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NOTE

Lernanthropus kroyeri infections in farmed sea bass *Dicentrarchus labrax*: pathological features

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ABSTRACT: Twenty sea bass *Dicentrarchus labrax* L. from a fish farm (floating cage) in Greece were examined for the presence of parasites. The gills of 7 (35%) fish were infected with adult female specimens of the parasitic copepod *Lernanthropus kroyeri* van Beneden, 1851, and the intensity of infection ranged from 1 to 24 parasites per host. The most infected portion of the gills appeared to be the primary lamellae. Erosion, desquamation and necrosis of the secondary lamellae were noticed near the site of copepod attachment; furthermore, the terminal claw of the second antennae lacerated tissue and vessels of infected gill. Parasitism by *L. kroyeri* affected the host's condition factor (mean ± SE in uninfected vs parasitized; 1.88 ± 0.04 vs 1.66 ± 0.12; $p < 0.05$).

KEY WORDS: *Dicentrarchus labrax* · Crustacean parasite · *Lernanthropus kroyeri* · Histopathology

INTRODUCTION

Dicentrarchus labrax L. is one of the most economically important species in Mediterranean mariculture; intensive farming for this species was developed in Italy between 1970 and the early 1980s, and then developed in other Mediterranean countries, such as Greece, which is the greatest producer and exporter of *D. labrax* in Europe (Franchetti 1983, Ravagnan 1992, FAO 2000). Despite its importance, there is little information on its pathology associated with ectoparasites, although there are records of other crustacean parasites on sea bass from Greece, which appear in an account by Papapanagiotou et al. (1999). These authors reported a cumulative mortality of 10.75% in sea bass as a consequence of cymothoid isopod infections. The present study forms part of our on-going study investigating the pathological effects of fish parasites on their hosts. A literature review of past data indicates that fish gill pathology is caused primarily by the copepod genera *Ergasilus* (Rogers 1969, Einszporn-Orecka 1973, Paperna & Zwerner 1982, Dezfuli et al. 2003) and *Dissonus* (Bennett & Bennett 1994, 2001), and little work has been undertaken on the effect of *Lernanthropus* Blainville, 1822 on host gills. This genus is among the most common genera of parasitic copepods, and its members are parasitic on the gills of marine teleosts, with a preference for those hosts inhabiting warmer waters (Kabata 1979).

Therefore, the main purpose of this investigation was to study the histopathological condition of sea bass gills infected with *Lernanthropus kroyeri* van Beneden, 1851 and to evaluate the potential detrimental effects on fish.

MATERIALS AND METHODS

Twenty sea bass *Dicentrarchus labrax* were randomly sampled from a Greek floating-cage farm. The standard length of the fish, the total body weight and the weight of the liver were recorded before a complete necropsy was performed on all fish organs, and condition factor (weight × standard

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length^{-3} × 100) and hepatosomatic index (liver weight × somatic weight^{-1} × 100) were then calculated. Pieces of gill tissue with the parasite attached, and pieces of uninfected gill tissue (as a control) were isolated, fixed in 10% buffered formalin, dehydrated through a graded ethanol series, paraffin-embedded, and 6 µm thick histological sections taken and stained following the Azan-Mallory method. Fresh and May-Grünwald Giemsa-stained smears were obtained by means of gentle tissue apposition on microscopic slides. Scrapes were taken from the intestine, liver, kidney and spleen, and examined for occurrence of protozoa, and the excised gills were examined for monogeneans with the aid of a stereomicroscope.

Part of the formalin-fixed tissue was then washed in 0.1 M cacodylate buffer, post-fixed for 2 h in 1% osmium tetroxide at 4°C, washed again in the same buffer for 5 h, and then dehydrated through a graded ethanol series and critical-point dried. After mounting, the material was sputter-coated with gold using an Edwards S 150 sputter coater and examined with a Cambridge Steroscan S 360 at an acceleration voltage of 20 kV. Biometrical data were analysed statistically (1-way ANOVA) in order to detect differences between parasitized and uninfected fish. The effect of infection intensity on the different measured and screened parameters was also evaluated by means of Pearson correlation.

