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STUDIES ON *HYSYTEROTHYLACIUM* SP. (NEMATODA: ANISAKIDAE) INFECTING KUWAITI FISHES.

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Key words: *Hysterothylacium*, Nematoda, Anisakidae, Fishes, Kuwait

ABSTRACT

A group of 186 fish specimens were obtained from Kuwait water including 37 silver pomfrets, (*Pampus argenteus*), 71 greasy groupers, (*Epinephelus tauvina*) and 78 Malabar blood snapper (*Lutjanus malabaricus*). They were examined during the period from December, 2001 to November, 2002 for helminth infection. Only one snapper fish and a small proportion of the silver pomfrets were found infected with the nematode, Hysterothylacium sp., but all the greasy groupers were free of infection with any species of helminthes. Thus no cestodes, trematodes, monogeneans or acanthocephalans were found yet in any of the examined fishes. The relationships between level of infection, sex of the infected fishes, and the environmental factors are hereby discussed.

INTRODUCTION

Kuwait is located on the North-Western part of the Arabian Gulf. It has an area of 18,000 km² and approximately 500 km coastline. The Kuwait marine environment is generally shallow with broad flats to the North, a gentle to steeply sloped shelf along the Southern coastline and a relatively deep trough inbetween (Al-Ghadban *et al.*, 2002).

Kuwait's marine waters cover approximately 8,700 km², equivalent to more than a half of the country's land area. These waters do not represent a uniform ecosystem. Rather, there is a number of distinct ecosystems which support a large diversity of marine animals and plants. Although the Arabian Gulf is adjacent to the Arabian Sea region of the Indian Ocean, it is almost separated from it by its location between the Arabian Peninsula and Iran, being virtually landlocked but for the Strait of Hormuz. Because of its isolated location, it is likely to provide a different environment from

that of the Arabian Sea and Indean Ocean for both free-living and parasitic faunas (Al-Yamani and Nahhas, 1981).

There are at least 337 species of fish belonging to 94 families in Kuwait marine waters, 44 of which are used commercially. A total of 124 species, (35%) of all Kuwait's fish fauna have been recorded from the coral reef ecosystem alone. The total production of commercial fishes from both local fishing and aquaculture meets only about 40-50% of the market demand (Al-Sarawi and Al-Obaid, 2002).

Three of the local fish species namely *Pampus argenteus* (Euphrasen) (Family: Stromateidae), known locally as "Zobaidy", *Epinephelus tauvina* (Forsskål) (Family: Serranidae), known locally as "Hamour", and *Lutjanus malabaricus*, Block and Schneider, 1801 (Family: Lutjanidae) (see Haedrich, 1967and Haedrich and Horn 1969) were chosen for this study because they are the most favoured of the commercial fishes in Kuwait (Al-Qattan, 1998). Previous records of *P. argenteus* from the Arabian Gulf include these of Misra (1947), Khalaf (1961), Mahdi (1962) and Kuronurma and Abe (1972, 1986). *E. tauvina* has been recorded by several authors from the Gulf, e.g. Regan (1905), Belgvad (1944), Khalaf (1961), Kuronurma and Abe (1972, 1986), Basson *et al.* (1977) and Sivasubramaniam and Ibrahim (1982).

Al-Yamani and Nahhas (1981) have studied adult digenetic trematodes which parasitize marine fishes of the Kuwait coast and of the Arabian Gulf. They collected 165 fishes belonging to 35 species and 19 families, and examined them for parasites. *P. argenteus* was one of the fishes examined, but it was listed among fish species not observed to harbour digeneans. *E. tauvina* was not included in that study.

Kuronurma and Abe (1972) mentioned that habitats of species of family Lutjanidae varied from shallow coastal waters including estuarine and around coral reef to offshore open waters. They also reported that such species are carnivorous in general, and include 14 or more species in the Arabian Gulf, among which *Lutjanus coccineus* is the commonest species of snapper in the Arabian Gulf and also in the market. The relationship of this species to *L. sanguineus* (Cuveir) needs clarification. According to Allen (1985), most species of *Lutjanus* feed mainly on fishes, stomatopods, gastropods, shrimps and crabs.

