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A case study on the environmental features associated with *Amyloodinium ocellatum* (Dinoflagellida) occurrences in a milkfish (*Chanos chanos*) hatchery

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Abstract. *Amyloodinium ocellatum*, an obligate parasitic dinoflagellate, occurrences in hatchery-reared milkfish (*Chanos chanos*) fry in Iloilo province was investigated. There were frequent anecdotal reports of *A. ocellatum* infestation during the wet season (June – September) of the year in Oton hatchery. On the other hand, there were no reports of *A. ocellatum* infestation for the past eight years in Tigbauan hatchery. Comparisons on water quality parameters (temperature, salinity, dissolved oxygen, and pH), fry microscopic examinations, and management practices in the two hatcheries were monitored during dry and wet seasons (May and June, respectively). Dissolved oxygen levels in both hatcheries were significantly lower during wet season (5.3 – 5.6 mg L^{-1}) than dry season (6.8 – 7.3 mg L^{-1}). Analysis showed significantly lower mean salinity in Oton hatchery (30.2 ppt) than in Tigbauan hatchery (30.9 ppt) during dry season. In Tigbauan hatchery, the rearing water had significantly higher mean salinity and mean pH in dry season (30.9 ppt and pH 8.7) than in wet season (29.9 ppt and pH 8.2). These significant differences however did not encourage any *A. ocellatum* occurrence. In July and September 2012, *A. ocellatum* infestations occurred in Oton hatchery. This validates the anecdotal reports that *A. ocellatum* infestations occur in wet season in Oton hatchery. The importance of good husbandry is discussed. Effective filtration system and sanitation are recommended to avoid the introduction of *A. ocellatum*-contaminated water which can be influenced by heavy rains.

Key Words: dinoflagellate, protozoan parasites, amyloodiniosis, water quality, hatchery practices.

Introduction. Aquaculture has been the fastest growing sector of food production meeting the demand of the world's growing population. With the technological advancement, intensification of existing facilities and expansion into new areas contributes largely to the development of aquaculture (Bondad-Reantaso et al 2005). However, accompanying this development in aquaculture system are problems that needs the aid of fish health management plans (Blayblock & Whelan 2000).

Fish health management plans specific to issues on the development of diseases should be given great importance since the aquaculture industry suffer great losses each year because of diseases. One of the major causes of mortalities in fish hatcheries are fish protozoan parasites (Noga & Levy 1995; Urawa 1996; Cruz-Lacierda et al 2004). *Amyloodinium ocellatum* (an ectoparasitic dinoflagellate) infestations have been recorded in temperate and tropical countries causing fish cultures to suffer (Baticados & Quinitio 1984; Cruz-Lacierda et al 2004; Pereira et al 2010). Trophonts, the feeding stage of *A. ocellatum*, commonly attached on skin, fins, eyes and gills, can interfere with gas exchange, osmoregulation, and tissue integrity thus resulting to spasmodic gasping, uncoordinated movements, and extensive mortalities of fishes (Masson et al 2011).

Disease development is greatly influenced by the interaction of three factors: the host, the pathogen, and the environment (Lavilla-Pitogo 2010). Management practices, considered as part of the environment, are correlated with diseases as in the case of soft-shell syndrome in prawn (Baticados et al 1986). Thus, the probability of having an *A.*

ocellatum infestation generally depends on its environmental circumstances, as well as management practices (Reno 1998).

A small-scale commercial fish hatchery in Oton reports frequent infestation of *A. ocellatum* among hatchery-reared milkfish fry during wet season in the Philippines on the months of June to September (A. Marte, personal communication). The said hatchery acquires milkfish eggs and larvae from other hatcheries with broodstock or spawning facilities. On the other hand, the Integrated Fish and Broodstock Hatchery Complex of Aquaculture Department, Southeast Asian Fisheries Development Center (SEAFDEC/AQD) in Tigbauan, a Research and Development Center, reports no *A. ocellatum* infestation on milkfish fry all year-round. This suggests that environmental and management factors may play a significant role in the onset of infection. The need for comprehensive and thorough ecological and parasitological investigations has always been stressed out (Dogiel et al 1970). However, there is less information about the ecology and the environmental conditions that favor the occurrences of this organism (Pereira et al 2010).

