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Video multitracking of fish behaviour: a synthesis and future perspectives

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Abstract

With the development of digital imaging techniques over the last decade, there are now new opportunities to study complex behavioural patterns in fish (e.g. schooling behaviour) and to track a very large number of individuals. These new technologies and methods provide valuable information to fundamental and applied science disciplines such as ethology, animal sociology, animal psychology, veterinary sciences, animal welfare sciences, statistical physics, pharmacology as well as neuro- and ecotoxicology. This paper presents a review of fish video multitracking techniques. It describes the possibilities of tracking individuals and groups at different scales, but also outlines the advantages and limitations of the detection methods. The problem of occlusions, during which errors of individual identifications are very frequent, is underlined. This paper summarizes different approaches to improving the quality of individual identification, notably by the development of three-dimensional tracking, image analysis and probabilistic applications. Finally, implications for fish research and future directions are presented.

Keywords *3D tracking; ethometry; fish tracking; occlusion; schools; video multitracking*

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Background

Aristotle first reported schooling behaviour in fish over 2400 years ago (Masuda and Tsukamoto 1999). However, the first real quantitative studies of their structures and dynamical properties were undertaken only <60 years ago (e.g. for the earlier paper : Breder 1954; Keenleyside 1955; Cullen *et al.* 1965; Pitcher 1973; Pitcher and Partridge 1979; Partridge *et al.* 1980). The initial measurements were manual and laborious. Since the 1980s and 1990s (e.g. for the earlier works: Aoki 1982, 1984; Reynolds 1987), many attempts have been made to replicate the patterns of moving animal groups using computer simulations (e.g. Parrish *et al.* 2002; Czirók and Vicsek 2006). Generally, this approach constituted an a posteriori study of these behaviours. It was based on predetermined interaction rules between individuals, which could be examined by comparisons with natural behavioural patterns. However, although a good fit was obtained between computed and natural patterns, the interaction rules in natural fish groups remained less well explained.

With the recent developments in image analyses and computer sciences, there are now powerful tools that can substitute or complement traditional behavioural observation tools. Video tracking, by definition, is the tracking of moving objects (here fish individuals) and the monitoring of their activities by image sequences obtained from video cameras (Maggio and Cavallaro 2011). It is an automatized procedure that determines animal position over time and gives the resulting tracks with a large array of data such as distance travelled, speed or space used (Noldus *et al.* 2001; Maggio and Cavallaro 2011). The quantitative approach of this methodology has made it possible to collect straightforward data in fields as varied as ecotoxicology (Kanen *et al.* 2005; Jakka *et al.* 2007; Denoël *et al.* 2010), neurotoxicology (Eddins *et al.* 2010), behavioural brain research (Mathur *et al.* 2010; Silverman *et al.* 2010), pharmacology (Pinhasov *et al.* 2005; Singh *et al.* 2009; Cachat *et al.* 2010), genetic and behavioural screening (Orger *et al.* 2004; Egan *et al.* 2009; Blaser *et al.* 2010), animal well-being studies (Navarro-Jover *et al.* 2009; Winandy and Denoël 2011), behavioural ontogeny (Fontaine *et al.* 2008; Fukuda *et al.* 2010), social behaviour (Newlands and Porcelli 2008; Salierno *et al.* 2008), cognition (Bisazza *et al.* 2010) and ethology and behavioural ecology (Pritchard *et al.* 2001; Speedie and Gerlai 2008).

With video multitracking, more than one individual can be tracked simultaneously. Sensu stricto, this refers to tracks of individuals within the same space, which is called an arena in video-tracking procedures. Video multitracking can tackle the new challenge of integrating the interactive component in animal behaviour. This has important outcomes, because animals interact with others during their lifetime, such as when defending territories, courting sexual partners and taking care of progeny. There are also a large number of species living in groups in which the individuals orient their behaviour according to that displayed by other members of the group. All these interactions between individuals within groups are fundamental in the processes of information transmission and social learning (Brown and Laland 2003; Hoare and Krause 2003), collective decision-making (Conradt and Roper 2003; Couzin *et al.* 2005), and in the function of social behaviours (Pitcher and Parrish 1993). Interactions and links between the individuals and 'higher' levels of biological organization (group, populations, species) are of utmost importance in the structures of social systems (Camazine *et al.* 2001; Anderson 2002), multispecies interactions (Ward *et al.* 2002a; Mathis and Chivers 2003) , self-organization (Camazine *et al.* 2001; Anderson 2002; Parrish *et al.* 2002), social synchronization and social amplification phenomena (Camazine *et al.* 2001; Anderson 2002; Canonge *et al.* 2009), and in the ontogeny of social behaviours (Masuda *et al.* 2003; Fukuda *et al.* 2010). Specifically, in situations where a large number of individuals are involved, the use of video-tracking data is essential, as manual analyses would be complicated, time-consuming and sometimes even impossible. Today, multitracking allows us to observe directly the behaviours of groups and to determine the real interaction rules by sampling data collected in nature or in the laboratory, without any a posteriori rules (e.g. Ballerini *et al.* 2008; Cavagna *et al.* 2010; Herbert-Read *et al.* 2011; Katz *et al.* 2011).

Among living organisms, fish frequently form shoals, defined as a voluntary association of individuals, in both fresh and salt water environments (Pitcher and Parrish 1993). It has been estimated that more than 25% of the approximately 27 000 species of teleosts adopt shoaling behaviours throughout their life, and over 50% do this as juveniles (Shaw 1978). These behaviours are also reported in many other animal taxa such as selachians (Klimley 1985), cephalopods (Boal and Gonzalez 1998), crustaceans (Evans *et al.* 2007) and amphibians (d'Heursel and Haddad 2002). In collective behaviours of fish, gradients from swarm (unpolarized shoals) to school (polarized shoals) have been identified depending on the degree of polarization and the synchronization of speed (Pitcher 1983; Couzin *et al.* 2002; Parrish *et al.* 2002). Small teleost fishes are used as laboratory models to study these collective behaviours (e.g. Wright and Krause 2006). For instance, the zebrafish (*Brachydanio rerio*, Cyprinidae), one of the most commonly used laboratory animals, which adopts shoaling and schooling behaviours, is the model organism par excellence for all the above-mentioned topics (e.g. Gerlai 2003; Guo 2004; Hill *et al.* 2005; Rubinstein 2006).

As happened when single video techniques were launched, video multitracking has been underused even though it could be a pertinent tool for understanding the mechanisms of animal interactions and particularly of shoaling behaviours. Several initiatives of multitracking have been developed independently, using a variety of technologies.

Aims and scope

Our objective is not to create a comparative list of all available video multitracking systems, commercial or otherwise. In the present review, we provide a general outline of video multitracking methods applied in fish studies. It is addressed to fish researchers, particularly those working on fish schools, to give them an overview of the available methodologies but also to support them in their choice of the most appropriate methodologies. In the first section, we define the different characteristics of video systems in fish studies, their advantages and drawbacks. In the second section, we tackle the problems of individual identifications of multitracking systems but also present directions towards optimization of these procedures, particularly with regard to occlusions as explained in the third section. Finally, the last section covers the applications of video multitracking in fish research, putting forward interesting applications of these techniques that should have a wider use.

