

Stomach Histological Decay of Milkfish, *Chanos Chanos* (Forsskal, 1775): Ontogeny, Environmental Stress, Shifting Food Composition, And Disease Infection

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Abstract

Monitoring of a traditional tambak (brackish-water pond) for milkfish culture was held in Sidoarjo, East Java, Indonesia from January to May 2012. The tambak was stocked with 14-days old fry at density of 0.5 fish m⁻². Pond owner decided to harvest the tambak after 98 days of culture period, 22 days earlier than the normal grow-out period in the region. Three heavy metals, Pb, Hg, and Cd were detected in the pond water system. High concentration of ammonia may result in the formation of free NH₃-N that toxic to fish. Histological examination of digestive tract showed abnormalities the caused by changes in the availability of natural food composition, dominated by cyanophyta toward the end of culture period. Also, the presence of parasite, Myxobolus, was observed inside stomach and intestine wall. Combination of all these factors resulted in slower growth, high mortality and lower biomass production of traditional milkfish culture of East Java in the last decade.

Key words: milkfish, histopathology, environmental stress, natural food, diseases

INTRODUCTION

Traditional brackish-water aquaculture (tambak) has been practiced in Indonesia for more than 700 years (Ronquillo, 1975), with main target species of milkfish, *Chanos chanos* (Forsskal, 1775). The first written record of tambak in Java appeared in the Kutara Menawa statues written during Mojopahit era, 14th through 15th centuries AD (Thorburn, 1982). Java's north coast is still Indonesia's largest tambak area up to now. Since 2010, total aquaculture production of Indonesia surpassed total capture fisheries (MMAF, 2012), and this has positioned Indonesia as the 4th important country as aquaculture-based fish producer (FAO, 2012). Boosting aquaculture is expected to compensate the loss from marine capture fisheries as a result of over-exploitation (Mous *et al.*, 2005). Milkfish aquaculture by far, contributes around 7.0% of total aquaculture production and ranked the second in volume, after tilapia (DGA, 2011).

Milkfish culture is particularly important in Indonesia, especially for domestic consumption, local economy and food security. Bandeng-asap and bandeng-presto (smoked- and pressed-cook milkfish) are two local and popular foods in the region. Since early 1980s, Government of Indonesia promoted a shrimp culture development program (Hariati *et al.*, 1996) to compensate the reduced shrimp export from marine capture fisheries due to complete trawl ban (Buchary, 1999). Tambaks, in many areas, were converted into intensive shrimp culture system whereas milkfish culture was maintained traditional. Since 2008, milkfish farmers observed and reported a slower growth and hence lower production. A reconnaissance survey held in 2010 showed an average mortality of 40 – 50% and size at harvest between 6 – 7 fish kg⁻¹, from normal size of 3 – 5 fish kg⁻¹ and average harvest of 550 kg ha⁻¹.

As traditional nature of milkfish culture in Indonesia, the system relies on the supply of natural food from the surrounding environment that came in through incoming water system. Changes in this natural environment, either natural food or environmental stress and diseases are suspected to be the main cause of the problems. This study focused on histological changes in the gastro-intestinal tract of milkfish during culture period in relation to changes in the availability of natural food in the pond system, environmentally-induced stress and diseases.

MATERIALS AND METHODS

Pond culture

A traditional tambak (brackish water pond) of ± 2 ha was selected based on willingness of a traditional farmer to cooperate. The tambak was located in District Sidoarjo, a typically area for traditional milkfish culture in North Java. This pond was partitioned into three smaller ponds; a reservoir pond of 0.15 ha, a fry-rearing of 0.3 ha, and

1.6 ha of grow-out pond. Periphery and diagonal to each pond, there is caren of 0.5 m thick and 0.5 m depth. As commonly practiced, the pond water source totally relies from nearby river that flow through gravity. Input water from the river can only flow to the pond during high tide. During low-tide, the reverse is avoided by closing the input-water dike.

Prior to culture operation, the pond was dried out for 14 days through pumping out the water using a 15 PK diesel machine. Farmer added eight tones of lime (CaCO_3) and 0.4 ton of urea fertilizer to pond bottom. With the help of high-lunar tide, the pond was watered and maintained for another 14 days to let natural food grow. It finally stocked with fry from nearby hatchery at a density of 0.5 fry m^{-2} . This fry was stocked in fry-rearing pond for a period of 30 days, by then released into the grow-out through opening-dike. After 90 days of culture, farmer observed some fish mortality and slower fish movement in the pond. The pond was dried and prematurely harvested after 98 days of culture.

