Spot Disease Identification using unsupervised Machine Learning based Image Segmentation with its Remedial Solution in Aquatic Fauna

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Abstract
Spot diseases are pre-eminent agent affecting fish mortality contribute substantial losses to the farmers. This research project is monopolizing to detect and discern different kinds of spots in aquatic fauna. This study gives the analytical data about how this spot disease has been caused and how these spots can be identified by taking fifteen species of aquatic fauna with the help of image processing method. Now a day's fish mortality is increased due to the spread of spot diseases. Thus, it is ineluctable to develop advance techniques to discover different kinds of spots so that dwindling fish mortality. Generally, three kinds of spot diseases have been found. They are black spot diseases, white spot diseases and red spot diseases. The enactment of image processing method in the fisheries efficacious which proffer fish protection, improvement in aquaculture. Canonical detection of spot plays a major role in its treatment. Clustering plays a major role in Image object segmentation both in Gray and RGB in this paper, appraisal of spot in aquaculture is done by manoeuvring various image processing techniques. This research allocates facile, sharp-witted and consummate result of detecting spot and recognition in aquaculture.

Keywords: - Spot disease, Black Spot Disease, White Spot Disease, Red Spot Disease, K-means Clustering, Gradient object detection

1. Introduction
This world inside hydrosphere has their own discrete living manner. Yet they are not untouched by diseases; fishes mostly affected when young carry pathogens which cause various infections naturally or due to environmental pollutants including chemical and hazardous waste. Common fish diseases like white spot disease, pop-eye, swim bladder disease etc. are frequent in fresh water aquaria. Recurrent use of antibiotic to control bacterial infections in fish have caused risk of developing antibiotic restraints strains of bacteria; as a result, herbs have become traditional medicine to control bacterial, fungal, viral and other diseases. Aquatic diseases offer extensive evidence on how they have detrimental effects on international trade and economy.

1.1 Existing Work
White spot disease (Ich disease) is caused by Ichthyophtirius multifiliis. Metacercaria and turbellarian are responsible for black spot diseases in aquatic fauna. Turbellarian disease affects the ornamental fishes and that displays black spot, which is easily noticed by naked eyes (Justine et al., 2009). Ichthyophthirius multifiliis is the large dsDNA parasite and sole member of Nimaviridae family. RNAi and VP28-siRNA have been shown effective against WSSV. Another path to prevent White spot disease is associated with reluctance in certain hosts that has been evolved through incorporation of viral DNA into genome of host cell, providing aversion to the inserted virus (Huang et al., 2017). Micro RNAs (miRNAs), are non-encoding RNAs that regulates the gene expression in transcription process. Silencing of miR-217 from Chinese mitten crab guide that it had the significant function in White spot syndrome virus replication by silencing the host tube gene inhibiting the expression of ampicillin genes. Host miR-9041 and miR-9850 played an important role in replication of WSSV by silencing STAT gene in Macrobrachium rosenbergii. MiRNA silence various genes in host-virus interactions (Ren et al. 2017; Verbruggen et al. 2016). With the help of differential gene expression analysis in white spot disease infection stage which results in absolute gene expression measurement than relative measurements. Early & immediate-early (IE) genes of White spot syndrome virus triggering the viral late expression & early genes, abolishing immune system of the host (Xue et al. 2013). In PK1 kinase activity, Mg2+ and ATP plays an important role (Lin SJ et al. 2014). In penaecid shrimp, an experiment was done which showed that immunostimulants has proven the

development of immune system of the shrimp. Immunostimulants also help the sustain fish aquaculture good sources of uncontaminated water and enough aeration can cure this (Sangamaheswaran and Jeyaseelan et al., 2001). Nowadays Marine virus plays a vital role in geochemical cycle. WSSV was first found in Taiwan in1992, and then in Japan and gradually in all Asian countries. Several white spots on appendages, inner side of epidermis & exoskeleton in shrimp with WSSV. This infection most widely seen in the crustacean. The interaction between marine viruses with their respective host gives opportunities to the scientists for research (Sánchez PA 2010). The main cultured species as Chinese carps, Indian major carps like grass carps in Vietnam. Red spot Disease (Epizootic Ulcerative Syndrome) is mostly found in fresh water aquaculture. After measured of the water quality parameters they concluded that RSD cause by poor water quality. PCR (Polymerase chain reaction) a technique to identify the presence of WSSV DNA. It was the most widely adopted because of its high sensitivity, especially the 2stepPCR can detect the presence of WSSV in asymptomatic carrier. This PCR method widely used for the identification of virus in wild shrimp, crabs other Arthropods. The most important thing is that the disease and shrimp health in generally has greatly improved in the last 10 years. Several host functions involving development, immunity, nutrition and disease resistance are influenced by Intestinal microorganisms.
Epsilonproteobacteria in antiviral protection plays an effective role in white spot infection. The class Epsilonproteobacteria inhibit many ecological niches (Ding ZF et al., 2017). Segregating infected fish from fresh fish image using supervised machine learning by Support Vector Machine is new area of focus recently among food and agricultural sector (Ahmed MS et al. 2022).