The present case study is done to provide information on the precursor of *A. ocellatum* outbreaks and seasonal behavior. Knowledge on when and how infestation happens will recommend effective management practices. With this, mass mortalities in hatcheries will be prevented.

Material and Method

Study site. This study was conducted on two milkfish hatcheries in the province of Iloilo which is about 10 km away from each other. One is a small-scale backyard hatchery characterized by 8 circular marine plywood tanks lined with canvas material with 10-ton water holding capacity and is located in Oton. The distance of seawater intake is about 50 meters away from the shore. Mass mortalities of milkfish fry have been frequently observed in this hatchery (A. Marte, personal communication). The other is the Integrated Fish Broodstock and Hatchery Complex, a production and research facility of SEAFDEC/AQD, characterized by rectangular concrete tanks with 10-ton water holding capacity and is located in Tigbauan. The distance of seawater intake is about 100 meters away from the shore. This hatchery has no *A. ocellatum* infestation on milkfish for the past eight years (B. Eullaran, personal communication).

Water quality measurements. Handheld Multiparameter Instrument (YSI 556 MPS) and Eutech EcoScan were used to measure water quality parameters (temperature, salinity, pH, and dissolved oxygen). Daily monitoring of water quality for two sampling periods, May 7-28, 2012 and June 4-25, 2012, from day 0 to day 21 of milkfish larval rearing were done to represent the dry and wet seasons in the Philippines.

Husbandry practices. Husbandry practices of both hatcheries were evaluated by regular observations and personal interviews. These include water management, diet and feeding practices, sanitation, and other larval rearing protocols.

Fish larval monitoring for A. ocellatum. At least ten milkfish larval samples were sacrificed daily for observation of the presence of *A. ocellatum*. Samples were fixed in 10% buffered formalin and examined under Motic compound microscope. This was done at the Fish Disease Laboratory of the College of Fisheries and Ocean Sciences, UP Visayas, Miagao, Iloilo. Prevalence and intensity of infection were calculated following Bush et al (1997).

Statistical analysis. Independent t-test using SPSS 20 was used to determine whether there was a statistically significant difference between the means of water quality parameters between the two hatcheries during the two sampling periods (wet and dry season). The SPSS t-test procedure uses Levene's test to test the equality of variances and the t-value for both equal- and unequal variance. This provides a relevant descriptive statistics.

Results and Discussion. Environmental and seasonal dynamics of *A. ocellatum* infestation is documented in literature (Kuperman & Matey 1999; Pereira et al 2010; Saraiva et al 2011). *A. ocellatum* is said to tolerate a wide range of temperature and salinity thus infecting over 100 species of marine and euryhaline fish (Paperna 1980; Reed & Francis-Floyd 1994; Noga & Levy 1995; Blayblock & Whelan 2000; Pereira et al 2010). Thus, a correlation study with environmental and biological factors as variables is conducted to explain the occurrences *per se* of *A. ocellatum* in cultivated gilthead seabream (Pereira et al 2010). The study concludes that salinity is positively related with trophont occurrences. Dissolved oxygen, water temperature, pH, and phytoplankton biomass have significant negative relationship with *A. ocellatum* trophonts.

The present study attempts to correlate environmental factors with *A. ocellatum* infection. The dissolved oxygen levels in both hatcheries are significantly lower during wet season (5.3 – 5.6 mg L^{-1}) than in dry season (6.8 – 7.3 mg L^{-1}). Oton hatchery has a significantly lower mean salinity (30.2 ppt) than Tigbauan hatchery (30.9 ppt) during dry season. Tigbauan hatchery has higher mean salinity (30.9 ppt) and pH (8.7) during dry season than in wet season (29.9 ppt and pH 8.2) (Table 1). The significant differences during dry and wet seasons on salinity and dissolved oxygen in the two hatcheries do not result to *A. ocellatum* infestation. Thus, it is difficult to correlate these differences with disease occurrence. This suggests that seasonal dynamics observed in *A. ocellatum* infestations may not be directly related to water quality parameters.

