

## Development of a comprehensive lesion severity classification model for largemouth bass(*Micropterus salmeoides*)ranavirus(LMBV)basedon machine vision system

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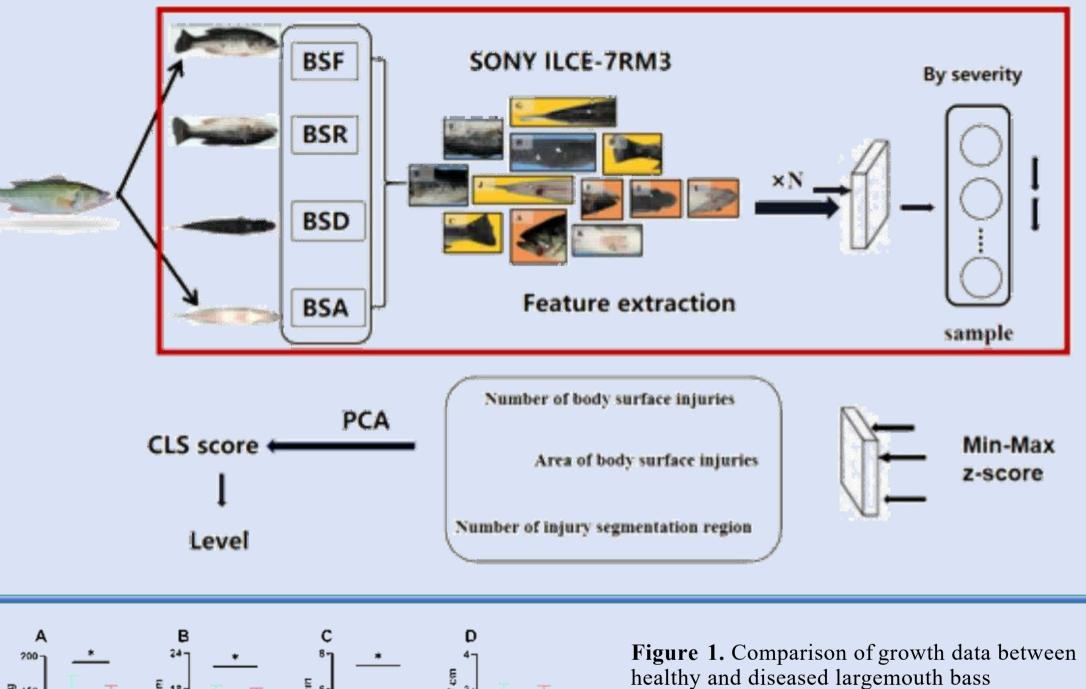
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Purpose -----

The main objective of this study is to construct an automated and quantitative grading model based on machine vision (MV) for assessing surface damage in largemouth bass (*Micropterus salmoides*) infected with largemouth bass virus (LMBV). This model aims to achieve rapid, objective, and non-invasive evaluation of the degree of LMBV infection, providing a scientific basis and technical support for early diagnosis and precise prevention and control of the disease.

## Methods

This study developed a quantitative evaluation method using machine vision to assess body surface damage in largemouth bass (Micropterus salmoides) infected with largemouth bass ranavirus (LMBV). High-resolution images (6000  $\times$  4000 pixels) of 239 infected specimens were captured with a SONY ILCE-7RM3 camera in a softbox. Key parameters—number of injury areas, lesion count, and total lesion area—were analyzed and integrated via principal component analysis (PCA) to create a damage scoring model. Severity was classified into four grades: uninjured (0), minor (1), moderate (2), and severe (3). Histopathology showed early infection (grade 1) involved localized hemorrhagic spots with limited lesions, while grades 2–3 exhibited extensive ulcers, muscle necrosis, and visceral damage. qRT-PCR revealed upregulated pro-inflammatory cytokines (*IL-6*, *IL-8*, *TNF-a*, *CXCL2*) and apoptosis-related genes (*CASP8*, *CYC*) in immune organs. This study provides a rapid, objective grading system for early diagnosis and control of LMBV.