Dadzie *et al.* (2001a) studied the food and feeding habits of *P. argenteus* in Kuwaiti waters. They examined the stomach contents of

738 specimens. Crustaceans were dominant in the diet, in the form of copepods and their eggs. The next major food group was the bacillariophytes, followed by molluscs, fish scales and finally fish eggs and larvae. Analysis of monthly variations in stomach contents indicated that feeding intensity fluctuated throughout the year being minimal during August and September, which coincide with spawning period.

Dadzie *et al.* (2000b) studied the length /weight relationships and condition factor of *P. argenteus* in Kuwait waters. They quoted that this species, which is highly priced in Kuwait, has an estimated annual landing for the early 1980s of 600 tonnes (Morgan, 1985). *P. argenteus* also contribute to the commercial fisheries of countries bordering the Arabian Sea (Mohamed and Ali, 1992, Ali and Mohamed, 1993) and Arabian Gulf (Aagren *et al.*, 1991). Davis and Wheeler (1985) reported that *P. argenteus* has also been recorded from the North Sea.

Notable work on *P. argenteus* in Kuwait's waters includes that on stock assessment (Morgan, 1981, 1982 and 1985) and both general biology and ecology (Hussein and Abdulah, 1977; Abu-Hakima *et al.*, 1981). More recent stock assessment work was undertaken by Lee (1990), and the latest data are those of Dadzie *et al.* (1998) on spawning periodicity.

A 12 month study was carried out by Al-Marzouk and Al-Qarablly (2001) to investigate and identify different types of monogeneans infesting marine cultured orange-spotted grouper *Epinephelus coioides* (hamour), silvery Black sea bream *Sparidentex hasta* ("sobaity") and yellow-finnd sea bream *Acanthopagrus latus* ("sheim"). This study appears to be the first to record the prevalence of these fish gill parasites in Kuwait waters and to describe their new geographical records.

Tareen (1981) showed that ecto-parasites imposed serious problems and cause mortalities among the different maricultured fishes in Kuwaiti. Teng *et al.* (1983) and Victoria and Tareen (1986) [both cited from Al- Marzouk and Al-Qarabally, 2001] studied methods for control of some monogeneans of the cultured *S. hasta* and *E. coioides*. More recently, Al-Marzouk and Al-Rifae (1997) have recorded a monogenean infesting the skin and gills of tank-cultured *E. coioides*.

Nikolaeva (1985) studied the distribution and biology of the trematode fauna (Didymozoidae) and mentioned the pathology of these parasites in the marine organisms.

Abdul-Salam *et al.* (1990a) reported that the adult trematodes of the family Didymozoidae Poche, 1907 parasitize various predatory fishes, with their larvae being found in small fishes and a wide range of invertebrates. They described a larval didymozoid from the muscles of the marine fish *Nemipterus peronili* from the Arabian Gulf (Abdul-Salam *et al.*, 1990b).

