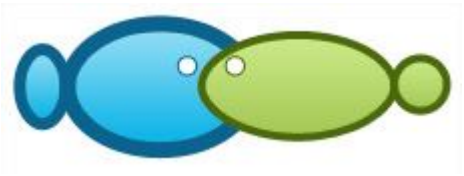

Parasites and histopathology of infected spiny lobster *Panulirus* spp. cultured in outer of Kendari Bay, Indonesia

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Abstract. Parasites infections increase the chance for potential disease outbreak in Indonesian lobster culture industry. Therefore, the aim of this study is to examine and identify parasites infection on larvae and adult lobsters including histology changes in infected organ. There were three species of lobsters examined in this research, namely *Panulirus versicolor*, *P. ornatus*, and *P. longipes*. One species of protozoan parasite (*Vorticella* sp.) was identified dominant from larvae of *P. versicolor*. Meanwhile, some samples adult lobsters of *P. versicolor* and *P. ornatus* cultured in floating net cage had tail rot and burning-like diseases on their pleopod, uropod, and telson. While, in *P. longipes* showed clinical signs as milky disease. Histopathological samples were collected for parasite and tissue changes. There were parasites found to infect all species adult lobsters on external body and gill. *Tokophrya* sp and *Cryptocaryon irritans* trophont were protozoans, *Octolasmis* sp. was crustacean, and one species of Anisakidae nematode. Histopathological examination showed some tissue changes including necrosis in the hepatopancreas, in the gills and in the uropods and telson.
Key Words: histopathology, lobster, microbial infection, tissue changes.

Introduction. The bamboo lobster, *Panulirus versicolor,* ornate lobster, *P. ornatus*, and longlegged lobster *P. longipes* are common lobsters in the ocean, especially around Kendari Bay, South East Sulawesi, Indonesia. The lobster has been captured and exploited in over 90 countries (Philips & Kittaka 2000) which has led to loss of spawning stock and overfishing. Now, in many fisheries lobsters have undergone a dramatic decline in population due to pollution, habitat loss, and susceptibility to infection (Behringer et al 2012); consequently, the lobsters are now considered as rare species.

The lobsters remain an important source of economic value to many communities and they are a preferred seafood in the world. To address the increasing demand for food, lobsters have become a major sector of fisheries production through aquaculture. Active research and development programs throughout the world have sought to develop this sector, but to date none have been successful. During the culture period, lobsters often experience many serious problems, particularly those caused by microbial infections. Emergent infectious diseases of lobster in some places in the world impact to the sustainability of this species (Stewart 1984; Cawthorn 2011; Hoenig et al 2017). Larval stage is the most vulnerable to infections (Kittaka & Booth 2000). To date, seed stock cannot yet be produced on demand.

It has been reported that black gills caused by fungus *Fusarium* sp. appears to have caused mortality in 69.5% of lobster in cage in Vietnam (Nha et al 2009). Transmission of PaV1 virus among lobsters also has been detected (Matthews & Maxwell 2007; Behringer et al 2012). Additionally, several types of bacteria have been isolated from lesions of affected lobsters of shell diseases and were known to be transmissible (Tlusty et al 2007; Tanaka et al 2017) including *Vibrio, Aeromonas, Pseudomonas, Cytophaga* and *Pseudo alteromonas* (Getchell 1989; Smolowitz et al 2005). Further examination of these lobsters revealed four parasite species: *Porospora gigantea*

(Apicomplexa: Sporozoea), *Polymorphus botulus* (Acanthocephala: Palaeacanthocephala), *Hysterothylacium* sp. (Nematoda: Secernentea), and *Stichocotyle nephropis* (Platyhelminthes: Trematoda) (Brattey & Campbell 1986). Therefore, the aim of this study was to examine and to characterize the infestation of parasites potentially cultured lobsters (larval stages and adult stage of the spiny lobsters), and also to look at the histopathological changes of their tissues. This is regarded as a major cause of low production in aquaculture system. Characterization and identification of pathogen is important in disease control and effective treatment.

Material and Method

Lobster sampling and maintaining. Wild lobsters were sampled from South East Sulawesi waters and Banda Sea, Indonesia (Figure 1). They were caught and maintained at outer of Kendari Bay, South East Sulawesi, Indonesia. The research was conducted from June to December 2016.