2. Materials & Methods

In this paper the image of different fishes suffering from life terminator disease is identified with various image processing method. Fish image dataset are collected then the simulation of Region of Interest (ROI) i.e., the spot disease area is identified using image processing and unsupervised Machine Learning method (K-means Clustering) by MATLAB. In general, an image comprises of two-dimensional matrices enclosing pixel as its elements. Image processing problems are always based on a raw image input which yields a knowledge-based image after the processing therefore providing valuable information for analysis. Essentially, 2-D image is amplification of reflectance & illumination function. (Barik and Agrawal, 2017).

$$f(x, y) = i(x, y) * r(x, y) \quad (1)$$

Where f (x, y) shows the pixel intensity, r (x, y) shows reflectance property, i(x, y) shows illumination property. Through empirical observation the image segmentation shown in the paper was implied in colour plane and Binary plane. Any full colour Images is a combination of 3 colour spectrum one for each primary colour (Red, Green, Blue) having 24-bit full image. Hence the total $(2^8)^3 = 16,777,216$ possible colours are obtained. The number of bits used to signify individual pixel in RGB space is termed as pixel depth. Whereas the secondary colour spectrum originated from primary colour is detonated as Cyan(C), Magenta (M) and Yellow(Y). Mathematically CMY colour model is obtained as follows:

$$\begin{bmatrix} C \\ M \\ Y \end{bmatrix} = \begin{bmatrix} 1 \\ 1 \\ 1 \end{bmatrix} - \begin{bmatrix} R \\ G \\ B \end{bmatrix}$$

In humans' view of things coloured objects are always articulated as Saturation, Value and Hue as luminance. Angle of distinction range between 0 to 360 degrees such as Red (0-60), Cyan (180-240), Green (120-180), Blue (240-300), Magenta (300-360), Yellow (60-120).
Superiority of Hue colour is called as saturation. Value is the quality of colour, i.e., how dark or how the light an image is. Hue Saturation Value colour constituent portrayed by model of primary colour (Red, Green & Blue) shown in equations 2 and 3:

$$H = \begin{cases} \theta & G \geq B \\ 360 - \theta & G < B \end{cases} \quad (2)$$

Where;

$$\theta = cos^{-1}\left\{\frac{[(0.5R - 0.5B) + (0.5R - 0.5G)]}{[(G - B)(R - B) + (R - G)^2]^{\frac{1}{2}}}\right\} \quad (3)$$

Saturation mathematical representation in equation 4 can be given as:

$$S = 1 - \frac{3}{(B+R+G)}[min\,(G, B, R)] \quad (4)$$

The mathematical representation of the value or Brightness component in equation 5 is given as:

$$V = max(B, R, G) \quad (5)$$

In equation 6 the RGB constituent is normalized from the scale 0-255 as, 0-1 by:

$$R=R/255$$
$$B=B/255$$
$$G=G/255 \quad (6)$$

L*a*b* Model signifies a model of 3D colour. Lightness of the colour is shown by L* L*=0 indicates dark colour and L*=100 shows white. a*, b* Scale does not depend upon the space of colour. Equation 7 shows L*a*b*(CIELAB) mathematically:

$$L^* = 116y\left(\frac{Y}{Y_n}\right) - 16$$

$$a^* = \left(500f\left(\frac{X}{X_n}\right) - 500f\left(\frac{Y}{Y_n}\right)\right)$$

$$b^* = 200\left(f\left(\frac{Y}{Y_n}\right) - f\left(\frac{Z}{Z_n}\right)\right) \quad (7)$$

X_n, Y_n, Z_n Shows CIE tristimulus values of white.

2.1 Image Normalization

This section embraces the upgradation of images in such way that keen details are polished and images are isolated from noise which also eliminates blurring effect. Typically, image smoothing is mathematically denoted as in equation 8.

$$g(x,y) = \frac{\sum_{i=-m}^{m}\sum_{j=-n}^{n} w_{i,j} \cdot f(x+i, y+j)}{\sum_{i=-m}^{m}\sum_{j=-n}^{n} w_{i,j}} \quad (8)$$

Revamped grey level pixel Intensity of image is denoted by (x, y), wi stands for weighted mask and grey level pixel Intensity function in both spatial x and y coordinate is represented by $(x+ i, y+ j)$. The value of m and n are dependent on the mask size of -1 to 1 for 3 × 3 mask, -2 to 2 for 5 × 5 mask, similarly -3 × 3 for 6 × 6 mask and so forth.

