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Polydora infestation on *Crassostrea madrasensis* : a study on the infestation rate and eradication methods

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Abstract

The Indian backwater oyster *Crassostrea madrasensis* (Preston) forms a significant bivalve resource of Ashtamudi Lake, India where several fishers have initiated oyster farming in the recent years. *Polydora ciliata* a common polychaete worm, which bores and resides within the oyster shells, was found in the oyster populations of this estuary. The percentage infestation in the oysters occurring in the natural bed and in different age classes of farmed oysters was studied. Infestation was low in the natural bed than in the farmed oysters. About 80% of small oysters (<6 month) were uninfested in the natural bed while in the farmed oysters of the same age group, only 44% were uninfested. The severity of infestation was observed to increase with age in the natural bed as well as in farmed oysters. Experiments were conducted to eradicate these worms by dip treatments in formalin, chlorine and freshwater. Formalin treatments in three different doses, 1000, 500 and 250 ppm for 30 minutes, 1 h and 2 h respectively were capable of removing 79.6%, 69.1% and 69.6% worms from oysters with minimum mortality (6.6, 1.6 and 0% mortality) to test oysters. Eradication treatment using chlorine at doses 1000, 700 and 500 ppm for 3, 5 and 6 h were successful in eliminating 78.3%, 65.1% and 57.7% worms from shells with test oyster mortality of 15%, 11.6% and 3.3%. Freshwater treatment for 3, 6, 9 and 12h and aerial exposure after brushing the oysters with formalin were not effective in eradicating the mudworm.

Introduction

Annelid worms of the genus *Polydora* are borers of several economically important molluscs throughout the world. Commonly known as the mud worms, these are known to use the host only as a refuge without feeding on its tissue. However, it is known that the bore holes can weaken the shells rendering the oyster shell brittle and easily broken during post harvest process like shucking, packing and transport. Though the mud worms never come in contact with the oyster meat, the oyster reacts to severe attack by forming blisters. Apart from the damage done on the shell, mud worm infestation gives a watery appearance and makes the condition of the meat poor (Skeel, 1979). Lauckner (1983) has suggested that the blisters produced by mussels in response to *Polydora ciliata* which burrows into the region of adductor muscle can result in atrophy and detachment of muscle tissue and can interfere with gamete production if the calcareous ridges press against the mantle. However, Medcof (1946) and Mohammed and Murad (1972) have reported on healthy fat oysters containing high mud worm infestation indicating that the mud worm attack does not affect the quality of

meat. Recent studies by Almeida *et al* (1996) have shown that zinc, iron and manganese contents of infested shells were significantly higher than the unifested oyster shell.

Handley (1995) has identified the different species of mud worms infesting bivalves. Considering the damage done to the oyster industry, attempts were made to eradicate them. (Thomson 1954, Baccon *et al.* 1991, Nel *et al.* 1996). In India it has been reported that mud worms infest the natural population of *Crassostrea madrasensis* (David, 1978) *C. gryphoides* (Durve, 1964) and the farmed oysters (Nayar and Mahadevan, 1983). Velayudhan (1983) tested various eradication methods for mud worms infesting the pearl oysters, *Pinctada fucata.*

Ashtamudi Lake situated between 8°45' - 9°28'N and 76°28' - 77°17'E in India supports a vast bivalve resource comprising mainly Venerid clams and oysters. The Indian backwater oyster, *C. madrasensis* and the rock oyster *Saccostrea cucullata* (Born) are regularly fished and sold in the local markets. Since 1995, farming of oysters has become an additional source of income for many fishers (Appukuttan *et al.* 2000). While studying the oyster resource in Ashtamudi Lake it was observed that *Polydora* infestation was present in both *C. madrasensis* and *S. cucullata* inhabiting the intertidal and subtidal regions (Kripa, 1998). Subsequent observations in the oyster farms also showed the infestation by mudworm. Considering the economic damage borers cause to oyster culture it was decided to study the intensity of *Polydora* infestation on *C. madrasensis* inhabiting Ashtamudi Lake, Kerala. The main objectives of the present study were 1) to understand the percentage of *Polydora* infestation in different age classes of farmed oysters and natural bed and 2) to evolve methods to eradicate *Polydora* infesting *C. madrasensis.* The results of the study were intended to provide information on successfully controlling the boring menance in commercial oyster farms.