Histology, parasites and stomach-food examination

Samplings of 15 fish were held bi-weekly (starting day-0) using cast net of 3 m diameter: 5 fish were selected for histological examination, another five fish for stomach-food study and the rest five fish for investigation on the presence of parasites. These samples need two hours transport before stored in laboratory, using ice for temporary preservative.

The first five-fish were dissected, all the digestive tract separated, fixed and processed with paraffin. The cross-sectional histology ($\pm 3.0 \mu\text{m}$) was H&E stained following Lillie (1965) and Luna (1968). Light microscope examinations were held at different enlargement-scales, depends on the size of the object; 40, 100, 400, and 1000 times. Of the second five-fish samples, stomach of each fish was dissected to identify natural-food composition, following Prescott (1964). The cyanophyta's group was further separated into different genus (Davis, 1955; Prescott, 1964). The last five-fish samples were prepared for parasites examination. The cross-sectional histology of digestive tract was H&E stained following procedure mentioned above (Lillie, 1965; Luna, 1968) and examined through light-microscope.

Water quality parameter

A set of water quality parameter was monitored on bi-weekly basis, and was sampled just prior to fish sampling. In-situ pH was measured using pH-meter (0.01 degree accuracy), dissolved oxygen (DO) with DO-meter (0.01 mg l^{-1}) together with water temperature ($0.1 \text{ }^\circ\text{C}$), salinity with refractometer (1.0 mg g^{-1}), and water transparency with secchi disk (accuracy of 1.0 cm). Ammonia (mg l^{-1}), Nitrate ($\text{NO}_3\text{-N}$) and orthophosphate ($\text{PO}_4\text{-P}$), free- CO_2 , and Total Organic Matter (TOM), all are in mg l^{-1} , were measured following standard water quality procedures (APHA, 1998). Sampling for water quality generally completed between 10:00 to 11:30 AM.

Data analysis

All the harvested milkfish was measured as total biomass production. A fish sampling was held to estimate total numbers of fish in one kg weight. The total number of fish at harvest was estimated by multiplying the total numbers of fish in a kg weight with total biomass production (kg). Finally, an estimate for total mortality was the estimated number of harvested fish divided by numbers of fry stocked at the beginning. Descriptive statistics analysis was done for all water quality parameters.

RESULT AND DISCUSSION

Fish harvest, mortality and size at harvest

The pond was harvested at culture period of 98 days, around 22 days earlier than the normal period. Farmer reached a total harvest of 367 kg ($\pm 158 \text{ kg ha}^{-1}$), with an average fish size of 13 fish kg^{-1} . Estimate of the total fish at harvest was 4,771 fish, resulted in an estimate of total mortality of 53%. This may a symptom of traditional milkfish culture has significantly reducing (Chen, 1976; Guanzon, 2004). This reduced productivity is due to slower growth (small individual size at harvest), higher mortality, and symptoms of disease outbreaks (Maciels *et al.*, 2011), such as environmental stress (Cruz & Pitogo, 1989; Chen, Tsao & Jiang, 1989).

Research held in Kendal, Central Java showed that traditional milkfish culture in late 1980s reached an average production of 530 kg ha^{-1} (Harun, 1992). When grown together with seaweed, the productivity varied between $500 - 900 \text{ kg ha}^{-1}$ (Guanson *et al.*, 2004)

Water quality

Significant concentration of heavy metals (Pb, Hg, and Cd) were detected in the pond used for milkfish culture (Table 1). The concentration even found a bit higher than that measured near Jakarta (Arifin, Puspitasari, & Miyazaki, 2012; Takarina *et al.*, 2012). Bio-accumulation of these metals in milkfish certainly needs to be investigated. Prior to fish stocking, farmer strived to maintain the pond with high natural food productivity as indicated from water visibility of $\pm 17 \text{ cm}$. These natural food consequently consumed by milkfish and water visibility was maintained around 24 cm. Some variations in visibility were observed during culture period; it probably caused by multi-factors, include rainfall that influence the availability of natural food.