2.1.1 Smoothing of Image

Smoothing of the image is used to reduce the noise to produce less-pixelated image. This is done with the help of non-linear & low pass linear filters.

2.1.2 Sharpening of Image

Sharpening of image is done to sharp edges that will guide to spot region. In image processing, first order of derivative is used with the help of magnitude of gradient. In equation 9 coordinates (x, y) are 2-D column vectors for f (x, y).

$$\Delta f = grad(f) = \begin{bmatrix} gx \\ gy \end{bmatrix} = \begin{bmatrix} \frac{\partial f}{\partial x} \\ \frac{\partial f}{\partial y} \end{bmatrix} \quad (9)$$

In Equation 10 change in rate of f at (x, y) is given.

$$_{Mag}(\nabla f) = \sqrt{g}x^2 + \sqrt{g}y^2 \quad (10)$$

Segmented or edge detected image is obtained with the filter mask of 3 × 3 & 2 × 2 weighted matrix.

Z_1	Z_2	Z_3
Z_4	Z_5	Z_6
Z_7	Z_8	Z_9

Z_1	Z_2
Z_3	Z_4

Roberts Method: Roberts mask as shown below having 2 × 2 matrix turn the whole image using vertically and horizontally in both y and x coordinates respectively.

-1	0
0	1

0	-1
1	0

Equation 11 shows Approximate of Horizontal derivative and as Equation 12 shows Approximate of vertical derivative.

$$gx = (z_4 - z_1) \quad (11)$$

$$gy = (z_3 - z_2) \quad (12)$$

Sobel Method: To increase the intensity of edge as shown below sobel mask with 3× 3 matrix turn the whole image.

-1	-2	-1
0	0	0
1	2	1

-1	0	1
-2	0	2
-1	0	1

Equation 13 shows Horizontal derivative approximates and Equation 14 shows vertical derivative approximate.

$$gx = (z_9 + z_3 + 2z_6) - (z_4 + z_7 + 2z_1) \quad (13)$$

$$gy = (z_9 + z_7 + 2z_8) - (z_3 + z_1 + 2z_2) \quad (14)$$

2.1.3 Elimination of Noise

Any external source contaminating or diluting the original signal is called noise. Different sources causing pest image to be noisy can be decreased by processing of image through high pass and low pass filters in both frequency and spatial domains.

2.2 Unsupervised Machine Learning

In case of Unsupervised Machine Learning the machine is forming clusters or groups based on the mean square distance between the mean data sample and each data sample based on different distance measure for example clustering. Most popularly utilized clustering is Kmeans clustering.

2.2.1 Segmentation using K-Means Clustering

Data Points of similar group are identical and allotting the unallied data in similar groups forming cluster is called Clustering. Whereas the process of extracting vital information from complex medical images is called Segmentation such that an image is isolated into commonly modified and exhausted regions and each significant region is spatially continuous along with homogenous pixels. K means is the most recurrent clustering methods due to its simple and easy computation.

K-means algorithm plays an extensive role in clustering by cataloguing the data based on the distance like Euclidean and Manhattan etc. as represented in equation 15 and 16. If two points $x(x_1, x_2, x_3 \cdots x_n)$ and $y(y_1, y_2, y_3 \cdots y_n)$ present in image space then the distance in n-space according to Euclidian representation can be:

$$D(x, y) = \sqrt{\sum_{i=1}^{n} (y_i - x_i)^2} \quad (15)$$

If two points $y(y_1, y_2, y_3 \cdots y_n)$ and $x(x_1, x_2, x_3 \cdots x_n)$ present in image space then the distance in n-space according to Manhattan representation can be:

$$D(x, y) = \|y_i - x_i\| \quad (16)$$

The points which are clustered around cluster centre or centroid $\mu_i \forall$ i = 1,2,3,4,5 … k is attained when distance is minimized as shown:

$$V = \sum_{i=1}^{k} \sum_{x_j \in s_i} (x_j - \mu_i)^2 \quad (17)$$

Where the k clusters s_i, i = 1,2,3,4,5 … k and μ_i is the mean point or centroid of all the points $x_j \in s_i$ [17].

When an image of resolution x × y is examined and the image assemble in k number of clusters. Let c_k be the cluster centres and the input pixel to be clustered is p (x, y). Then for k-means clustering the algorithm is:

Step 1: Computing the centre and number of cluster k.

Step 2: Calculating the Euclidian distance ‘d’ connecting centre & pixel of the image in equation 18.

$$d = \|p(x, y) - c_k\| \quad (18)$$

Step 3: Pixels are assigned to the closest centre based on distance ‘d’ and afterwards the selection of required pixels, the new centre position is recalculated using the below relation:

$$c_k = K^{-1} \sum \in c_k \boldsymbol{y} \sum \boldsymbol{x} \in c_k (\boldsymbol{x}, y) \quad (19)$$

Step 4: Process is repeated until resistance or error value is obtained and then the cluster pixels are reformed back into image.