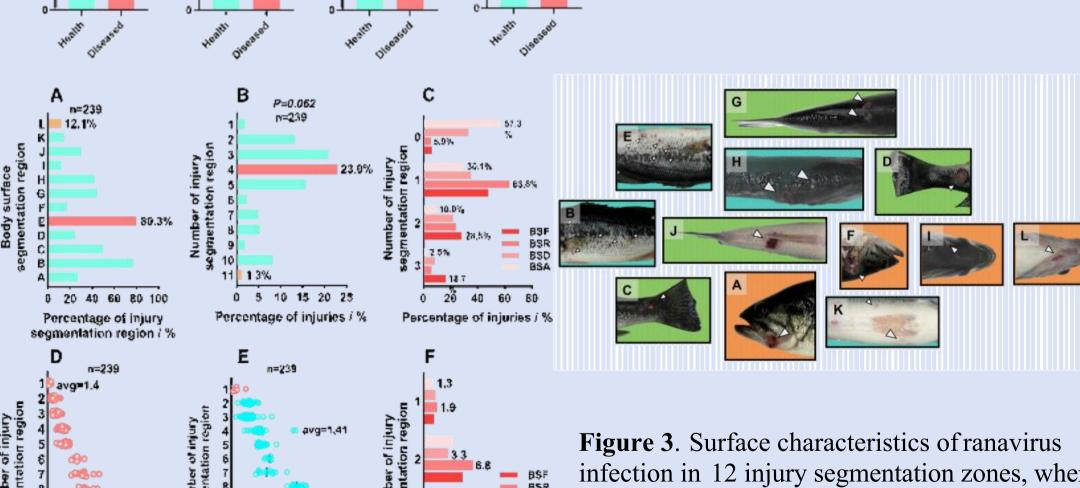


Figure 3. Surface characteristics of ranavirus infection in 12 injury segmentation zones, where white capital letters (A-L) indicate the 12 specific anatomical regions defined in Figure 3. White arrows point to examples of red hemorrhagic spots.

Number of body sortage injuries

individuals, including: (A): Body weight (B):

Body length (C): Body height; (D): Body

thickness

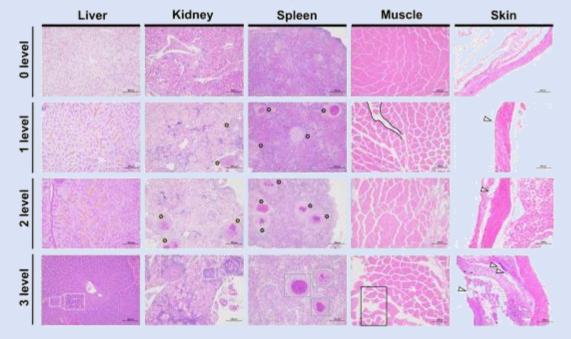
Number of injury segmentation region

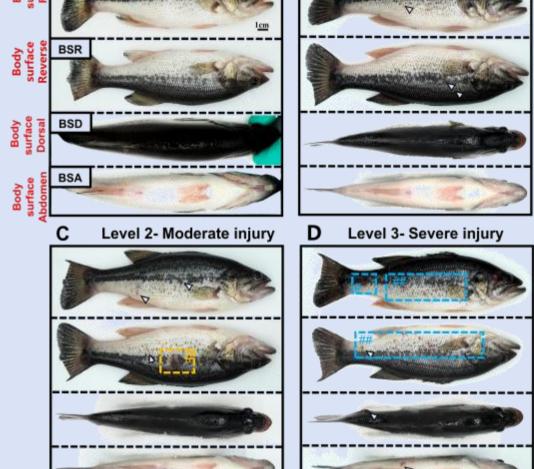
Figure 2. Surface injury image data.

Number of injury segreptation region

**Figure 4.** Correlation analysis between three variables: number of injury segmentation regions, number of body surface injuries, and areas of body surface injuries

**Figure 5.** Damage grading criteria for largemouth bass infected with LMBV, including (A): Level-0, (B): Level-1, (C): Level-2, (D): and Level-3, where # indicates the severity of damage and white arrow pointing to a Example of red bleeding spot.