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Material and methods

Oyster samples from the subtidal natural beds and from the oyster farm (Rack and ren) of Central Marine Fisheries Research Institute (CMFRI) were collected and analyzed during the period from January to June 1998. To study the intensity of infestation relative to age, oysters of 3 age classes viz. less than one year, between 1 to 2 year and above 2 year old were selected. The age group of cultured oysters was fixed based on the time of spat settlement on the ren, which were suspended from the farm (rack). The rens, which are suspended from the wooden rack during the spatfall season are farmed at the same site till they reach harvestable size. The data relevant to the age of oys-

ter, based on the time of spatfall was obtained from the Molluscan Fisheries Division of CMFRI.

Oysters from the natural beds were collected from the fishery sample, which represented both the intertidal and subtidal populations. This gave a representative sample of the oyster resource of the estuary. The length (in nearest mm) and shell on weight (nearest mg) of these oysters were measured. Since the age of the oysters could not be assessed directly, their age was back calculated using the formula

$$\text{Age} = t_0 - \frac{1}{K} \times \ln\left(1 - \frac{L}{K}\right)$$

where K the growth parameter was 0.179 and t_0 1.2. (Nair, 1987). The analysis showed that oysters from the natural beds belonged to all three age groups (Fig. 1). The smallest group of farmed oysters, less than one year had an average length of 31 mm and shell-on weight of 4.5 g. The average length and weight of oysters of age 1 to 2 year and above 2 year old was 65.4 mm, 47.6 g and 94.7 mm, 121.3 g respectively. The length of the oysters collected from the natural bed was between 32 and 130.5 mm while the shell on weight ranged between 5 and 225 g indicating the representation of all age classes.

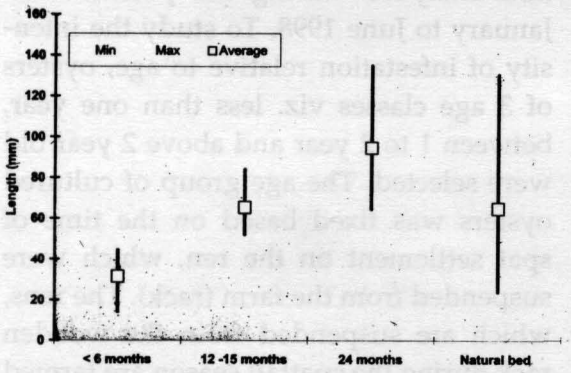

Fig. 1. *Length range of oysters analyzed for observing infestation rates.*

The oysters for analyses were declumped, cleaned and then graded by observing the inner valves. To minimize error in the larger oyster groups, the small oysters, *i. e.* - subsequent recruits on the periphery of the ren were discarded and only the initial settlers were retained. Intensity of infestation was decided according to the area occupied by the mud blister on the inner surface of the shell valves. The criterion based on which the intensity of infestation was grouped is given in Table 1.

Experiment on the eradication of Polydora infesting oyster in shell.

Formalin and chlorine are two chemicals, which are widely used for antiparasitic external treatment of fishes, crustaceans and other shellfishes. In the present experiment commercial formalin (40% formaldehyde) and chlorine solution (5%) in different doses and duration were used. Before subjecting the oysters to the test doses of formalin and chlorine, trial experiments were carried out to determine the approximate time of exposure and dosage of chemicals, which could eliminate more than 50% of the infested worms. Based on the results, the experimental doses of formalin were i) 1000 ppm for 30 min ii) 500 ppm for 60 min and iii) 250 ppm for 120 min. The test doses and

Table 1. *Grading index of Polydora infestation based on visual examination of inner side of shell valves*

Grade	Description of shell valve of oyster
Nil	No evidence of mud blisters or boring visible on the inner surface of the shells
Mild	<25% of the internal shell surface as mud blister, or any discoloration giving evidence of boring beneath the shell surface.
Moderate	>25% but < 50% of the internal shell surface as mud blister
Severe	> 50% of the internal shell surface as mud blister

duration using chlorine were i) 1000 ppm for 3 hrs, ii) 700 ppm for 5 hrs and iii) 300 ppm for 6 hrs. Apart from these two chemical treatments, the infested oysters were placed in freshwater for 3, 6, 9 and 12 h to observe its effect on a *Polydora*. Trial experiments on aerial exposure of oysters were conducted after brushing the oysters with formalin.