Knowledge of the parasite fauna of the Arabian Gulf fishes is relatively poor in comparison with the information available for the Indian Ocean. Until recently, only scattered reports were available for both ecto- and endo-parasites from different localities. From Kuwaiti waters, Al-Yamani and Nahhas (1981) described some digenetic trematodes and obtained the acanthocolpid *Stephanostomum* from *Lutjanus coccineus*. Amin *et al.* (1984) presented a brief note on three acanthocephalan parasites, Khalil and Abu-Hakima (1985) revised one cestode parasite, Hussey (1986), Khalil and Abdul-Salam (1992), Kristsky *et al.* (2000) described monogenean parasites from the Arabian Gulf including new genera and species. Nematodes from marine fishes of Kuwait were described by Petter and Sey (1997). They reported the nematode, *Hysterothylacium* sp., as larva and *Hysterothylacium reliquens*, as adult in *Epinephelus tauvina*. They also reported the nematode *Dichelyne (D.)* sp., in *Lutjanus coccineus*. In 1998, Sey and Petter recorded the prevalence of ascaridoid larvae in Kuwait food fishes. Al-Kawari *et al.* (1994) studied some helminth parasites (Cestoda) of fishes from the Arabian Gulf, and in 1996, they studied the biodiversity of helminth parasites in the Arabian Gulf. Eslami and Mokhayer (1977) and Kardousha (1992) presented surveys of nematode parasites from Qatar. Al-Ghais and Kardousha (1994) studied some helminth parasitic larvae common in the West and East coasts of Arabian Gulf fish. Saoud *et al.* (1986) gave a preliminary survey of the digenea from fishes of Iran, Mirzayans (1970) recorded cestode parasites. Gonzales-Solis *et al.* (1997) reported some parasites of fishes from southeastern Iran. Kardousha (1992 & 1999) collected helminth parasitic larvae from the Arabian Gulf fishes and recorded for the first time some Trypanorhynch cestodes from economically important fishes.

El-Naffar *et al.* (1992) published a comprehensive study along the western and eastern coasts of the UAE between 1986 and 1988 in which they surveyed the helminth parasites of the economically important fishes. Five families of Monogenea including six genera, 11 families of digenetic trematodes including 17 genera, four families of cestodes represented by five genera, two families of nematodes

represented by three genera and two families of acanthocephalans with three genera were recorded. In Kuwaiti waters, Abdul-Salam and Khalil (1987) reported two digeneans from the intestine of the needlefish *Albennes hians* (Valenciennes) and in 1988 and 1992, Khalil and Abdul-Salam described some Monogenea from Kuwaiti waters.

Our knowledge of the digenean trematode fauna of fish from the Arabian Gulf is yet limited. Currently, only 15 species of adult trematodes have been recorded (Al-Yamani and Nahhas, 1981; Abdul-Salam and Khalil, 1987; Abdul-Salam *et al.*, 1988, 1989, 1990a,b,2000; Abdul-Salam and Sreelatha, 1992a, b, 1993a, b, 1995a, b, 1996, 1999; Bray and Cribb, 1989, 2001; Kalantan *et al.*, 1992; Saoud *et al.*, 1986, 1987, 1988a,b. 1989,Sey,1996; Sey and Nahhas, 1997; Nahhas *et al.*, 1998; Sey *et al.*, 1998; Cribb *et al.*, 1999; Nahhas and Sey, 1998, 2002; Al-Kawari, 1992; al-Kawari *et al.*, 2001) despite there are more than 465 species of fishes in the Arabian Gulf (Kuronuma and Abe, 1986) and Siddeek *et al.* (1999) reported over 350 commercial fish species from Oman, United Arab Emirates, Qatar, Saudi Arabia, Bahrain, Kuwait, Iraq and Iran. There are also more than 374 species of bivalves and gastropods (Glayzer et al.,1984) native to Arabian Gulf, Canada and Mediterranean.

Several studies were made dealing with the helminth parasites of fishes and other aquatic organisms in Europe and Atlantic areas (Margolis and Arthur, 1979; Beverley-Burton, 1984; Kabata, 1988; Arai, 1989; McDonald and Margolis, 1995; Gibson, 1991,1993, 1995, 1996a,b; Bray and Gibson, 1991a,b,1995, 1998; Bray *et al.*, 1999; Bartoli and Gibson, 1991, 1995, 1998, 2001; Taskinen *et al.*, 1991Gibson *et al.*, 1992, 1998; Bartoli *et al.*, 1993; Arthur and Albert, 1994; Valtonen and Gibson 1997; Gibson and Costa, 1997; Santoz and Gibson, 1998; Dimitrov *et al.*, 1999; Kohn *et al.*, 1999; Wayland *et al.*, 1997, 1999 and Costadinova and Gibson, 2001).

