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Screening black tiger shrimp seedstock for WSSV, MBV

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Samples analyzed by malachite green staining and nested polymerase chain reaction assay

The shrimp-farming industry faces challenging issues that include the sometimes limited availability of healthy broodstock animals and postlarvae (PL), and the emergence of various viral pathogens. Strict biosecurity measures are essential to protect the shrimp in hatcheries and ensure successful production in grow-out systems. The PL produced must of high quality and health, and free of pathogens.

Postlarvae are evaluated for MBV by staining wet tissue mounts and viewing occlusion bodies under a microscope.

Long-term study

To assist area farmers in stocking healthy fry, the authors carried out a study from November 2000 to March 2003 to assess the health of shrimp seedstock produced in hatcheries around Tamilnadu in southeastern India, and the incidence of white spot syndrome virus (WSSV) and monodon baculovirus (MBV).

During this time, 2,999 samples of black tiger shrimp (*Penaeus monodon*) postlarvae of various ages were received from shrimp farmers. The samples were analyzed following the method used by Systems Aquaculture Management Inc. of the Philippines, which was adopted from the SEAFDEC format.

Samples for MBV were analyzed by malachite green staining, which is part of the overall shrimp fry health analysis. Samples for WSSV were tested by the nested polymerase chain reaction (PCR) assay method.

Fry health analysis

The fry health analysis included assessment for MBV and examination of body length, rostral spine, swollen hind-gut, muscle:gut ratio, necrosis, presence of filamentous bacteria, and free and attached protozoans. Based on these parameters, the shrimp fry received an overall score indicating their health status.

An overall score greater than 93 percent, representative of predominantly healthy animals, was used as a benchmark to determine the health of different seedstock lots. MBV detection identified samples as “not satisfactory” and unfit for stocking. Other parameters like swollen hind-gut syndrome and very high necrosis also led to nonsatisfactory

scores in some of the samples analyzed.

Results

Results are presented in Table 1 and Figs. 1 and 2. The muscle:gut ratio was predominantly acceptable. Monthly averages of the samples' health status scored 93 to 97 percent during the study period. There were only 102 samples, spread through different months, with low or poor health.

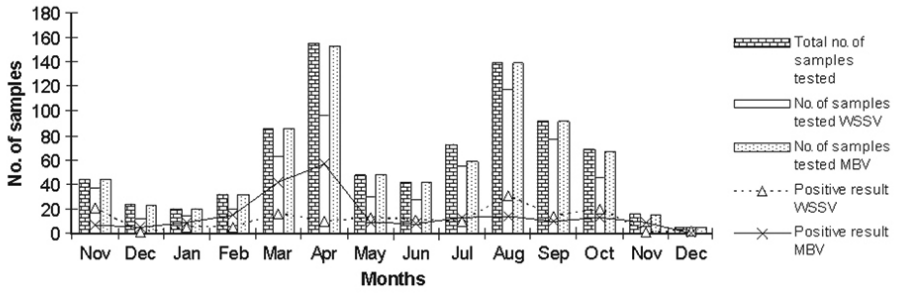

Fig. 1: WSSV and MBV incidence in shrimp fry samples, November 2000-December 2001.

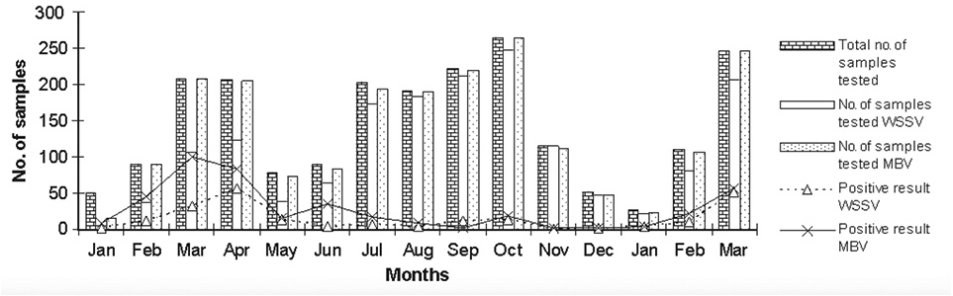

Fig. 2: WSSV, MBV incidence in shrimp fry samples, January 2002-March 2003.

Although free and attached protozoans under acceptable levels were generally observed, no rejections were made due to high protozoan infestation. It is also interesting to note that, except for the December 2001 samples, all samples had incidences of swollen hind-gut. But only 5 percent of rejections were based on swollen hind-gut syndrome, which nevertheless affected the overall health scores. Shrimp farmers in the study area now use a health score index over 93 percent to consider a seedstock lot suitable for stocking.

Table 1. Comparison of WSV and MBV occurrence in shrimp seedstock samples.