The oysters for the study were collected from the same site and were cleaned to remove epibionts and silt and then acclimatized for 10 days. The oysters were maintained at a stocking density of 15 no/40 l with daily water change. Salinity and temperature during this period were 29 ± 2 ppt and 30 ± 2°C. They were fed either *Chlorella* (3 to 6 million cells/ml) or mixed phytoplankton culture @ 11./oyster/day. While cleaning, the mud tubes made by the mudworm worms projecting outside the shell were also eliminated but they reappeared within 5 to 6 days. Infested oysters were identified by the presence of these external tubes and those, which showed good filtering activity, were selected. During the treatment period aeration was discontinued. Prior to the treatment the number of mudworm present in each oyster was determined by counting the prostomial tentacles of the worm which was seen on the external surface of the oyster shell.

For every treatment three replicates of 20 oysters each were kept and observations made after 15 minutes, 30 minutes, 1 h, 2 h, 24 h, and 48 h. Each group of 20 infested oysters were maintained separately in two groups, first group consisting of 17 oysters in which number of mudworm in each oyster were not counted and the second group of 3 oysters in which the number of mudworm in each oyster were counted. Behaviour of the mudworm like movement from the shell and tentacular activity, and oyster mortality were noted at the predetermined time. The test oysters were placed back after treatment in ambient seawater and the reappearance of tentacular activity and oyster moratlity (if any) were observed for two weeks. The results obtained were compared by standard analysis of variance (ANOVA) (Zar, 1974)

Results

Polydora infestation relative to age in the natural bed and in the farmed oysters.

In the natural oyster beds of Ashtamudi Lake, 80% of oysters in the age group less

than 6 months were not infested by the mud worms *Polydora ciliata* while only 44% farmed oysters of same age where uninfested. In oysters between 12 to 15 months age, the percentage of uninfected oysters was 48 and 17.4 in natural bed and in farmed oysters respectively. All farmed oysters above 2 years had *Polydora* infestation while in the natural bed 2% were still uninfected. The intensity of infestation in the same age group of oysters was found to be more in the farmed oysters than in the natural bed (Fig. 2.).

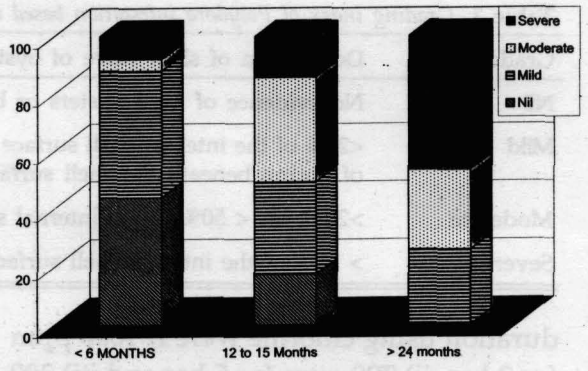

Fig. 2. *Infestation rate (%) in different age groups of farmed and natural beds*

The intensity of infestation was found to increase with age in both natural bed and farmed oysters. Servere infestation was not observed in small oysters (less than 6 month) in the natural bed while in the farm 8% of the same age group were severely infested. In the farm, the percentage of oysters with severe infestation increased from 14.3 in the first year to 46.5 after 24 months. At the same time in the natural bed in oysters above 24 months only 38% were severely infested. In mild and moderately infested oysters the meat of the oyster was not affected. In a few severely infested oysters, apart from the damage done on the shell, the meat was found to be watery while in others, firm mantle with mature gonad was observed.