Functional flow of Proposed Methodology

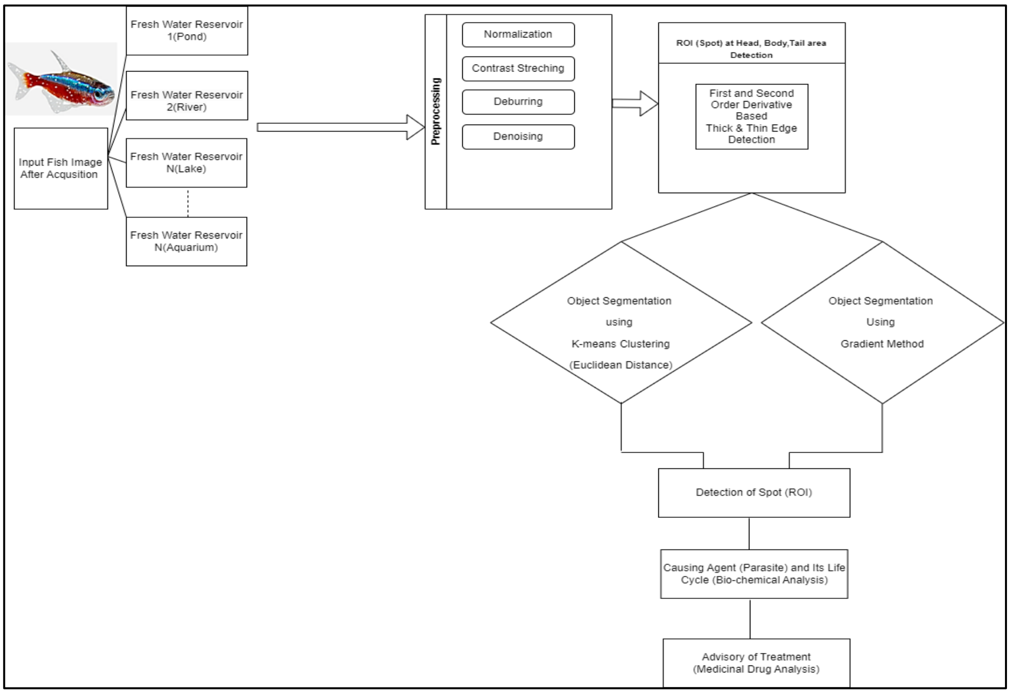

Figure 1. Demonstrates the Segmentation of methodology with the help of k-means Clustering and Gradient method.

3. Result and Discussions

Spot Diseases are pre-eminent agents affecting fish mortality. In this section spot diseases and their primitive categories such as white spot, red spot and black spot along with the individual spot diseases and their causing agents are analysed.

3.1 White Spot Disease Diagnosis and its Causing Agent

Ichthyophthirius multifiliis is the causing agent of WSD. Figure 2 & Figure 4 shows white spot in Fish RGB image, grey scale image, gradient magnitude. Figure 3 & Figure 5shows unsupervised clustering (K-means) of white spot showing different classes and mask detecting the region of study and identifying white spot.

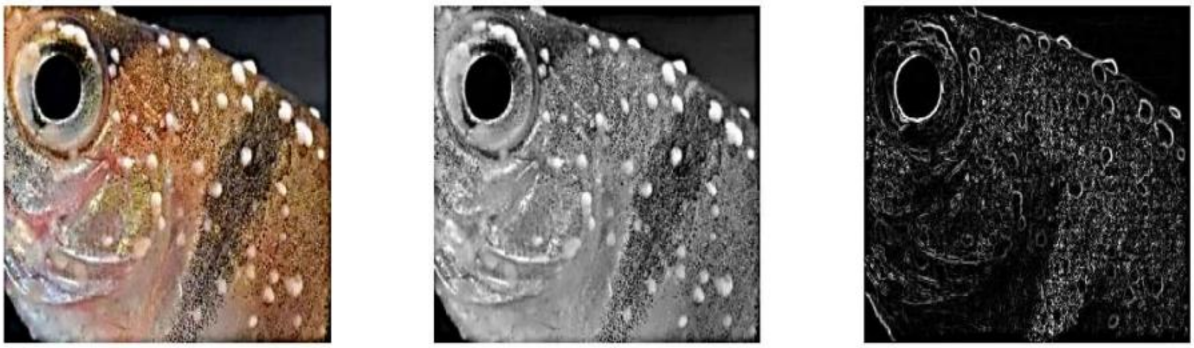

Figure 2: Fish Head region showing a) white spot in RGB image b) white spots Grey scale image c) gradient magnitude clearly segmenting white spots.

