A case study on mortality of neon tetra (*Paracheirodon innesi*) associated with the seasonal climate transitions in West Java – Indonesia

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**Abstract.** This study aimed to identify pathological agent and determine the impact of water quality during climate transitions on mass mortalities of neon tetra (*Paracheirodon innesi*) in the West Java region of Indonesia. Ten thousand fishes reported died each day during the seasonal. Samples of 10 fish/tank were taken and tested for parasites, fungal, and bacteria. The investigations tests showed that the primary disease was caused by parasite from protozoa *Pleistophora* spp. (Microsporidia). It was confirmed by taxonomic descriptions and histopathological diagnosis. This parasite causes histozoic infection. Fungus was not detected, and bacteria were identified as *Aeromonas* sp. and *Pseudomonas* sp.. The water temperature fluctuation was recorded higher than 7°C between daylight and night. Mortality was recorded in every size of fish. The clinical signs of fish on the outbreak were pale body, discoloration of the lateral line, the appearance of white patches under the skin, lethargy, swimming disorder, and losing weight. The recommendations due to the outbreaks were total eradication for infected fish populations, separating newly arrived fish (quarantine) at least 2 weeks, maintaining water quality in the optimal range, and proper cleaning (sanitizing) must be performed.

**Keywords:** Neon tetra, mortalities, clinical sign, disease

1. Introduction

Neon tetra *Paracheirodon innesi* is a small freshwater fish that is native to Northern South America [1]. The history of *P. innesi* invasiveness to Indonesia did not well documented. Neon tetra has specific characters with a green to blue bright iridescent stripe from head to the tail and red band. It has silver belly and, and a slender body with slightly laterally compressed. The photoresponse of iridophores of neon tetra skin depends on the light intensity, and that light near 500 nm is most effective [2]. *Paracheirodon innesi* is one of the most popular fish among the aquarium hobbyist and most valuable species in the ornamental fish market [3]. Neon tetra requires a tropical climate that can support the establishment of a good population.

Several factors had contributed to the loss of ornamental fish market, such as high mortality rates of wild-caught fish, and low quality of fish. The market prices decrease caused by lack of genetic
diversity of fish and infection disease which disruptive the coloration or fish patterns. There have been many reports on the degradations of ornamental fish quality and mass mortalities cases of neon tetra during seasonal climate transitions in the West Java region of Indonesia. This study aimed to evaluate the causative agent due to mass mortality cases of neon tetra in West-Java - Indonesia.

2. Materials and methods

2.1. Case histories
West Java – Indonesia is a center for ornamental fish cultivation. Neon tetra examined from five locations of fish farms that reported an outbreak of the diseases in Depok and Bogor – West Java. Research Station for Fish Disease Control laboratories in Depok – West Java – Indonesia conducted analyses of the fish samples. The fish included in all samples were taken randomly from the aquarium that showed external signs of the disease. Ten fish from each aquarium were tested for parasites, fungal, and bacteria isolation.

2.2. Screening examinations
Sampling methods used to observe the fish mortality, clinical sign, microscopic examination for parasites and biochemical tests to identify bacteria. The fish were examined for ectoparasites and endoparasites based on standard methods [4-6]. All the parasites sections were stained with Giemsa [7]. The bacteria were isolated in pure cultures from the kidney, blood, liver, and skin ulcers. The sample was inoculated directly onto specific medium Rimler-Shotts (RS) Medium Base and Trypticase Soy Agar (TSA). The agar plates incubated at 28°C for 24 hours and re-streaked the grown colonies for pure cultures. Biochemical profiles were determined using Analytical Profile Index (API) 20E kits (Biomerieux).

3. Results
Neon Tetra (Paracheirodon innesi) cultivation was concentrated in Sawangan and Bojongsari Districts of West Java - Indonesia. In early April 2014, many fish farmers have reported an outbreak of fish disease that caused mass mortality more than 70 percent in each population.