Basis of the video-tracking techniques

Experimental set-up and principles of video tracking

The basic set-up of a video-tracking system consists in filming organisms, such as fish in an aquarium, with a video camera. The signal from the camera is then either transformed into a numerical video file through a frame grabber linking the camera to a computer or is directly available if the camera is digital. The signal can be processed in real time by video-tracking software. It can also be stored first and analysed later, which is safer as this avoids a system crash or experimenter errors of calibration (see later).

Individual fish are free to move anywhere in the experimental space. This filmed experimental space is called the arena. Often it corresponds to the limits of the aquarium on the video frames. The experimenter can define this surface of analysis in the video-tracking programme. Thus, pixels outside the arena are not taken into account in the analysis. From this defined arena, the experimenter can define different zones such as shelters and feeding areas, inside as related to the experimental protocol (see notably Noldus *et al.* 2001).

Video tracking consists in recognizing and following spatially over time moving objects or organisms on the basis of typical features, which could be body shape, body colour or body greyscale level and which are visible in each frame of a video sequence. An automatically detected characteristic must not be present as part of the background. If it is not possible to distinguish fish from the background, an easily visible and detectable tag can be used on the animal's body. Marks can take the form of coloured tags such as a bead fixed on the body, as done by Ylieff (2002), or a subcutaneous injection of Visible Implant Elastomer, as in Delcourt *et al.* (2011). Further information on marks is discussed later.

The tracking system analyses the incoming video signal and in each frame distinguishes from the background pixels the pixels belonging to the fish image or to the tag if the fish is marked. Another means of obtaining a fish image is to eliminate all pixels characteristic of the background. This can be achieved by the subtraction method that compares a reference image of the arena without the fish with the incoming video signal, and in each frame, it identifies all pixels with different values from the reference image as deriving from the live tracked targets. There are different ways to obtain a reference image of the background. The easiest is to take a picture of the arena without the animals. A second possibility is to choose randomly several frames from the experimental video sequence over a relatively long period of time during which the fish changed position, and afterwards to calculate for each pixel the value with the highest luminosity level. If the fish are darker than the background, an image with only the pixels from the background is obtained. Another possibility is to calculate the mean value (grey or colour levels) of pixels. In this case, the fish is expected not to be in the same place for a significant period of time. With the last two methods, dynamic subtraction can be applied. This consists in using the subtraction method, but the reference image is updated over time, preventing any change in the background image (displacement of gravel, faeces, etc.). Another method is motion detection. This method assumes that a change in the value of pixels (colour or greyscale) is a consequence of the movement of a tracked animal (Lipton *et al.* 1998; Bogomolov *et al.* 2003). In this case, the current frame is compared with the previous frame. This detection technique allows for a more heterogeneous environment, but there must always be sufficient contrast between the animal and the background.

In a treated frame in which only the detected pixels appear, which correspond to the tracked target, each individual represents an island of detected pixels. The programme then considers only these islands of pixels. It analyses the size of each island using a filtering processing, as discussed later, and determines the coordinates of each individual within the arena for each image for a given time. The coordinates can be the centre of gravity of the pixel island defining an individual, but other coordinates can also be used, such as the beginning of the snout.

Detection thresholds

Different thresholds can be defined to improve the quality of detection and data collection. They can refine the background noise detected, improve the definition of the fish image and take into account fish displacement features.

Minimum threshold size

The image of the arena can include several pixels that are not the focal organisms such as faeces or a shadow, but all the objects creating an image have a similar contrast with the background. Although shadow problems can be avoided with good lighting, the presence of artefacts in the image needs to be dealt with accordingly to avoid errors in detection and identification. To this end, a minimum threshold size can be imposed on the system, so that it automatically fails to register all surfaces smaller than the designated pixel size for the detection of the focal organism.

Body size and arena size

The available image resolution can determine the ideal dimension of the arena without the risk of losing the tracked animal because it is too small. For example, if on one axis, the resolution of a video file is 480 pixels and if the image of the fish has a length of 15 pixels (minimum threshold size to be sure to detect a fish) corresponding to a real size of 1 cm, then determined by a ratio, the maximum length of the arena would be 32 cm. Making the same calculation with the other axis gives the second dimension of the arena. The real detected size is generally smaller than in the theoretical calculation because the fins and the edges of the fish body are not detected, and thus a smaller arena may be needed. Generally, these pixels have an intermediate characteristic between the colour (or greyscale) of the fish and the background (Fig. 1). However, detection of fish on the basis of 3–4 pixels as body size is possible. In this case, a larger arena can be used, but the background noise must be very weak.

Activity threshold and frame rate

The displacement measured is the result of the real displacement of the fish and the displacement noise resulting from the crude measurement of the fish position. This noise increases when the apparent image of the fish is highly pixelated. If the fish swims at a low speed or is inactive, the effect of measuring the distance and the orientation of fish displacements can become biased. A threshold of activity

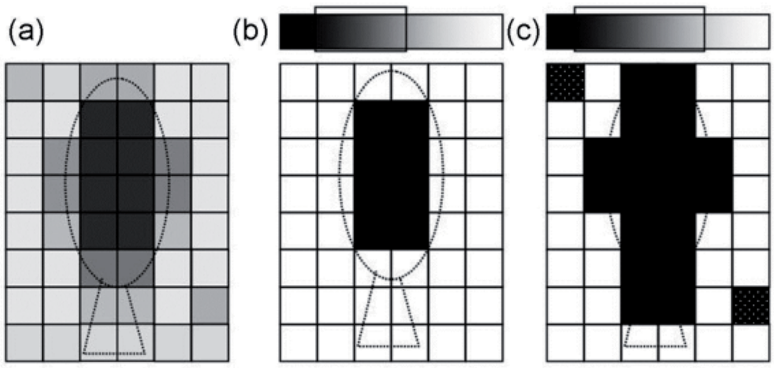

Figure 1 Theoretical example of effect on pixelization on the detected size and filtering of background noise. (a) Recorded frame where each square is a pixel with a different intensity along a greyscale. (b) Using a small detection range, only the darker pixels are detected, the pixels from the fish outline are not detected. The size of detected island is significantly smaller than the real body size of the fish. (c) Using a large detection range, the detected grey range is larger, allowing the detection of more pixels from the fish image but also a detection of a background noise (represented here in black with white points). The fish shape is represented in dotted line.

can be determined to filter data when the animal is inactive: if the speed does not reach a minimum value, which is often proportional to fish body size, the fish is considered to have a null speed, thus removing the artefact.

Moreover, the frame rate used by the recording unit is also very important. If the sample rate is too high, the noise caused by small movements of the animal will be picked up and give an overestimate of parameters such as distance moved and velocity. If the sample is too low, data would be lost, giving an underestimate of the above parameters. The determination of the optimal sampling rate depends on the speed and complexity of the movements adopted by the tracked animals. Plotting the length of the pathway or velocity for different values of the frame rate with the same video file can help to decide on the best sampling rate (Fig. 2).