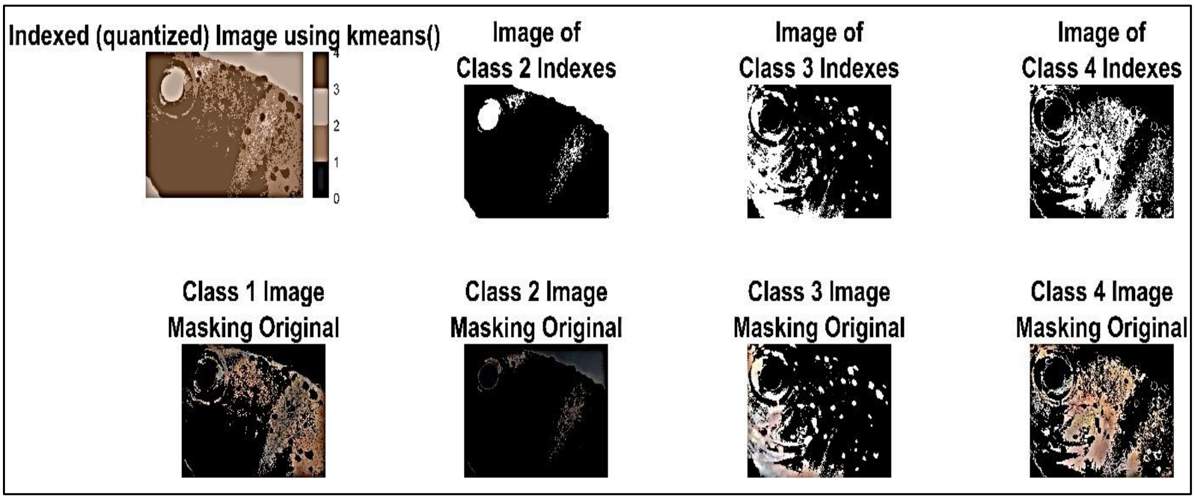

Figure 3: Unsupervised clustering (K-means) of white spot Fish Especially Head region showing different classes and mask

Figure 4: Fish showing a) white spot in RGB image b) white spots Grey scale image c) gradient magnitude clearly segmenting white spots.

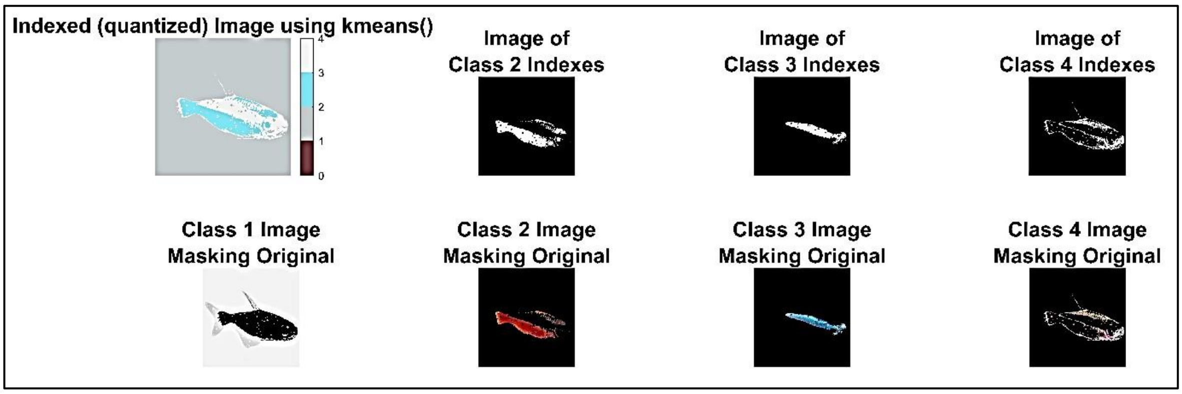

Figure 5: Unsupervised clustering (K-means) of white spot Fish showing different classes and mask

3.1.1 Life Cycle

Ichthyophthirius multifiliis belongs to sub-kingdom protozoa. It is is transferred by an infected fish. Figure 6 shows the lifecycle of Ichthyophthirius mutlifilis in aquatic fauna.