With a tropical climate, April was the dry season commenced in West Java Indonesia. In this seasons typically experienced dry and sunny days with the water temperature fluctuations. The high temperature fluctuation was recorded more than 7°C between daylight and night, while the pH, ammonia, and nitrite were in the optimal range (Table 1).

Each stage of fish growth usually takes 2-3 weeks. However, after being infected by infectious disease and got the treatment the fish growth are defined as stunted. Based on observation results, there were two factors of suspected causes of neon tetra mortality, the first due to environmental conditions (temperature fluctuations), and the second was administrations of unhygienic water fleas (Daphnia) for natural feed that suspected as a potential vector.

| Parameter        | Range of water quality | Kucharczyk et al. [8] |
|------------------|------------------------|-----------------------|
| Temperature (°C) | 22-29                  | 22                    |
| pH               | 7-8                    | 6.9-7.4               |
| NH₃, NO₂ (mg dm⁻³)| < 1.0                  | <1.0                  |

The results of a thorough examination showed that fungus was not detected and bacteria were identified as Aeromonas sp. and Pseudomonas sp. Wet mount preparations of skeletal muscle and whole body area surface from infected fish revealed numerous sporophorous vesicles and contained fully developed spores. Based on the morphological observations of stained preparation, those characteristics lead to protozoa Pleistophora spp. (Microsporidia). Furthermore, there are several other types of parasites found in this study, i.e. Dactylogyrus sp., Gyrodactylus sp., Trichodina sp., and Ichthyophthirius multifiliis (Table 2).
Mortality was recorded in every size of fish from five locations of fish farm, starting from S (<1 cm), SM (1 - 1.5 cm), M (1.5 - 2 cm), ML (2 - 2.5 cm), L (± 2.5 cm ready for harvest), and XL (> 2.5 cm rejected size or used as a prospective parent). Clinical signs of fish on the outbreak were pale body and discoloration of the lateral line. Some fish displayed no clinical symptoms of the disease in mild infections. Otherwise, moderate to severe infection was marked by the appearance of white patches under the skin, lethargy, swimming disorder, and losing weight. The white patches indicated damage to the deeper fish muscle tissues.

**Table 2. Data related to the sampling sites in this study**

| Location     | Number of samples (fish) | Cumulative mortality (%) | Source of disease          |
|--------------|--------------------------|--------------------------|----------------------------|
|              |                          |                          | Fungi | Species | Infections | Parasites | Bacteria            |
| Parung       | 10 [2]                   | > 70                     | -     | Pleistophora spp. | Moderate   | -          |
|              |                          |                          |       | Dactylogyrus sp.  | Light      | -          |
|              |                          |                          |       | Trichodina sp.    | Light      | -          |
| Pondok Petir| 10 [2]                   | > 90                     | -     | Pleistophora spp. | Severe     | Aeromonas hydrophila |
|              |                          |                          |       | Ichthyophthirius multifilis | Moderate | Pseudomonas sp. |
|              |                          |                          |       | Gyrodactylus sp.  | Light      |            |
| Sawangan     | 10 [3]                   | > 95                     | -     | Pleistophora spp. | Severe     | Aeromonas hydrophila |
|              |                          |                          |       | Ichthyophthirius multifilis | Light      | Pseudomonas sp. |
| Pancoran Mas | 10 [1]                   | > 80                     | -     | Pleistophora spp. | Severe     | Aeromonas hydrophila |
|              |                          |                          |       | Gyrodactylus sp.  | Light      |            |
|              |                          |                          |       | Trichodina sp.    | Light      |            |
| Bojong Sari  | 10 [4]                   | > 90                     | -     | Pleistophora spp. | Severe     | Aeromonas sp. |
|              |                          |                          |       | Ichthyophthirius multifilis | Light      | Pseudomonas sp. |

Notes: “-” = not detected

4. **Discussion**

Lethal Time 50 (LT$_{50}$) of water temperature tolerance at above 35°C and below 15°C of *Paracheirodon* sp. caused total fish mortality [9]. Temperature can affect the fish in multiple ways, such as biochemical, physiological activities, and become a lethal factor [10,11]. Neon tets have ionoregulation in exceptionally acid tolerant which are native to the ion-poor at pH 3.5 and high pH 6.5 [12]. Nitrite concentrations above 1.1 mg/L may interfere fish survival, especially during the long exposure involved in shipping and maintenance at the wholesaler facilities [8].