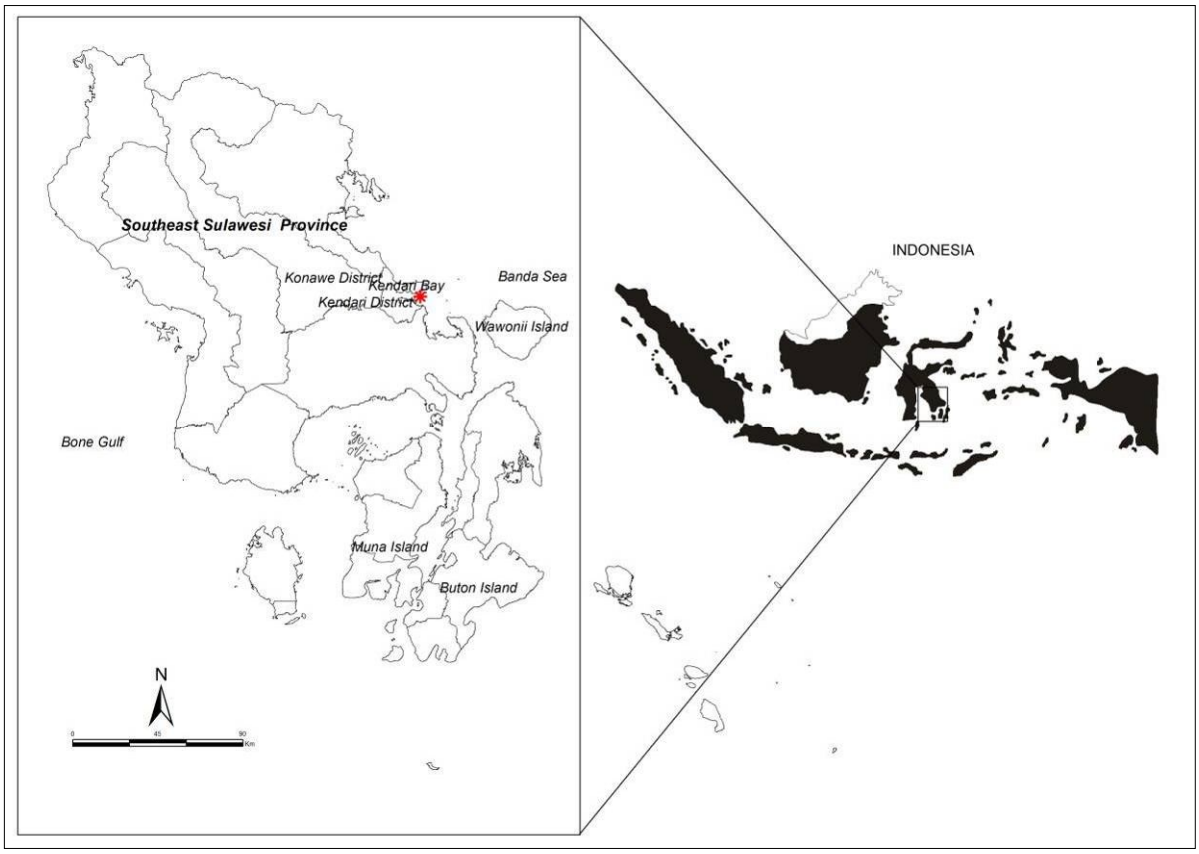

Figure 1. The location of study area (*).

Juvenile lobsters were cultured in 3.5 m x 3.5 m x 3.5 m polyethylene floating net cage with mesh size of net of 1 inch. The measurements of water quality were temperature (26-29^{o}C), pH (7.0–8.0), and salinity (27-31 ppt). During the culture period, the lobsters were fed once per day with sardine fish fresh 10-15% of the body weight of lobster.

Broodstock and larva. Female lobsters that carried eggs under their abdomen were transported to the land using air circulation system. After the egg bearing females arrived at the hatchery, the lobsters were kept in the concrete tank for varying periods of time depending on the development stage of their eggs. The larvae were sampled at 1-6 days post hatching (dph).