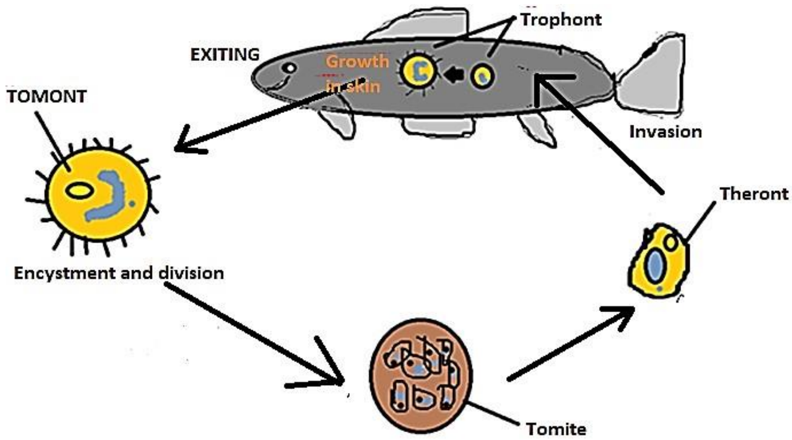

Figure 6: Demonstrates the structural interpretation of *Ichthyophthirius multifiliis* life cycle.

3.1.2 Diagnosis and Treatment

Treatment can only work during the multiplication phase of parasite known as free phase. At the infection phase, treatment does not function. Malachite green & anti-parasitic medicines are efficacious medicines.

3.2 Black Spot Disease Diagnosis and Causing Agent

Neascus is a digenean trematode. infected. Figure 7 & Figure 9 shows black spot in Fish RGB image, grey scale image, gradient magnitude. Figure 8 & Figure 10 shows unsupervised clustering (K-means) of black spot showing different classes and mask detecting the region of study and identifying black spot.

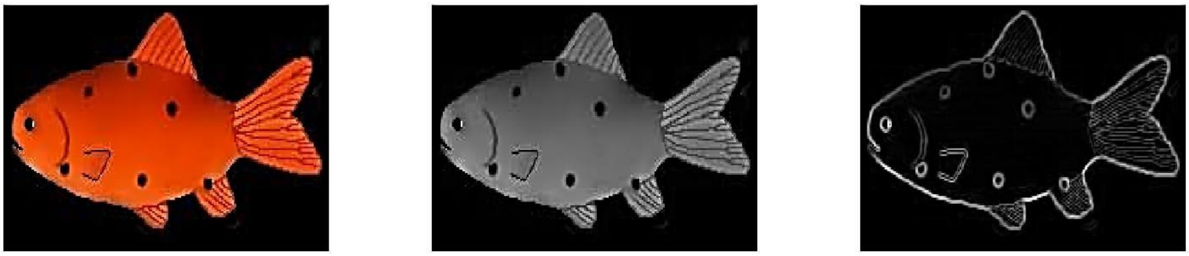

Figure 7: Fish showing a) Black spot in RGB image b) Black spots Grey scale image c) gradient magnitude clearly segmenting Black spots

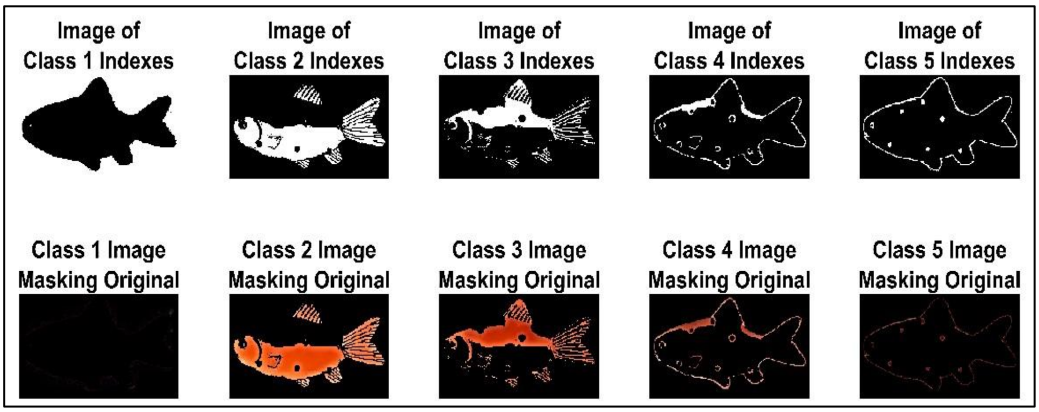

Figure 8: Unsupervised clustering (K-means) of black spot Fish showing different classes and mask

Figure 9: Fish showing a) Black spot in RGB image b) Black spots Grey scale image c) gradient magnitude clearly segmenting Black spots

