

Investigating a mortality in hatchery cultured tropical abalone, *Haliotis asinina* Linnaeus, 1758 in Malaysia

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ABSTRACT

Abnormal mortality rates ranging from 4-5 individuals/day were reported for abalone cultured in fibreglass tanks at the hatchery facility of the Fisheries Research Institute, Penang, Malaysia. Previously, mortality was only in the range of 1-3 abalone/month. Histological and bacteriological examination of the samples collected over a three-month period showed systemic bacterial infection by Gram-negative bacterial rods with a presumptive identification to the genera *Vibrio* spp. and *Pasteurella* spp. The main histological feature observed was severe enteritis. Further investigation confirmed that the disease was transmitted from the seaweed (*Gracilaria changii*) used as food for the abalone. The *Gracilaria changii* stock was procured from abandoned shrimp ponds located on the north western coasts of the peninsula. This case study highlights the importance of good farming and management practices and as well as appropriate abalone husbandry procedures.

Key words: tropical abalone, *Haliotis asinina*, vibriosis, good management practices, *Gracilaria changii*.

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INTRODUCTION

Abalone are among the top five most expensive food items on the Chinese cuisine market. They are usually sold fresh, frozen, canned or dried and are consumed raw (sashimi) or cooked. Although abalone are slow-growing molluscs, they are a new candidate for aquaculture in many countries as wild fisheries have progressively been declining. These factors have prompted the Fisheries Research Institute (FRI) in Malaysia to embark on an abalone breeding program which was first initiated in 2000.

Haliotis asinina L. is a tropical abalone species, commonly known as the donkey's or ass's ear abalone. It is native to Australasia, Thailand, the Philippines, Malaysia, Vietnam and New Zealand. Despite its moderate size (8-10 cm shell length) and dubbed the 'cocktail abalone', it is a species with great potential for aquaculture in Southeast Asia. Hatchery propagation and farming of this species has been proven to be successful in Thailand, the Philippines, Australia, New Zealand and recently in Malaysia.

Adult *H. asinina* broodstock were procured from the northern waters of East Malaysia, off Sabah (from Kota Belud, Pulau Matanani and off Sempoerna Island). They were air freighted to Penang, and subsequently transported by land to the mollusc hatchery at FRI. Upon arrival the abalone were conditioned in 2-tonne (2000 l) fibreglass tanks. The adults spawn after about 2-3 months of conditioning. These tropical abalones need not be induced to spawn as they spawn naturally coinciding with the lunar cycles (new and full moon period). The larvae settle within 2-4 days and they feed on pre-cultured diatoms collected on acrylic culture plates. The abalone spat usually take about 18 months to attain marketable size of 6-7 cm. During the culture period, increased mortality rates were noted for both the adult and juvenile abalones. This paper reports the findings of the study conducted to investigate the cause of mortality of *H. asinina* in the rearing facility.

MATERIALS AND METHODS

Rearing conditions – Abalone broodstock were held in 2000 l tanks with flow-through sea water (4 l/min) at a stocking density of 200 adult abalone (6-8cm/tank). Juveniles were stocked at 1,500 pieces per 2000 l tank. The hatchery facility consisted of six 2000 l tanks: three tanks for adult abalone and three tanks for juveniles. The adults were fed seaweed, *Gracilaria changii* while the juveniles were fed seaweed and mixed diatoms. The seaweed stock was held in a separate tank and used on demand to feed the abalone. Similar rearing conditions were provided for the adults and the juveniles: tanks were supplied with flow-through seawater and provided aeration. Water change was carried out at 50% and 100% on alternate days. Every fortnight (i.e. after 14 days), the abalone juveniles were transferred manually, using a hard plastic strip to loosen their grip, into a new tank with fresh diatoms. Waste was siphoned out routinely and uneaten seaweed was collected and washed before returning it to the culture tanks.

Case history – An unusually high mortality rate was reported in January 2005. The facility management noted 4-5 abalone dying daily as compared to only 1-3 dying monthly, previously. Healthy and sick individuals were collected on the 17th and 23rd February and 21st March, 2005. Sampled abalone were subjected to clinical observation and further bacteriological isolation and histological examination.

Investigation - Fresh smears from the gills and tegument lesions were taken to assess parasitic infections. Necropsies were conducted under a dissecting microscope (4 – 40X magnification).