Metazoan parasites such as nematodes and Monogenea (Gyrodactylidae and Dactylogyridae) found in the intestine, liver, stomach, and other internal organs. Potentially pathogenic Gram-negative rods such as *Aeromonas sobria* and *A. hydrophila* found in several samples taken from wounds and gills [13].

*Pleistophora* spp. was the causative agent of disease that targeted the skeletal muscle. The primary and type host is the neon tetra *Paracheirodoninnesi* (Characiformes: Characidae) [9]. Microsporidia (non xenoma-inducing) in skeletal muscle of neon tetra, heavy infections may be grossly visible as whitish patches under the skin as parasites replace muscle fibres [14]. A typical microsporidian cycle involved three phases: the infective phase, which is characterized by polar tube extrusion and injection of the sporoplasm into the cytoplasm of a host cell, the proliferation phase or merogony, and the spore-forming phase or sporogony [5]. *Pleistophorasp.* revealed contained fully developed spores were 7 by 4 μm (n = 20), contained a prominent posterior vacuole measuring 3 by 3 μm, and muscle myofibers contained from 2 to 30 at different development stages, ranging from rounded-up multinucleated meronts to sporophorous vesicles containing sporoblast mother cells and vesicles with mature spores [9].
5. Conclusion
Thermal stress on fish triggered the diseases occurrence and fish mortality. Multi infections from several disease agents caused mortality in neon tetra. The main agent of the disease was *Pleistophora* spp. (Microsporidia). There was no effective drug to treat disease caused by Microsporidia. Total eradication for fish populations that suspected of being infected, separating newly arrived fish (quarantine), maintaining water quality in the optimal range, and routine disinfection of containers were recommended as preventive action.

References
[1] Chapman F A. Fitz-Coy S A, Thunberg E M and Adams C M 1997 *Journal of the World Aquaculture Society* 28 1
[2] Sanaye S V, Singh H and Tibile R M 2012 *Annals of Biological Research* 3 5665
[3] Kasai A and Oshima N 2006 *Zoological Science* 23 815
[4] Kabata Z 1985 *Parasites and diseases of fish cultured in the tropics* (London and Philadelphia: Taylor & Francis) p 318
[5] Bruno D W, Nowak B and Elliott D G 2006 *Disease of Aquatic Organisms* 70 1
[6] Lom J and Dykova I 2006 *Folia Parasitologica* 53 1
[7] Arkush K D, Frasca Jr S and Hendrick R P 1998 *Journal of Aquatic Animal Health* 10 1
[8] Kucharczyk D, Targonska K, Zarski D, Krejszeff S, Kupren K, Luczynski J M and Szczerbowski A 2010 *Polish Journal of Natural Sciences* 25 81
[9] Sanders J L, Lawrence C, Nichols D K, Brubaker J F, Peterson T S, Murray K N and Kent M L 2010 *Diseases of Aquatic Organisms* 91 47
[10] Oliveira S R D, Souza R T Y B D, Nunes E D S, Carvalho C S M D, Menezes G C D, Morcon J L, Roubach R, Ono E A and Affonso E G 2008 *Acta Amazonica* 38 773
[11] Currie R J, Bennett W A and Beiting T L 1998 *Environmental Biology of Fishes* 51 187
[12] Gonzalez R J and Preet M R 1999 *Physiological and Biochemical Zoology* 72 156
[13] Hongslo T and Jansson E 2009 *Bulletin of the European Association of Fish Pathologists* 29 163
[14] Weiss L M and Becnel J J 2014 *Microsporidia Pathogens of Opportunity: First Edition* (Oxford: Wiley Blackwell) p 709