Generally, the pond was found to be over-saturated with Dissolved oxygen (DO) since 10:00 AM. With respect to DO supplies from natural system, stocking traditional tambaks with milkfish at 0.5 fish m⁻², still allow surplus in DO supply for the fish (Boyd, 1989). High concentration of ammonia leads to the formation of free NH₃-N that toxic for fish, especially in low salinity or freshwater with high pH and temperature (Boyd, 1989; Almendras, 1987).

Table 1 Results of bi-weekly water quality measurements (eight replicates) during culture period taken from grow-out pond

Water quality parameters	Mean	Min	Max	STDEV	CV
Temperature (°C)	31.71	29.67	33.67	1.57	4.95
Secchi visibility (cm)	24.09	16.50	28.50	4.18	17.36
Water pH	9.0	7.6	10.0	0.9	10.1
Dissolved Oxygen (mg l ⁻¹)	9.62	7.92	11.71	1.27	13.18
TOM (mg l ⁻¹)	11.47	3.16	32.88	9.75	84.97
Ammonia (mg l ⁻¹)	0.788	0.075	3.320	1.112	142.940
Nitrate (mg l ⁻¹)	1.83	1.34	2.86	0.53	29.08
Orthophosphate (mg l ⁻¹)	0.08	0.04	0.15	0.04	45.28
Soil pH	4.7	4.0	5.5	0.4	8.0
Pb (mg l ⁻¹)	0.082	0.048	0.116	0.022	27.022
Hg (mg l ⁻¹)	0.015	0.002	0.054	0.016	105.767
Cd (mg l ⁻¹)	0.008	0.001	0.018	0.005	62.874

Note: free-CO₂ was undetectable; water salinity was nearly zero (undetectable) all the time, so it rather considered as freshwater pond

Natural food composition

Stomach composition of the milkfish was dominated by cyanophyta, also called cyanobacteria (Fig. 1A), especially toward the end of culture period. Cyanophyta are prokaryotic organisms with non-membrane bound organelles, and hence more closely related to bacteria than algae. The dominance of cyanophyta in the water is determined by salinity (Kuhlmann *et al.*, 2009). For almost freshwater tambak, it is not surprising for the dominance of this cyanophyta found in the pond. Individual cell in this group typically has a thick cell wall but flexible, usually covered with mucus. When ingested, this type of cell is difficult to be digested by fish (Segner *et al.*, 1987). Blooming of cyanophyta can be influenced by water quality and other climatic condition. El-Shehawey *et al* (2012) predicted that global warming stress may induce blooming of this phytoplankton, and can be toxic to fish (Bhaskar & Rao, 1990; Villaluz & Unggui, 1983).

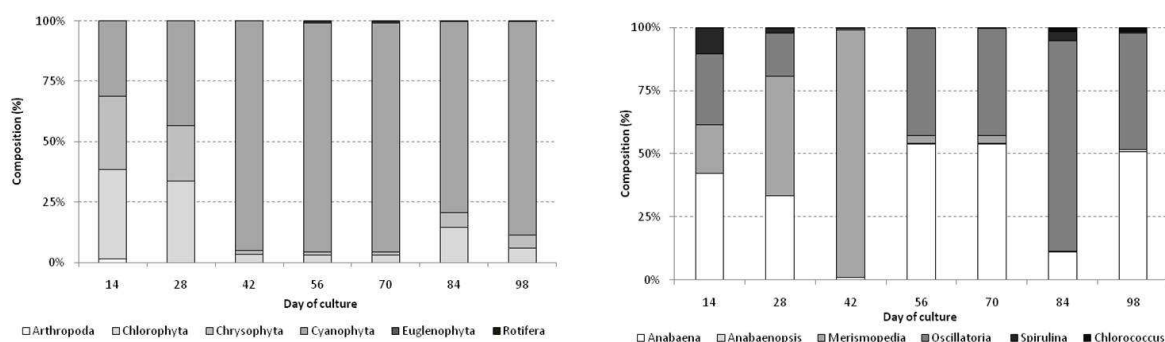


Figure 1 Changes in natural food composition of milkfish at difference day of culture (A); genera composition that construct up the cyanophyta's group (B)

Further investigation of this group (cyanophyta) resulted in six different genera (Fig. 1B): Anabaena, Anabaenopsis, Merismopedia, Oscillatoria, Spirulina, and Chlorococcus. Merismopedia was found to be dominating in the beginning of the culture followed by Anabaena and Oscillatoria in the end.

