Monogenean disease in cultured grouper (*Epinephelus* spp.) and snapper (*Lutjanus argentimaculatus*) in Khanh Hoa province, Vietnam

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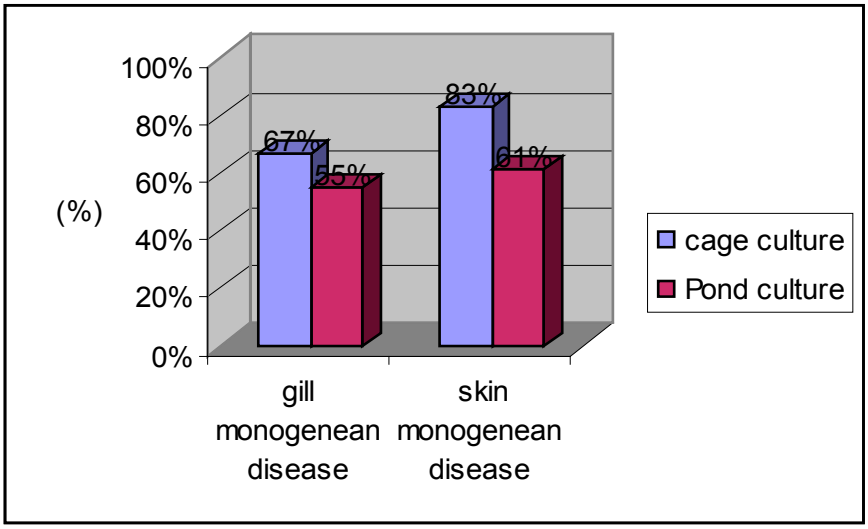
Marine fish culture has developed relatively recently in Khanh Hoa province with several high-value species such as grouper (*Epinephelus* spp), snapper (*Lutjanus argentimaculatus*), sea bass (*Lates calcarifer*), and cobia (*Rachycentron canadum*). However, these have suffered from serious disease issues under culture. Monogenean disease causes heavy losses for farmers. The disease can cause mortalities in cultured groupers and snappers, especially in small size fish (<20cm). Parasitic monogenean disease affecting the gills and skin of marine cultured finfish species have been studied by researchers such as Fris Jonhn, Des Roze (2002), Isti Koesharyani, Zafran, Kei Yuasa, Kishio Hatai (1999), Leong tak Seng (1994) and Yaowanit Danayadol (1994).
In this article, we present several epidemiologic characters of the disease and the monogenean species that are pathogens of the disease in cultured marine fish in Khanh Hoa province, Vietnam. In addition, we also report on tests of chemicals and treatments including formalin, hydrogen peroxide, Hadaclean and fresh water baths.

Prevalence of monogenean disease in skin and gill of cultured groupers and snappers in cages and ponds in Khanh Hoa province (n = 63).



Materials and methods

Data was collected from the local Aquaculture Department on the marine fish cultural areas in the province. For epidemiological investigation, 63 marine fish farms (of which 30 were cage fish culture farms and 33 were pond fish culture farms) were interviewed. In addition, we directly collected diseased and healthy fish specimens in order to examine the clinical signs and causative agents, allowing us to determine the frequency, clinical symptoms, harmful effect, seasonality and which life stages are affected the most. Fish specimens (both healthy and diseased) were collected randomly (8-10 fish/ cage or pond). Overall, 210 fish were collected (of which 55 skin disease samples, 75 gill diseasd samples and 80 healthy fish samples were obtained). Fish were moved to laboratory under aeration. The samples were divided into two groups by size; smaller (< 200g/individuals) and larger (>200g/individuals). Parasitic determination on/in fish was made following the methods of Dogiel, 1929 and Haky, 1993. Monogenean classification based on morphological characteristics. Infectious level of the parasite in fish was assessed based on two indicators:

Infection rate (%) = the amount of infected fish/tested fish x 100

Infection intensity: In skin parasites, the number of parasites per cover glass of skin mucus calculates infection intensity. In gill parasites, the number of parasites per gill sheet calculates infection intensity. The amount of parasites was determined by stereo dissecting microscope.