Examination for parasites on larvae and adult lobster. The live lobsters were maintained in aerated containers and transferred to the laboratory where they were kept in aquaria for a maximum of two days until they were euthanized to look for parasite examination. Prior to dissection, all lobsters were weighed (Digital Weighing Sigma 1000 g/0.1 g). Larvae were investigated on outer layers attached to the body. Adult lobsters were examined externally (carapace, gills, and fins) and internally (hepatopancreas) for clinical signs of diseases and abnormalities. Lobsters were dissected using general methods (Noga 2010). Briefly, each lobster was systematically searched for parasites. First, the carapace, gills and uropod/telson were examined using a combination of eye and microscope. The outer layer of the carapace was scraped with scalpel and then the scraping was examined using microscope. The gill filaments were removed, and examined individually under microscope and then the scraping into a petri dish filled with clean water to dislodge the parasites and examined under a stereo microscope. Hepatopancreas were dissected and examined for parasites using a microscope. Squash preparations were prepared and examined under a microscope. All detected metazoan parasites were removed and treated with physiological saline (PBS). Photographs were taken of live specimens and then they were killed, fixed and preserved in 70% ethanol. Photomicrographs were taken on a light microscope (Leica, HC) equipped with a digital camera, and observed at 100-400 magnifications. Parasites were classed as ectoparasites or endoparasites.

Identification and determination of prevalence and intensity. External and internal parasites were identified using the relevant works (e.g. Kabata 1985; Smith & Noga 1992; Roberts 1989). Prevalence and mean intensity for selected parasites were determined according to (Margolis et al 1982). All parasites were identified to at least genus or species level and enumerated.

Histological examination. After examination for parasites, the skin, gills, and hepatopancreas were removed from each lobster. Tissues were cut into small sections and fixed in 10% formaldehyde, especially for tail fans were decalcified in a standard decalcification fluid first. Fixed tissues then were processed for histological preparation (under guidance of Takashima & Hibiya (1995) and Mumford (2007)). Briefly, water from the tissues was removed by dehydration and then followed by clearing. Dehydration was done with a series of alcohols: 70% to 95% to 100%, while clearing was done with xylene. Finally, the tissue is infiltrated with the embedding agent. Embedding process was done through placing the tissue in fresh paraffin wax and the latter allowed to cool paraffin, then cut into sections that can be placed on a slide. Slides were stained with haematoxylin and eosin (H & E). Prepared sections and stained were examined under a light microscope (Olympus BX53), photograph was taken (Olympus DP21 camera with Stream program), and histopathological alterations were evaluated.

Results

Parasites recovered from larvae. Parasites found during this study on larvae 3-6 dph was Protozoan *Vorticella* sp., which were observed in outer layers of larvae (Figure 2), therefore it was included in ectoparasites. *Vorticella* sp. are small - unicellular ciliates often found on carapace and skin of many fish species. *Vorticella* sp. have low host specificity and therefore are widely distributed. Most families of freshwater fish harbor *Vorticella* sp. (Smith & Noga 1992; Hoffman 1999). They have also been reported on amphibians, crustaceans, mollusks and coelenterates. According to Hoffman (1999), some *Vorticella* sp. are pathogenic. Classification of *Vorticella* sp. in detail were: Phylum Ciliophora; Order Peritrichida; Sub Order Sessilina; Family Vorticellidae; Genus *Vorticella* (Kabata 1985).

There are various species of Protozoan parasites, which are distinguished by their cillia. Phylum Ciliophora is characterized with short cillia. Genus *Vorticella* also consisted of numerous species, attach to the substrate (host) with stalked-like, namely slender, cylindrical, or contractile stalk (Kabata 1985; Lom 1995).

Vorticella is bell shaped with big mouth (peristome). Cell color varies, but it is usually yellow or green. They live in freshwater and sea water, and the adult stage is attached to an object, animal, or aquatic plant. Peritrich and suctorian ciliates are often found as commensals on the external surfaces of crustacean embryos, such as *Corthunia* sp., *Epistylis* sp., and *Vorticella* sp. which usually infect lobster cultures (Shields 2011). They attacked the body including the eyestalk, antenna, uropod, and eggs. In this research, we found that the stalked of parasite *Vorticella* penetrated the inner part of the larvae body.

