Veterinary Parasitology 174 (2010) 359–365

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Short Communication

Cellular response in semi-intensively cultured sea bream gills to *Ergasilus sieboldi* (Copepoda) with emphasis on the distribution, histochemistry and fine structure of mucous cells

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ARTICLE INFO

Article history:
Received 23 March 2010
Received in revised form 4 August 2010
Accepted 19 August 2010

Keywords:
Aquaculture
Sea bream
Crustacean ectoparasite
Gill cell responses
Mucous cells

ABSTRACT

Light and ultrastructure studies were carried out on gill of sea bream, *Sparus aurata* L., naturally infected with *Ergasilus sieboldi* (Copepoda) to assess pathology and host cell responses. Thirty *S. aurata* were examined, and 23 (74%) were infected, the intensity of infection ranging from 3 to 50 parasites per host. The copepod encircled gill lamellae with its second antennae, occluded arteries, compressed the epithelium, provoked hyperplasia and haemorrhage, and often caused tissue disruption. Adjacent to the site of attachment, rodlet cells (RCs), mast cells (MCs) and mucous cells were observed. In parasitized fish, mucous cells were more abundant in infected gills than in uninfected (*t*-test, $P < 0.01$), while no significant differences were encountered in the numbers of RCs and MCs between gill of infected and uninfected fish (*t*-test, $P > 0.01$). In both infected and uninfected gill, the RCs were within the primary lamella and also sometimes occurred in secondary lamella. In healthy and infected gill, MCs were free within the connective tissue inside and outside the blood vessels of the primary lamellae and made close contact with vascular endothelial cells. Infected and uninfected gill mucous cells stained positively for neutral muco-substances (PAS positive). In all sea bream, gill mucous cells presented a central or eccentric electron-dense core within the mucus granules.

1. Introduction

Intensive aquaculture has an important role in supplying food to a growing world population, but is also faced with disease problems in fish farms and to transmission of diseases between wild and farmed fish (Guo and Woo, 2009). The most common marine ectoparasites of fish are crustaceans belonging to the order Copepoda. These crustaceans are damaging parasites on both farmed and wild fish (Costello, 2006).

The attachment and feeding activity of parasitic copepods on the skin and gills can induce localized damage, the pathological effects of which ranges in severity depending upon the species and the intensity of infection (Noga, 1996). Cell infiltration and hyperplasia at the site of attachment has been reported (Bennet and Bennet, 1994, 2001; Roubal, 1999; Covello et al., 2009; Andrews et al., 2010).

The Ergasilidae is a family of parasitic copepods with about 140 species, more than 40 of which occur on fish from estuarine and marine habitats (Abdelhalim et al., 1993). Ergasilid copepods have been recorded in a variety of non-salmonid finfish reared in marine and brackish waters. Outbreaks of disease due to ergasilids are a major source of copepod-induced mortality in brackish and freshwater fish culture (Johnson et al., 2004). Host mortalities have been reported in fish in Israel (Paperna, 1975), Taiwan (Lin and Ho, 1998), Japan (Yamashita, 1980), China (Wang et al., 2002), the USA (Benetti et al., 2001), South Africa (Tsotetsi et al., 2005), Hungary (Molnar and Székely, 2004), and Italy (Dezfuli, unpublished data).

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doi:10.1016/j.vetpar.2010.08.024

Investigation of sea bream immune responses to *Ergasilus sieboldi* will help estimate the level of damage that may occur in the event of an epizootic affecting commercially cultured fish and will be a first step towards developing potential mitigation strategies. The rodlet cell is an inflammatory cell type closely linked to other piscine inflammatory cells (Dezfuli et al., 2000; Manera and Dezfuli, 2004; Reite, 2005; Vigliano et al., 2009). Published records on the presence of RCs in relation to ectoparasites are rare, with the available information referring to protozoans (Leino, 1979), monogeneans (Dezfuli et al., 2007b) and to copepods (Dezfuli et al., 2003). Mast cells, also known as eosinophilic granular cells, have been recognized in gills, skin, and alimentary canal of several species of fish, and in fish infected with metazoan parasites (Reite and Evensen, 2006; Dezfuli et al., 2007a, 2008, 2009, 2010b). It is known that they play a role in fish immune responses (Silphaduang and Noga, 2001; Colorni et al., 2008; Dezfuli and Giari, 2008; Mulero et al., 2008; Andrews et al., 2010).

Fish mucus is involved in a wide range of functions, including ionic and osmotic regulation, excretion, respiration, feeding, reproduction, and in protection against, and resistance to, disease (Shephard, 1994; Yan et al., 2007; Schroers et al., 2009). An increase in mucus secretion of fish has been shown to be linked with an increase in mucous cell density (Bosi et al., 2005; Covello et al., 2009; Dezfuli et al., 2010a). Mucous cells were encountered in both infected and uninfected gills of *Sparus aurata* and they appear atypical. The main aim of this investigation was to examine the cellular responses of *S. aurata* to *E. sieboldi* infection.