RESULTS

Seven out of the 20 (35%) sea bass gills sampled were infected with *Lernanthropus kroyeri*. Adult female copepods were attached to the flat, lamellae-bearing sides of the primary lamellae (gill filaments) by their second antennae and third legs. The body of each parasite was positioned between the hemibranchs, attached to the internal face, with their axis parallel to the primary lamellae axis and with their cephalic extremities oriented towards the gill arch. The intensity of infection ranged from 1 to 24 parasites per host (mean ± SE; 10.86 ± 3.19). Enhanced mucus production, congestion, haemorrhages and primary lamellae erosions resulted from parasitic infection. No other ecto-/endoparasites were found in the examined tissue.

Biometrical data and analyses resulting in significant differences are given in Table 1. Although parasitized fish were, on average, longer and heavier, they appeared more emaciated, and their condition factor was reduced. The intensity of parasitic infection was found to be correlated with the body weight of the fish (Pearson correlation: 0.98; $p < 0.05$).

Table 1. *Dicentrarchus labrax*. Biometrical data (mean ± SE) of uninfected sea bass, and of individuals parasitized by *Lernanthropus kroyeri*. ANOVA, $p < 0.05$. Different letters in the same line indicate significant differences ($p < 0.05$)

	Uninfected	Parasitized
Standard length (cm)	25.88 ± 0.59^a	29.57 ± 1.45^b
Body weight (g)	330.18 ± 20.33^a	422.51 ± 34.98^b
Hepatic weight (g)	8.87 ± 0.72^a	12.56 ± 1.57^b
Condition factor	1.88 ± 0.04^a	1.66 ± 0.12^b
Hepatosomatic index	2.72 ± 0.21	3.02 ± 0.33

Scanning electron microscopy (SEM) observations provided useful information on the mode of *Lernanthropus kroyeri* attachment. Specifically, parasites anchored themselves to the primary lamellae through the piercing action of their second antennae. The security of attachment is reinforced through the action of the maxillipeds and the third pair of legs. The latter, through their clasping action on the lamella, cause erosion of the tissue (Fig. 1). Hyperplasia of the interlamellar epithelium and partial fusion of the secondary lamellae was also observed in close proximity to the site of attachment (Fig. 1).

Histologically, at the site of parasite attachment, regressive phenomena prevailed: complete superficial tissue erosion with exposure of the primary lamellae cartilage, exposure of blood vessels and haemorrhaging resulting from the grasping action of the mandibles and the maxillae (Fig. 2). Erosion, desquamation and necrosis of the secondary (respiratory) lamellae also occurred near the site of attachment. In the distal part of the infected primary lamellae, proliferation of the interlamellar epithelium resulted in lamellar fusion and massive mucus cell proliferation. Compression by the parasite's cephalic extremity also affected the branchial afferent artery (lumen reduction) and the hemibranch adductor muscle (compression atrophy) (Fig. 3). The piercing action of the terminal claw of the second antennae resulted in tissue and vessel lacerations (Fig. 4).

DISCUSSION

Crustacean parasites are numerous and have a worldwide distribution, in fresh, brackish and salt waters (Kabata 1970). Copepods comprise the largest group of crustacean parasites on fish, numbering more than 1000 species (Kabata 1970, 1979). With regard to damage caused to fish, local and general effects have been reported (Kabata 1970). Data on the structural and functional properties of host tissue, as well as the anchorage modality and feeding habits of parasitic crustacean copepods, are available (Kabata 1970, Pike