For experimental treatment, we used diseased grouper specimens (13–15 cm length). All had been highly infected by monogeneans in the gills with typical clinical signs. The monogenean infection intensity and prevalence before and after treatment were measured to evaluate the effect of the treatment method. Some chemicals were tested such as formalin (200; 250 and 300 ppm), hydrogen peroxide 30% (300, 450 and 600 ppm), Hadaclean (5, 10 and 15 ppm). The tested chemical concentrations for the experiments were based on LC_{50} of every chemical. Each experiment was repeated two times in 120 liter resin tanks. Diseased fish were bathed by chemical solutions for 30 minutes with different concentrations, two times in a week. Health of fish, survival rate, infection intensity and prevalence after chemical treatment were examined and discussed.

Results and discussion

Clinical signs: The diseased fish often displayed lethargic swimming; loss of appetite, and secretion of mucous fluid from damaged gills and skin. If monogenean intensity in skin was high, diseased fish showed ulcerative lesions in skin in final stage of the disease. Conversely, if parasite density was high in the gills the operculum would be swollen, a lot of mucous fluid secreted, and the skin would be dark and in the two cases, mass mortality occurred in small fish (<200g/ind). The parasites in fish skin were flat, oval shape

monogeneans, look like sesame seeds while and the other parasites in fish gills were thin, flexible monogeneans. In the diseased fish, there were no other parasitic pathogens observed in internal organs.

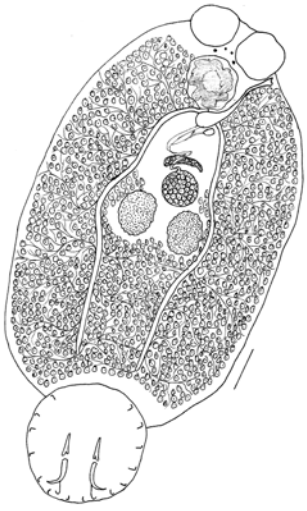
Epidemic character: In marine fish cultured ponds/cages in Khanh Hoa, the frequency of the disease is very high, 71.4% the disease in skin and 60.3% of disease in the gills of fish (with n=63). Outbreak of the disease in marine fish cages was higher than pond culture systems. Investigation showed that occurrence of the disease was year-round but it often concentrated in the dry season (from April to Sept) when temperatures were high (30 – 34°C). The disease affected mainly small fish (less than 200gram per individual).

Causative agent: From skin diseased fish specimens with ulcerative lesions on the bodies (40 groupers and 15 snappers), we detected two parasitic genera belong to class Monogenea. They were *Benedenia* and *Neobenedenia* (Capsalidae) affecting the skin and fins of diseased fish. Of these, *Benedenia epinepheli* were found on the skin of diseased grouper, other species also belong to two the genera, but the names of species were not determined yet. In diseased fish, the infection level of the parasites was very high: prevalence was 100% and the intensity was 5–15 parasites/cover glass of skin mucus. We also detected some species of monogenean in skin of healthy fish samples (n = 80), but infection level was low, prevalence was 16,6%; intensity was 0-1 parasite/cover glass of skin mucus in groupers (n=60) and 15% prevalence; 0-1 parasite/cover glass of skin mucus in snappers (n=20).