In Egypt, numerous studies were conducted concerning the helminth parasites of fishes (Hassan *et al.*, 1990a, b, 1996, 1997, 1998; Soliman and El-Damarany, 1995; Shalaby and Hassanine, 1997a, b and Al-Bassel, 1997).

The grouper *Epinephelus tauvina*, known locally as "hamour", is a highly valued food fish in all countries of the Arabian Gulf and appears to be a promising candidate for commercial fish culture. In a preliminary study on the helminth parasites of *E. tauvina* from Kuwait, Abdul-Salam and Khalil (1987) reported the presence of a long filamentous, tightly packed didymozoid inside a characteristic

yellow capsule on the gills. The didymozoid was found to represent a new species belonging to the genus *Gonapodasmius* Ishii, 1935.

Petter and Sey (1997) made a survey on nematode parasites of marine fishes from Kuwait. They reported *Hysterothylacium* sp. as larvae most frequently encountered with other anisakid larvae. They also reported *Hysterothylacium reliquens* infecting *E. tauvina*.

In 1998, Frantisek and Kazuy described a new anisakid nematode, *Hysterothylacium japonicum* sp. n. from a single female specimen collected from the stomach of the rare marine fish *Trachipterus inshikaeai* Jordan et Snyder, from the sea of Japan (Toyama Bay). Torres *et al.* (1998) described *Hysterothylacium geschei* n. sp. from the intestine of *Cauque mauleanum* (Steindachner) (Atherinidae) from lake Panguipuli, Chile.

However, up to the present, about 55 species of the genus *Hysterothylacium* have been yet described from marine, estuarine and freshwater fishes (Bruce *et al.*, 1994; Moravec *et al.*, 1997). Carpenter *et al.* (1997) considered *Lutjanus malabaricus* as synonym misidentification of *Lutjanus sanguises* (non Cuvier-1828) and *L. coccineus* (non Cuvier-1828) and they mentioned that the common name for this fish is malabar blood snapper (Al-Hamara).

MATERIAL AND METHODS

From October 2001 to September 2002, 186 specimens of three economically important fishes were collected. These were *Pampus argenteus* (37 specimens), *Epinephelus tauvina* (71specimens) and *Lutjanus malabaricus* (78 specimens). The fishes were obtained from local fishermen (at Souk-Sharaq), Kuwait.

All fishes were taken to the laboratory in ice boxes, where they were examined both macroscopically and microscopically for helminth parasites. Most tissues and organs were examined, including the gills, muscles, digestive tract, body cavity and gall bladder. They were examined for Monogenea, Digenea, Cestoda, Nematoda and Acanthocephala. Nematodes were fixed in hot 70% ethyl alcohol, cleared in lactophenol and investigated directly without staining.

The fishes were identified using guides of Kuronuma and Abe (1972, 1986), Carpenter *et al.* (1997) and with the assistance of ichthyologists of the Mariculture and Fisheries Department of the Kuwait Institute for Scientific Research.

RESULTS

Out of the 37 examined specimens of *Pampus argenteus* for parasites, only one female fish was found infected (6.7%). Such fish was caught during March 2002, harbouring a single adult nematode. Unfortunately, this specimen was in a poor condition to identify, due to the long period between fish catching and bringing them to the laboratory for investigation.

No helminthes were found in any of the examined specimens (71) of *Epinephelus tauvina*. On the other hand, in *Lutjanus malabaricus*, nematode larvae were found in Summer (20% male and 22.2% female), Spring (11.8% male and 14.3% female) and Fall (10% male) of these, were third-stage larvae of *Hysterothylacium*, some resembled *Hysterothylacium* type KD of Petter and Sey (1997). One specimen was a small adult male *Hysterothylacium*.

The rate of infection by the nematode *Hysterothylacium* sp. in male and female fishes during the study period is presented in Table1. A higher infection rate was reported in female (22.2%) than in male (20%) of *L. malabaricus* during Summer followed by Spring (Female, 14.3% and males 11.8%).

