are the two species, roach and perch (Guillard et al. 2006b). Other fish present in this upper layer were individuals making brief incursions from the deeper layers for foraging: mainly salmonids, older perch, or from time to time, pike (*Esox lucius*). This spatial distribution of fish, coupled with the fact that only two species exhibit schooling behavior, in such a restricted ecosystem, provided ideal conditions to study fish school morphology (versus the open sea).

Schooling in fish (Shaw 1961) is an anti-predator vigilance device, which also offers hydrodynamic advantages (Magurran et al. 1985; Pitcher and Parrish 1993; Pitcher et al. 1996; Parrish et al. 2002). In Lake Annecy, from June to mid-autumn, above the thermocline, perch and roach live in school structures during the daytime, and disperse at sunset to feed (Thorpe 1977; Azzali et al. 1985), leading to local dispersion throughout the area they occupy (Masson et al. 2001). Our trawl sampling, carried out on the same day, just after the acoustic observations, began during sunset when the schools dispersed. Several attempts were made to catch schools by day, using the same fishing device, but these were all unsuccessful. We were, therefore, unable to check the precise species composition for each school detected (ground truth for each school), and had to determine the species occurrence as the probability for each school to belong to one or another species in the pelagic areas surveyed. The number of trawl samples was increased in 2008 to determine accurately the proportion.
of the two species in the pelagic area. The results were patent in terms of species composition: two species were present in 2004; whereas in 2008, perch was almost the mainly dominant species (93% of the catch).

Schools of Y-O-Y fish are commonly monospecific (Fréon and Misund 1999) and are composed of individuals of the same size. In our case, the two species are predator (P. fluviatilis) and prey (R. rutilus) (Turesson and Brönmark 2004) and they school in different aggregations. Furthermore, these two species exhibit different behaviors (Eklöv and Persson 1995; Kahl and Radke 2006) and physiology (Thorpe 1977). Eklöv and Persson (1995) have shown that juvenile roach form denser schools in the presence of predators, and have a higher swimming speed in the open water than juvenile perch. Thus, we can expect that these species exhibit different internal school structure and dynamics (Tien et al. 2004). Internal school structure is typically not uniform and is characterized by the presence of many vacuoles (Fréon and Misund 1999; Gerlotto and Paramo 2003), correlated with the school volume (Guillard et al. 2006a). In 2004, the significant positive correlations showed two distinct modes, similar to the occurrence of the two species found in the lake. Whereas in 2008 only one relationship existed, similar to the relationship attributed to perch in 2004, and confirmed by trawl samples that caught 93% of perch. The statistical significance of this correspondence is sensitive to extremes of the data, i.e., very large schools. These larger schools were present due to the great perch density in 2008, where fish densities were up to 150 kg ha⁻¹ (Guillard et al. 2006b).

The relationship between school volume and number of vacuoles in the FG1 and FG3 datasets were close but not identical; this is likely to be due to some environmental variability in schooling behavior. Such variability could be explained by different assumptions. In 2008 (FG3), the relationship could have been affected by the presence of a small number of R. rutilus schools (7% of the fish caught) in the sonar dataset. Furthermore, the individual size of perch was smaller in 2008 (FG3) than in 2004 (FG1), and this could have an additional impact on schooling behavior. Lastly, the roach population may have had a different spatial distribution in 2008, distributed more towards the littoral areas during the day, whilst at night, some of them may have moved toward deeper areas (i.e. depths greater than 10 m) as scattered fish available to the trawl. Thus the schools of R. rutilus were not observed by sonar, but some individual fish caught in the trawl.

Several studies (Coetzee 2000; Brehmer et al. 2007) have shown that changes in school behavior depend on environmental conditions. The external school morphology can vary according to the variation of the environment; there is, however, a lack of information on the effect of the environment on the internal spatial structure of a school. In our study, the environmental conditions have remained rather stable over the study period: this is typical of an oligotrophic freshwater lake (Domaizon et al. 2010; Perga et al. 2010). It is reasonable to assume that such small variations have not had an impact on internal morphological school parameters. Other parameters may also be involved in such changes (Brehmer et al. 2007); nevertheless we assume that they do not play a significant role in our case study as they are likely to change at a rate that is slow enough to not have had any effect. Thus, we propose that the school internal morphology, as described by the relationship between the school volume and the number of vacuoles, is a phenotypic expression therefore characteristic of a species (Brehmer et al. 2007). Further investigations are planned, with a focus on ground truth protocols to establish the true composition of the schools. Using fishing devices such as a purse seine to catch schools during daylight will be beneficial. It would also be useful to examine schooling fish in other ecosystems, particularly marine ones, to extend our hypothesis to other populations. These relationships between internal school morphology and their species composition will be an efficient tool for fish species discrimination and thus of useful help to study the aquatic ecosystem and so improve the management of pelagic fish populations.

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