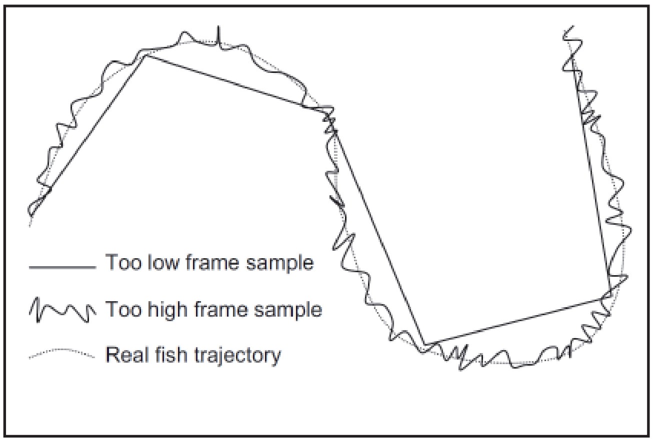

Figure 2 Theoretical example of trajectory of a fish at different frame rates. When the frame sample is too low, both distance and speed are underestimated; when the frame is too high, the trajectory is overestimated because the imprecision to measure the referent point of fish location plays a too significant effect. In this latter case, the small movements are not the real behaviour of fish.

The specificity of multitracking: tracking groups

General comments

One of the new challenges in video tracking is the possibility to track the largest number of individuals at the same time. The interest here lies mainly in producing rapidly a battery of synchronized tests. Some of these video-tracking systems, called multiple arena video tracking, often represent 'false' multitracking because individuals are isolated from each other; individuals moving in separate arenas are analysed simultaneously. This isolation greatly limits or excludes the study of social behaviours. A typical example comes from the multiwell plates used in zebrafish research (Baraban *et al.* 2005; Prober *et al.* 2006). Such systems, for example, EthoVision® (Noldus Information Technology, Wageningen, The Netherlands) or VideoTrack® (ViewPoint, Lyon, France), are now able to track simultaneously more than 100 isolated individuals.

Real video multitracking systems, which track several individuals within a single arena, are a newly available technique offering great potential but there are also certain difficulties that need to be adequately dealt with. The first problem with these programmes is detecting each animal in the same arena individually and not merging individuals that are close to each other. The second is to clearly differentiate the identity of each animal from the others and to retain this identity throughout the recording process. Besides these specific problems in multitracking, the detection techniques have a direct influence on the possibility to track individuals, subgroups and the entire group.

Detection

The first systems developed using black-and-white image analyses could track two individuals on the basis of their relative size (Noldus *et al.* 2001), thus requiring that one individual was smaller than the other. With this method, Hansen *et al.* (2008), studying the aggressive behaviours and the effects of food in Atlantic cod (*Gadus morhua*, Gadidae), were able to track two fish of significantly different size together in an arena.

Video multitracking based on colour detection can follow different individuals with different body pigmentations. An example is presented in Fig. 3, where two goldfish (*Carassius auratus*, Cyprinidae), a red one and a white one (in this case the detected pixels are light pink, because white is not a colour) are tracked using this feature (Ylieff 2002). However, different body pigmentations in

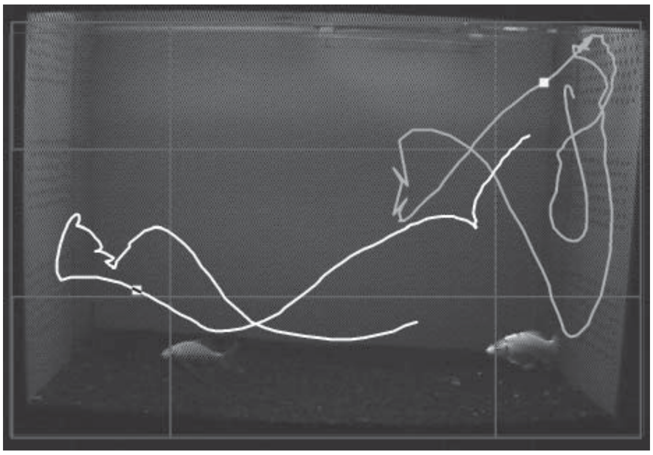

Figure 3 Two goldfish (*Carassius auratus*) are individually tracked in an aquarium based on their different pigmentation; one fish is red (black track) and the other is white (white track) (Ylieff 2002, Ylieff and Poncin 2003). However, white is not a colour, the tracking of white fish is based on the detection of very light pink pixels.

a species are rare, and when this is the case, the level of colour differentiation is often not sufficient for the system to differentiate the individuals. Consequently, the number of individuals that can be tracked simultanesousły is very low.

With individual coloured tags, detecting the animal's body is not necessary. If a colour is clearly associated with only one individual, identification is easy without any other data required. With systems like Swistrack® (open source, holded by SourceForge) and EthoVision Color-Pro®(Noldus Information Technology, Wageningen, The Netherlands), the number of tracked individuals on the basis of differential colour tags can rise to 10 and 16, respectively. However, because of the optical characteristics in water and the heterogeneity of light conditions within an aquarium, this number is usually lower than in theory. Ylieff (2002; Ylieff and Poncin 2003; Jadot *et al.* 2005) managed to track two damselfishes (*Chromis chromis*, Pomacentridae) or three salema porgy (Sarpa salpa, Sparidae) in the same tank using only an individual-specific coloured bead attached on the dorsal fin of each fish (Fig. 4). Delcourt *et al.* (2011) succeeded in tracking up to four translucent glass eels (*Anguilla anguilla*, Anguillidae) at low luminosity using various fluorescent visible implant elastomer (VIE) tags.

Even though the number of tracked fish is very limited, it is possible to follow several individuals in a larger group. In this case, it is impossible to track the entirety of the shoal, but individual behaviour can be studied within a social context. An example is shown in Fig. 4, where two of the four damselfish are marked by a coloured bead attached to their backs (Ylieff 2002).

The possibility to track simultaneously a large number of unmarked individuals is a very recent development. Before this, and even recently, some researchers used manual detection consisting in a manual click on the screen at the position of the fish, frame by frame (e.g. Miller and Gerlai 2007, 2008). Often, these researchers did not attribute an identity to each individual, their study being based on a global spatial parameter such as the average nearest neighbour distance for example. With EthoVision Multi-Pro®, Buma *et al.* (1996, 1998) tracked up to 16 fish. Suzuki *et al.* (2003) used another home-made system and tracked 25 individuals, but without explaining its functioning and its limits. Recently, Delcourt *et al.* (2006, 2009); Becco *et al.* 2006; Delcourt *et al.* 2008) were able to track up to 100 individuals for several minutes (see example in Fig. 5).

In systems such as EthoVision®, VideoTrack® or SwisTrack®, the track of fish given as a displacement during a time period is traced by the connection over time of the unique position of the animal as detected in each frame. This is the case for simple tracking with one individual in one arena or for multitracking based on different tag detection. In individual-based unmarked multitracking systems, such detailed tracking is not possible because of the larger number of coordinates corresponding to each fish for each image. Frame after frame, the tracking programme must identify correctly each individual in all detected positions. However, perfect identification without error does not exist because of occlusions.

Occlusions: difficulties and solutions

An occlusion is the phenomenon of two or more tracked target images becoming one during a time period. This mergence–splitting phenomenon leads to many identification problems. These are particularly frequent when the targets are more similar in appearance, which is often the case in animal groups.