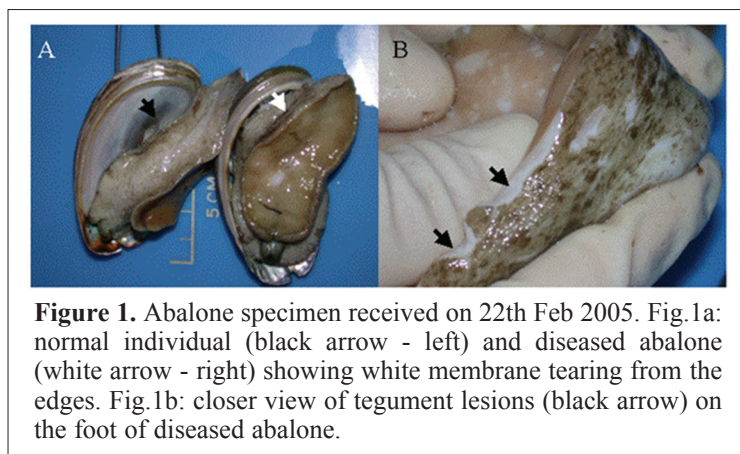
Primary isolations of bacteria from the lesions on abalone and the seaweed (*Gracilaria changii*) were carried out on Tryptone Soya Agar, TSA (OXOID) with additional 1.5% salt for total heterotrophic flora and Thiosulfate Citrate Bile Sucrose agar, (TCBS) (OXOID) for *Vibrio* spp. at ambient temperature (30°C). The plates were incubated at 30°C for 24 to 48 hrs. The bacteria isolated were identified using the API 20E kit (BioMérieux).

Normal and abnormal specimens were dissected and lesions from the foot margin, ulcer-type lesions from the tegument and samples of 1 cm thickness of the cross-sectioned internal organs consisting of gonad, gills, intestine and stomach, were fixed in 10% buffered formalin in filtered seawater for 24-48 hrs, followed by storage in 70% alcohol. Samples were subsequently processed for histology according to standard methods (Bondad-Reantaso *et al.*, 2001), stained with either hematoxylin-eosin (H&E), Gram's stain or methylene blue and mounted for light microscopy (Zeiss Axioplan).

RESULTS

The mortality rate was 4-5 abalone/day over a six-month period, resulting in high cumulative mortality. Affected individuals were generally weak with the following clinical signs:

- i) The mantle was attached loosely to the shell with development of a white pseudo-membrane in some instances (Fig. 1a).



- ii) Some specimens showed ulcer-like lesions of the tegument. In the most severe cases, individuals displayed white patches at the foot margins (Fig. 1b).
- iii) No parasites were found from fresh smears and squashes. Gram-negative bacteria, presumptively identified as *Pasteurella* spp. and *Vibrio* spp. were isolated from the foot lesions (Fig. 1b). and characterised by biochemical tests (Table 1).

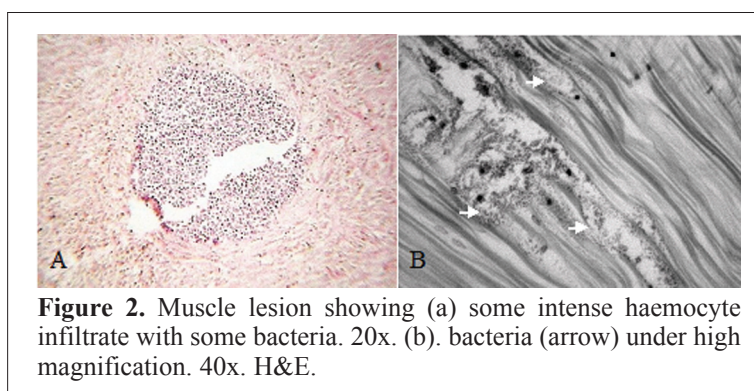
Table 1

Biochemical tests on the Gram-negative bacteria isolated from the foot lesions of abalone

