Bull. Eur. Ass. Fish Pathol., 24(3) 2004, 144

Digenea of Adriatic cage-reared northern bluefin tuna (*Thunnus thynnus thynnus)*

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Abstract

Cage rearing of northern bluefin tuna (*Thunnus thynnus thynnus*) in the Adriatic Sea is a relatively novel branch of aquaculture, with scarce literature on fish pathologies. In this study, viscera and gills of 52 specimens of 13 kg tuna showed an interesting composition of digenean parasitofauna. Most abundant and prevalent were the members of the Didymozoidae family - *Coeliodidymocystis abdominalis, Didymocystis wedli, Platocystis alalongae, Koellikerioides internogastricus, Koellikerioides intestinalis* and *Koellikerioides apicalis*, isolated from various tissues. Ventricular, kidney and gill tissues showed disseminated granulomas, presumed to be the eggs of the blood trematode *Cardicola forsteri*, however no adult forms were found. This is the first report on tuna digenean parasitofauna in the Adriatic Sea.

Introduction

In Australian captive southern bluefin tuna (*Thunnus maccoyii*), Rough et al. (1995) stated that mortality rate induced by diverse pathological factors was up to 15 %. Today there are still a limited number of studies regarding the health status of cage-reared northern bluefin tuna (*T. thynnus*). Sporadic findings, usually related to parasitological examinations, are the most frequently reported in wild tuna populations. Even though such findings seldom lead to mortality in natural environments, mortalities can be expected in confined, highly productive and stressful rearing systems.

Detailed and elaborate review of diseases of economically important tuna species brings up a long list of isolated pathogens from wild tuna populations (Munday et al., 2003). Any of these agents are in a position to become potential threats for aquaculture.

Even in captive environments, with high stock density, artificial feeding and other anthropogenic and abiotic stimuli, which are known to induce stress, the development of disease in captive bluefin tuna is only responsible for a relatively low percentage of mortality.

During a 3.5 year period of tank-rearing yellowfin tuna (*T. albacores*) Wexler et al. (2002) observed that the majority of fish remained in good health with very low incidence of external parasitic infections. This is also reported by Sawada et al. (2002) who assigned most of the mortalities that occurred to improper management procedures of seedling production of *T. orientalis*.

The presumed high resistance of tunas against pathogens could be related to specificities of their immune system that becomes sensibilized and competitive earlier than in other fish (Sawada et al., 2002) and

posses at least two different molecular weight populations of immunoglobulins (Watts and Munday, 2001). Tuna's body temperature is markedly elevated from the ambient temperature (Stevans et al., 2000) and this could also contribute to an adequate synthesis of antibodies throughout the whole year. The only compromising factor in such a highly functional immune system is the high content of oxidized oils in tuna diets, which in other fish species act as immunosuppressors (Obach and Baudin Laurencin, 1992).

Nevertheless, with the huge growth and enormous economic profit achieved from cage-reared tuna and the quantity of input by researchers towards sophisticated and efficient seedling production, a background for possible emerging diseases has to be created, which can provide knowledge in epizootiology and pathogenesis of potential agents. As is the case for all reared species, the first investigated pathologies are related to parasitic infections.

This study is the first report on the findings of specific digenean parasitofauna in cage-reared Adriatic bluefin tuna in the Adriatic Sea.

Materials and methods

During the harvesting period of bluefin tuna in January 2003, from the facility on the NW part of Island of Braè, 52 fish were eviscerated and weighed. Samples of visceral organs and gill archs were collected. The body surface was inspected for the presence of any changes prior to technical washing of fish trunks. Because of the value of carcasses, no incisions of fins, skin or eyes were allowed.

Prior to rearing, fish were caught in May 2002, in the waters of Island of Jabuka, trawled to the farm and reared until January 2003. During that period fish were fed with a variety of fish from small trawling boats, combined with frozen imported herrings.

Samples of viscera were individually collected in plastic bags and transported to the laboratory. Blood samples were collected during the procedure before the evisceration, from the incision made beneath pectoral fins, through the heart. Sodium citrate was used as the anticoagulant.