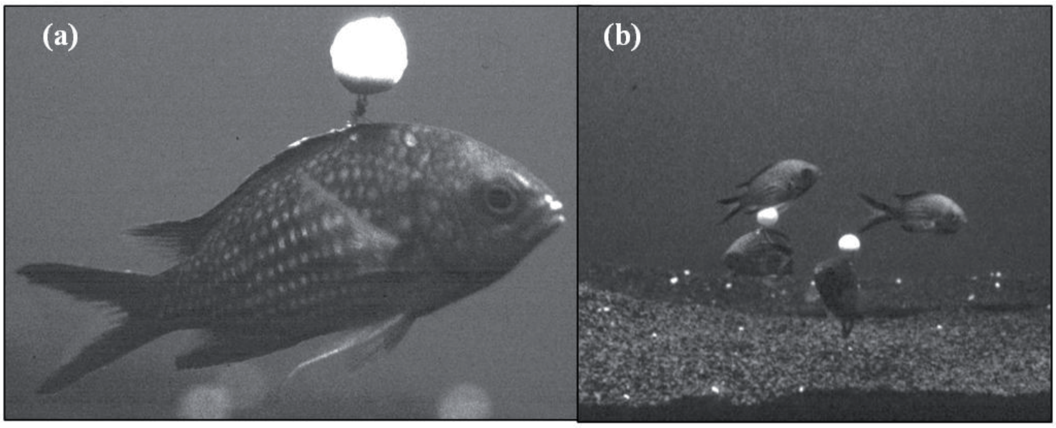

Figure 4 (a) Example of colour tags with a pearl fixed on the back (attached by surgical thread just in the front of the dorsal fin) of damselfish (*Chromis chromis*), a species with dark pigmentation (Ylieff 2002). (b) Example of two marked individuals in a group of four individuals, showing the possibility to track several individuals in a social context without the need to track the entire group. In this example, one bead is pink, the other one is blue.

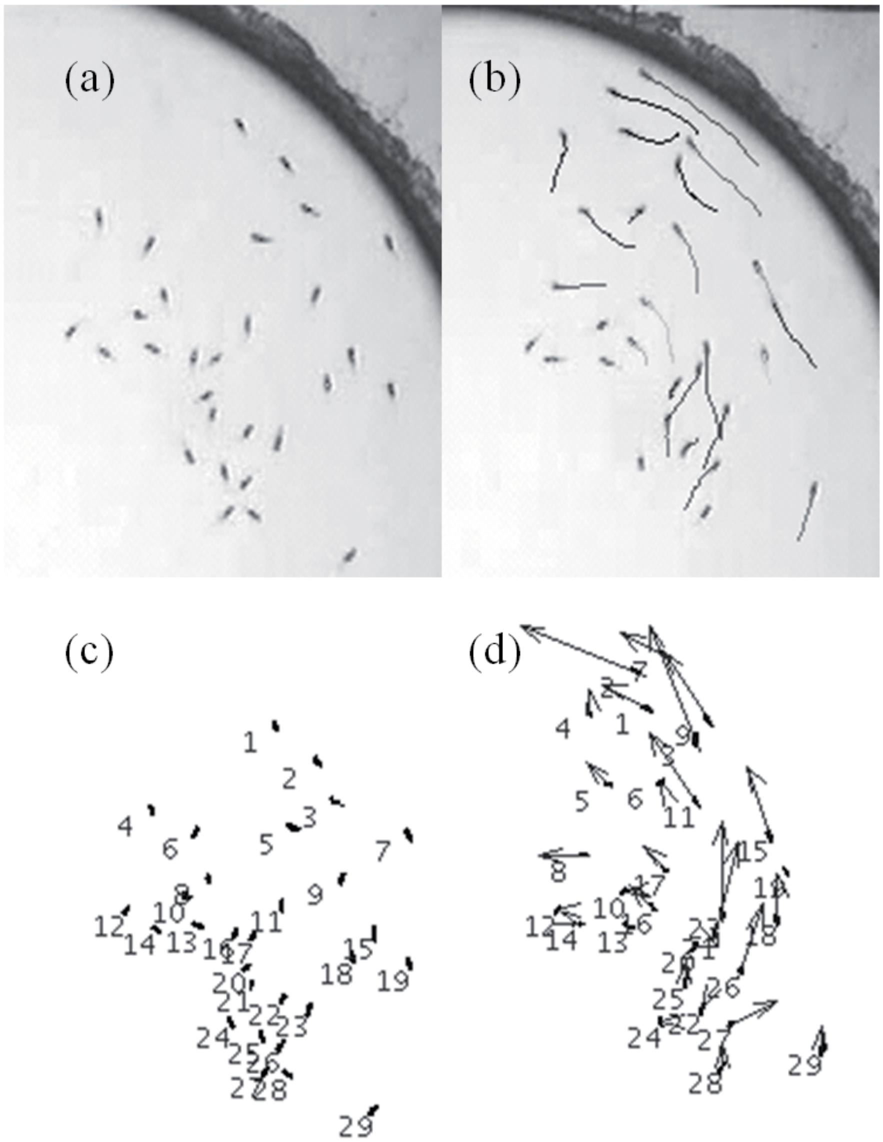

Figure 5 (a) Partial view of a circular arena with a shoal of 29 individuals of early juvenile Nile tilapia (*Oreochromis niloticus*): (a) initial frame of the sequence; (b) frame 30 (the previous individual trajectories are shown); (c) initial frame where only the detected pixels are shown, each individual is identified by a number; (d) treated frame 30: individuals are identified by a unique number and their speed is shown by the length of the arrows with the largest being for the fastest fish. (Delcourt *et al.* 2008, 2009).

The main problem of all video multitracking systems is the error of individual identification. These errors lead to two types of misidentification: loss of fish identity and swapping identity between individuals (Delcourt *et al.* 2009). Generally, when the fish are clearly distant from each other, and the frame sample rates are high, the errors are very rare or absent. The two successive individual positions are very close to each other and distant from other fish positions, so it is easy to correctly identify individuals over time. However, during occlusion events, identification errors can arise (i.e. Delcourt *et al.* 2009). Indeed, when the trajectories of two fish cross each other and their images merge, termed occlusion, it is difficult to identify who is who after crossing. This makes these automatized systems without marked individuals imperfect, leading to possible identification errors if no corrections are made (Buma *et al.* 1996, 1998; Khan *et al.* 2005, 2006; Delcourt *et al.* 2009).

With EthoVision Multi-Pro®, the programme is unable to attribute the correct identity to each fish after crossing (Buma *et al.* 1996, 1998). In a recent study, Delcourt *et al.* (2009) use two parameters to determine the successful identification: (i) the Recognition ratio of individual fish (see also Kato *et al.* 2004) = $A*TO + B*TS + C*TN$, where TO = number of identity assignments in the context of occlusion/ total number of identity assignments; TS = number of identity assignments when separation occurs/ total number of identity assignments; TN = number of identity assignments in other cases/total number of identity assignments; A = successful identification ratio when there is occlusion; B = successful identification ratio when there is separation; and C = successful identification ratio in other cases; (ii) the Separation ratio (after occlusion) = successful number of separations/total number of occlusions. In Kato *et al.* (2004), separation was defined during the occlusion, using a method of erosion/dilatation of fish images. In Delcourt *et al.* (2009), the recognition ratio was >99.5%, but successful identification (separation ratio) immediately after occlusion was very poor at between 50% and 85%. If the goal of the experiment is a statistical analysis of individual performance, the results of recent systems are excellent; the errors are submerged in the data and have little effect. In contrast, if the goal is a rigorous analysis of the individual behaviours, notably to detect differences between fish, a single error can affect the results dramatically. This implies the need to be able to edit data manually to correct the errors if this is necessary.