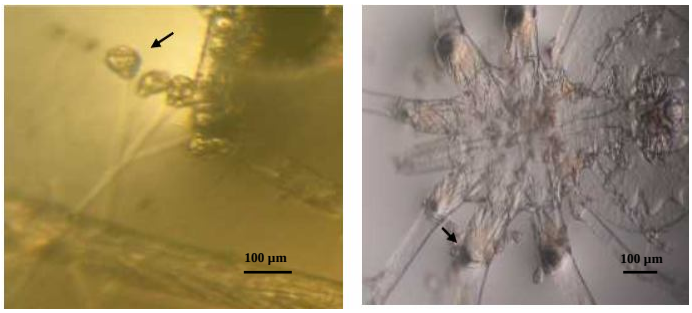

Figure 2. *Vorticella* sp. (arrow) on larvae ornate lobster aged 3-6 dph.

Parasites recovered from adult lobster. Four genera of ectoparasites were found, which were classified in three phyla. The first phylum was Arthropoda (Crustacean) with *Octolasmis* sp. (Figure 3). The second phylum was Nemathelminthes, with the class Nematoda, Family Anisakidae at low intensity found on gill (Figure 4). The third and fourth parasites found from adult lobsters were ciliated protozoans, consisting in two genera such as *Tokophrya* and *Cryptocaryon* at high intensity (*Tokophrya* from Order Suctorida, while *Cryptocaryon* is included in Order Prorodontida). The parasites can be seen in Figures 3-5.

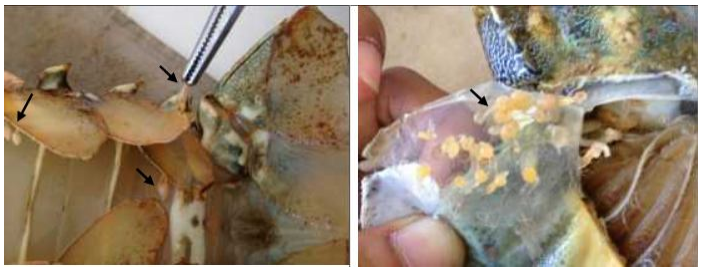

Figure 3. *Octolasmis* sp. on exoskeletons and gill chamber of adult lobster.

Figure 4. Nematode on exoskeletons of adult lobster.

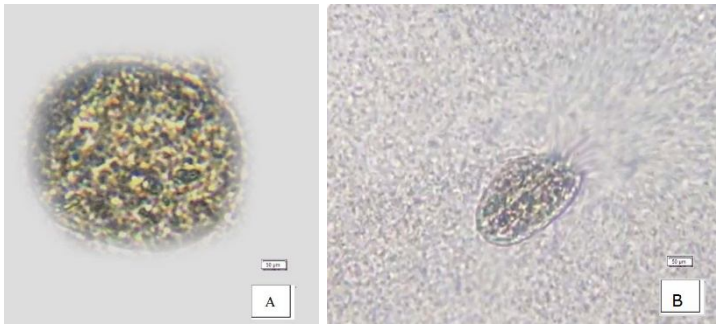

Figure 5. *Cryptocaryon irritans* trophont (A) and *Trycophrya* sp. (B) protozoan parasites of fresh mount gill filaments of adult lobsters.

Octolasmis sp. is more commonly found attached to the outer body of a lobster and on its gills (Figure 3). These organisms are also commonly found attached to other crustaceans, but are more numerous on crab. It can be explained that due to crabs have a terminal molt (stop sheding), the infestation by *Octolasmis* barnacles can not be eliminated in the future moults. Some of them are used as an indicator of the host health (Shields 2011). Barnacle *Octolasmis* sp. use a number of crustaceans as its host, including lobsters *Panulirus* and crabs *Scylla* (Zafran et al 1998). The barnacle lives on the exoskeletons and gill chamber of its host. They are very adaptable to the host life cycle. The species *Octolasmis bullata* reach 48% in the prevalence of lobster *P. polyphagus* in Singapore (Jeffries et al 1982). From our data, it is known at the prevalence of 50% and high intensity (64 individuals lobster^{-1}). Moreover, lobsters were infected with *Trycophrya* sp. by prevalence of 100% and high intensity (69 individuals lobster^{-1}), besides uncounted number of *C. irritans* trophont (Figure 5). Heavy infestation of *Octolasmis* sp., *C. irritans*, and *Trycophrya* sp. (with or without tentacles) on gill samples may indicate that the organisms in this study had significant pathology, particularly those involving the respiration system of lobsters, they can cause alterations to host behaviours and even mortality. In some cases, *Trycophrya* sp. showed commensal association with fish, but in high intensity it can cause mortality by blocking the flow of oxygen (Durborow 2003). In large numbers of trophont stage of *C. irritans* ciliate parasite attached to these lobster gills would cause harmful effects, as its effects on feeding, respiratory rate and ionic regulation of marbled rockfish *Sebastiscus marmoratus* (Yin et al 2014). The effects of parasites infection in many cases have been observed to reduce growth between molts. Host should get priority for treatment so that the infection will not spread.