Upon arrival in the laboratory, fresh smears of gills, kidney, spleen, liver, gall bladder, intestinal and stomach mucosa and endocardium from the ventricles were examined under the light microscope. Digenean cysts from gills, cartilaginous parts of gill arch, stomach layers, pyloric caecae, skin and intestine were collected, measured and ruptured with fine needles under the stereomicroscope. Individuals were fixed under coverslip pressure in a small quantity of Bouin's fluid, stained in Borax carmine and mounted in Canada balsam. Remaining cysts were collected and fixed in 70 % alcohol.

Results

Digenea isolated from cage-reared bluefin tuna with their prevalence, abundance, site of infection and gross pathology are shown in table 1.

Eggs of the blood fluke *Cardicola forsteri* (Sanguinicolidae) were isolated mostly from kidney, less from heart and gills. No adult were observed *in vivo* from the ventricular myocardium.

Parasite	Reference	(sub)Family	Prevalence %	Abundance	Form	Site	Gross pathology
Cardicola forsteri	Cribb et al, 2000	Sanguinicolidae	63,34	14	Eggs	Kidney, heart, gill	Granuloma
Coeliodidymocystis abdominalis	Yamaguti, 1970	Didymozoinae	63,16	3,79	Adult	Among pyloric caeca	None
Didymocystis wedli	Ariola, 1902	Didymozoinae	73,68	13,26	Adult	Gill	None
Platocystis alalongae	Yamaguti, 1938	Didymozoinae	21,05	0,95	Adult	Skin	Ishaemic area
Koellikerioides internogastricus	Yamaguti, 1970	Koellikerinae	21,05	0,32	Adult	Stomach layers	None
Koellikerioides intestinalis	Yamaguti, 1970	Koellikerinae	57,89	12,47	Adult	Intestinal serosa	None
Kolllikerioides apicalis	Yamaguti, 1970	Koellikerinae	73,68	6,79	Adult	Branch catilage	None

Table 1: Digeneans isolated from cage-reared bluefin tuna, their prevalence, abundance, site of infection and gross pathology.

Eggs were of irregular spherical shape and differing in size. They were rarely observed free in the lumen of the ventricular chamber, but mostly seen encapsulated in the tissue and surrounded by granulomatous material of different width. Granulomas measured from 25.3 x 11.8 to 65.5 x 55.2 ìm. The demarcation line between epithelioid cells and lymphocytes and healthy tissue was well defined. The inflammatory reaction in the kidney was evident by aggregation of melano-macrophage centres around the egg granuloma, while the tissue reaction in gills was not so marked.

Coeliodidymocystis abdominalis (Didymozoinae) were encapsulated in pairs under the serosa of pyloric caecae, which is an important characteristic feature of the species. Cysts of *C. abdominalis* obviously differ from cysts of other Didimozoidae members, as they are of dark ochre colour, elongated and sausage-like, presumably because of pressure imposed from neighbouring tissue. They are thin-walled and covered by pyloric serosa. In reared tuna, where the pyloric caecae are embedded deep in adipose tissue, cysts are almost hidden and not evident at first sight.

The forebody of the parasite is cylindrical and elongated, broader aborally with a rounded globular hindbody (figure 1). The oral sucker is well defined, longer than wide; a pharynx is present which is muscular and cylindrical. The oesophagus is long and bifurcation of the caecae is apparent, with broad and dark branches ending deep in the hindbody of the parasite. Two testes in the hindbody are tubular, running the full length of the hindbody. The ovary is tubular, winding and branching till the caudodorsal area of the hindbody. Vitelaria is branched and filling the remaining part of the hindbody. The uterus is filled with countless embrionated eggs. Metraterm opens at the genital pore near the oral sucker, which is slightly sigmoid.