Myxobolus Parasite

Stomach histological examination of fish on day-56 indicated the presence of strange cysts (Fig. 2A). Further investigation under microscope confirmed the adult stage of *Myxobolus* (Fig. 2B) identified by the presence of two-polar capsule (Maciel *et al.*, 2011). In the form cysts, infection of *Myxobolus* parasite may not lethal to fish that cause acute mortality. However, the adult *Myxobolus* were found in almost connective tissue surrounding the cysts. The time cysts change into adult it cause rupture of connective tissue and lead to gastritis (Maciel *et al.*, 2011).

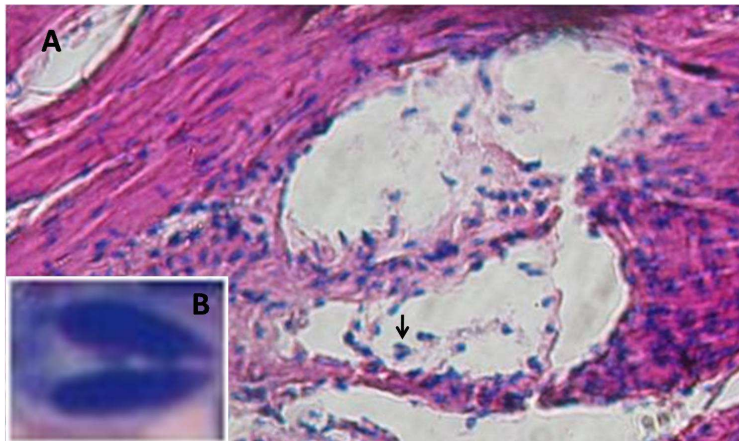


Figure 2 Stomach histology of sampled milkfish at day-70 of culture (400X enlargement) indicating the presence of parasite (A); confirmation of parasite *Myxobolus* (two-polar capsule) under microscope of 1.000X enlargement (B)

Stomach Histology

The developmental status of the digestive system of first-feeding larvae dictates the possibility or not the larvae to digest the food ingested (Lavens & Sorgeloos, 1996). The stomach and intestine of milkfish were histologically distinguishable after day-14 of culture period (Fig. 3A and 3B). However, the complete structure of digestive tract was observed on day-28 (Fig. 3C). It composed of mucosal epithelium (Me), lamina propria (Lp), sub-mucosa (Sm); muscularis (M), serous membrane (S), and Goblet-Cell (Ge) (Ferraris, Tan, & Cruz, 1987).

Starting on day-42 mucosal epithelium (M) of the stomach undergone hypertrophy (Fig. 3D) and continued with metaplasia on day-56 (Fig. 3E). The shape changed from monolayer-cylindrical epithelium became multilayer-rod shape (epithelium stratum squamosum). On day-70, this Me experienced atrophy with inflammable muscularis layer and many broken blood-vessels. Finally, the damages expanded into almost all of the stomach cell lines (Fig. 3G & 3H).

Environmental stress, chemicals or diseases have shown to be pathologically changing sensitive organs of milkfish (Cruz & Pitogo, 1989), such as gills, kidney and digestive tract. This study clearly showed the damage of digestive tract of milkfish. This damage can be linked to one or combination of the following factors: water quality (NH₃-N or Nitrite), natural food that toxic to fish, and/or *Myxobolus* parasite. These factors have triggered stress that lead to slower growth and finally fish mortality.