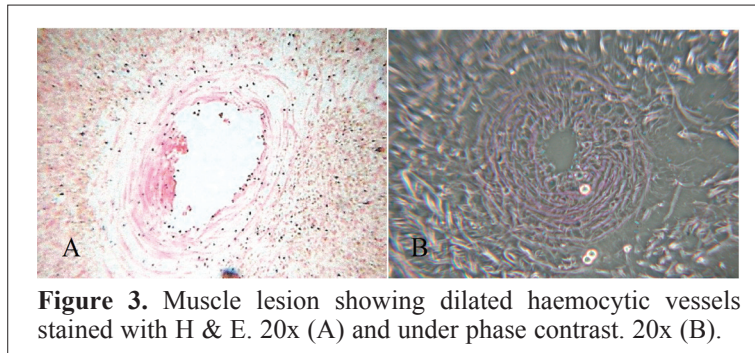
Test	Gram negative bacteria	
	<i>Pasteurella</i> species	<i>Vibrio</i> species
TSA	Yes	Yes
Colony colour on TCBS	Green	Yellowish
Gram stain	Negative	Negative
Shape	Rod	Rod
Oxidate/Fermentative	Positive/Positive	Positive/Positive
Motility	Negative	Positive
Oxidase	Positive	Positive
McConkey	Negative	Negative
O/129	Sensitive	Resistant
API20E	<i>Pasteurella pneumohaemolyticus</i>	<i>Vibrio alginolyticus</i>

- iv) Gram-negative bacteria identified as *Vibrio* spp. (*Vibrio vulnificus* and *Vibrio alginolyticus*) were isolated from the seaweed.

Histology revealed systemic bacterial infection with intense haemocytic infiltrations and abscess-like lesions in the foot (Fig. 2). Some haemolymph sinuses were abnormally dilated with haemocytes adhering to the vessel wall. This was interpreted as an early sign of a



systemic inflammatory response (Fig. 3). Inflammation was also noted in the nervous tissue. Some specimens had severe hemorrhagic enteritis associated with septicaemia.



DISCUSSION

The high mortality rate recorded in the rearing facility led to a comprehensive investigation of the possible origin and remediation options. The investigation concluded that the outbreak was caused by bacterial infections of *Vibrio* and *Pasteurella*. Histopathological examination revealed abscesses, enteritis and severe haemocyte infiltration in response to systemic bacterial infection.

Bacterial infections of abalone are well documented. Vibriosis has been reported to be caused by *Vibrio harveyi*, *V. splendidus* I, and *V. alginolyticus* (Dixon *et al.*, 1991; Elston and Lockwood, 1983; Handler *et al.*, 2002; Reuter and McOrist, 1999; Lee *et al.*, 2001; Nishimori *et al.*, 1998). Among these, *V. harveyi* and *V. carchariae* (a junior synonym of *V. harveyi*, see Pedersen *et al.*, 1998) are more prevalent. Nishimori *et al.* (1998) reported mass mortalities in Japanese abalone *Haliotis diversicolor supertexta* Lischke, 1870 due to *V. harveyi*. Bacterial infection such as *V. harveyi* in *H. tuberculata* L. has been reported in both wild and cultured abalone in Brittany and Normandy, France (Nicolas *et al.*, 2002). In Tasmania, Australia, *V. harveyi* and *V. splendidus* I were found in abalone showing septicaemia. In addition, *Flavobacterium*-like bacteria have been identified during disease outbreaks in cultured *H. rubra* Leach, 1814, *H. laevigata* Donovan, 1808 and their hybrids (Matsunaga, 1967). *Clostridium lituseberense* has also been reported to occur in abalone (Bower, 2003 and 2004). However, infection by *Pasteurella* sp. in abalone has not previously been reported. In this case, *H. asinina* L. with necrosis and white lesions on the foot had shown infection caused by two bacteria types, presumptively identified as *Pasteurella* sp and *Vibrio* spp. In this study, we were able to identify the bacteria up to the genus level, however, confirmation of the bacterial species needs to be pursued in future.

The mortality could be associated with poor management practices in the hatchery, such as direct use of seaweed from the prawn farms (introduction of anthropogenic pollutants such as bacteria, parasites and mud) and returning uneaten seaweed to the tanks which

further fouled up the water due to presence of waste matter. The manual removal of the abalone could also have inflicted lesions on the body. No specific treatment was given at the time of the mortalities. Mitigation measures undertaken were frequent exchange of water in the abalone and seaweed culture tanks and quarantine of the seaweed prior to feeding, which showed a notable decrease in the number of mortalities.

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