Didymocystis wedli (Didymozoinae) was isolated from the lower third of gill lamellae, from yellow-whitish, thin-walled, ellipsoid

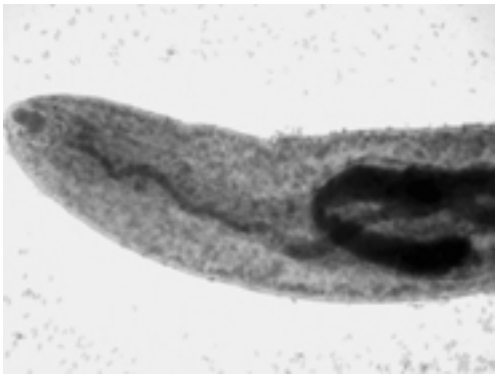

Figure 1: Cranial part of forebody of *Coeliodidymocystis abdominalis*.

cysts (figure 2). Cysts and individuals differ in size according to their age. Cysts ranged from 3 to 12 mm. They were situated in the subepithelial connective tissue of gill lamellae. The forbody is slim and elongated, with a somewhat sharp oral part. The oral sucker is piriform, strong, opening at globular and muscular pharynx. The oesophagus is long, entering wide, robust caecal bifurcation and filled with reddish ingest. The metraterm is winding parallel to the oesophagus and opening anteriorly near the oral sucker. The hindbody is voluminous and heart shaped with a curved and elevated caudal part. In the anterior part of the hindbody are located two compact, tubular testes. The ovary is tubular and branching and located near the testes. The rest of the hindbody comprises the uterus filled with eggs. Eggs embryonate after being held in seawater overnight at room temperature.

No inflammation, haemorrhages, necrosis or deletion of gill lamellae was found associated with mass infection with *D. wedli*.

Platocystis alalongae (Didymozoinae) was isolated from the laterocaudal part of the host's flanks. The parasite is non-encapsulated and attached in parallel rows of individuals, with the forbody set in the direction of swimming. It is easily noticeable by the dark orange colour (figure 3). The oral sucker is small and globular with a tiny pharynx and the genital pore opens ventrally near the oral sucker. The oesophagus is long and slightly winding. The caeca is long and slim. The hindbody is strongly dorso-ventrally flattened and fan-like. The forbody attaches between the first one-third and the rest of the anterior hindbody edge. Vitelaria is branched and tu-

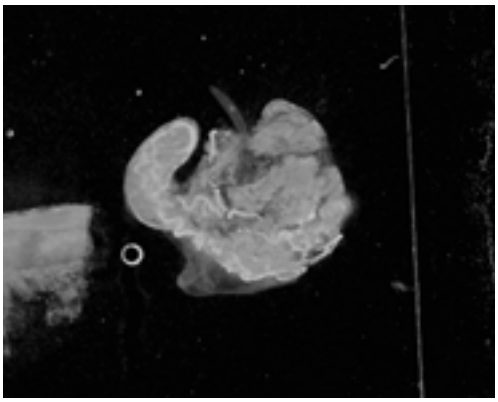

Figure 2: *Didymocystis wedli* with smashed hindbody.

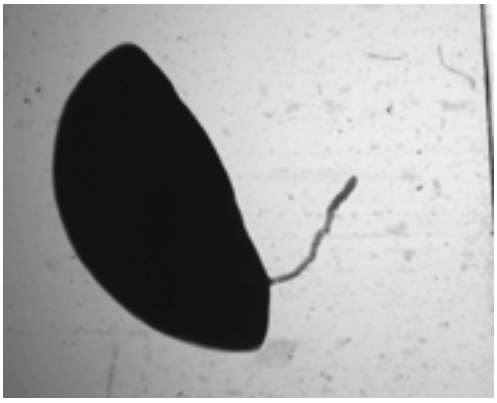

Figure 3: Adult of *Platocystis alalongae* from skin of bluefin tuna.

bular, winding caudally from the anterior part of the hindbody. Testes are near the anterior end of the hindbody, convoluted and in the vicinity of the ovary. The rest of the hindbody is filled with the uterus. Eggs are more spherical than bean-shaped.

Koellikerioides internogastricus (Koellikeriine) was isolated from the inner muscularis mucosa of the stomach wall. Cysts are oval, whitish and measured 2060.31 x 1718.55 mm (figure 4). The parasite is gonochoristic, with the female twice as big as the male. The male is situated together with the female at the transmission of her forbody into the hindbody. The forbody is spoon-like with a pronounced oral sucker, while the pharynx is feebly visible. The oesophagus is long and slender, the caecae saccular in their distal part. The genital pore opens near the ventral part of the oral sucker. The ovary is tubular and located in the anterior part of the voluminous, saccular hindbody. The male has one curved testis.

