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RESEARCH PAPER

Mortality of the abalone *Haliotis mariae* (Haliotidae: Mollusca) in aquaculture

A case study in Oman

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نفوق رخويات أذن البحر (Mollusca Haliotidae) *Haliotis mariae*: في الإستزراع السمكي في سلطنة عمان: دراسة حالة

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Abstract. The Omani abalone, *Haliotis mariae*, is the only species of abalone found in Omani waters. Given the rarity of this species and the high price it can fetch on the market (US$ 150 kg^{-1} dry weight), the wild abalone fishery in the Dhofar region has been regarded as a valuable income source for the past decade. The present study was undertaken set to investigate the mortality of abalone held at the Mirbat Research Center, through bacteriological and histopathological based investigations and challenge tests. Only the adult wild abalone that had been kept for a year in the hatchery, visually, appeared to be clear of disease symptoms. Infected individuals typically were swollen around the mouth, had fluid tinged with blood, bubbles in the intestines, and, very weak adhesive strength. The foot area (muscle) of diseased animals was noticeably very soft and individuals that were seen lying upside down on the bottom of the tank subsequently died. On dissection, the intestinal organs released bubbles and a foul smelling odour. Identification of the isolated bacteria using various identification methods indicated that individuals were infected with *Staphylococcus sciuri*. Histopathology of infected individuals revealed spongiosis of the tissues with evident bacterial infection. Neither of these histopathological conditions were seen in healthy abalone. The study concludes that the bacterium *Staphylococcus sciuri* may be the likely cause of abalone mortalities,.

Keywords:

المستخلص: أذن البحر العماني ، *Haliotis mariae* هو النوع الوحيد من أذان البحر الذي يوجد في المياه العمانية . ونظراً لندرة هذا النوع وارتفاع سعره ، فمن الممكن ان يباع في السوق بـ ١٥٠ دولار أمريكي لكل واحد كيلوجرام من الوزن الجاف ، فقد كان ينظر الى مصائد أذن البحر البرية في منطقة ظفار كمصدر دخل قيم على مدى العقد الماضي . يعنى البحث الحالي بدراسة أسباب نفوق أذن البحر والذي أجري في مركز البحوث في مرباط من خلال الفحوص الجرثومية والنسيجية واختبارات التحدي. وجد أن أذن البحر البرية البالغة التي ظلت لمدة عام في التفريخ فقط هي التي لم تظهر عليها اعراض المرض. أما الأفراد المصابة فقد تورمت حول الفم و قد شاب دمها سائل كما وجدت فقاعات في الأمعاء ، و ضعفت قدرتها على الالتصاق . كانت منطقة القدم (العضلات) في الحيوانات المريضة لينة جدا بشكل ملحوظ وقد نفقت اذن البحر شوهدت تقف رأساً على عقب في خزان الماء لاحقاً . وعند التشريح ، أفرزت الاعضاء المعوية فقاعات وروائح كريهة. وقد أظهر الطرق المختلفة للتعرف على البكتيريا المعزولة بأنها كانت مصابة بالمكورات العنقودية السنجابية *Staphylococcus sciuri*. كشفت الدراسات النسيجية عند تشريح الأفراد المصابة عن ظهور أنسجة اسفنجية مع وجود واضح للعدوى البكتيرية. لم تتم مشاهدة أي من هذه الحالات النسيجية المرضية في اذن البحر السليمة. وخلصت الدراسة إلى أن بكتيريا المكورات العنقودية السنجابية *Staphylococcus sciuri* ربما تكون المسبب المحتمل في نفوق أذن البحر.

الكلمات المفتاحية: أذن البحر العماني، مرض بكتيري، المكورات العنقودية

Introduction

Abalone are among the most commercially important marine gastropods, valued for their high market value and nutrient content. However, natural stocks are in a serious decline because of overexploitation and the slow growth rate of populations in their natural habitat. Omani abalone, *Haliotis mariae* is the only abalone species found in Oman. Given the rarity of this species and its high market demand, the wild abalone fishery in the Dhofar region has been regarded as a valuable source of income for the past decade (Al-Rashdi and Iwao, 2008). Dried abalone, for example, can fetch up to US$ 150 kg-1 dry weight. Despite