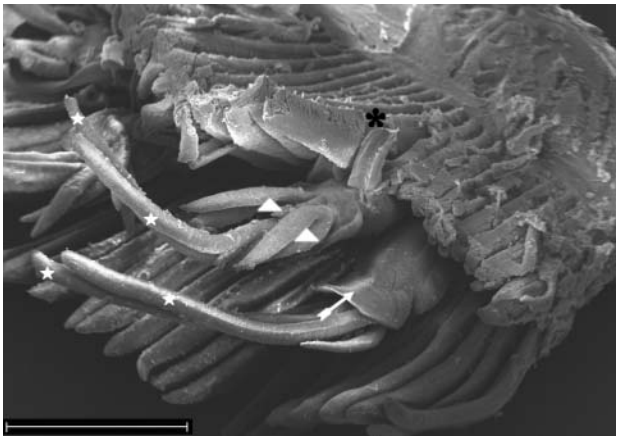

Fig. 1. *Dicentrarchus labrax* parasitized by *Lernanthropus kroyeri*. Scanning electron micrograph of an excised sea bass gill with 2 mature female specimens of *L. kroyeri* attached *in situ* between the hemibranchs. The action of the third pair of legs of the parasite effects a secure attachment on the host and causes an annular lamellar erosion about the attachment site (black asterisk). White arrowheads: 4th pair of bi-lobed and flattened legs; white arrow: dorsal shield; white stars: egg sacs. (Scale bar = 2 mm)

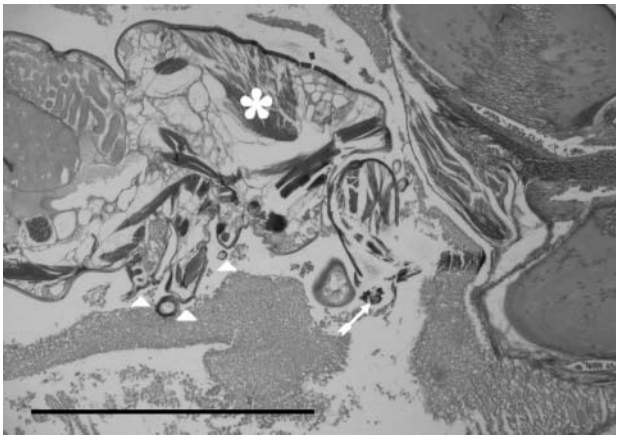

Fig. 2. *Dicentrarchus labrax* parasitized by *Lernanthropus kroyeri*. Azan-Mallory stained tissue section through sea bass gill tissue. White asterisk: cephalic extremity of *L. kroyeri*; white arrow: 2nd antenna; white arrowheads: maxillipeds. Note the extent of erosion both dorsally and ventrally. (Scale bar = 1 mm)

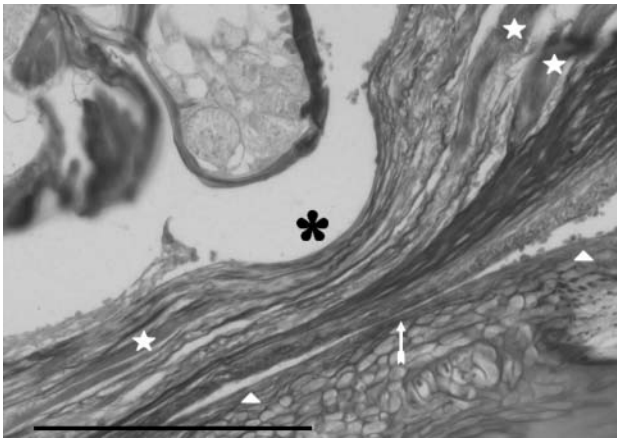

Fig. 3. *Dicentrarchus labrax* parasitized by *Lernanthropus kroyeri*. Azan-Mallory stained tissue section through sea bass gill tissue. Compression exerted by the cephalic extremity of *L. kroyeri* has resulted in erosion of the branchial lamellar epithelium (black asterisk), atrophy of the lamellar abductor muscle (white stars) and partial occlusion (white arrow) of the branchial afferent artery (white arrowheads). (Scale bar = 500 µm)