Adult spiny lobsters were observed to have higher rate of parasites, diseases and symbiosis than the larval stage this far. The disease is more frequent and serious in post larva and early juvenile stage (Shields 2011). For example, mortality due to *P. argus* virus (PaV1) attacked was highly correlated with smaller sized lobsters (Butler et al 2008). *Vibrio* infection can cause serious problems in larval culture of spiny lobster (Bourne et al 2004; Goulden et al 2012). The clinical signs of infected larva were hepatopancreas opaqueness and had small red spots troughout the body with bacterial proliferation in the midgut gland (hepatopancreas).

Histopathology examination. A variety of histopathological lesions were observed in all organs examined. Histopathological changes, including hemorrhage, tubular epithelial cell vacuolization, cell necrosis and mineralization were found in uropod, telson, gill and hepatopancreas tissue. Often, these were generalised reactions, but in many cases they were closely associated with, and likely to be a response to, parasitic infection. This is suggestion not only from histological findings of parasites surrounded but also by pathological changes in host tissue, but also may be in relation with environment or water quality.

Macroscopically, hepatopancreas was seen colour changes to pale and brown due to pigment loss in the connective tissue (Figure 6). Moreover, histologically hepatopancreas of lobsters showed hemorrhage, increased vacuoles with phagocytic content in the cytoplasm, tubules were disfigured and the lumen size was enlarged

(Figure 7). The epithelial cells of the hepatopancreatic tubules, were also seen. This research is in line with Sakhare & Kamble (2014) who found that hepatopancreas was the sensitive organ to aquatic pollution in crabs. These tissue changes might affect the lobster metabolisms as tubule plays a role in filtration by active transport (Smith et al 2006).

Figure 6. Severe degeneration of lobster hepatopancreas which appears pale to brown when necropsied.

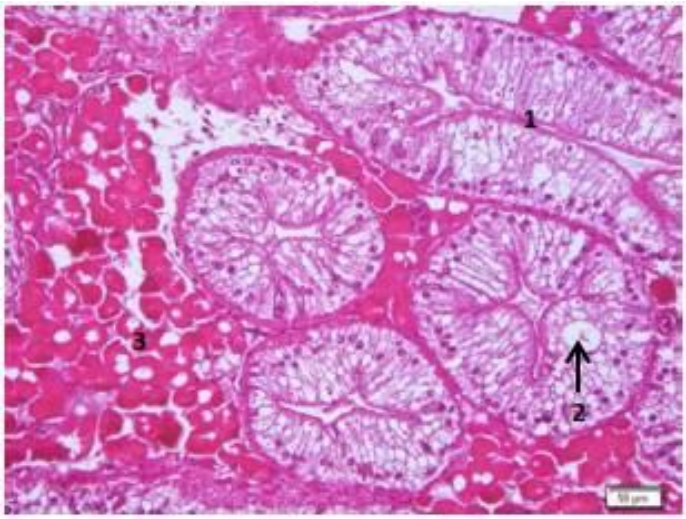

Figure 7. Lobster hepatopancreas histopathology shows haemorrhagica nephritis: (1) tubules, (2) vacuolization tubular epithelial cells, (3) haemorrhage (HE.200x).

In some samples in this study were found infected lobsters with the disease symptoms of soft tissue and milky-colored hemolymph (Figure 8). Disease called milky haemolymph disease (MHD) is also often seen as a symptom to mass mortality occurred at lobster farms in Gerupuk Bay of Lombok, Indonesia (Koesharyani et al 2016). MHD was not infected only in lobster, but also in other decapod crustaceans such as shrimp *Penaeus monodon* and crab *Carcinus maenas* (Nunan et al 2010) with the pathology appearing similar.