Koellikerioides intestinalis (Koellikeriine) is isolated from lengthwise submucosa of the intestine. Cysts are small (984.76 x 486.78 μm), bean-shaped, dark yellow and disseminated along the length of the intestine. Particular aggregation of cysts is noticeable in the rectal part of the intestine. The male body is half the size of the female. The pharynx is feebly visible. The oesophagus is long and slender, with elongated caecae. The male is deeply embedded in the female's hindbody. The hindbody is granular and voluminous. The ovary is situated in first third of the anterior part of the hindbody, along with one testis. No granulomatous changes or vascularisation of cysts was noted.

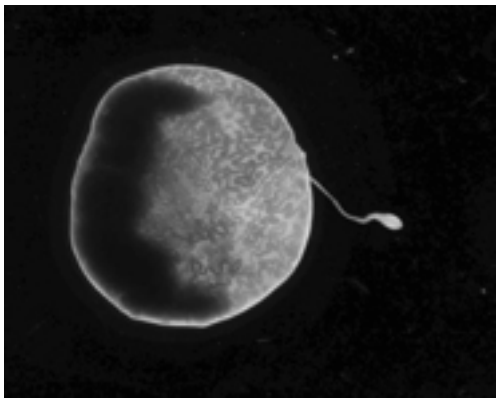

Figure 4: *Koellikerioides internogastricus* from stomach layers of bluefin tuna.

Koellikerioides apicalis (Koellikeriine) was isolated from the apical part of the pseudobranch (spherical appearance) or the cartilaginous part of the basis of the gill arch (oval). Cysts measured 2768.79 x 1174.1 μm. The oral sucker is well developed with an unpronounced pharynx. In morphology it resembles the former species, except it is confined to the cartilaginous structure of the host.

Discussion

Cardicola forsteri is a newly reported species of Sanguinicolidae that parasitizes the Australian population of southern bluefin tuna (Cribb et al., 2000). The parasite cannot be directly connected with mortalities of grown tuna, unless in combination with other drastic changes like toxic algal bloom (Munday and Hallegraeff, 1998) or when juvenile stock are heavily infected. Colquitt et al. (2001) assumed that the invasion is present in wild tuna at low level, however it is exacerbated during the rearing cycle in confined conditions. This assumption coincides with the finding in Adriatic bluefin tuna, where only reared fish at harvest show high level of in-

fection, while newly caught tunas are free (unpublished data).

Even though Adriatic tuna shows a high number of disseminated granulomas with marked inflammatory response of host tissue, without an isolation of adult parasite or development of PCR primers for *C. forsteri*, this finding remains only an assumption of possible *Cardicola* sp. infection. Nevertheless, pathology even of disputable aetiology showing high prevalence and abundance, in the most active and burdened tissue, certainly will induce high pressure on the physiological oxygen consumption that will reflect on a wide number of other physiological processes. The problem must not be neglected in the future when the rearing cycle of tunas will probably be prolonged to two years.

Didyozoids were far the most abundant and prevalent group of Digenea, with six different species isolated, belonging to two subfamilies. This is a first record of Didymozoidae in the Adriatic Sea.

The genus *Celiodidymocystis* consists of only two members: *C. abdominalis* and *C. kamegaii*, easily recognizable by the absence of the pharynx and very short oesophagus in the latter species (Yamaguti, 1970). The name of the genus points to the localizations of the cysts in the coeloma or visceral cavity, that is unusual for the Didymoziodae. However this genus along with genus *Univitellodidymocystis* once belonged to the genus *Didymocystis* (Kamegai & Araki, 1995), separated today into different genera, showing how confusing and unresolved the classification of Didymozoidae still is.

Cysts are hard to notice in well-fed reared tuna, where numerous pyloric caecae are embedded in adipose mesenterial tissue. In such conditions they assume an elongated sausage-like form imposed by the pressure of neighbouring tissue, however no evident inflammatory reaction was observed at the sight of their localization even though cysts sometimes reached up to 2 cm. Morphology of the cysts or individuals is not a base for identification of the digenean but it can provide useful information about the age of the infection.

