Invariant digital color correlation for the identification of worm parasites from bullseye pufferfish

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ABSTRACT

The secure identification of parasites can be problematic and yet it is of prime importance. However, the specific identification of the parasites with traditional techniques can be slow and time consuming, requiring quality preparations where each taxonomically important structure can be clearly observed. Color information becomes an important discriminant feature, which we need to include in the whole identification process. Digital images of the monogeneans: \textit{Heterobothrium ecuadori} and \textit{Neobenedenia melleni} and, the digeneans \textit{Lintonium vibex}, \textit{Homalometron longisinosum}, \textit{Bianium plicitum} and \textit{Phyllodistomum mirandai} were processed to obtain their diffraction patterns. A numerical simulation was performed in order to correlate diffraction patterns of parasites species with phase only filters. The position, scale and rotation invariant image recognition was made through the scale transform.

Keywords: invariant correlation, parasites, automatic systems, scale transform.

1. INTRODUCTION

The culture of marine fish represents an alternative to increase the diversification in production from shrimp to other species and utilise unused shrimp culture facilities on the Mexican coast. The “botete diana” fish (Sphoeroides annulatus) is an important species in local fisheries in the East coast of Mexican Pacific with a high market value ($6.0-8.0 \text{ US dollars per Kg}$). Recent studies about the biology of the bullseye pufferfish of the family Tetraodontidae show that this species can be cultured. (Duncan and Rodríguez, 2001).

However, the development of aquaculture has been affected by parasite problems that decrease the fish production. In addition to the direct losses due to mortality, parasites have a considerable impact on growth and behaviour of fish, reducing their resistance to other stressing factors, increasing their susceptibility to predation and affecting the marketing of the fish (Scholz, 1999). The parasitic problems are generally the principal causes of outbreaks of diseases in the culture of fish in cages and aquaculture systems. In the Fish Health Diagnostic Laboratory at Virginia State University, parasites represented 30 – 40 \% of diagnosed disease cases from 1993 to 1999 (Crosby, 2001).

The tetraodontid species are susceptible to parasitic infections; amongst these are the monogeneans that represent a problem for the development of the culture of this family (Ogawa and Inouye, 1997). The first results from studies of the
parasitology of the wild “botete diana” show the existence of a diverse parasitic fauna. Monogeneans and digeneans have been identified and when the fish are transferred to captivity, parasites with a direct life - cycle reproduce rapidly resulting in parasitic diseases that can lead to fish mortalities if not treated (Fajer-Ávila and Chávez-Sánchez, 1999).

The secure identification of parasites can be problematic but it is of prime importance. However, the specific identification of the parasites with traditional techniques can be slow and time consuming, requiring quality preparations where each taxonomically important structure can be clearly observed, the analysis of these structures and comparison with keys, consultation of relevant literature, recording of morphometric data from the parasites and structures and possibly the use of molecular biology techniques.

The application of new techniques in biosystematics is a real possibility. Studies show that image processing is a technique that can potentially give reliable and precise results.

Zavala-Hamz et al., (1996) and Zavala-Hamz and Álvarez-Borrego (1997) completed a study using diffraction patterns and circular harmonic filters as a tool to identify copepods and showed that these techniques discriminated between two species, the sexes of each species and could identify the species from fragments of the organism. Later Pech-Pacheco and Álvarez-Borrego (1998) with an optical-digital system identified 5 species of phytoplankton important as bio-indicators of red tides. The precision of the technique was 90% irrelevant of the size, position or orientation of the sample. Álvarez-Borrego and Chávez-Sánchez (2001) demonstrated that color digital correlation can be used to detect the virus IHHN in shrimp tissues. Mouriño-Pérez (1999) evaluated the utility of a coherent optical system with color correlation for the recognition of Vibrio cholerae 01 and Alvarez-Borrego et al., (2002) shows a new invariant algorithm to recognize Vibrio cholerae 01 from polychromatic images.

In this study, the diffraction pattern is used to discriminate 2 species of helminth parasites.

2. MATERIALS AND METHODS

The parasites to be used in this study were collected from 226 wild Sphoeroides annulatus, Jenyns, 1853 (“botete diana” fish) caught from two different locations in the State of Sinaloa, Mexico (Teacapan 22° 32’N-105° 44’W and Mazatlan 23° 11’N-106° 25’W) during three spawning seasons (1999, 2000 and 2001). Each fish was surveyed for helminths ectoparasites on gills, fins and scraped off the body surface and endoparasites from the internal organs (air sacs, intestine, and urinary bladder). All helminthes recovered were flattened between a cover slip and a glass slide, fixed with 70% alcohol, stained with Semichon’s acetocarmine and mounted in Canada balsam. For this study we selected the following parasites identified by traditional methods. The parasites were the monogeneans: Heterobothrium ecuadori and Neobenedenia melleni, ectoparasites from the gills and skin respectively, the trematodes Lintonium vibex and Phyllodistomum mirandai endoparasites from the air sacs and urinary bladder respectively, and Bianium plicitum and Homalometron longisinosum from the intestine of the “botete diana”.

We selected between 10 and 30 whole mounts with different qualities in relation to the details of the taxonomic structure of each parasite but that allow us an specific identification. By means of a high resolution video camera (Sony CCD IRIS) connected to a microscope (LEICA DMLB10) and the program Pixel View installed in a computer (Dell Dimension 4100), images of the parasites, f(x,y), were enlarged and recorded (40x). The images were 256 x 256 pixels in size.