During this study, gill tissue was the most heavily changed by bacteria and parasite infection. Gill lesions consisted of severe inflammatory reactions with necrosis. It is well known that as biomarker, gills are among the most sensitive organs, which react first in impaired environmental conditions in the water since respiration, osmoregulation and excretion are performed through the gills (Camargo & Martinez 2007; Raskovic et al 2010). Gill necrosis (Figure 9) was detected in this investigation. Necrosis is an irreversible damage to the gills that occurs following exposure to toxicants and/or chronic irritants (Takashima & Hibiya 1995; Mallatt 1985). Necrosis was prominent on the gills of

lobsters. These changes were always more severe at the site of parasitic and bacterial infection. Outer layer lesions were principally associated with infections parasite, which led to necrosis of the epidermal layer.

Figure 8. Milky appearance in muscle and hemolymph of *P. longipes.* Basil negative bacteria were found in hemolymph.

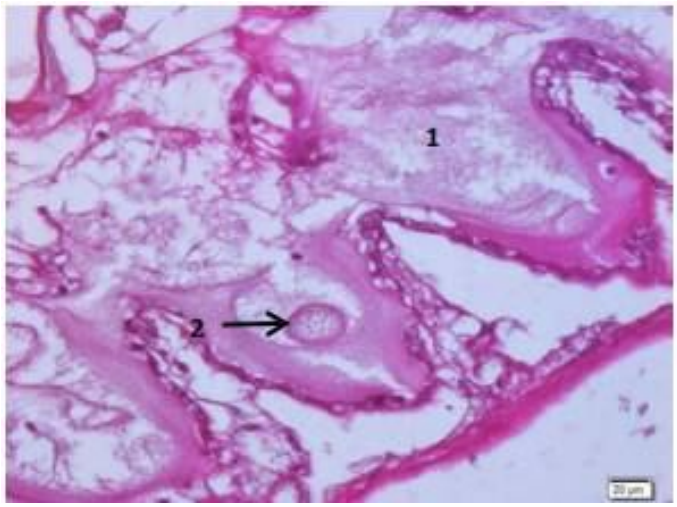

Figure 9. Gill necrosis: (1) a colony of bacteria (coccus) and (2) parasite (HE.400x).

Some infected lobsters also showed tail fan erosion. In the telson section that appears erosive lesions and such burns then were made histologic specimen (Figure 10). The histological evaluation showed necrosis and the presence parasite around tissues. Some of the characteristics seen in this sample showed similarities with shell diseases syndrome as disclosed by Mancuso et al (2010).

Parasites abundance can be explained by environmental conditions in the cage such as stocking density and/or stress (Roberts 1989; Raskovic et al 2010). Since organic matter becomes a substrate for resident microorganisms, once it goes up, the number of parasites will also increase. Further, the causes of diseases are in most cases attributable to feed quality and feeding management (Jones 2015). Elevated organic pollution in lobster culture cage is often a result of excess feed and its decomposition. This affects lobster gills making them more sensitive to pathogens and parasites. On the other hand, hepatopancreas tissue reaction to organic pollution showed gross signs of disease included brown focal necrotic lesions.

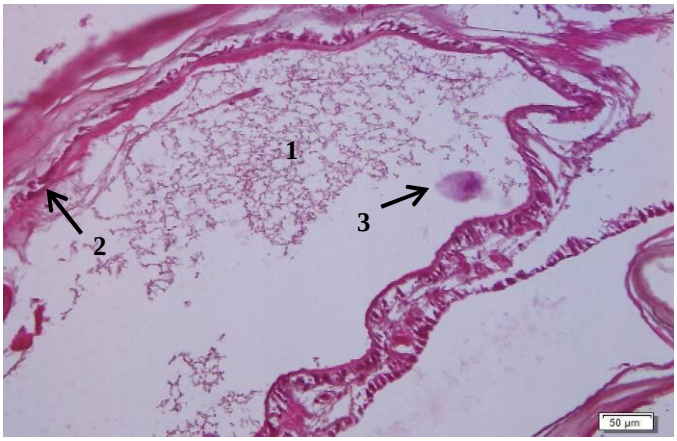

Figure 10. Telson lobster necrosis: (1) bone marrow, (2) necrosis, and (3) parasite (HE. 200x).

Conclusions. Both larvae and adult lobster were infected by parasites at high levels on gill filaments and bodies. Dominant species of parasites are protozoan and crustacean. The main effect of parasite load was that it growth between molts. Necrosis, haemorrhage and vacuolization of hepatopancreas tubule epithelial reduced cells and gill are among the principal histopathological changes due to disease. Environmental management of cage lobster culture is needed to reduce mortality and to increase production.

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