Didymocystis wedli is maybe the best know and described didymozoid (Kohn et al., 2001). It is synonymous with *D. katsuwonicola* and *D. crassa* (Yamaguti, 1935). Based on the presence of the ventral sulcus of the hindbody, for this and 16 other similar didymozoids, a new genus *Didymosulcus* was established (Kamegai & Araki, 1995), however the old genus name is still in use (Kohn et al., 2001).

D. wedli has the highest prevalence in Adriatic bluefin tuna. A similar situation is present in yellowfin tuna *T. albacares*, where the prevalence of *D. wedli* was 36 % and the intensity ranged from 4 to 86 individuals per fish.

Its affinity for the lamellae of first gill arch supports the theory of metacercarian attachment directly from the water current in-flow, and not via the vascular routes to gills after the ingestion of infected intermediate hosts. A similar mechanism was suggested in a model for monogenean infection (Gutiérez & Martorelli, 1999).

The cysts, although not inducing any apparent inflammatory response, decrease the functional respiratory surface of gills, which in

heavy infections can markedly lower the capacity of gas exchange and induce asphyxia, especially in warmer months where manipulation with fish (tagging) often takes place. In a long-term rearing cycle this didymozoid could thus provoke significant health distress.

Platocystis alalongae is the only didymozoid found on the body surface of its host. It is non-encysted and attached as single, separate individuals. It was the first ectoparasitic didymozoid described (Yamaguti, 1958); however Yamaguti (1970) suggested a new genus *Dermatodidymocystis* for *D. viviparis* and *D. viviparoides* as two other ectoparasitic forms. The main difference between *P. alalongae* and both species of *Dermatodidymocystis* genus is that the later are viviparous, while *P. alalongae* is not. Only recently did Abdul-Salam & Sreelath (1993) isolate another flat ectoparasitic didymozoid – *Platocystoides poliaster,* but from another host fish: barracuda (*Sphyraena obtusata*). Even though the parasite does not have a well-developed ventral sucker, its hindbody is strongly attached to the dermal surface of the host. After removing the parasite, a small pallid ishemic impression is noticeable, but without erosion or haemorrhages, which suggests that the didymozoid does not feed on blood and thus does not present a major discomfort for the host.

The subfamily Koellikeriinae does not have a completely elucidated classification. Yamaguti (1935) include gonochoristic parasites in this group, which he divided into the genus *Koellikeria* (with oral and ventral sucker) and *Wedlia* (with only oral sucker). Afterwards the genus *Koellikeria* became synonymous with *Wedlia* (Yamaguti, 1958) making it impossible to distinguish the two genera. However, Yamaguti (1970) suggested the new genus *Koellikerioides* for didymozoids with only one testis and the absence of a ventral sucker. Nikolaeva (1978) described in more details the differences between *Koellikeria* and *Wedlia*, characterizing *Koellikeria* with marked sexual dimorphism and the presence of the ventral sucker, while *Wedlia* has the same morphology for males and females, which are attached at the same part of the hindbody and have a ventral sucker.

Koellikerioides internogastricus is one of two species parasitizing the inner layers of the gastric mucosa, however, Yamaguti (1970) distinguished it from *K. exsternogastricus* by the feebly branched vitelaria and localization in the inner muscularis mucosa of the stomach. Even though the globular hindbody reached a size up to 2 cm, gross pathology in stomach layers were not noticed, so except for mechanical decrease of gastric secretory and absorption surfaces, digestion processes do not seem to be affected.

Koellikerioides intestinalis showed the smallest cysts among all didymozoids, but were disseminated mostly in the caudal part of the intestine, which presumably could be an adaptation of the parasite for easier dispersion of embrionated eggs into the environment. No inflammatory response was observed in the intestinal mucosa.

Koellikerioides apicalis does not significantly differ from the previous species, except for the particular affinity for cartilaginous tissue, site of infection and the number of ovary' branches. No tissue response was induced at its localization, however, in the long term,

with growing of the cysts, mechanical pressure and tissue atrophy can be presumed to occur.

Decreased functionality of infected tissues caused by the mechanical obstacles, that the cysts represent, certainly is the primary effect of Didymozoidae on the host. However, in rearing systems where the fish is daily exposed to stress, such changes have to be investigated over longer periods to gain accurate insight into the pathology and its effect on the fish.

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