Eradication of Polydora

In the present study dip treatments except freshwater were effective in eradicating *Polydora*. Experiments conducted to eradicate the worms using different chemicals gave varied mortality rates of *Polydora* (Fig. 3). Immediately on exposure to formalin and chlorine the tentacular activity of mud worms ceased and they started coming out of the shells. The tentacular activity reappeared within 24 to 48 hrs, in the first two formalin treatment while in the third and in the chlorine treatments the activity was resumed within 12 hours. In some replicates of formalin treatments the tentacular activities did not reappear even after two weeks suggesting that the mudworm suffered mortality within the shell of the oyster. Mortality of *Polydora* was highest, 79.6%

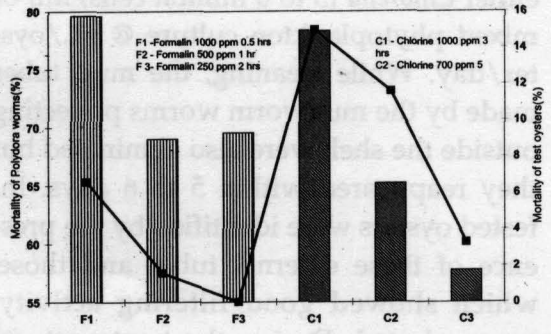

Fig. 3. *Percentage mortality of Polydora and test oysters in different treatments*

Table 2. *Percentage mortality of Polydora and test oysters*

Chemical used	Dose ppm	Duration Hours	Average mortality of *Polydora*	Average mortality of test oysters	Reappearance of tentacular movements
Formalin	1000	0.5	79.62	6.6	24 hrs to nil
Formalin	500	1	69.07	1.6	48 hrs to nil
Formalin	250	2	69.55	0	12 hrs to nil
Chlorine	1000	3	78.33	15	12 to 24 hrs
Chlorine	700	5	65.19	11.6	24 to 24 hrs
Chlorine	500	6	57.76	3.3	24 to 24 hrs
Fresh water	nil	3,6,9 & 12	nil	nil	Observed throughout
Control	nil	0.5 to 12	nil	nil	Observed throughout

in the first treatment, 1000 ppm for 30 minutes while in the 2nd and 3rd treatments the moralities were nearly 10% less than in the first treatment. ANOVA showed that there was no significant difference between the different treatments (Table 3). In the chlorine treatment the maximum mortality of worms was observed in 1000 ppm for 3 hrs while in the other two treatments the mortality was comparatively lower. The effect of chlorine treatment between the three doses showed significant difference. However considering the comparatively higher rates of test oysters it is felt that formalin treatments will be more viable commercially. Aerial exposure of oysters after brushing with formalin did not have any effect on eliminating *Polydora*. Average mortality of test oysters was 6.6% in the first treatment of formalin while in the second and third treatments the mortality was still lower.

Table 3. *Results of Analysis of Variance*

Variable	Source	Df	SS	Mean Square	F ratio	F prob
Formalin	Between groups	2	212.7269	106.364	2.5894	0.1546
	Within groups	6	249.4587	41.07 65		
	Total	8	459.1856			
	Bartlet-box F = 1.407, P = 0.251, Variance = 19.649, P = 0.358					
Chlorine	Between groups	2	650.6162	325.3081	59.4336	0.0001
	Within groups	6	32.8408	5.4735		
	Total	8	683.457			
	Bartlet - box F = 1.407, P = 0.251, Variance = 24.116					

Discussion

Mud worm infestation has been reported from the wild oyster population (Galtsoff, 1969) and in cultured oysters (Quayle and Newkirk, 1989). In the present study also mudworms were observed in oysters inhabiting the natural beds of Ashtamudi Lake as well as in the off bottom culture systems. The study revealed that the oyster population was infested by *Polydora ciliata*. The rate of infestation at the present site was much lower than infestation reported in other oyster beds. Some of the oyster populations in the temperate regions are completely infested by *Polydora* (Owen, 1957). In the pearl oyster beds of Gulf of Mannar, Alagarswami and Chellam (1976) have observed 78.4% infestation by *Polydora*. In mussel beds (Kent, 1979) and scallop populations (Mori *et al.* 1985) high degree of mud worm attack has been observed. In southern Australia, five species of polychaetes, *Polydora haswelli, P. hoplura, P. websteri, Boccardia chilensis* and *B. polybranchia* were observed in 95% of *Mytilus edulis*. Although the intensity of infection was generally low, about 15% of the mussels had serious damage caused by mudworm (Pregenzer, 1983).