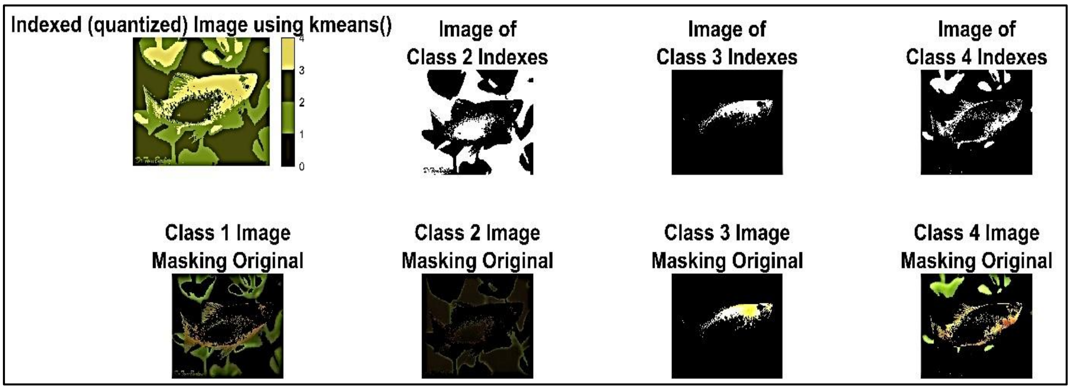

Figure 10: Unsupervised clustering (K-means) of black spot Fish showing different classes and mask

3.2.1 Life Cycle

Black spot disease caused by genus Neascus. Spots are found on the fins and skin of fish. This lifecycle contains three hosts such as fish-eating birds such as Great blue heron and kingfisher snail, fish. Figure 11 shows life cycle of trematode causing blackspot in aquatic fauna.

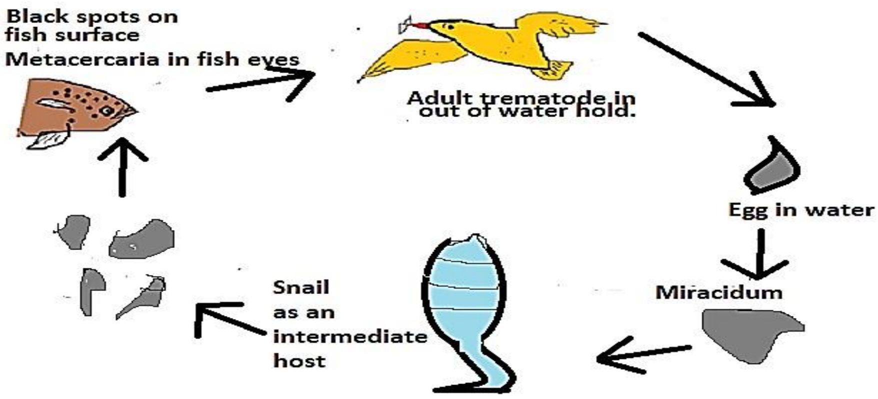

Figure 11: Demonstrates the life cycle of trematode that cause black spot disease containing 3 hosts i.e. bird, snail and fish

3.1.2 Diagnosis and Treatment

Hypothetical diagnosis is done by observing of small black spots in fish body or fins. Freezing fish at -20°C for seven days or boiling at 67°C about five minutes' kill parasite. There is no treatment for complete cure of this disease. Though some of the treatments are as below:

a) A gold fish has developed black smudge due to parasitic in aquarium then it can be treated with anti-parasitic water treatment.

b) Test ammonia levels in the aquarium. If ammonia levels are high, then water should be changed regularly to reduce ammonia levels.

3.3 Red Spot Disease Diagnosis and Causing agent

Red spot disease also known as Epizootic ulcerative syndrome and Mycotic granulomatosis. Figure 12 & Figure 14 shows red spot in Fish RGB image, grey scale image, gradient magnitude. Figure 13 & Figure 15 shows unsupervised clustering (K-means) of red spot showing different classes and mask detecting the region of study and identifying red spot.

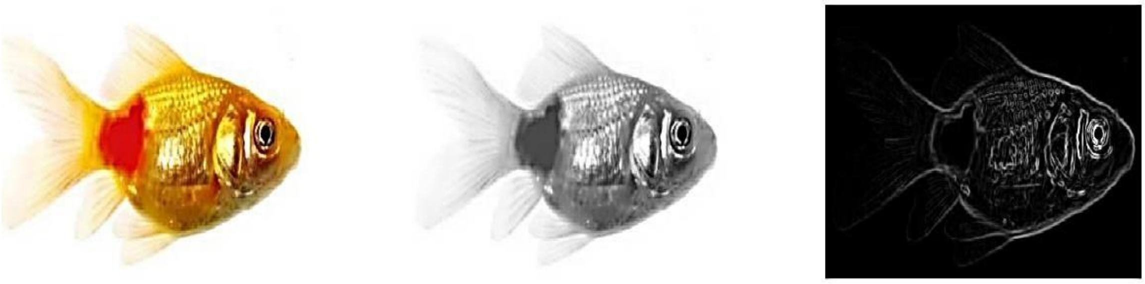

Figure 12: Fish showing a) Red spot in RGB image b) Red spots Grey scale image c) gradient magnitude clearly segmenting red spots