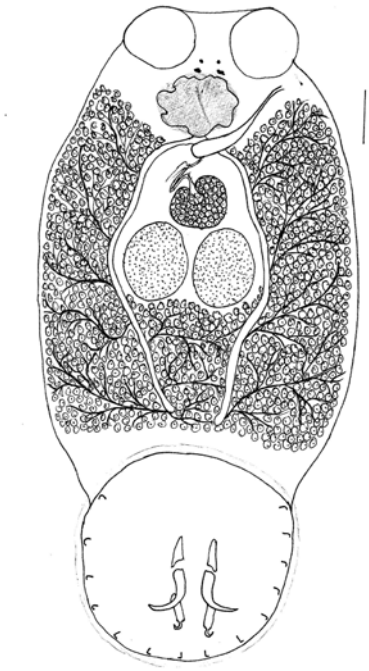
From gill diseased fish specimens (45 groupers and 30 snappers) with swollen operculums we detected some other species of monogenean. They were *Pseudorhabdosynochus epinepheli*, *Pseudorhabdosynochus* sp; *Diplectanum* sp and *Ancyrocephalus* sp; in grouper these had 100% prevalence; intensity was 55-253 parasites/gill sheet; in snapper they had 100% prevalence; intensity was 40-130 parasites/gill sheet. We also found the same species of monogenean in the gills of healthy fish samples (n=80), but at a low level of infection: 33.3% prevalence; 0-6 parasites/gill sheet in grouper and 25%, 1-5 parasites/ gill sheet in snapper. We believe that *Benedenia epinepheli*, *Neobenedenia* spp (Capsalidae) are primarily skin parasites and *Psedorhabdosynochus epinepheli*, *Psedorhabdosynochus* sp, *Diplectamum* sp and *Ancyrocephalus* sp. are primarily gill parasites cauing monogenean disease of cultured marine fish species in Khanh Hoa province.

Treatment: The fish samples used in the experimental treatment were infected with monogenean parasites on the gills. The average infection intensity was 2.5 parasites per gill sheet. We found that formalin 200-250ppm, hydrogen peroxide 100 – 200ppm and Hadaclean 5-10ppm were effective for killing monogenean parasites on the gills of small grouper (13-15 cm) after two bath treatments in a week. The treatment was carried out in conditions of water temperature 28 – 30°C, salinity 30-33% and pH 7.9-8.2. These treatments killed from 85.8 to 100% of monogenea in the gills of diseased fish. The experimental fish were still healthy after the treatment. Our research also showed that formalin at 300 ppm and Hadaclean at 15 ppm could kill 100%

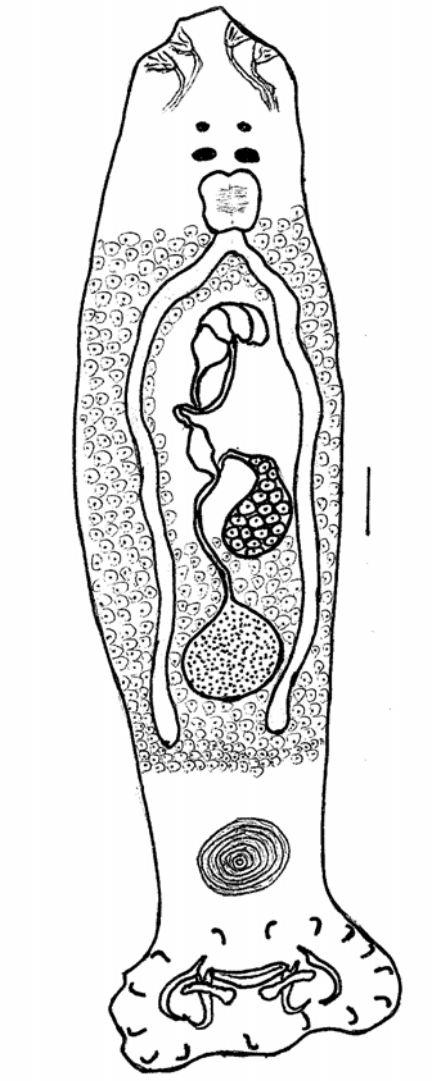
Monogenean species affecting the skin (below) and gills (right) of cultured grouper and snapper in Khanh Hoa, Vietnam