Occlusions cause problems in two steps: first, during the occlusion; second, after the occlusion when the fish are clearly separated on the image. To solve these problems, different solutions have been proposed such as the addition of three-dimensional information (Isard and MacCormick 2001; Zhao and Nevatia 2004) or using the animals' characteristics on the screen, for example the shape of the target (Isard and Blake 1996; MacCormick and Blake 1999; Branson and Belongie 2005). Using the change in shape and/or the specific topology makes the system more robust (Rasmussen and Hager 2001; Sanchez and Dibos 2004; Sigal *et al.* 2004; Khan *et al.* 2006), notably by application of a predictive statistical model.

Detection of occlusions

The first difficulty is to detect the occlusion events automatically. In an experiment with a fixed number of fish, an individual lost by the tracking programme will be lost because the system is detecting two fish as one so long as there are no detection faults in the system itself. Another way of detecting occlusions is based on the apparent size of fish image. The tracking programme can detect occlusions by the analysis of the size of the pixel island defining the fish images. If the size of the image of a detected fish increases significantly, it is because the programme detects two or more fish together.

Three-dimensional tracking

An interesting perspective that can be used to study social animals is tracking in three spatial dimensions. The movement of an aerial or pelagic animal is rarely limited to a simple two-dimensional plan. The structure of animal groups such as a fish school (Partridge *et al.* 1980; Axelsen *et al.* 2001; Paramo *et al.* 2010), a bird flock (Ballerini *et al.* 2008) or an insect swarm (Ikawa *et al.* 1994) is typically three-dimensional.

Contrary to manual methods, three-dimensional automatic video multitracking systems are only in their first developmental stages (Grünbaum 2003; Viscido *et al.* 2004; Hemelrijk *et al.* 2010). These new systems are keenly awaited particularly because they can improve the quantification of displacements by taking into account the three axes of the entire space used by the fish, but also because they can resolve the large majority of occlusions.

In constrast, numerous manual methods have been developed since the 1960s with which to study the three-dimensional structure of schools (examples of significant earlier works: Cullen *et al.* 1965; Hunter 1966; Graves 1977; Pitcher 1973, 1975; Partridge *et al.* 1980). These methods can give information needed for automatic three-dimensional tracking.

Graves (1977), who assumed invariant fish size, used the size of each individual's image on the screen or on a photograph as a measure of its distance from the camera, so including the third dimension. However, generally, the individual size composition in a group is variable.

The other techniques are of two major types: 'shadow' and 'stereo' (Fig. 6). The shadow method uses the shadows of the fish projected onto the substrate as a second point of view of the school. This method needs to use only one camera. For example, Laurel *et al.* (2005) used the projection of fish shadows (Fig. 6a): two spotlights placed slightly on the side of the aquarium projected two shadows per individual on the substratum. Two light sources were redundant in most circumstances, but it assured that one shadow was cast on the substratum as objects approached the aquarium walls. With the two-dimensional position of these shadows and the two-dimensional position of the fish, it is possible to know the three-dimensional position of the fish using trigonometric computations. This method can be applied to track several fish simultaneously (Cullen *et al.* 1965; Partridge *et al.* 1980; Laurel *et al.* 2005). Video analysis needs to detect each fish and each shadow, and must accurately connect each fish with its shadows. When the number of fish increases, this analysis quickly becomes very difficult, notably because the shadows can be in occlusion, and several fish can hide the shadows with their body.

The alternative stereo method is to use stereo-cinematography techniques, which requires two simultaneous images from different angular positions. This is possible with two or more video cameras (Aoki *et al.* 1986; Pereira and Oliveira 1994; Hughes and Kelly 1996, Zhu and Weng 2007), one video camera and a mirror (Fig. 6b,c), a periscope (Pitcher 1975) or a stereo prism lens (Cullen *et al.* 1965) based on the parallax principle of different view angles. Studies have multitracked groups of 30 giant danios (Devario aequipinnatus, Cyprinidae) using two cameras (Grünbaum 2003; Viscido *et al.* 2004). Hemelrijk *et al.* (2010) used

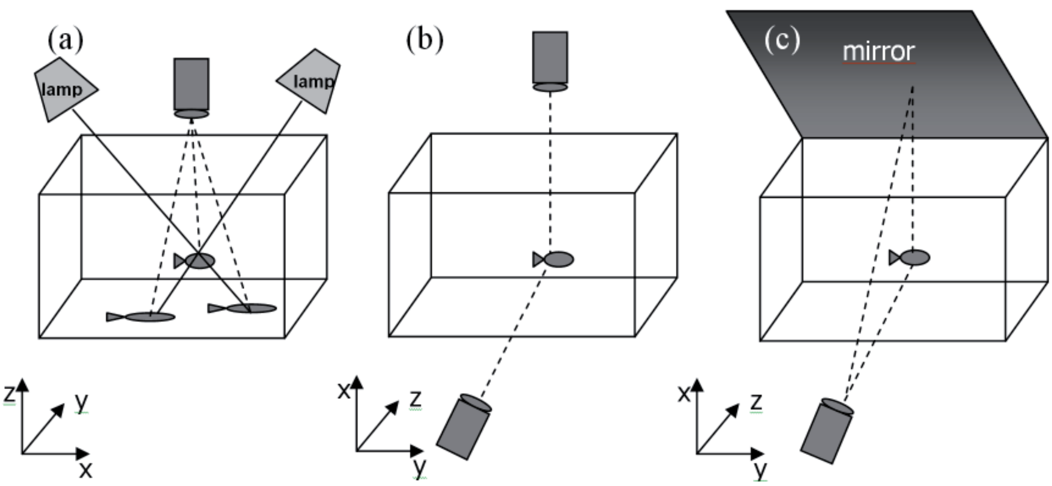

Figure 6 Three methods to measure the 3D position of a fish in an aquarium. (a) measuring the position of the fish and its shadows produced by two lamps (inspired by Laurel et al. 2005); (b) stereo-cinematography using cameras; (c) using a mirror and a camera.