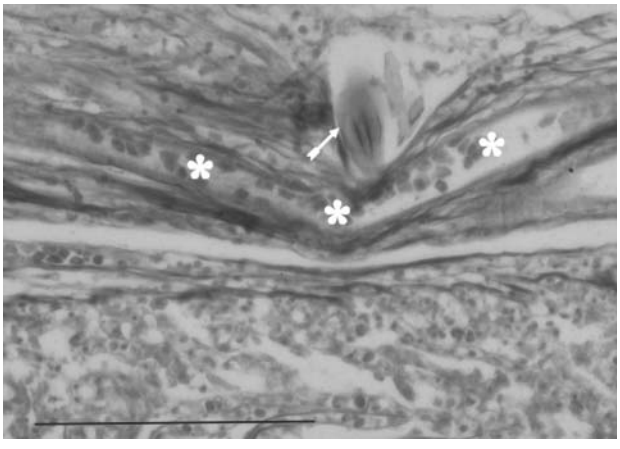

Fig. 4. *Dicentrarchus labrax* parasitized by *Lernanthropus kroyeri*. Azan-Mallory stained tissue section through sea bass gill tissue. Uncinate extremity of the second antennae (white arrow) lacerates tissue and damages vessels (white asterisks). (Scale bar = 250 µm)

& Wadworth 2000). *Lernanthropus* spp. are morphologically suited to attach to the host gill by means of the piercing action of the second antennae, which is assisted by the action of maxillipeds and the modified third pair of legs (Kabata 1979). Unfortunately, there is no specific reference concerning the local and general effects exerted by *L. kroyeri* on *Dicentrarchus labrax*, but there is a great deal of literature covering the pathological damage inflicted by crustacean genera (reviewed by Kabata 1970). Recently, Pike & Wadworth (2000) published a review on the species of sea lice parasitising salmonids. Kabata (1970) listed 3 types of local effect that crustaceans can effect on the gills of fish: occlusion of the branchial circulation, destruction due to the pressure of feeding, and hypertrophy. In this study, all 3 types of damage were detected; however, occlusion of gill circulation was the most severe type observed.

In Kabata's (1970) monograph concerning the superfamily Dichelesthioidea, which included the genus *Lernanthropus*, the author stated: 'Nothing is known about their biology', and that '… there is no evidence

that they (the members of the group with the exception of *Anthosoma*) cause excessive damage.' Nevertheless, Kabata listed a number of effects that parasitic copepods can exert on their fish hosts, e.g. changes in condition factor, hepatosomatic index, fat content, haemoglobin content, retardation in growth and of gonad maturation, metabolic disturbances, and secondary infections as a result of their feeding and attachment activity. Furthermore, Roubal (1989) reported 'compression, deformation, hyperplasia, oedema, cellular infiltration and haemorrhage' in the epithelium, and 'haemorrhage, oedema and infiltration' in the subepithelial region of the gill filaments of *Acanthopagrus australis* infected with *L. atrox*. The author attributed the massive disruption of tissue and extensive haemorrhaging to the blood-feeding activity of this copepod. Based on the evidence presented in this paper, and on the above-mentioned effects, it has been demonstrated that copepods can cause excessive damage to fish.

With regard to host size and ectoparasite settlement in *Dicentrarchus labrax* parasitized with *Lernanthropus kroyeri*, an increase in the intensity of infection in 2 yr old fish was recorded from February to July. This phenomenon was attributed to a recruitment of parasites to the host population (Davey 1980). Furthermore, the author provided detailed information regarding the distribution of this copepod on the gills, with female parasites showing a clear preference for the internal face of the medial sector of the posterior hemibranch of the second gill.

According to Davey (1980), the female's preference for this site results from an adaptation in her respiration system allowing her to exploit the strong branchial ventilation currents in this region. In conclusion, more data is required before conclusive statements as to the precise distribution of *Lernanthropus kroyeri* can be made.

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