In July and September 2012, considered as the peak of wet season in the country, mass mortalities of 21-day old milkfish fry due to *A. ocellatum* occur in Oton hatchery. This validates the anecdotal reports that *A. ocellatum* infestations on milkfish fry occurs during the wet season. The occurrences of *Amyloodiniosis* in July and September in Oton hatchery may be due to the introduction of *A. ocellatum*-contaminated water which is influenced by the tropical storms that affected Iloilo province. The Philippines Star Online Newspaper reported that a Low Pressure Area (LPA) developed into tropical storm "*Gener*" on July 28 and a tropical storm intensified into typhoon "*Karen*" on September 15. The seawater in the culture tanks becomes turbid during heavy rains (A. Marte, personal communication). In contrast, there is no reported *A. ocellatum* infection in Tigbauan hatchery.

Light microscopy observations of moribund and dead fry showed brownish or yellowish, round to oval-shaped trophonts attached to the body of the fish (Figures 1 & 2). The infestation on July resulted to a sudden mass mortality. Lower prevalence and intensity of infection at 14% and 1 respectively, are attributed to the nature of the fry samples examined (Table 2). Some trophonts might have completed its feeding stage and detached to become a tomont (Schwarz & Smith 2009).

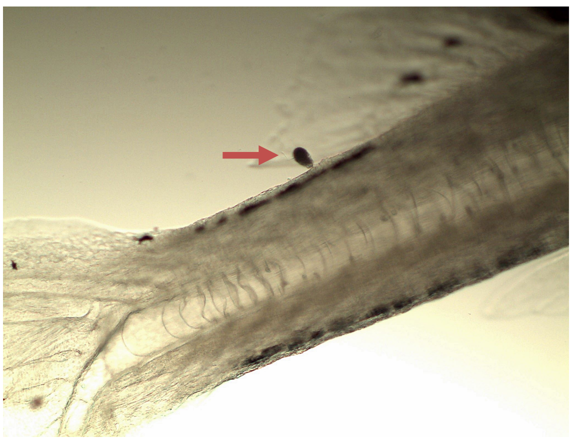

Figure 1. *Amyloodinium ocellatum* trophont (arrow) attached posterior to the dorsal fin of 21-day old milkfish fry (July 2012 infestation; fresh mount, 100X).

Table 1

Mean values of the water quality parameters[+] during dry and wet season in Oton and Tigbauan fish hatcheries. Values are means ± standard deviation

Hatchery	Temperature (^0C) Mean±SD		Salinity (ppt) Mean±SD		Dissolved oxygen (mg L^{-1}) Mean±SD		pH Mean±SD	
	Dry	*Wet*	*Dry*	*Wet*	*Dry*	*Wet*	*Dry*	*Wet*
Oton hatchery	27.98±0.98[a]	28.55±1.24[a]	30.21±0.93 [a]	29.92±0.78[a]	7.27±1.47[a]	5.30±0.43[b]	8.40±0.46[a]	8.14±0.48[a]
Tigbauan hatchery	28.13±0.61[a]	28.57±0.99[a]	30.94±1.31[b]	29.90±1.06[a]	6.84±1.57[a]	5.55±0.88[b]	8.74±0.36[a]	8.18±0.56[a*]

[+]Mean from 21 days of sampling, 3 readings per day; *significant difference within a row only; SD = standard deviation; values for each parameter in a column or row with the same superscript are not significantly different (p = 0.05).