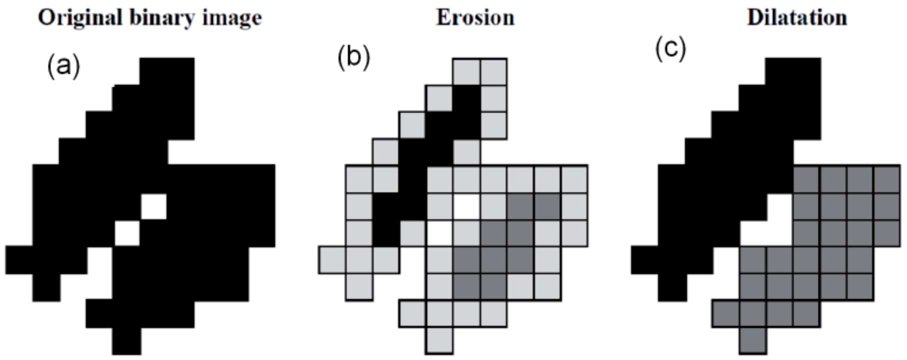

Figure 7 Process of erosion–dilatation to resolve an occlusion in 2D (inspired by Kato et al. 2004). (a) Case where the image of two fish is occluded; (b) the occluded image is eroded to obtain two significant pixels islands (erosion process); (c) if the process allows to obtain clearly two pixels islands, each spot is considered as a fish and each is enlarged to previous eroded close pixels (dilatation process) to obtain the original image size.

a mirror to find the three-dimensional positions of individuals in a shoal with one camera. Finally, another possibility consists in creating light flashes. These light flashes reveal the objects present in a single plane at a time. The flashes appear with a rapid variation in the third dimension. The technique requires a high-speed camera and must not be invasive for the fish. This laser flash technique is already used in multitracking inanimate particles, notably in the hydrodynamic study of fish locomotion (e.g. Nauen and Lauder 2002; Wilga and Launder 2002). The three-dimensional spatial coordinates can also be determined using holographic techniques with laser (Malkiel *et al.* 2006; Hobson *et al.*, 2000; Sheng *et al.* 2007). Here, the method is based on the interference between two laser beams: the referent beam is perceived directly by the measurement machine, and the object beam is perceived indirectly by the diffusion of the beam by the object. In measuring the phase and amplitude of the object beam, it is possible to obtain the three-dimensional structure of this object, in this case a group of individuals.

Merge–split and straight-through approaches

Several methods not employing three-dimensional analysis were developed to resolve the identification problems created by occlusions. Two approaches were undertaken: the merge–split approach and the straight-through approach (Gabriel *et al.* 2003). In the former, the characteristic of the occlusion state, such as its shape for example, is taken into account to identify the different tracked objects (e.g. Kato *et al.* 2004). In the latter, information is used just before the occlusion event, which might include the direction and speed of the tracked objects (Delcourt *et al.* 2009). However, interaction between individuals is an important parameter in the multitracking study (Ying 2004; Khan *et al.* 2005, 2006), as an individual can modify its behaviours drastically during and after an interaction. Simple projection of behaviours is not neccessarily a sufficient approach, and more in-depth analysis is needed by a statistical approach. These methods are discussed in the next few sections.

Identifying individuals during occlusion

Kato *et al.* (2004), using an erosion–dilatation process, managed to resolve the occlusion issue in several cases. In this process, the occluded image was reduced by its body edge until it was divided into two segments, and the separated images were labelled as two objects. If the separation is successful, the image is individually labelled as two fish and enlarged (dilatation) again to the original image size (Fig. 7). This system is accurate when the movements of animals are strictly limited to two dimensions. In a three-dimensional shoal, when the number of fish increases up to 4, the efficiency of this process decreases dramatically. Delcourt *et al.* (2009) suggested attributing to the merged spot the identity of both fish during the occlusion. The major difficulty is to identify correctly each individual after uncrossing.

A way to improve the quality of individual detection during occlusion is by using a system based on the shape-identification characteristics of the animal body. This shape can be a particular geometric shape or a reference image (see below). In occlusions, the visible parts of one fish behind another can be used to reconstitute the complete shape of the fish (Fig. 8). However, the efficiency of this method depends on the image resolution (Fig. 8).

Probabilistic applications

Another way to resolve the identification errors caused by occlusions is to apply a probabilistic approach. This method generally presents two essential processes: a predictive or dynamic part and an observational or corrective part (Ying 2004; Egerstedt *et al.* 2005; Khan *et al.* 2005, 2006).

In the studies reported by Becco *et al.* (2006) and Delcourt *et al.* (2006, 2009), the identity of each fish was determined by extrapolating the previous movement of fish (Fig. 5). The programme begins to detect the position, without taking into account the identity of each fish. Then, with the data of the previous positions of each individual, the programme assigns the identity number. How is this done? At instant t_0, the fish is at position (X_0, Y_0); at instant t_1, the fish is at position (X_1, Y_1). At instant t_2, the system detects numerous targets: each one is potentially the tracked fish. Then, the system estimates a theoretical position at instant t_2, because the direction of movement and the speed of the fish between t_0 and t_1 are known (predictive part). By this extrapolation, the computer finds a theoretical point that can be compared with the real detected positions. The nearest real position to the theoretical position is attributed to the tracked fish (observational part). To improve the video-processing time, the software searches within a fixed circular area parameterized by the user. The limits of this searching area can be adapted and refined in relation to the previous movement. For a given velocity, a searching surface can be obtained where the peripheral limit corresponds to a threshold of occurrence probability. This

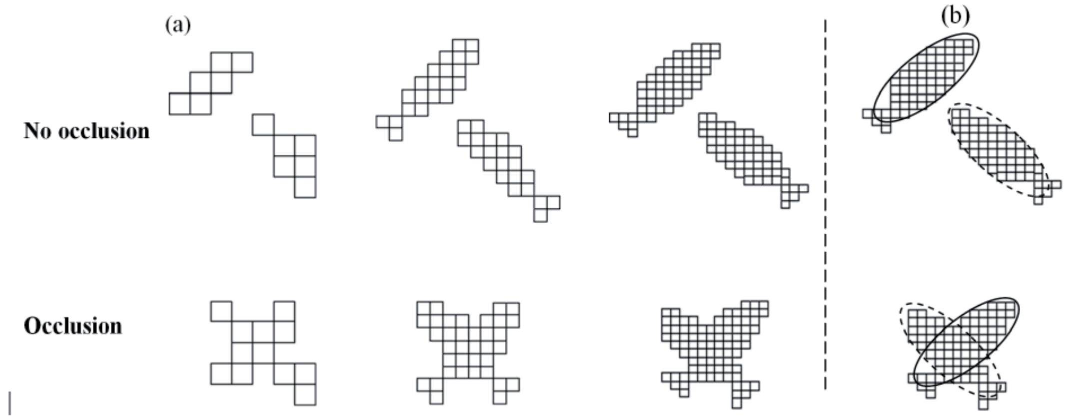

Figure 8 Increasing image resolution provides better evaluation of fish shape during the occlusion. Above, cases when the images of two individuals are clearly separated, and beneath, when the two images are occluded, for an increasing in resolution from the left to the right (a). (b) Theoretical example of identification of individuals on the basis of the shape of their image, notably during the occlusion.

method is very accurate when fish are not occluded. However, when they are occluded, the system produces errors, thus requiring manual corrections for each case of occlusion (Delcourt *et al.* 2009).

A more sophisticated system was developed for insect tracking (Ying 2004; Egerstedt *et al.* 2005; Khan *et al.* 2005, 2006). It applies the succession of observational and predictive parts. The basic idea of the approach is to use the information from the previous observations to predict the position of targets in a statistical way in subsequent observations. To be more precise, the predictive part employs a Markovian model, that is, a model that uses information on a target from only the immediately preceding observation (Lawler 1996; Ying 2004), to predict possible locations, or 'sampling zones' for a target or individual. In the observational part, each of the sampling zones is compared with a reference image of a target (e.g. an image of an individual), and the sampling zone that best agrees with the reference image (by some measure of correlation between images) is considered to be the present location of the tracked target. Figure 9 illustrates this approach.