DISCUSSION

The present study was carried out for clarifying the present status of the helminth parasitic fauna of fishes caught off the coasts of Kuwait. It was found that helminth infection of the two studied fishes, *Pampus argenteus* and *Epinephelus tauvina*, is very low, as only one specimen of *P. argenteus* was found to be infected with nematode. This result accords with that of Al-Yamani and Nahhas (1981), who reported that the latter fish did not harbour trematodes. Nevertheless, El-Naffar *et al.* (1992) examined 105 fishes belonging to family Serranidae from the Arabian Gulf, where they reported 47.6% total prevalence of helminth parasites including trematodes (26.7%) cestodes (27.2%) and nematodes (29.7%). No monogeneans or acanthocephalans were found. They also examined 30 fishes belonging to family Stromateidae in the same region where they found 40% total prevalence of helminth parasites. The results of the present study agree with the above reports in that the serranid *E. tauvina* tends to be free from monogeneans and acanthocephalans, but differs in that no digeneans, nematodes or cestodes were found. Similarly, the infection of the stromateid *P. argenteus* with a

nematode support that of El-Naffar *et al.* (1992), but differs in that no cestodes were found. Moreover, the present results concerning infection of Kuwaiti fishes with larvae of *Hysterothylacium* sp. agree with that of Petter and Sey (1997) although *E. tauvina* was not infected with larvae of the nematode as they mentioned.

El-Naffar *et al.*(1992) reported, in their general survey of helminth parasites of Arabian Gulf fishes off the coast of United Arab Emirates, that all of the examined fish species were found infected with at least one group of parasite, usually a cestode or a nematode. They note an obvious relationship between the parasite fauna of fishes of the UAE coasts and that of adjacent areas, such as Qatar, Kuwait and Iran. They also mentioned that the most heavily infected fish are omnivorous or carnivorous, emphasizing the effect of host feeding habits on their parasitic infections. They examined fishes from families Lutjanidae, Serranidae and Stromatidae and reported that no acanthocephalans in their examined fishes. This is in accordance with the results of the present study. They also reported infection of *Lutjanus coccineus* with trematodes cestodes and nematodes. *E. tauvina* was infected with trematodes and nematodes while *P. argenteus* was found infected only with trematodes.

These findings do not agree with the present results as the nematode *Hysterothylacium* was found infecting only *P. argenteus* and *L. malabaricus*.

Al Qattan *et al.* (1998) reported that fishes exhibit a spectacular diversity in relation to their dentition, since tooth structure and arrangement are intimately related to the nature of the diet and the methods utilized for the acquisition of food items. They exhibit a wide adaptive radiation in their feeding habits. There is often a correlation between morphological traits and trophy role, because morphology determines how a fish can feed (Wootton,1990).

It is unequivocal that *Pampus aregnteus* feeds principally on crustaceans, with copepods constituting the dominant group (Kulkarni, 1958; Rege, 1958; Kuthalingam, 1967 and Pati, 1980). Other crustaceans were reportd in the diet of the species including ostracods and amphipods (Rege 1958; Kuthalingam, 1967) and isopods (Kulkarni, 1958). Dadzie *et al.* (2000a) investigated the feeding habits of *P. argenteus* in Kuwaiti waters by examining the stomach contents of 738 specimens. Their diet consisted of a broad spectrum of food types, but crustaceans were dominant, with copepods and their eggs constituting 39% of the total, with other, non-copepod crustaceans constituting 16%. The next major food

group was the bacillariophyta (21%), followed by molluscs (11%), fish scales (10%), and finally, fish eggs and larvae (3%). In Summer, this fish fed on a wide variety of food items than during winter. They concluded that its feeding intensity fluctuated throughout the year, with a low level during August and September, which corresponded to spawning period.