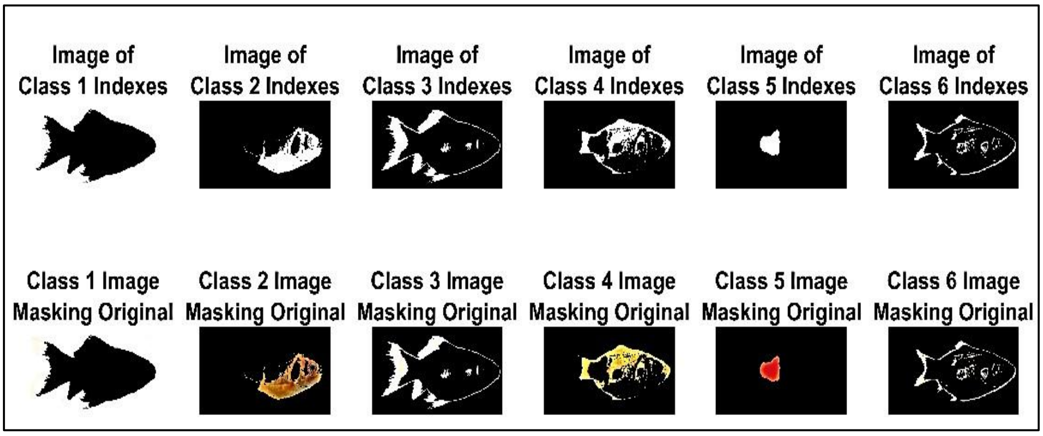

Figure 13: Unsupervised clustering (K-means) of Red Spot Fish showing different classes and mask

Figure 14: Fish showing a) Red spot in RGB image b) Red spots Grey scale image c) gradient magnitude clearly segmenting red spots

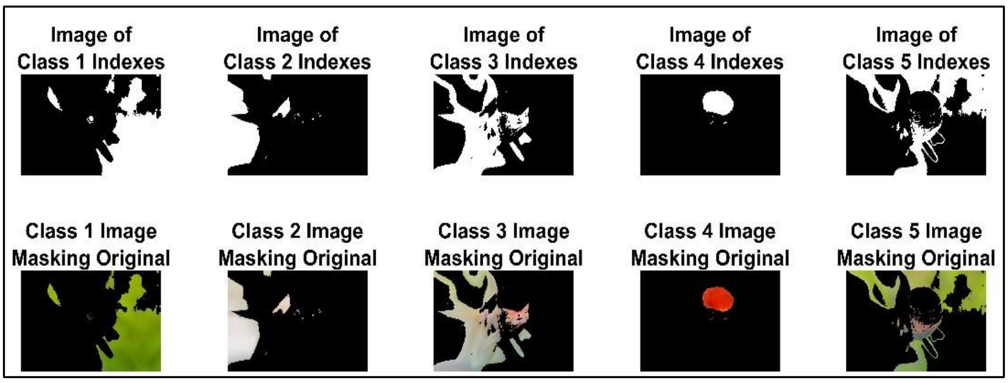

Figure 15: Unsupervised clustering (K-means) of Red Spot Fish showing different classes and mask

This disease is instigated by the Aphanomyces invadans. The favourable conditions for this disease are low temperature and heavy rainfall seen in the tropical and sub-tropical region infecting many fresh waters as well as brackish water fishes.

3.3.1 Life Cycle

The virulent stage of A. invadans is a zoospore that is free-swimming and attaches to its host, encysts, germinating vegetative aseptate hyphae marauding host tissues. A. invadans is transmitted from infected to host via the genesis of a sporangium thriving from the mycelium, forming primary spores on the surface of host tissue. The biflagellate are reformed in structures that are meticulous swimmers after the primary spores are overhauled into secondary spores. The life cycle is padlocked when the secondary zoospore is recognized by a new host otherwise it may encyst. Early symptoms showing skin damage due to the penetration in susceptible hosts.

3.3.2 Diagnosis and Treatment

RSD is treated through many techniques such as Immersion treatment involve dips, baths, dips, flowing treatments and flushes. Prophylaxis and hygienic procedures comprise of good husbandry practices. Remedial processes involve use of salt, lime, bleaching powder and potassium permanganate.

4. Conclusion and Future Scope

A pragmatic investigation has been done to imitate the spot region existing in the spot diseased fishes (Red, White and Black) in successful manner which significantly help farming communities to prevent fish mortality during aquaculture. Novelty of this research contribution depicts the Edge based segmentation, grey threshold-based segmentation, Hue saturation and value-based colour segmentation using k-means clustering applied over fish sampling to detect the spot regions spontaneously. This work can be extended to detect diseases related to fish scales.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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