The predictive model used can be updated and improved throughout the tracking process by incorporating additional observations and data that become available. Repeated corrections produce a more refined and robust predictive model (Ying 2004; Khan *et al.* 2005).

Predicting many different possible sampling zones for large numbers of tracked individuals quickly becomes computationally demanding. Sophisticated filtering processess that assess the likelihood of sampling zones in the predictive part in more detail have been suggested to reduce the computational load (see Ying 2004 and Khan *et al.* 2005 for details).

The approach outlined above is highly successful in tracking large groups of ants (up to 100 individuals). Unfortunately, there remain errors of occlusion despite the high tracking efficiency (>99% correct identifications).

Tracking fish in this way would provide an interesting perspective. However, the dynamic properties of fish appearance and fish movements are very different to those of an insect such as an ant. First, the apparent image of an ant is relatively constant with the tagmata, or body segments, clearly evident in contrast to the moving legs. When observed from the side, the shape of fish is highly variable (Fig. 10). Observed from above the aquarium, the apparent image of the fish is more constant but the animal shape is not easily identifiable. In fact, during swimming, the body shape of the fish undulates along the body axis and varies according to the type of swimming adopted by the fish (e.g. Sfakiotakis *et al.* 1999). Butail and Paley (2010) have developed a probabilistic model able to estimate the shape and position of several fish in a school. This model is based on the capacity to identify a fish shape as an ellipsoid with a curvature coefficient that can incorporate bending of the fish body. This system is original in that it allows one to develop an observational model of a three-dimensional shape projected on a plane accompanied by the stereo cinematographic methods with two cameras. Second, fish movement can be more difficult to predict than insect movement because the fish adopts a large range of speed values, and the fish can employ a rapid burst of swimming at anytime. This is particularly the case for species adopting mainly a burst-and-glide swimming mode (e.g. Gadidae and Clupeidae, in Blake 1983). Using a high frame rate would improve the capacity of such systems. However, other species, with a more constant swimming speed such as members of the Tetraodontidae, or Diodontidae (Blake 1983), have more predictable movements.

Applications of multitracking

Scale of analysis: from individual to group patterns

With the multitracking system, animal groups can be studied at multiscale levels, where the focus is on one individual within a group (Ylieff and Poncin 2003; Delcourt *et al.* 2011) or considering the group as a single entity (Miller and Gerlai 2007, 2008) or as several subgroups (Couzin *et al.* 2002; Ward *et al.* 2002b; Hemelrijk and Kunz 2005). For the two latter cases, global analysis consists of measuring a parameter that is characteristic of the group but not of the individual. This would be characterized by the displacement of the central position of the group, its speed and the area covered by the group.

Analysing individuals provides more detailed information on two levels. First, it gives information on the variability in the inter-individual behaviours, and second, it allows detection of subgroups. For instance, in self-organization theories, if two animal subgroups are characterized by different degrees of attractive social force, segregation can appear (Couzin *et al.* 2002; Grégoire and Chaté 2004; Hemelrijk and Kunz 2005).

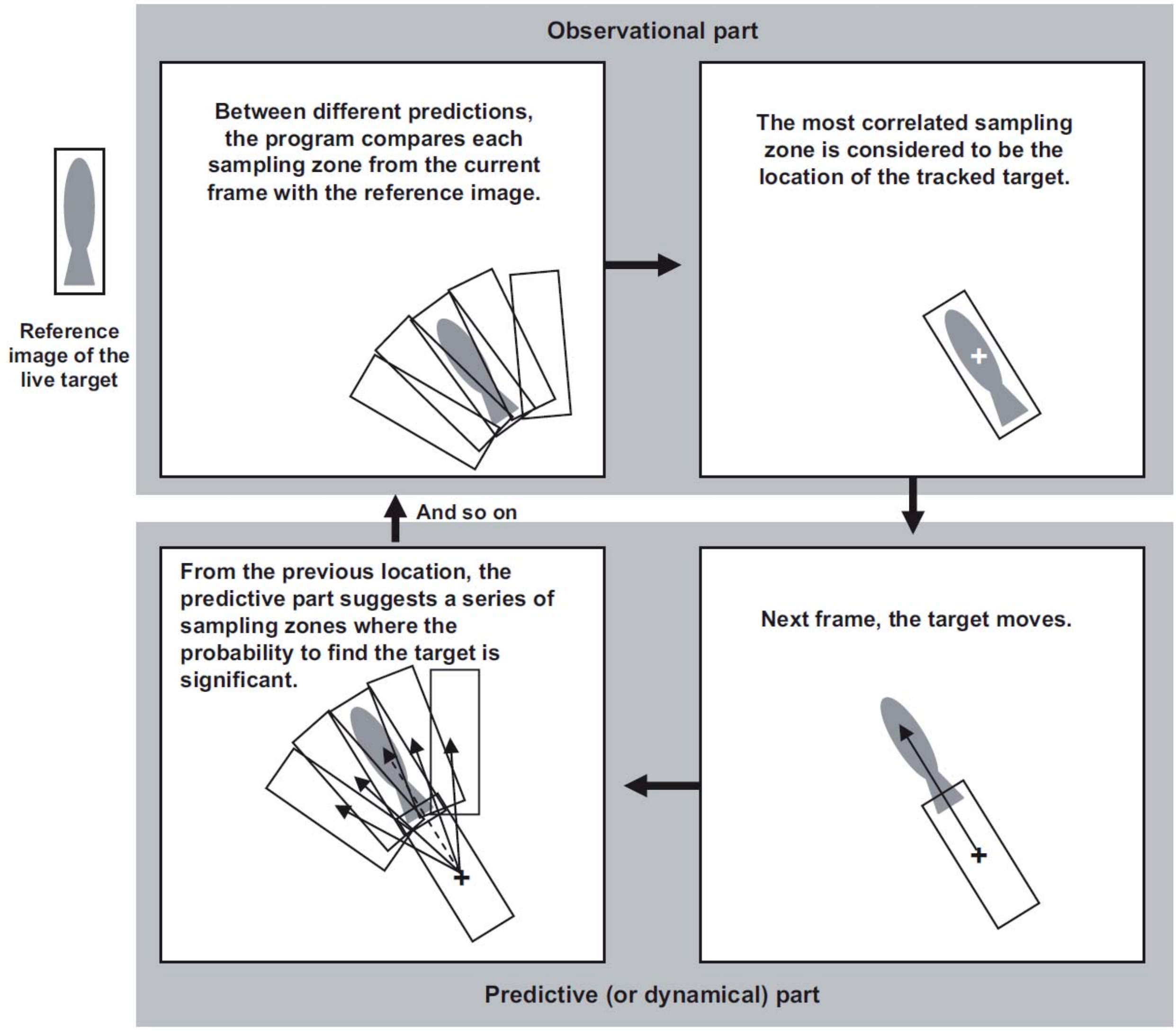

Figure 9 Example of a system based on statistical observation and prediction (inspired by Egerstedt *et al.* 2005; Khan *et al.* 2005, 2006).

The central zone of the group is occupied by the individuals adopting a strong attractive social force defined as an individual's tendency to be attracted by other individuals, and the peripheral zone where individuals adopt the weakest attractive social force. When simplification is required, most notably in computer simulations, a group may be considered to be composed of identical individuals. However, in a natural group, heterogeneity in group composition is very frequent (e.g. Peuhkuri *et al.* 1997; Krause *et al.* 2000a,b; Ward *et al.* 2003).