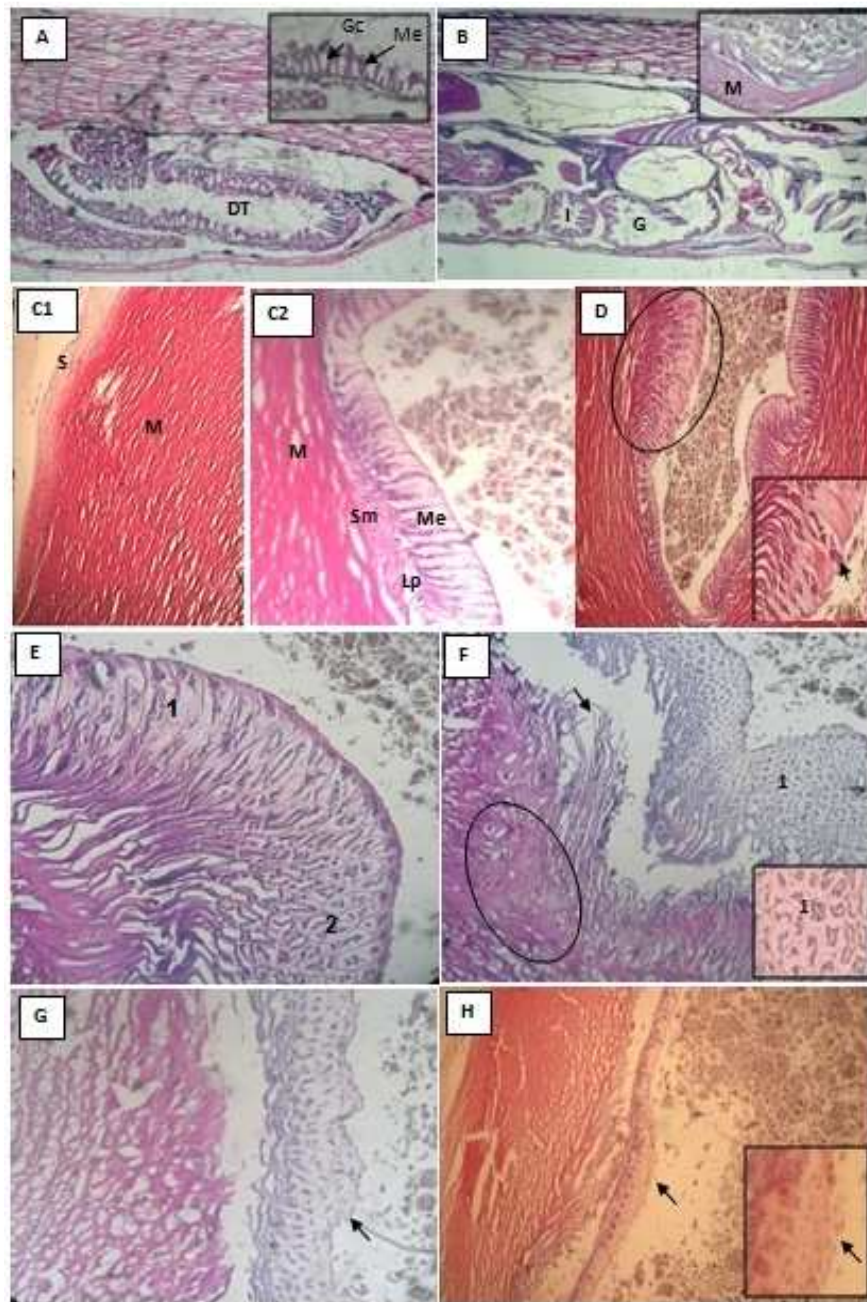


Figure 3 development of milkfish digestive-organ during culture period – (A) stomach and intestine of the fry when stocked to pond are still undistinguishable. The presence of digestive organ can be seen from Ge, and Me; (B) stomach (G) and intestine (I) are clearly distinguishable after day-14 of culture. The stomach is indicated by the presence of muscularis (M); (C1) serous membrane (S) and mucosal (M) of stomach are clearly distinguishable at day-28; (C2) normal layer of sub-mucosal (Sm), lamina-propria (Lp), and mucosal epithelium (Me) identifiable inside muscularis starting from day-28 of culture; (D) hypertrophy of Lp and Me (are inside black circle) was shown after day-42 of culture, but still produce mucus (inset arrow sign); (E) Mucosal epithelium (Me) experienced metaplasia since day-70, from mono-layer of cylindrical epithelium (1), shifted into multi-layer oval-shaped epithelium, *epithelium stratificatum squamosum*, (2); (F) atrophy of Me was shown starting on day-84 of culture (1) with inflammable muscularis layer (black circle) and with many broken blood-vessels (arrow). Sign of atrophy was indicated by the form of the cells (inset 1); (G) almost all Me were observed to eroding (arrow sign). Note: Me = mucosal epithelium; Lp = lamina propria; Sm = sub-mucosa; M = muscularis; S = serous membrane; G = gastric (stomach); I = intestine; DT = digestive tract; Ge = Goblet-Cell.

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