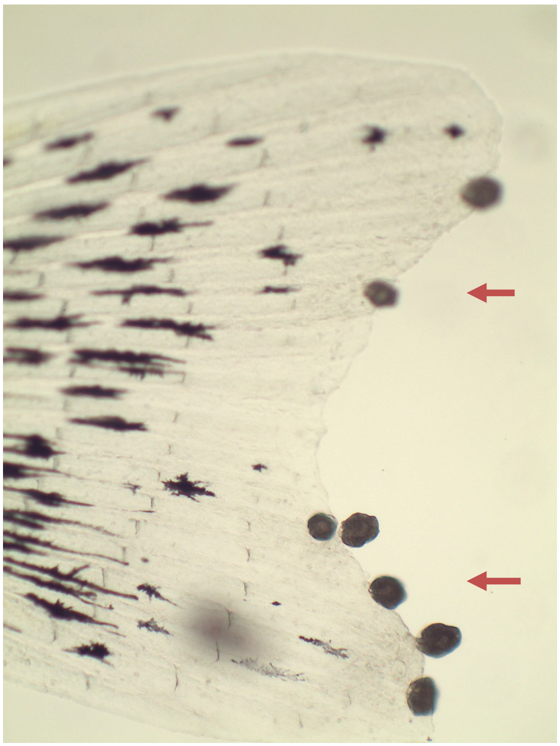

Figure 2. Several trophonts of *Amyloodinium ocellatum* (arrows) attached on the caudal fin of 21-day old milkfish fry (September 2012 Infestation; fresh mount, 100X).

Table 2

Mean intensity and prevalence of infection during July and September *Amyloodinium ocellatum* outbreak in Oton Milkfish Hatchery

Month	*Total number of fish examined*	*Mean intensity of infection Mean±SD*	*Prevalence of infection*
July	100[a]	1.2±0.84	14%
September	15[b]	27±8.52	93%

[a]dead fry; [b]live fry; SD = standard deviation.

Tigbauan and Oton hatcheries practice the same hatchery management procedures such as having a stocking density of 15 larvae per liter, feeding with green algae at day 2 and rotifer plus artificial diet at day 10, and begin water change at day 6 (Table 3). However, they differ in water management, filtration system, availability of fresh water source, and disinfection protocols (Table 3). Tigbauan hatchery changes the water every day starting at day 6 at 50% and siphons every other day. The pre-filtered seawater is stocked in a reservoir tank with sand filter. It is then distributed into the larval tanks by gravitational force and is finally filtered by a polyester or cotton cloth. It has a direct freshwater source. Hatchery paraphernalia are disinfected such as washing them with detergent before and after use. Plankton count is regularly monitored. On the other hand, water change and siphoning in Oton hatchery do not follow a regular scheme and are dependent upon the culturist. The pre-filtered seawater goes directly to each tank filtered by a satin cloth. There is no direct freshwater source thus it has to acquire freshwater from nearby community. Available freshwater is stocked in plastic drums. There is no protocol followed in terms of disinfection of hatchery paraphernalia.

Table 3
Summary on hatchery management practices in Oton and Tigbauan fish hatcheries

Specification	*Oton hatchery*	*Tigbauan hatchery*
Distance of water intake from the shoreline	~50 meters	~100 meters
Stocking density	15 larvae L^{-1}	15 larvae L^{-1}
Feeding management	Green algae starting at day 2; rotifer + artificial diet at day 10	Green algae starting at day 2; rotifer + artificial diet at day 10
Water management	Irregular; about thrice a week	50% water change daily
Filtration system	Pre-filter; satin cloth as filter bag	Pre-filter; reservoir tank with sand filter; cotton or polyester cloth as filter bag
Availability of freshwater	None; source is outside the facility; available water is stored in plastic drums	Direct source available
Disinfection of hatchery paraphernalia	None	Washing with detergent before and after use

Water source contaminated with tomonts and dinospores is the possible cause of *A. ocellatum* infestation in Oton hatchery which can be a result of poor filtration and sanitation practices since trophonts and resting cysts (tomonts) can cause recurrence of *Amyloodiniosis* (Reed & Francis-Floyd 1994; Abreu et al 2005; Francis-Floyd & Floyd 2011).

Conclusions. Although the present study was not able to show the relationship of water quality parameters to *A. ocellatum* occurrence, observations suggest that *A. ocellatum* infestations on milkfish fry in Oton hatchery during July and September may be attributed by the introduction of parasite-contaminated water which was influenced by heavy rains. Effective filtration system and proper sanitation procedures must be applied to prevent disease outbreak. Further information on the ecology and environmental conditions that favor the occurrences of this parasite can be obtained following these recommendations: to monitor environmental parameters during the height of rainy season (July – September) and to record plankton count as well.

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