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the high value of the wild abalone harvest, the industry is faced by many problems including low abundance, small harvest sizes and impacts of toxic blooms of algae (Al-Gheilani, 2009). For the sustainable production of Omani abalone products, the government emphasized the importance of aquaculture with support for initiatives to increase the size of the wild population by funding projects to look at the habitat preferences of abalone (Wall *et al.*, 2012). Currently, there is no health monitoring or surveillance of wild abalone in Omani waters. A broad spectrum of disease agents have been reported to cause mortalities of both wild and cultured abalone (Sawabe *et al.*, 2007). Among the causative agents, Vibrios have been frequently highlighted (Nishimori *et al.*, 1998; Cheng *et al.*, 2004). *Vibrio parahaemolyticus* and *Vibrio alginolyticus* isolated from the haemolymph of moribund abalone have been demonstrated to cause outbreaks of vibriosis in warm water environments (Liu *et al.*, 2000; Lee *et al.*, 2001). According to Muroga (2001), epizootic mortalities in juvenile black abalone, *Nordotis discus discus*, and in *Ezo abalone*, *N. discus hannai*, during seed production and the subsequent nursery stages have been occurring within several hatcheries since the early 1980s. Other reports include that by Moore *et al.* (2001) on the Withering Syndrome of Abalone (WSA) caused by the bacterium *Candiatus Xenohaliotis californiensis* that is reported to kill most of the species of *Haliotis*. Infections are reported to infect the digestive system of abalone with a subsequent loss of body mass.

The Mirbat Abalone Hatchery Research Center in Salalah is a unique research station which has focused on production and the stock enhancement of the Omani abalone, *H. mariae*. When the center was initiated it began by collecting specimens from the wild. They were maintained in land based raceway tanks fed with local seaweed and a commercially formulated diet from Iran. While wild abalone have been stocked in the hatchery, occasional mortality events leading to the loss of more than 50% of the stock are reported. A bacterial infection as the cause of mortality events was suspected. The aim of the present study was to investigate the mortality of abalone at Mirbat Research Center through a comprehensive bacteriological and histopathological evaluation, with subsequent challenge tests to determine whether bacteria were the cause of mortality.

Materials and methods

Abalone collection

Omani abalone, *Haliotis mariae*, used in the present study were supplied by the Mirbat Abalone Research Center. Three different samples were used: 1. Adult specimens from the wild supplied just before the start of the trial; 2. Adults collected from the wild one year ago and then maintained on artificial and natural feed in a raceway system within the Mirbat Research Center; and, 3. Juvenile abalone originating from the hatchery. All samples were maintained in triplicate sets of tanks and the post-mortem evaluation of samples was conducted at the Mirbat Research Center.

Diagnosis and bacteria culture

The external and internal appearance of apparently healthy and diseased abalone were compared and features such as shell appearance and muscle appearance were documented before the abalone were dissected for bacteriology and histopathology. Bacteriology samples from the muscle, intestine and the mouthparts of each abalone were inoculated on TSA + 1.5% NaCl agar plates.

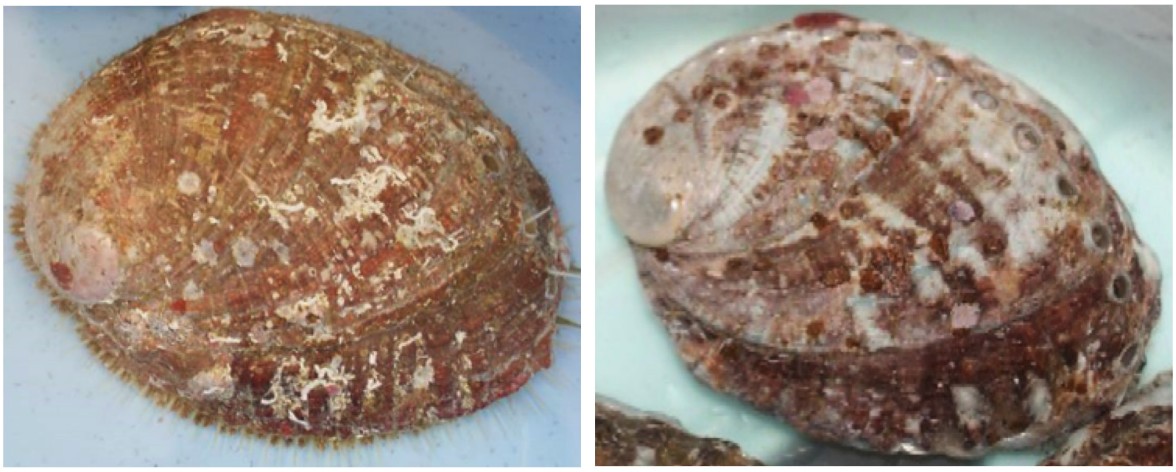

Figure 1. A direct comparison between healthy (left) and diseased (right) abalone specimens. Note that the diseased animal is lighter in appearance, its tentacles and frill are not visible, and this animal has a weak or no attachment to the substrate.

Figure 2. An infected abalone which has some haemorrhaging around the mouth, has shrunken muscle, a swollen intestine with bubbles inside that release a foul odour.

Identification of bacteria

The bacterial colonies were isolated and sub-cultured for identification. Isolated pure colonies were stained with Gram stain and subjected to API Staph biochemical tests. Results were subsequently confirmed by PCR and sequencing of the amplified products.