A numerical simulation was performed in order to correlate diffraction patterns (DP) of parasites species with phase-only filters (Horner & Gianino, 1984). Figure 1 shows six steps for rotation, scale and position invariant correlation. All steps were developed digitally.

In figure 1 the method for obtaining the composite filters is presented in blocks. Due to some parasites have different morphology it is necessary to include these information in the filters. So the first step (Step 1), it is to choose all the different
morphologies, $f_1(x,y), f_2(x,y),..., f_n(x,y)$, and to obtain the modulus of the Fourier transform of each one (rigorously speaking, the DP intensity is the square module of the Fourier transform, but in this case we are using the modulus of the Fourier transform only), $|F_1(w_x,w_y)|, |F_2(w_x,w_y)|, ..., |F_n(w_x,w_y)|$ (Step 2), after this, to sum all the modulus for obtaining only one image (Step 3). Then, the high frequencies are enhanced using a high-pass filtering. Of this way, the high frequencies are now observed in more detail. This better definition in the high frequencies will help, by consequence, to obtain a good identification of the object to be recognized. Then, the scale factor $\sqrt{r}$ is applied. This process is what differentiates the scale transform from the Mellin-transform. After these steps, the Cartesian coordinates were mapped to polar coordinates (Step 4). In step 5, a bilinear interpolation of the first data of coordinate conversion is introduced (Pech-Pacheco et al., 2002). This is done to minimize sampling errors, which in one way or another affects the identification of the objects. The resulting image (Step 6) is the composite filters which will be used in the invariant correlation (filters which have information about species to be recognized). Steps 3, 4, 5 and 6 from fig. 1 are necessary to ensure that the information contained in the diffraction pattern is invariant to rotation, scale, and position, thus obtaining correlation invariant of the diffraction patterns.

To maintain not only the scale but the rotational invariance we use the nonseparable scale transform, by taking the log of the radial coordinate $\lambda = \ln \left( x^2 + y^2 \right)^{1/2}$

$$S(u_\rho, v_\theta) = \frac{1}{\sqrt{2\pi}} \int_0^{\pi} \int_0^{2\pi} f(\lambda, \theta) \exp \left( -\frac{\lambda}{2} \right) \exp \left( -j \lambda c_\rho - j \theta c_\theta \right) d\lambda d\theta.$$

(9)
To discriminate among parasite species, all the images to be recognized were transformed as indicated in figure 2 which is very similar to figure 1, except that in the Step 1 we have only an image in the input. In step 6 we can see the result obtained using the steps of figure 1. In the step 7 of the figure 2 we make the invariant digital correlation using the phase only filter with the phase of the image to recognize.

The recognition of particular species of parasites is a complex issue. Color information becomes an important discriminative feature, which we need to include in the whole identification process.

Process shown in figure 2 will be repeated for each channel (R,G, and B). In each channel, the composite filter to be used is matched to the corresponding component of the target. In general, objects which have a determined component similar to component of the target will give a maximum of correlation in this channel ($\lambda_i$). But only the target will simultaneously give a correlation maximum in each channel. So, an object is detected as the target if it simultaneously produces a correlation peak in the three channels.
3. RESULTS AND DISCUSSION

Figure 3 shows the parasite studied in this paper. The nomenclature used was the following: Hl = Homalometron longisinosum, Bp = Bianium plicitum, He = Heterobothrium ecuadori, Lv = Lintonium vibex, Nm = Neobenedenia melleni, and Pm = Phyllodistomum mirandai.

In order to show the method used in this study, two parasites species were analysed among the five species above mentioned.

The correlation values (RGB) obtained for each composite filter against all other diffraction patterns were box plotted and for each group of organisms the mean, standard error (SE) and two times the standard error (2*SE) are represented (Fig. 4). Correlation values are non-normalized.

The results for Hl and for He are shown (Fig. 4). Letters a, b and c show the results for the three channels (RGB) respectively, the same for letters d, e and f. X-axis shows, in each figure, the species studied. Y-axis give us the invariant correlation value obtained for each channel. For Homalometron longisinosum mean values of $0.42 \pm 0.01$, of $0.42 \pm 0.01$ and of $0.43 \pm 0.01$ were for channels red, green and blue respectively, when the filter Hl was correlated with all the other five parasite species (Fig. 4a, 4b and 4c). These values were separated from the correlation values obtained for other species. In figures 4d, 4e and 4f the correlation values obtained for Heterobothrium ecuadori with the He filter are represented, mean values of $0.33 \pm 0.01$, of $0.35 \pm 0.01$ and of $0.41 \pm 0.01$ were obtained for channels red, green and blue respectively, which values are completely separated from the values of the other parasite species.
The results obtained are very good, considering the complexity of the morphologies of the parasites. However, we selected these parasites because we accomplish their previous identification and we have the numbers of specimens necessary to work with this new methodology. We must to remember that if you want to recognize a specific parasite you

![Graphs showing correlation values for different channels and parasites]

Fig. 4. Invariant correlation results for *Homalometron longisinosum* (Hl) in a) red channel, b) green channel and c) blue channel. Invariant correlation results for *Heterobothrium ecuadori* (He) in d) red channel, e) green channel and f) blue channel.
must to have the previous information of the organisms in order to obtain the composite filter. Specifically, this method will be used for the separation of pufferfish parasites.

In summary, an adequate method for the discrimination between parasites species has now been developed. However, the generation of an image catalogue is necessary, which should include the digital images of all parasite species in order to apply the technique and to continue with this research. This work is a basis from which new techniques can be applied for the development of an automated system for the identification of parasites.

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