The current multitracking systems allow the simultaneous tracking of a large number of individuals. In their fish study, Delcourt *et al.* (2006, 2009), Delcourt (2008) and Becco *et al.* (2006) studied up to 100 individuals within the same aquarium. The experimenter usually prefers to track an entire shoal, but in some cases focuses rather on a marked fraction of the group (e.g. Ylieff 2002; Delcourt *et al.* 2011).

Implications in fish research and future directions

Video multitracking tools have several implications in basic research of social behaviours. These implications are reflected in the four fundamental ethological questions determining proximal and ultimate causes of behaviour (Tinbergen 1963; Dewsburry 1999): (i) the mechanisms of social behaviour as physiological, cognitive and stochastic processes; (ii) the ontogenetic processes; (iii) the adaptive significance; and (iv) the evolution of the organisms. Multitracking could be used to study the genesis (evolution, cultural inheritance and development), control (external and internal to the group and to the individual) and consequences (for the individual, environment, and differential reproduction between individuals, and between groups) of social behaviours adopted by the individuals.

Studying the relationship between the positions of individuals in the group would significantly improve our knowledge of whether the schooling behaviours have a hydrodynamic function using the inverted von Kármán street (succession of turbulences produced by the swimming of other conspecifics) (Weihs 1973; Weihs and Webb 1983; Sfakiotakis *et al.* 1999). Video multitracking opens interesting study perspectives on the acquisition (Brown and Laland 2003; Hoare and Krause 2003) and transmission of information in a group (Treherne and Foster 1981; Godin and Morgan 1985). It could improve our understanding of the rules of synchronized movements, notably during prey–predator interactions (Pitcher and Wyche 1983; Pitcher and Parrish 1993; Axelsen *et al.* 2001) and in the interaction between parents and fingerlings within species (e.g. Keenleyside 1991).

Studying the synchronization of individual spatial positions during shoaling behaviours and the synchronization of speed and orientation in schooling behaviours (Pitcher 1983; Pitcher and Parrish 1993) would

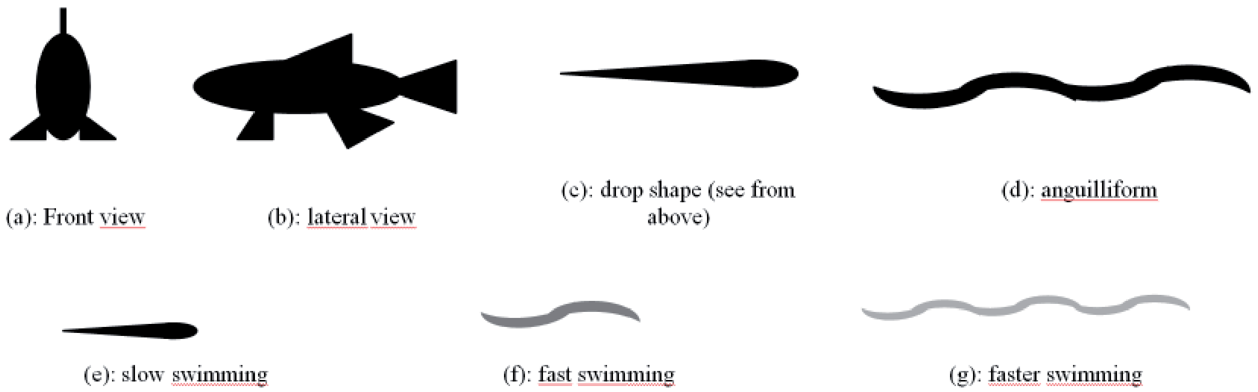

Figure 10 The apparent image of a fish varies with the orientation of the individual and the position of the observer (a–c), with fish morphology and locomotion mode (c, d), and with the shutter speed of the camera and the swimming speed of the fish (e–g). For a given shutter speed, the more a fish swims rapidly, the more its apparent image is deformed (like a retinal impregnation) and the less visible it is (represented here by the greyscale).

make it possible to test and verify the theoretical results of artificial computer simulations (Parrish *et al.* 2002; Viscido *et al.* 2004; Hemelrijk *et al.* 2010; Katz *et al.* 2011) so as to understand whether the mechanisms of these behaviours are based on global or external information (rheotaxis, luminotaxis, etc.) (Camazine *et al.* 2001) or on a self-organization process where individuals take into account only the nearest individuals of the group (Camazine *et al.* 2001; Anderson 2002).

Moreover, studying the group at the different scales of the individual, the subgroup and group, would explain the connection between individual behaviours, subgroup behaviours and group behaviours. It would be possible to test whether the global behaviours are a direct consequence of individual behaviours or whether they are emergent, properties contingent on the probabilistic interactions between individuals. Investigations could be conducted to determine the likelihood of the super-organism theory, a theory that considers the group as a unified entity (Marshall 2002; Hölldobbler and Wilson 2008).

Tracking whole groups would make it possible to understand the despotic, egoistic or democratic decision-making processes in an animal group (Conradt and Roper 2003; Couzin *et al.* 2005) and to identify possible leaders in shoals (Krause *et al.* 2000a,b; Camazine *et al.* 2001; Reebs 2001; Leblond and Reebs 2006). The impact of the interactions between individuals in relation to the characteristics of a group could be better highlighted: features that could be recorded are group size, the types of interactions in adopted behaviours, the heterogeneity in the composition of a group or the relative size of each subgroup (e.g. Couzin and Krause 2003).

The multitracking tool offers new perspectives for the study of the ontogeny of collective behaviours and for the phenotyping of social animals in the laboratory, particularly using interesting strains in aquaculture. Video tracking could become a valuable tool in medical research for studying diseases in social behaviours (Guo 2004) using social animal models in the laboratory.

Video multitracking can also make important contributions to the applied sciences. First, it can be used to test potential drugs or pollutants at the social level. Second, it can be used to characterize the parameters controlling social behaviours. In fact, the possibility to modify animal social behaviours could contribute towards the management of fish stocks, with possible applications in fisheries and aquaculture. One interesting future perspective in such applications is to create interactions between animal individuals and robotic individuals so as to drive the social behaviours in a given direction (Corell *et al.* 2006; Halloy *et al.* 2007). Third, multitracking would allow for phenotyping of strains of model species on the basis of social behaviour. Fourth, by monitoring the social behaviours, one could study quantitatively the well-being of social species, notably in the laboratory, in fish farms and in zoos.

A final challenge would be leaving the laboratory to track fish and other animals individually in nature. The researcher could solve the problem of calibration of distance to measure the individual's position using reference marks in the environment (metric distance) or measuring relative distance (topologic distance) (Hoare *et al.* 2001; Ballerini *et al.* 2008; Newlands and Porcelli 2008). The problem of noise detection caused by the heterogeneity of the environmental background, particularly if the species is cryptic, would also need to be solved.

The implications and applications of multitracking concern all social animals, not only fishes. However, fishes are among the more frequently studied organisms for understanding social behaviours. Moreover, fishes are a diversified group with more species than any other vertebrate class. And, last but not least, the third most important laboratory animal, after mice and rats, is the zebrafish, a cyprinid fish adopting shoaling and schooling behaviours.

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