Histopathology

After taking bacteriological samples, abalone were dissected. Soft tissues were removed from the shell and all viscera, as well as a transverse section of the adductor muscle (foot muscle), and were placed into 10% seawater formalin to fix for at least 24 h. Sections of viscera, mouth parts and foot muscle were placed into plastic cassettes and processed routinely then made 5 µm thick paraffin embedded tissue sections, which were stained with haematoxylin and eosin and cover-slipped.

Challenge test

To confirm that the bacterium isolated from the moribund abalone was responsible for the abalone mortalities, a challenge test was performed. Eighteen similar sized abalone were weighed individually and allocated to one of two 50 litre tanks (i.e. 50 cm × 50 cm × 20 cm; 9 specimens/treatment) filled with aerated 34 ppt salt water. Each abalone was subsequently injected with a bacterial suspension containing 8 × 106 cfu ml/l live isolated bacteria using 1 ml sterile syringe with a 22-gauge needle. The dose administered to each specimen was adjusted to the individual weight of the abalone so that each received an equal dose. The abalone were maintained in the aerated test tanks; 50% of the tank water was replaced daily. Mortalities were recorded hourly over the 72 h challenge period after which all abalone, i.e. both the mortalities and survivors, were screened for the bacteria

Results and discussion

From the gross external and internal morphological appearance, only the adult abalone collected from the wild and maintained in the hatchery unit for a year presented clear signs of disease. The appearance of both healthy and diseased abalone is presented in figure 1. Diseased individuals typically had swollen tissues around the mouth, had only a weak attachment to the substrate, produced a fluid tinged with blood and had air bubbles within their intestine which were evident before post-mortem. The foot muscle region was very soft and diseased specimens were commonly found lying upside down on the bottom of the tank; specimens displaying this behavior subsequently died. On post-mortem dissection, the intestinal organs contained bubbles and these specimens gave off a foul odour (Fig. 2). The symptoms of this case were very similar to a *Vibrio* infection of abalone described by Cai *et al.* (2006). In this later report, the authors remarked that the diseased abalone were lethargic, had a typically white body colour and could not attach to biofilm covered substrates. The bodies of these abalone were visibly shrunken within their shells. By comparison, healthy individuals were active, dark in colour and the body tissues filled the shell space. The same bacterium isolated from the diseased abalone were also recovered from the specimens recently caught from the wild suggesting that the bacterium is ubiquitous but can become virulent when triggered by certain environmental conditions. The factors triggering this virulence though are not known at this stage and further research is required to identify potential factors or activators. The pure, dominant cultures isolated from the abalone were Gram positive and the results from a standard API test (see Table 1) suggested that the cultures were *Staphylococcus sciuri*. Subsequent DNA sequencing of these isolates (see Table 2) confirmed their identity (100% confidence). This is,

Table 1. Biochemical test results of isolated bacteria from the diseased Omani abalone.

Test	Response
Gram stain	+
Oxidase	+
Catalase	+
D-Glucose	+
D-Fructose	+
D-Mannitol	+
Maltose	+
Lactose	+
D-Trihalose	+
D-Mannitol	+
Xylitol	-
D-melibiose	-
Potassium nitrate	+
α-methyl phosphate	+
Sodium pyruvate	-
Raffinose	-
Xylose	+
Saccharose	+
α-methyl glucoside	+
N-acetyl-glucosamine	+
Arginine	-
Urea	-

Table 2. DNA sequence from isolated bacteria from a diseased Omani abalone.

Sequence
TGATCTACGATTACTAGCGATTCCAGCTTCATGTAGTCGAGTTG
CAGACTACAATCCGAACTGAGAATAATTTTATGGGATTTGCTTG
GCCTCGCGGATTCGCTGCCCTTTGTATTATCCATTGTAGCACGT
GTGTAGCCCAAATCATAAGGGGCATGATGATTTGACGTCATCCC
CACCTTCCTCCGGTTTGTCACCGGCAGTCAACCTAGAGTGCCCA
ACTTAATGATGGCAACTAAGCTTAAGGGTTGCGCTCGTTGCGGG
ACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCA
CCACCTGTCACTTTGTCCCCCGAAGGGGAAGACTCTATCTCTAG
AGCGGTCAAAGGATGTCAAGATTTGGTAAGGTTCTTCGCGTTGC
TTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGTCCCCGTC
AATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGA
GTGCTTAATGCGTTAGCTGCAGCACTAAGGGGCGGAAACCCCCT
AACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATC
TAATCCTGTTTGATCCCCACGCTTTCGCACATCAGCGTCAGTTA
CAGACCAGAGAGCCGCCTTCGCCACTGGTGTTCCTCCATATCTC
TGCGCATTTCACCGCTACACATGGAATTCCACTCTCCTCTTCTG
CACTCAAGTTTCCCAGTTTCCAATGACCCTCCACGGTTGAGCCG
TGGGCTTTCACATCAGACTTAAGAAACCGCCTACGCGCGCTTTA
CGCCCAATAATTCCGGATAACGCTTGCCACCTACGTATTACCGC
GGCTGCTGGCACGTAGTTAGCCGTGGCT