Date	Total Samples Tested	WSSV Samples	Positive Results	Occurrence	MBV Samples	Positive Results	Occurrence	Total Score
November 2000	44	37	21	56.7%	44	7	15.9%	95.05%
December 2000	24	12	1	8.33%	23	5	21.7%	96.38%
January 2001	20	14	5	35.7%	20	9	45.0%	97.01%
February 2001	32	20	5	25.0%	32	15	46.8%	94.95%
March 2001	86	63	16	25.3%	86	42	48.8%	96.21%
April 2001	155	96	10	10.4%	153	57	37.2%	94.02%
May 2001	48	30	13	43.3%	48	9	18.7%	95.48%
June 2001	42	28	11	39.2%	42	8	19.0%	96.52%
July 2001	73	55	10	18.1%	59	13	22.0%	95.38%
August 2001	139	117	31	26.4%	139	14	10.0%	93.00%
September 2001	91	77	14	18.1%	91	10	10.9%	93.06%
Octover 2001	69	46	20	43.4%	67	13	19.4%	95.18%
November 2001	16	6	1	16.6%	15	9	60.0%	94.21%
December 2001	5	5	2	40.0%	5	–	–	97.82%
January 2002	50	5	2	40.0%	15	8	53.3%	97.01%
February 2002	90	37	12	32.4%	90	45	50.0%	93.81%
March 2002	209	107	31	20.9%	209	100	47.8%	97.23%
April 2002	207	124	56	45.9%	205	84	40.9%	95.63%
May 2002	78	39	14	35.8%	74	15	20.2%	96.53%
June 2002	90	64	3	4.66%	84	35	41.6%	96.04%
July 2002	203	173	7	4.04%	193	17	8.80%	95.48%
August 2002	191	183	3	1.63%	190	8	4.21%	94.91%
September 2002	222	212	12	5.06%	220	1	0.45%	96.01%
October 2002	265	249	14	5.60%	265	18	6.70%	96.54%
November 2002	115	115	–	–	112	1	0.89%	96.77%
December 2002	51	47	1	2.12%	47	1	2.12%	97.01%
January 2003	27	22	3	13.6%	24	4	16.6%	97.15%
February 2003	110	80	10	12.5%	106	22	20.7%	96.72%
March 2003	247	207	51	24.6%	246	56	22.7%	94.94%

MBV incidence

Of 2,904 samples tested for MBV in the study, 626 were found positive with varied degrees of infection. These were termed light, moderate, or high, based on the presence of occlusion bodies in their hepatopancreatic smears.

The least occurrences of MBV were observed during September, November, and December 2002. Although no samples tested positive for MBV in December 2001, the five samples tested that month provided inconclusive information. The maximum MBV occurrence of 60 percent was found in November 2001, but this was another month with a low number of samples.

Approximately 21.5 percent of the samples were MBV-positive during the period of study. During April 2001, 37.2 percent of 153 samples were MBV-positive, with 10 percent of 130 samples found positive in August 2001. In 2002 and 2003, the maximum number of

Samples were tested for WSSV using nested PCR assays.

samples tested during September and October
were 220 and 265 samples, with MBV infection at 0.45 and 6.70 percent, respectively.

With 106 and 246 samples in February and March 2003, an increasing trend was observed, with 20.7 and 22.7 percent MBV positives, respectively. The lowest incidences were found in September 2002 (0.45 percent) and November 2002 (0.89 percent).

WSSV incidence

The maximum occurrence of WSSV-positive samples was recorded for November 2000, when almost 56.7 percent of the 37 samples received tested positive. In November 2001, only one of six samples received for testing was positive. Interestingly, in November 2002, all 115 samples tested were found WSSV-negative.

When representative individual fry health scores were determined to compare WSSV occurrence, 69 percent of the WSSV-positive samples had high health scores over 93 percent, and the remainder had very poor scores. It appears that even shrimp fry infected with WSSV can receive a high health score based on microscopic examination. Some WSSV-negative fry also had very poor health characteristics.

Observations

In the study, the maximum WSSV occurrence was 56.7 percent in November 2000, although WSSV was generally higher during 2001. MBV occurrence was higher during the months of January to June in both years, with 20 percent higher occurrence recorded during 2001. MBV occurrence varied 0 to 60 percent by month. The average MBV occurrence for the entire period of study was 21 percent, which was better than the results of an earlier study by the authors in the same region.

While observing the variation in MBV occurrence, an ascending trend started in January, peaked in April, and descended in May during 2001. A generally similar trend was noted in 2002, with a peak in March.

Among the fry with health scores over 93 percent, 31 percent of the samples tested positive for WSSV. Among the fry with lower scores, 20 percent were found positive. This has important implications for stocking individual seedstock lots.

Seedstock that test negative for WSSV may not be suitable for stocking if their general health score is poor, because chances are high they will become infected with WSSV after stocking. Based on these observations, it is clear that WSSV occurrence is not necessarily related to the overall health status of shrimp seedstock.

Conclusion

There is no harmonized, standard assessment available to evaluate select shrimp postlarvae. Different health standards for PL quality – from a simple observation for protozoan parasites to even *Vibrio* enumeration – are adopted by different diagnostic centers. Although these standards are varied, their goal is to comprehensively assess seedstock health as well as WSSV incidence.

Just checking for WSSV and stocking WSSV-negative seedstock do not guarantee healthy animals. Many farmers lose crops to WSSV in the first three weeks after stocking apparently WSSV-negative fry. This may be caused by post-larval carriers with low levels of undetected WSSV or poststocking infection due to disease vectors in the pond.

These incidents lead farmers to lose confidence in the PCR screening of fry, a generally accepted first step toward best risk management. Education is needed to help farmers understand that WSSV screening and general fry health analysis are both prerequisites for good pond yields.

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