In *Polydora* infested oyster shells the inner surface of shell had irregular shaped dark brown blisters. Since the parasite resides within the mud blister and does not come into contact with the actual flesh of the oyster, it would be natural to expect the effects of infestation by the worm on an oyster would be less severe than in the case of an active inter or intracellular parasite. In a general way, *Polydora* lives with an oyster, incurring advantage from the protection of the oyster shell of calcareous material (Narain, 1973). Mori *et al.* (1985) have reported that in the Japanese scallop *Patinopectin yessoensis*, the burrows penetrate the adductor muscle which may result in slow growth of the scallops. In *C. madrasensis* the damage done to the shell was very evident in the severely infested oysters.

In the present study, it was observed that the infestation rate is more in older oysters both in the farm and in the natural bed. These observations are similar to that made were by De Keyser (1987) in *C. gigas* with *Polydora* infestation. He has observed that in 2 month old *C. gigas Polydora* infestation is incidental while 60 week old oysters were nearly 100% infested. Mohammed and Murad (1972) and Mori *et al.* (1985) have recorded similar high rates in other bivalves. In the Japanese scallop *P. yessoensis*, the *Polydora* infestation was mild in one year group, while in the two year old scallops, the shells were very heavily infested by mud worms (Mori *et* al. 1985). In the pearl oyster *Pinctada margaritifera* also higher infestation was observed in older oysters (Mohammed and Murad 1972). Studies related to *Polydora* infestation and age of oysters showed that it is better to harvest the oysters within a year of growth since prolonged culture may result in oysters with higher degree of infestation. The duration of oyster culture under Indian

conditions is between 6 to 12 months. Hence the chances of extensive shell damage due to *Polydora* infestation becomes negligible. In marine mussels especially in specimens above 6 cm shell length *Polydora* infestation leads to considerable reduction in shell strength thereby increasing susceptibility to predation by crabs and birds (Kent, 1981).

Formalin treatments in pearl oyster *Pinctada fucata* were effective in removing 87.7% of infection (Velayudhan, 1983). Chemicals such as phenol (Owen, 1957) and dichlorobenzene (Mc Kensie and Shearer, 1961) have been tried to eradicate *Polydora* with little success. In chlorine treatments the mortality ranged between 3.3 and 15%. The mortality of oysters observed in the formalin and chlorine treatments is lower than the observation made in other eradication experiments (Nel *et al.* 1996).

Foulers on bivalves can be removed by keeping them submerged in hot water, in freshwater or by dipping in brine (Avault, 1998). In most bivalves exposure to freshwater has been observed to be fatal to mud worms. The annual variation in salinity in the natural oyster beds of Kerala is high, often falling below 5 ppt for 12 to 24h. during periods of heavy rain. This variation in salinity must have rendered both the oyster and the borer tolerant to freshwater exposure for periods of less than 24 hrs. Increasing the immersion time will be uneconomical and not feasible. David (1978) observed that in *Crassostrea madrasensis* population in Mulky Estuary in Karnataka, India, mud worm infestation was very low, almost nil during the monsoon months. He attributed the reason for low infestation to the almost fresh water condition of the estuary during monsoon period indicating that continued submergence in fresh water conditions for prolonged period is detrimental to the mudworm. Fresh settlement of mudworm was observed with the increase in salinity. At the farm site in Ashtamudi Lake such low saline condition does not persist for long periods due to the proximity to the sea.

The thick shell of *C. madrasensis* provides a good refuge to the borers, unlike thin-shelled bivalves like pearl oysters and mussel. Trial experiments conducted by brushing the oyster surface with concentrated formalin and keeping them exposed for 24 hrs, instead of dip-treatment were unsuccessful. This method gave good results in pearl oysters (Velayudhan, 1983) where the shell is thin and the formalin can easily penetrate into the borehole. The present study indicates that both formalin and chlorine treatments are effective methods for eradicating mud worms. Hecht and Brits (1992) pointed out that it is necessary to eliminate borers and encrusting foulers of farmed oysters to ensure high quality with respect to cup shape, taste and colour. The worm is entirely walled out from oyster meat and infestation by *Polydora* in no way renders oysters unfit for food. However oyster shells containing mud blisters have an undesirable appearance and the shells are easily broken during transport making

them unsuitable for half shell/live oyster trade. At present farmed oysters are heat shucked and the meat is marketed as a frozen product in India. With good appearance and clean shell the farmed oysters can enter the international live oyster market, which is more remunerative.

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