to the *authors' knowledge, the first time that a Staphylococcus* infection has resulted in the mortality of abalone. Although species belonging to the *Staphylococcus* group are generally considered to be harmless commensals and spoilage members, Nemeghaire *et al.* (2014), however, emphasised the ecological importance of the *Staphylococcus sciuri*-species group which can act as a reservoir for resistance and virulence genes. The authors concluded, however, that further studies investigating the role of the *S. sciuri*-species group as commensal and pathogenic bacteria were required to fully assess their medical and veterinary importance since certain species belonging to this group have been found to carry multiple virulence and resistance genes including genes implicated in biofilm formation or coding for toxins responsible for toxic shock syndrome and multi-resistance. The findings from the current study lend support to the suggestions made by the latter authors that further studies investigating the potential virulence of *Staphylococcus* species should be undertaken. Histopathology of muscle samples taken from infected abalone muscle reveals numerous bacteria and spongiosis, quite unlike the densely packed tissues seen in uninfected specimens (Fig. 3). The histopathology results suggest that the presence of this bacterium is the cause of the spoilage condition, i.e. a deterioration in muscle quality, air bubbles within the body organs and the production of a foul smelling odour. The response of the abalone following experimental infection by injection was pronounced, with the abalone becoming weak and losing their ability to attach to the sides of their tanks within 24 h post-infection. Within 48 h, all the infected abalone had died whilst there were no losses in the control group. This result lends further support to the proposal that this bacterium was the cause of the original mortality event.

Conclusions

This study concludes that a bacterium isolated from wild caught specimens that were maintained at the Mirbat Research Center for a year and from recently harvested wild abalone was identified as *Staphylococcus sciuri* following standard bacteriology tests and molecular sequencing. Only specimens that had been taken from the wild were infected and exhibited the following symptoms: a swollen mouth; blood tinged fluid; air bubbles within the intestine that gave off a foul odour; softening of the foot muscle; and, very weak adhesive strength. Infected animals were frequently found lying upside down at the bottom of the tank shortly before they perished. Histologically, there was spongiosis of the foot muscle with an evident bacterial infection present. Injection of healthy individuals with the isolated bacterium resulted in mortalities within 48 h. As the bacterium was recovered from a range of specimens taken from the wild, it would appear to be ubiquitous but the conditions triggering its virulence are not yet known. Species belonging to the Staphylococcus group are typically harmless commensals and spoilage members, but the findings from the present study support the suggestion that certain species may serve as a reservoir for resistance and virulence genes but this requires further study.

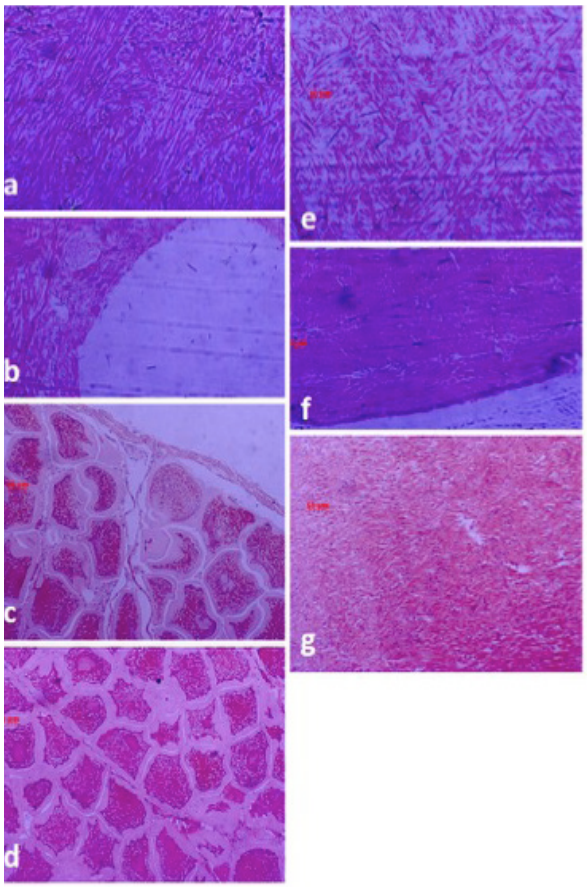

Figure 3. a-g. The histopathology results present clear differences between diseased (a-d) and healthy (e-g) abalone. Note that the healthy specimen has dense muscle whilst the tissues of the diseased abalone appear sponge-like in appearance with evident bacterial growth. .

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