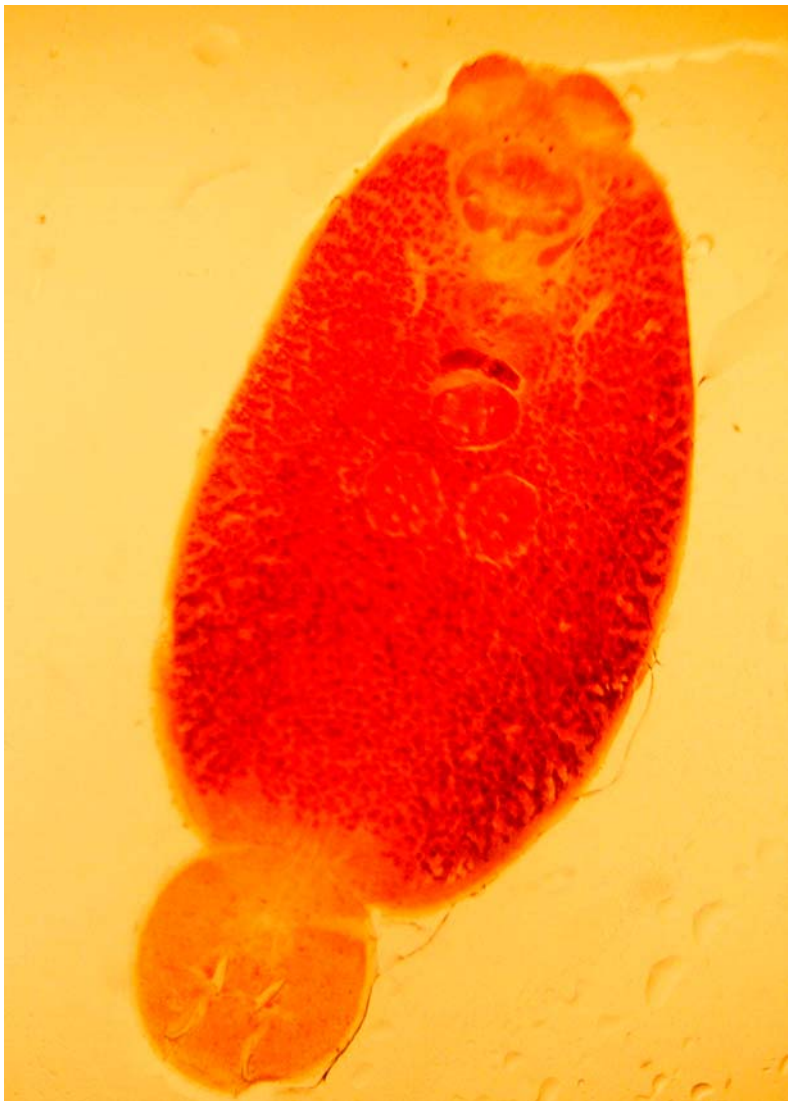
Neobenedenia sp.



Benedenia epinepheli



Pseudorhabdosynochus epinepheli



Neobenedenia sp. with carmine stain.

monogeneans on the gills of fish very quickly, but this concentration impacted badly on the health of fish.

Conclusions

In Khanh Hoa province, monogenean disease often occurrs in cage/pond cultured grouper and snapper. The frequency of the disease is high at 71.4% with disease in skin and 60.3% with disease in the gills of fish (with n =63). Prevalence of disease in cage cultured fish was higher than in pond cultured fish. The disease occurs all year round but is worst during the dry season from April to September, and mainly affects small fish less than 200g in weight. In diseased fish samples, some parasitic genera belong to Monogenea have been detected. *Benedenia* spp and *Neobenedenia* spp (Capsalidae) often parasitized the skin and caused ulcerative lesions on the body of fish. Other genera like *Pseudorhabdosynochus* spp; *Diplectanum* spp and *Ancyrocephalus* spp parasitized the gills causing difficulty in respiration, dark skin, swollen operculum and mortality, especially in small fish.

Using formalin (200–250 ppm), hydrogen peroxide 30% (300–600ppm) or Hadaclean (5–10ppm) to bath diseased fish for 30 minutes twice a week was effective to treat this parasitic disease in grouper.

Acknowledgment

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