In the present study, the digestive tracts of all studied fishes (*P. argenteus, E. tauvina and L. malabaricus*) were found to contain very little food. As *P. argenteus* is considered as an omnivorous or partly herbivorous fish [as zooplankton form the major part of its diet]. Its stomach is small (Wootton, 1990) and in the present study, very few food items were found and in some cases the stomach was completely empty. Occasionally, some copepods and other crustaceans in addition to other zooplankton were detected in the stomach of this fish, which agrees with Dadzie *et al.* (2000a). Remnants of fish bodies were found in the stomach of *E. tauvina*, a carnivorous fish that has a large stomach (Wootton,1990). Bond (1979) indicated that a fish stomach generally acts to store food and initiate digestion by mixing the ingested material with the gastric juice. Depending on the feeding habits of the fish species, this organ can be large, distensible and able to deal with large prey, or small and capable of processing only small food items acquired during an extended feeding period each day. The motility of the stomach in fishes is in many instances related to its degree of fullness, so that ingesta are removed more rapidly from a full stomach than from one that is partly full.

The infection of one specimen of *P. argenteus* with the nematode (*Hysterothylacium sp.*) may be due to the feeding habits and the nature of food, which, as indicated above, is likely to consist mainly of crustaceans.

These can harbour nematode larvae, as that found together with an adult nematode in the infected fish.

The third-stage larvae of *Hysterothylacium* sp. and a single adult specimen from the intestine of specimens of *L. malabaricus* were likely acquired with their diet of fish and benthic invertebrates (Froese and Pauly, 2002). This is indicated by the presence of the larvae in the gut of these fishes. The single adult could be a permanent parasite of this fish, but the small numbers involved suggest that these worms do not normally develop in this host.

Seasonal changes in food availability could be due to changes in the habitats available for foraging, or due to the life-history

patterns of food organisms or caused by feeding activities of the fishes themselves (Wootton, 1990). This may explain the infection of one specimen of *P. argenteus* with a nematode during March. (Spring in Kuwait). At this time, the environmental factors help increase the density and variety of organisms which comprise the diet of fishes; leading to an increase possibility of infection by parasites.

However, the Gulf is considered to be highly polluted, by several pollutants which are discharged into the coastal waters. Some of the discharged pollutants may accumulate, while others represent a direct hazard due to their toxicity. Gristaldi *et al.* (1986) considered that harmful substance resulting from pollution become included in food webs and cause toxicological, oncogenic and teratogentic diseases as well as environmental mutagenesis. They also mentioned that harmful substances, such as pesticides, radioactive elements, solvents and heavy metals carried by water, cause changes in the environment with a consequent drop in indices of diversity and an increase in eutrophication phenomena, in addition to the red tide and the sudden death of thousands of the wild mullet, *Liza macrolepis* which occur frequently Bay of Kuwait, It is a benthic feeder which normally ingests large quantity of mud and food organisms such as diatoms together with frominiferan shells and crustacean fragments, (Waldock and Algoet, 2002), This fish which is known locally as the `maid` in Kuwaiti waters, may be the reason for the paucity of the helminth fauna of the studied fishes, due to a species of bacteria which lives in the sewage effluent discharged into the seawater off Kuwait.

The higher infection rates with nematode in female fish compared to males *L. malabaricus*, during all seasons of the study period, needs investigation and this is very interesting point to be studied.

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Table (1): Rate of Hysterothylacium infection of male and female fishes in Kuwaiti waters (December 2001- November 2002)

season	*Pampus argenteus*					*Epinephelus tauvina*					*Lutjana malabaricus*				
	Tot. No	male	%	female	%	Tot. No	male	%	female	%	Tot. No	male	%	female	%
Winter	9	4	-	5	-	16	14	-	2	-	11	7	-	4	-
Spring	18	3	-	15	6.7	16	14	-	2	-	24	17	11.8	7	14.3
Summer	-	-	-	-	-	31	13	-	18	-	33	15	20	18	22.2
Fall	10	2	-	8	-	8	8	-	-	-	10	1	10	-	-