2. Material and methods

From September to November 2009, 30 specimens of semi-intensively cultured sea bream measuring 17.70 ± 2.20 cm in total length (mean ± SD) and weighing 79.90 ± 26.15 g (mean ± SD), were obtained from a local semi-intensive fish farm "Valle Ca' Zuliani" (Pila di Porto Tolle, Rovigo, Italy). The fish were transported live to the laboratory where they were anaesthetized using MS222 (Sandoz) and their spinal cords severed. The gills were examined for the presence of ectoparasites, and infected filaments were removed and fixed in chilled (4 °C) 10% neutral buffered formalin for 8 h. The samples were then wax embedded following standard procedures and 5 μm sections were stained with Hematoxylin–Eosin, Giemsa or Alcian Blue-PAS. For light and electron microscopy, infected gill filaments up to 7 mm in diameter were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer for 3 h at 4° C before post-fixing them in 1% osmium tetroxide in the same buffer for 3 h. The specimens were dehydrated through a graded acetone series before being embedded in Epoxy resin (Durcupan ACM, Fluka). Semi-thin sections (1.5 μm) were cut on a Reichert Om U 2 ultramicrotome using glass knives and then stained with toluidine blue. Ultra-thin sections (90 nm) were stained with a 4% uranyl acetate solution in 50% ethanol and Reynold's lead citrate and examined using a Hitachi H-800 electron microscope.

For comparative purposes, gills and pieces of middle intestine of 7 uninfected sea bream were similarly processed.

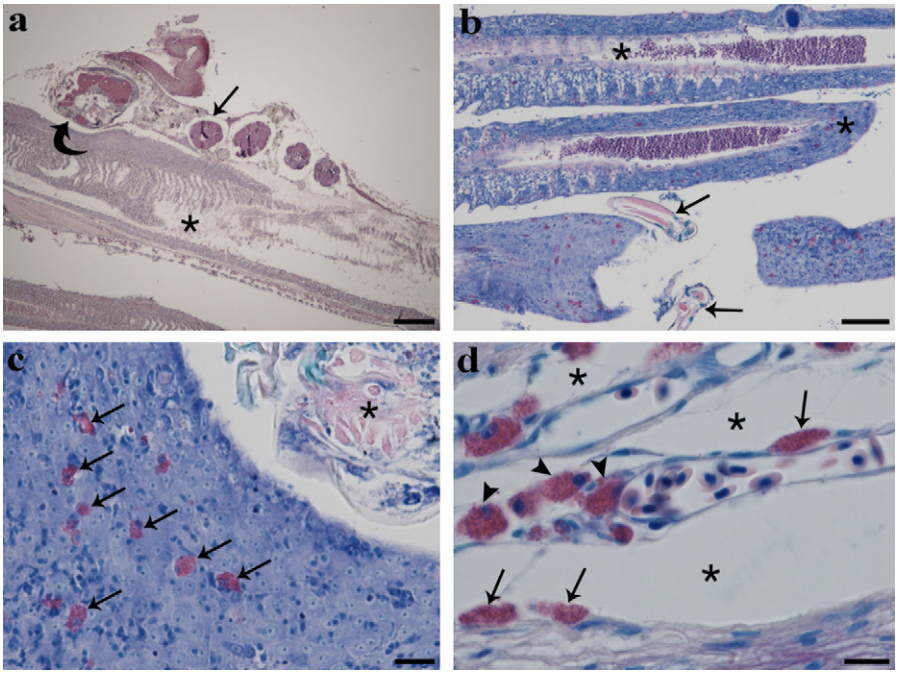

Fig. 1. Histological sections of gill of *Sparus aurata* infected with *Ergasilus sieboldi*. (a) Attachment of adult female *E. sieboldi*, the body lay between the hemibranchs with the axis (arrow) parallel to the primary lamella. Note destruction, desquamation and necrosis of the secondary lamellae (asterisk), curved arrow shows mouth parts of the copepod, scale bar = 200 μm, Hematoxylin–Eosin. (b) Two uninfected lamellae (asterisks) and one with a copepod are visible, second antennae of copepod (arrows) disrupted the infected gill filament, scale bar = 100 μm, Giemsa. (c) Within the disrupted gill filament the presence of several mast cells (arrows) is noticeable, asterisk shows the copepod, scale bar = 20 μm, Giemsa. (d) Micrograph shows several mast cells in vicinity (arrowheads) and inside (arrows) the vessel lumen (asterisks), scale bar = 10 μm, Giemsa.