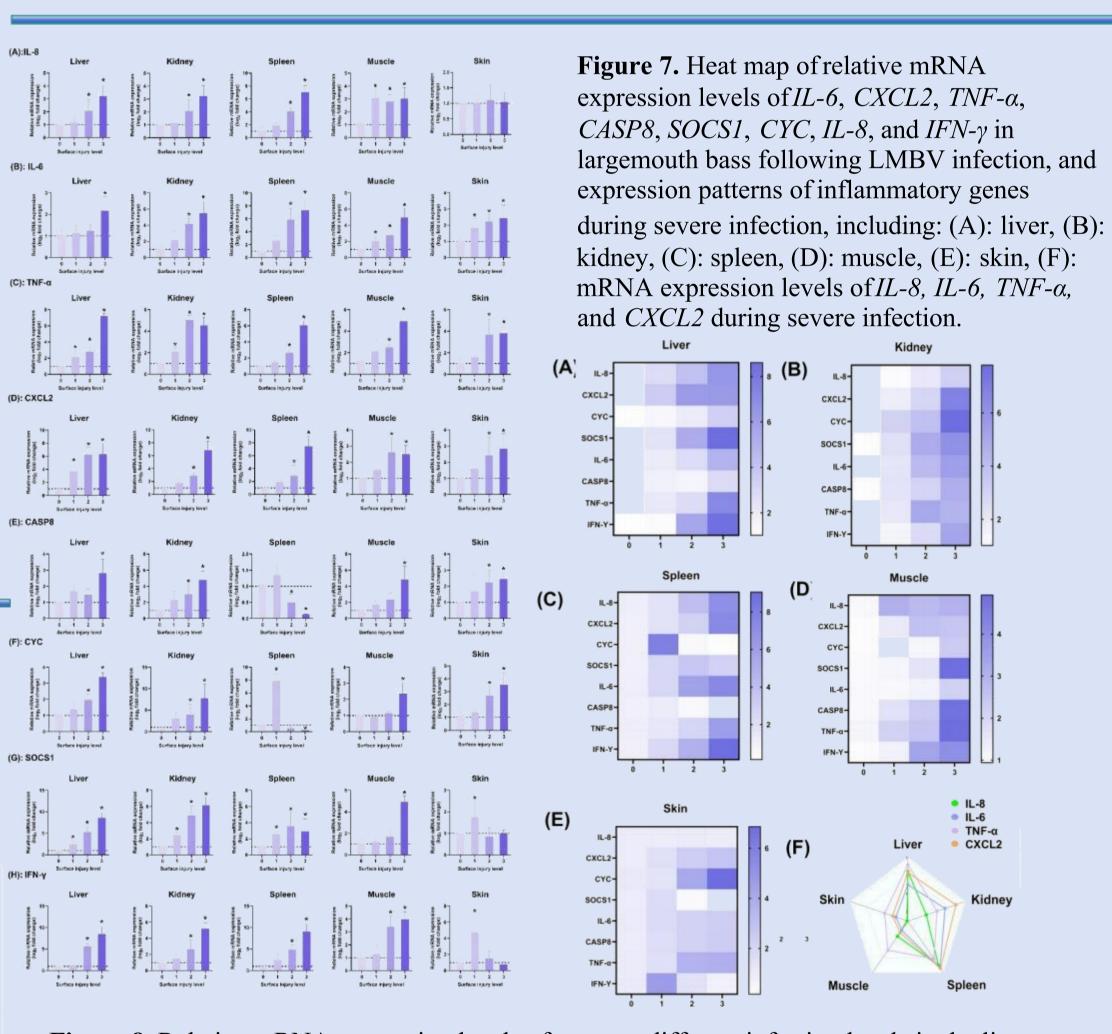




A Level 0- Uninjured

B Level 1- Minor injury

**Figure 6.** Histological observations (H&E) of the liver, kidneys, spleen, muscles, and skin of largemouth bass infected with LMBV at different levels of damage.



**Figure 8.** Relative mRNA expression levels of genes at different infection levels in the liver, spleen, kidneys, muscles, and skin of largemouth bass infected with LMBV, including: (A): IL-8, (B): IL-6, (C): TNF- $\alpha$ , (D): CXCL2, (E): CASP8, (F): CYC, (G): SOCS1, and (H): IFN- $\gamma$ .

\*Indicates significant differences between the uninjured (0) group and different severity groups after LMBV infection (P < 0.05) (n = 9).

Conclusion

In summary, this study successfully established a machine vision-based grading model for LMBV lesions. By integrating three quantitative variables—number of damaged areas, lesions, and lesion area—through PCA, LMBV infection in largemouth bass was classified into four severity levels: undamaged (0), mild (1), moderate (2), and severe (3). Histopathological and qRT-PCR analyses revealed that the severity of external injury correlated with visceral organ pathology and the expression levels of immune genes (e.g., *IL-6*). The MV comprehensive injury score effectively reflected the entire process from early local immune activation to late systemic inflammation and immune escape, further validating the model's reliability. this study provides scientific evidence for the early prevention and control of largemouth black bass iridovirus, holding significant implications for advancing the intelligent development of aquaculture in the future.